# ANIMAL-MEDIATED DISPERSAL IN UNDERSTUDIED SYSTEMS

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PUBLISHED IN: Frontiers in Ecology and Evolution

frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-88963-477-4
DOI 10.3389/978-2-88963-477-4

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# ANIMAL-MEDIATED DISPERSAL IN UNDERSTUDIED SYSTEMS

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**Citation:** Van Leeuwen, C. H. A., Tella, J. L., Green, A. J., eds. (2020). Animal-Mediated Dispersal in Understudied Systems. Lausanne: Frontiers Media SA.
doi: 10.3389/978-2-88963-477-4

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frontiers
in Ecology and Evolution

EDITORIAL
published: 10 January 2020
doi: 10.3389/fevo.2019.00508

# Editorial: Animal-Mediated Dispersal in Understudied Systems

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**Keywords: dispersal vector, dispersal syndrome, endozoochory, epizoochory, mutualism, plant-animal interaction, propagule, regurgitation**

**Editorial on the Research Topic**

**Animal-Mediated Dispersal in Understudied Systems**

Animals disperse many smaller organisms by ingesting, transporting and egesting propagules (endozoochory) or by carrying propagules attached to their exterior (epizoochory). Both forms of animal-mediated dispersal are generally well-studied, but most previous work focused only on a few kinds of species interactions. For example, seed dispersal by frugivorous birds and mammals, scatter-hoarding by small mammals, seed dispersal by ants, and dispersal of grasses and herbs by large herbivores have been investigated in detail. In contrast, other kinds of zoochory remain relatively unexplored, such as dispersal of propagules of aquatic invertebrates, or dispersal by vectors such as granivorous birds, fish, and reptiles. Our current knowledge on zoochory may be biased, overlooking important yet unidentified species interactions.

This Research Topic provides 14 new studies on zoochory in understudied dispersal systems to fill this gap. This collection includes reviews, statistical modeling, network analyses, field observations, and analyses of historical data. This identifies new interactions, and presents new methods and ideas for future work. The publications in this Research Topic highlight seven key points or lessons.

First, much of the plant dispersal literature is dominated by dispersal syndromes assigned based on the morphology of seeds and fruits. However, many of the studies collected here show that syndromes are not reliable and should not be assumed to reflect actual dispersal mechanisms in the absence of field studies. The "endozoochory syndrome" is generally applied exclusively to plants with a fleshy fruit and equated with "frugivory," thereby ignoring that many non-fleshy fruits may also be dispersed by endozoochory. This collection demonstrates how a wide variety of plant species generally assumed to rely on abiotic dispersal can be dispersed by endozoochory: Corvids (Green et al.), Cyprinidae fish (Boedeltje et al.), and ungulates (Baltzinger et al.) all disperse seeds without fleshy fruits. Additionally, the epizoochory syndrome often fails to predict what plants are actually dispersed via epizoochory by mammals (Baltzinger et al.).

Second, our dispersal topic shows that zoochory is not exclusive to plants, but also applies to an understudied range of other organisms—including animal propagules. Hessen et al. remind us how important zoochory of invertebrates such as cladocerans and copepods by migratory birds is, especially in areas such as the Arctic where species need to shift their distributions quickly due to climate change. Okamura et al. show us in their review that bryozoans have proved to be an excellent model of invertebrate zoochory by waterbirds, since these organisms are detected with

OPEN ACCESS

***Edited and reviewed by:***
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*British Antarctic Survey (BAS),*
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***Specialty section:***
*This article was submitted to*
*Biogeography and Macroecology,*
*a section of the journal*
*Frontiers in Ecology and Evolution*

***Received:*** *09 December 2019*
***Accepted:*** *16 December 2019*
***Published:*** *10 January 2020*

***Citation:***
*van Leeuwen CHA, Tella JL and*
*Green AJ (2020) Editorial:*
*Animal-Mediated Dispersal in*
*Understudied Systems.*
*Front. Ecol. Evol. 7:508.*
*doi: 10.3389/fevo.2019.00508*

remarkable regularity in field studies on many continents. Ironically, this taxonomic group (moss animals)—so poorly known by the general public and even by most biologists—has proved to be perhaps the best example of animal-mediated dispersal of other animals.

Third, a wide range of often-overlooked animal dispersers is identified. Parrots—often deemed only seed predators—are identified as key vectors of palm seeds and large nut-like seeds (Blanco et al.; Tella et al.), European Corvidae are rediscovered as endozoochorous vectors of over 150 plant species of which the majority lacks fleshy fruits (Green et al.), three temperate fish species disperse vegetative fragments of many vascular plants, mosses, and charophytes (Boedeltje et al.), and fleshy fruits are consumed by 470 different lizard species (Valido and Olesen). Several studies highlight that zoochory can occur by introduced animals, including ungulates (Baltzinger et al.), goats (*Capra hircus*), and pine martens (*Martes martes*) (Muñoz-Gallego et al.). This collection of studies therefore emphasizes the wide taxonomic range of vectors involved in zoochory.

A fourth key lesson is that current species interactions should be viewed in an evolutionary context (Blanco et al.; González-Castro et al.; Muñoz-Gallego et al.; Tella et al.). Plant-animal mutualisms may have evolved and then later have been disrupted by extinctions of the disperser animals. Historical dispersal interactions can be rescued by new interactions with new disperser species. Muñoz-Gallego et al. describe how two invasive mammals currently disperse a dwarf palm species, after its original dispersal vector went extinct. Blanco et al. investigate the potential of livestock to replace extinct megafauna, and González-Castro et al. identify two present-day vectors for the almost extinct plant Canary Islands dragon tree *Dracaena*.

A fifth lesson we can learn is that zoochory seems omnipresent across biomes and continents. While zoochory is most extensively studied in tropical forests and Mediterranean ecosystems, it also seems frequent for example in aquatic ecosystems, at high latitudes and in urbanized areas (Boedeltje et al.; Gelmi-Candusso and Hämäläinen; Hessen et al.; Okamura et al.). Studying species dispersal in freshwater ecosystems and at higher latitudes such as the Arctic and Antarctic is increasingly important due to the relatively strong impacts of global change there. Zoochory may be a key mechanism for species to cope with habitat reduction and fragmentation, but still more research is needed.

A sixth lesson is that zoochory can take many forms. Baltzinger et al. review the importance of seed dispersal by ungulates via endozoochory compared to epizoochory, and secondary dispersal compared to primary dispersal. They distinguish primary epizoochory (direct adhesion to fur) from secondary epizoochory (seed-containing mud adhering to animals, or transfer through contact with conspecifics), and show both overlap and complementarity of the different mechanisms. Thinking of endozoochory we usually assume seed passage through the entire alimentary canal and egestion in feces. However, also regurgitation is an important and understudied endozoochory process, both in mammals (Baltzinger et al.; Delibes et al.) and in birds whether as loose seeds or in pellets (González-Castro et al.; Green et al.). Delibes et al. focus on the spitting of seeds from the cud that occurs in mammalian ruminants, identifying at least 48 plant species belonging to 21 families that are dispersed this way. Spitting and regurgitation of seeds before digestion seems an especially important mechanism for larger-sized seeds, and it is here reported for ruminants (Blanco et al.; Delibes et al.) and birds (González-Castro et al.). For parrots and Eurasian blackcaps (*Sylvia atricapilla*), another dispersal mechanism (estomatochory) is also reported: these birds handle the fruits for consumption and disperse the seeds without having ingested them (Blanco et al.; González-Castro et al.; Tella et al.). Such synzoochory is also particularly relevant for large-seeded plants.

A final lesson we can learn from this collection of studies is that there are many new directions and technical advances that can benefit future studies. Hessen et al. highlights the importance of taking into account local species sorting and spatial scales. Even though zoochory may be frequent, community structures are importantly determined by many confounding parameters and even extensive zoochory does not have to affect communities e.g., owing to priority effects (Hessen et al.). Kleyheeg et al. estimated seed rain based on tracking data of migratory mallards (*Anas platyrhynchos*) and their experimental seed retention times. A comprehensive modeling exercise estimates how many seeds are deposited in aquatic habitats along their migratory flyways. Coughlan et al. provide a model that can be used to quantify the role of different dispersers, or intraspecific differences among animals in dispersal importance, and rank species along an axis of importance. New approaches advocated include genetic tools for assessing waterbird-mediated transport of bryozoans (Okamura et al.), and the use of dynamic seed dispersal networks to assess seed dispersal in fragmented and rapidly changing urban landscapes (Gelmi-Candusso and Hämäläinen). These new approaches will further expand the studied taxonomic range, for instance by facilitating the detection and tracking of microbial propagules such as moss spores or pathogens. All publications include many suggestions for future research directions.

In conclusion, these 14 publications jointly illustrate the extensive taxonomic range of zoochory, its omnipresence across biomes and the many ways by which animals can disperse a variety of animal and plant propagules. We hope that this Research Topic will function as a useful reference for future work on the importance of zoochory in its broadest sense, helping to emphasize its importance as a cosmopolitan source of connectivity. With global change and human pressure on ecosystems increasing, it is important to understand the contribution of natural and anthropogenic connectivity to the survival of native species and the spread of alien species worldwide. We hope this Research Topic provides an improved understanding of the contribution of zoochory to this connectivity – and hope it stimulates further investigation of zoochory in understudied systems.

## AUTHOR CONTRIBUTIONS

CL wrote the first draft of the manuscript. JT and AG edited and contributed additional sections to the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

## FUNDING

This study was supported by Marie S-Curie Actions–H2020 grant 750240 of the EU to CL, and Spanish National Plan project CGL2016-76067-P (AEI/FEDER, EU) to AG.

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Ecology and Evolution

PERSPECTIVE
published: 15 February 2019
doi: 10.3389/fevo.2019.00029

# Waterbird-Mediated Dispersal and Freshwater Biodiversity: General Insights From Bryozoans

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**Specialty section:**
*This article was submitted to
Biogeography and Macroecology,
a section of the journal
Frontiers in Ecology and Evolution*

**Received:** *28 September 2018*
**Accepted:** *28 January 2019*
**Published:** *15 February 2019*

**Citation:**
*Okamura B, Hartikainen H and Trew J
(2019) Waterbird-Mediated Dispersal
and Freshwater Biodiversity: General
Insights From Bryozoans.
Front. Ecol. Evol. 7:29.
doi: 10.3389/fevo.2019.00029*

Freshwater environments are fragmented and heterogeneous in space and time. Long term persistence thus necessitates at least occasional dispersal of aquatic organisms to locate suitable habitats. However, the insubstantial movements of many require zoochory—hitchhiking a ride with more mobile animals. We review evidence for waterbird-mediated zoochory of freshwater bryozoans, a group that provides an excellent model for addressing this issue. The feasibility of long distance transport by waterbirds of bryozoan propagules (statoblasts) is evaluated in relation to statoblast resistance to extreme conditions and waterbird gut retention times, flight durations and distances. We highlight genetic evidence for colonization following waterbird-mediated transport. The consequences of zoochory for biodiversity are manifold. Taxa that release statoblasts show lower levels of genetic differentiation, genetic divergence and haplotype diversity than those whose statoblasts are retained *in situ* (hence less available for zoochory). Zoochory may also disseminate pathogens and parasites when infected host stages are transported. Such co-dispersal may explain some disease distributions and is supported by viability of infected statoblasts. Zoochory can also be expected to influence local and regional population and community dynamics, food web structure and stability, and organismal distributions, and abundances. Finally, zoochory may influence host-parasite coevolution and disease dynamics across the landscape with the benefits to parasites depending on their life history (e.g., simple vs. complex life cycles, generalists vs. specialists). Our synthesis highlights the complex ecological and evolutionary impacts of zoochory of freshwater organisms and raises questions for future research.

**Keywords: waterbirds, migration, statoblasts, dispersal potential, evolutionary consequences, genetic flow, divergence, parasite co-dispersal**

## INTRODUCTION

Freshwater environments are patchy in space and time and surrounded by an inhospitable landscape. Such patchiness can challenge aquatic organisms when their habitats deteriorate. Larger mobile animals may actively escape such conditions, colonizing distant more suitable sites by flying, swimming walking, hopping, or crawling. Many freshwater invertebrates, however, are incapable of undertaking sufficiently large-scale movements to ensure successful dispersal. Instead they rely on animals with shared habitats to transport resistant stages. Such passive dispersal (Bilton et al., 2001) to new sites mitigates against local extinction and entails potential impacts on biodiversity. Freshwater bryozoans have proven to be an excellent model system to reveal how aquatic invertebrates depend on transport by mobile animals (zoochory) to ensure their long term persistence in dynamic and patchy freshwater environments (Okamura and Freeland, 2002) and the consequences of such dispersal for biodiversity and evolution.

Here we review evidence for zoochory by waterbirds and evaluate the feasibility of occasional long distance transport by migratory birds. We examine how rates of zoochory may vary amongst bryozoan taxa and associated clade-dependent patterns of diversification. Finally, we consider the consequences of zoochory for community development and biodiversity revealed by the potential for co-dispersal of infectious agents in dormant dispersive propagules. The introduction of parasites via zoochory of such infected propagules could impact multiple host populations, food webs and metacommunity dynamics across the landscape. Our collective evidence simultaneously illustrates the importance of waterbird-mediated zoochory for ecological and evolutionary processes in inland waters and raises many general questions for future research.

## THE LIFE HISTORY OF FRESHWATER BRYOZOANS

Freshwater bryozoans (Phylum Bryozoa: Class Phylactolaemata) comprise a relatively small group of exclusively freshwater taxa, with <100 described species (Massard and Geimer, 2008). During favorable conditions, bryozoans grow as colonies of physiologically-connected individual modules called zooids. Although not widely recognized, colonies are commonly found on macrophytes, submerged branches and tree roots, rocks, and man-made surfaces such as piers, buoys, floats, plastic, and boat hulls in both lotic and lentic environments. Each zooid possesses a tentacular crown (the lophophore) whose ciliary currents enable feeding on suspended particles. Budding of new zooids increases colony size and determines a range of colony morphologies (**Figure 1A**).

The life history of freshwater bryozoans entails extensive clonal reproduction in the form of colony growth, colony fragmentation or fission, and the production of specialized stages (statoblasts) that enable persistence during adverse conditions (**Figure 1A**). Statoblasts are asexual propagules with germinal tissue enclosed within protective chitinous valves. Low metabolism enables dormancy and survival during unfavorable times. When favorable conditions return statoblasts "hatch" and the first zooid of a new colony emerges from separated valves. Non-dormant statoblasts can contribute to overlapping generations within a single growing season (Brown, 1933; Wood and Okamura, 2005). Dormant statoblasts overwinter in temperate regions and may enable persistence during dry seasons in tropical environments (Wood, 2002).

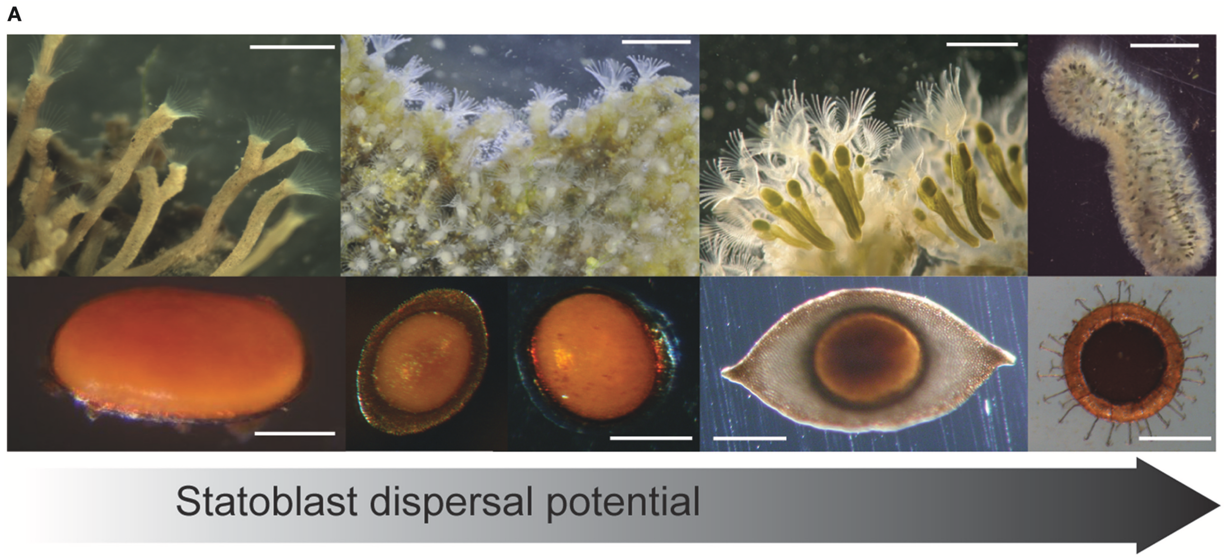
Many statoblasts are buoyant and float after release from bryozoans. Some of these "floatoblasts" have projections such as hooks and spines (e.g., *Cristatella mucedo*; **Figure 1A**) enabling attachment to various surfaces, including feathers (Figure 1 in Bilton et al., 2001). Other statoblasts are not released, remaining quiescent in the previously favorable parental microhabitat ("sessoblasts" in *Plumatella* and "piptoblasts" in *Fredericella*; **Figure 1A**). *Plumatella* species produce both floatoblasts and sessoblasts—a dispersal polymorphism (Karlson, 1992).

Statoblasts production varies from <1 to up to 27 per zooid dependent on species (Bushnell, 1973; Wood, 1973; Karlson, 1992) and typically peaks at the end of the growing season (e.g., in late summer/early autumn in temperate regions). Brown (1933) estimated that some 80,000 statoblasts could be released from *Plumatella repens* colonies growing on an average-sized *Potomageton natans* plant. Collectively huge numbers of statoblasts can be produced within sites. For example, a 0.3–1.2 m-wide shore drift of statoblasts extended for 0.8 km along a bay of Douglas Lake, Michigan (Brown, 1933). These various statoblast features support another critical function: dispersal amongst sites.

## WATERBIRD-MEDIATED STATOBLAST TRANSPORT: THE EVIDENCE

Statoblasts are repeatedly documented in feces and digestive tracts or on external surfaces of waterbirds (**Table 1**). Their transport could be achieved externally (ectozoochory) if they are attached to feathers, reside in mud clinging to birds, or adhere by surface tension or electrostatic attraction. Alternatively statoblasts may be ingested and excreted by birds (endozoochory). The majority of birds providing evidence for statoblast transport are dabbling and diving ducks, but coots, plovers (killdeer), and piscivorous birds (cormorants and pelicans) are also implicated. Bird prey may be relevant as statoblasts are recorded as fish dietary items (e.g., Osburn, 1921; Dendy, 1963; Marković et al., 2009). This collective evidence suggests that a diversity of birds could mediate both local and long distance dispersal.

For effective dispersal statoblasts must survive adverse conditions during transit. Statoblasts can hatch following exposure to desiccating conditions, freezing temperatures, and repetitive freezing and thawing (Brown, 1933; Hengherr and Schill, 2011; Abd-Elfattah et al., 2017). A proportion is typically also viable after passing through the digestive tracts of salamanders, frogs, fish, turtles, and ducks (Brown, 1933; Charalambidou et al., 2003a; Green et al., 2008; Brochet et al., 2010; Abd-Elfattah et al., 2017; Van Leeuwen et al., 2017) although, if assessed, hatching is reduced relative to controls. Intact statoblasts of four *Plumatella* species have been observed in 7.9% of 228 lower digestive tracts (ceca and intestine, where little digestion occurs) of 10 waterbird species (Figuerola et al., 2004). Bird species with heavier (potentially more destructive) gizzards and shorter ceca had a lower incidence and abundance of statoblasts in the lower gut. These results suggest that statoblast dispersal is more likely in birds with lighter gizzards and that longer ceca will entail a longer passage time which, in turn, may favor long distance dispersal. There is limited evidence that endozoochory is more common than ectozoochory (but viability was not tested) and that some waterbird species are more important as vectors, however sample sizes were low and analyses based on pooling statoblasts and cladoceran ephippia



**B**

| | *F. sultana* | | | | *C. mucedo* | | | |
|---|---|---|---|---|---|---|---|---|
| | Variance | % total | Φ-statistic | *P* | Variance | % total | Φ-statistic | *P* |
| Among countries ($V_a$) | 4.021 | 33.04 | $\Phi_{CT}$ = 0.331 | 0.000 | -0.011 | -1.15 | $\Phi_{CT}$ = -0.012 | 0.255 |
| Among populations within countries ($V_b$) | 4.212 | 34.61 | $\Phi_{SC}$ = 0.517 | 0.000 | 0.416 | 43.44 | $\Phi_{SC}$ = 0.429 | 0.000 |
| Within populations ($V_c$) | 3.938 | 32.35 | $\Phi_{ST}$ = 0.676 | 0.000 | 0.553 | 57.71 | $\Phi_{ST}$ = 0.423 | 0.000 |

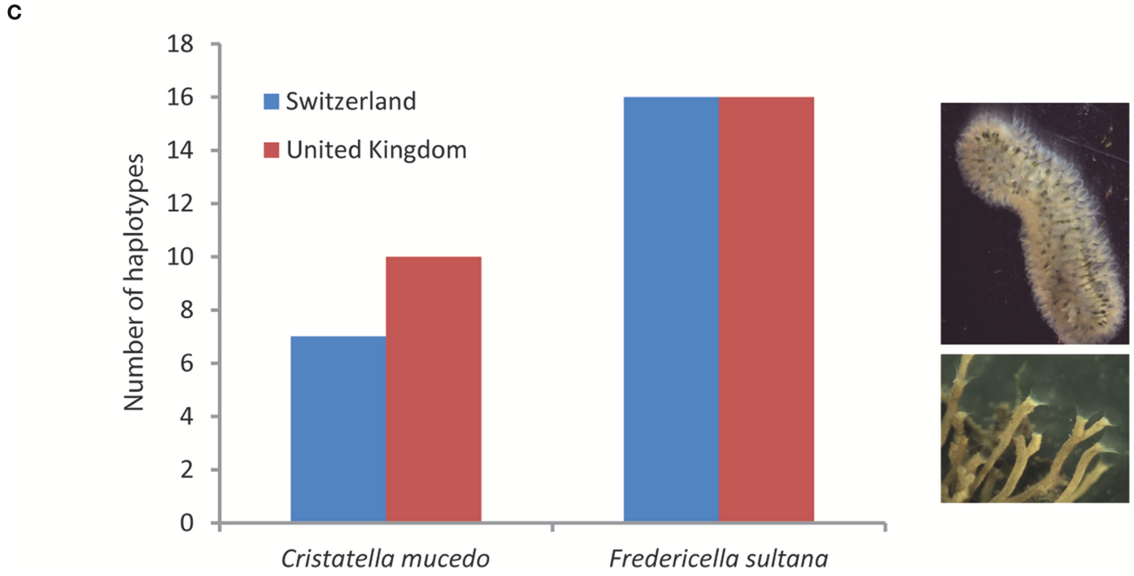


**FIGURE 1 |** Variation in dispersal potential (in terms of statoblast release, buoyancy, presence of hooks and spines) of freshwater bryozoans by waterbirds and its consequences. **(A)** Bryozoan colonies (upper panel from left to right: *Fredericella* sp.; *Plumatella casmiana* [image courtesy of T. Schwaha]; *Lophopus crystallinus*; *Cristatella mucedo*) and their associated statoblasts (lower panel from left to right: *Fredericella* piptoblast [image curtesy of T. Wood]; *Plumatella* floatoblast and sessoblast; *Lophopus* floatoblast; *Cristatella* floatoblast with hooks and spines) arranged according to increasing dispersal potential. Scale bars upper panel from left to right: 2 mm, 1.2 mm, 0.8 mm, 6 mm. Scale bars lower panel from left to right: 0.3 mm, 0.4 mm, 0.4 mm, 0.5 mm. **(B)** Analysis of molecular variance (AMOVA) of the mitochondrial DNA (nad4/H region) for *Fredericella sultana* and *Cristatella mucedo* in Switzerland and the UK (see **Supplementary Tables 1, 2** for summary of population data). **(C)** Numbers of haplotypes of *Fredericella sultana* and *Cristatella mucedo* in Switzerland and the UK. Produced in Arlequin v3.5.1.2 (Excoffier and Lischer, 2010).

**TABLE 1 |** Statoblasts present in feces or digestive tracts of waterbirds or collected externally from plumage or feet according to bird species and geographic region.

| Waterbird species | Geographic region | Statoblast collection | References |
|---|---|---|---|
| Blue-winged teal (*Anas discors*) | Illinois | DT | Anderson, 1959 |
| American pintail (*Anas acuta*) | Illinois, USA | DT | Anderson, 1959 |
| Green-winged teal (*Anas carolinensis*) | Illinois, USA | DT | Anderson, 1959 |
| American wigeon (Baldpate) (*Mareca americana*) | Illinois, USA | DT | Anderson, 1959 |
| Lesser scaup (*Aythya affinis*) | Illinois, USA | DT | Anderson, 1959 |
| Ring-necked duck (*Aythya collaris*) | Illinois, USA | DT | Anderson, 1959 |
| Redhead (*Aythya americana*) | Illinois, USA | DT | Anderson, 1959 |
| Shoveler (*Spatula clypeata*) | Illinois, USA | DT | Anderson, 1959 |
| Eurasian teal (*Anas crecca*) | Southern France; Northeast France | E[a], DT[a], DT[b] | Mouronval et al., 2007[b]; Brochet et al., 2010[a] |
| Gray teal (*Anas gracilis*) | New South Wales, Australia | F | Green et al., 2008 |
| Eurasian coot (*Fulica atra*) | Australia[a], southern Spain[b]; Northeast France[c] | F[a], DT[b], DT[c] | Figuerola et al., 2004[b]; Mouronval et al., 2007[c]; Green et al., 2008[a] |
| Black swan (*Cygnus atratus*) | New South Wales, Australia | F | Green et al., 2008 |
| Australian pelican (*Pelecanus conspicillatus*) | New South Wales, Australia | F | Green et al., 2008 |
| Northern shoveler (*Anas clypeata*) | Southern Spain | DT | Figuerola et al., 2004 |
| Mallard (*Anas platyrhynchos*) | Southern Spain; Northeastern France | DT[a], DT[b] | Figuerola et al., 2004[a]; Mouronval et al., 2007[b] |
| Red-crested pochard (*Netta rufina*) | Southern Spain | DT | Figuerola et al., 2004 |
| Greylag goose (*Anser anser*) | Southern Spain | DT | Figuerola et al., 2004 |
| Ruddy duck (*Oxyura jamaicensis*) | Southern Spain | DT | Sánchez et al., 2000 |
| Ruddy duck (*Oxyura jamaicensis*)/White-headed duck (*O. leucocephala*) hybrids | Southern Spain | DT | Sánchez et al., 2000 |
| Marbled teal (*Marmaronetta angustirostris*) | Southern Spain | F, F | Green and Sánchez, 2003; Fuentes et al., 2004 |
| Great cormorant (*Phalacrocorax carbo*) | Southern Sweden; The Netherlands | F | Van Leeuwen et al., 2017 |
| Widgeon (*Anas penelope*) | Northeast France | DT | Mouronval et al., 2007 |
| Pochard (*Aythya farina*) | Northeast France | DT | Mouronval et al., 2007 |
| Goldeneye (*Bucephala clangula*) | Northeast France | DT | Mouronval et al., 2007 |
| Tufted duck (*Aythya fuligula*) | Northeast France | DT | Mouronval et al., 2007 |
| Killdeer (*Charadrius vociferous*) | Oklahoma, USA | F | Green et al., 2013 |
| Egyptian goose (*Alopochen aegyptiaca*) | Southern Africa | F, E | Reynolds and Cumming, 2015 |
| Spur-winged goose (*Plectropterus gambensis*) | Southern Africa | F | Reynolds and Cumming, 2015 |
| Yellow-billed duck (*Anas undulata*) | Southern Africa | F | Reynolds and Cumming, 2015 |
| White-faced duck (*Dendrocygna viduata*) | Southern Africa | F, E | Reynolds and Cumming, 2015 |
| Cape shoveler (*Anas smithii*) | Southern Africa | F | Reynolds and Cumming, 2015 |
| Lesser-black backed gull (*Larus fuscus*) | Southern Africa | F, DT | Lovas-Kiss et al., 2018a |

*Statoblasts were generally intact (not fragments). Material collected as feces excreted in the environment or sampled from birds caught for ringing, from birds shot during the hunting season, or opportunistically (availability of dead birds). Where statoblasts were collected (Statoblast collection) identified as: F, in fecal samples; DT, in digestive tract sample; E, on external surfaces (plumage or feet). Superscripts a–c link statoblast collection information with reference.*

(Reynolds and Cumming, 2015). We must stress that zoochory is likely to be hazardous for statoblasts. Statoblast fragments in waterbird digestive tracts (e.g., Brown, 1933; Figuerola et al., 2004) suggest some break down and hatching of intact statoblasts retrieved from feces is reduced (unlike in some plant seeds; Jaganathan et al., 2016). Finally, zoochory is likely to be a relatively rare event, as most statoblasts will overwinter as uningested propagules.

Retention time in digestive tracts will critically determine potential dispersal distances and can vary with material ingested. Ten to 26 and greater 72 h have been estimated for mallard (*Anas platyrhynchos*) and blue-winged teal (*Anas discors*), respectively (Brown, 1933; Swanson and Bartonek, 1970; Agami and Waisel, 1986). Charalambidou et al. (2003a) found most *Cristatella mucedo* statoblasts were retained for 4 h but maximum retention times were 44 and 32 h in pintail (*Anas acuta*) and shoveler (*Spatula clypeata*), respectively. Although increased retention times may decrease viability as demonstrated for seeds (e.g., Charalambidou et al., 2003b), an early study concluded there was no relationship between length of time statoblasts were retained and subsequent hatching (Brown, 1933). Insights on waterbird flight speeds, distances traveled and measured retention times could enable occasional long distance dispersal of viable statoblasts. For example, a telemetry-based study found a maximum non-stop distance for pintail of 2,926 km over 38 h (using an average groundspeed of 77 km/h for a flight from southern Oregon to the Kenai Peninsula, Alaska; Miller et al., 2005). Some mallards appear to fly non-stop from Arkansas to the Prairie Pothole Region in Canada (Krementz et al., 2011). We estimate this would also take around 38 h [considering a mean mallard airspeed of 70.9 km/h (Pennycuick et al., 2013) and a linear distance of 2,675 km that modestly spans the migratory route]. These estimated non-stop flight times might enable dispersal over thousands of kilometers. However, many migrating waterbirds stop to feed. Average distances of such mallard migratory "legs" were 757, 446, and 664 km in 2004, 2005, and 2006, respectively (Krementz et al., 2011), translating to some 11, 6, and 9 h of flight time (based on the above mean mallard airspeed). Pintail migratory legs times ranged from 1.64 to 5.12 h with associated travel distances of 99.8–393.3 km (Miller et al., 2005). Greater numbers of viable statoblast are likely to be introduced by such migratory legs.

The association of statoblasts with many waterbirds and the potential for transport given their resistant nature along with gut retention and bird migration times suggest that occasional long distance dispersal over hundreds to thousands of kilometers is feasible given the huge numbers of waterbirds undergoing such regular movements. In view of the disproportionate influence of such rare events on colonization, gene flow and population genetic structure, a recent operational definition for long distance dispersal involves crossing geographic boundaries of populations and contributing to effective gene flow (Jordano, 2017). While long distance dispersal is difficult to robustly characterize it is indeed specifically proposed to explain the broad geographic distributions of many freshwater bryozoans along waterbird migratory routes (Wood, 2002; Wood et al., 2006). The movements of more sedentary birds may contribute to short distance dispersal across local landscapes. Evidence that waterbird-mediated dispersal actually effects colonization comes in the form of genetic studies.

## GENETIC EVIDENCE THAT WATERBIRDS PROMOTE COLONIZATION

The freshwater bryozoan, *Cristatella mucedo* (**Figure 1A**), has been especially valuable in demonstrating ongoing waterbird-mediated dispersal of freshwater organisms (De Meester et al., 2002; Okamura and Freeland, 2002). Freeland et al. (2000a) used microsatellites to characterize 14 populations collected along a major waterbird migratory route traversing northwestern Europe. Low levels of gene flow linked populations across the region and colonies with identical genotypes were collected from several sites, including two sites separated by 700 km of land and sea (in Sweden and The Netherlands). The latter result implies long distance transport of statoblasts by waterbirds. In addition, discriminant function analyses assigned 14% of individual colonies to populations other than those from which they were collected. The highest number of such cases was recorded for the Nationaal Park de Biesbosch—an important stopover site in The Netherlands for migratory waterfowl. In contrast, microsatellite analysis of 10 North American *C. mucedo* populations revealed highly differentiated populations with little evidence of recent gene flow across regions not linked by common waterbird migratory routes (Freeland et al., 2000b). Discriminant function analysis mis-assigned 8% of individual colonies to other populations and no clonal genotypes were shared between populations. More direct comparisons based on microsatellite markers common to both studies (Freeland et al., 2000c) revealed higher gene flow amongst European populations and mis-assignment of 32.5% of European colonies compared to 18.3% of colonies from North America. Finally, band recovery data (Figuerola et al., 2005) were shown to explain a significant proportion of variation in both genetic distance and gene flow amongst North American *C. mucedo* populations (and in two of three cladoceran species), even when controlling for geographic distance.

Collectively this body of evidence implies that waterbirds regularly disperse statoblasts and contribute to the metapopulation dynamics *C. mucedo* in both Europe and North America.

## DIFFERENTIAL DISPERSAL AND EVOLUTIONARY CONSEQUENCES

The frequency and magnitude of waterbird-mediated dispersal and associated gene flow can be expected to influence genetic differentiation. Panmixia and low genetic differentiation between sites should result from high dispersal rates. As dispersal rates decrease, genetic differentiation will increase due to processes such as founder effects, genetic drift and natural selection. Genetic differentiation may ultimately lead to speciation if dispersal rates are very low and/or selection pressure is very high.

Accordingly, statoblast trait-linked influences on zoochory (e.g., buoyancy, hooks, and spines) appear to explain some patterns of genetic differentiation and divergence in freshwater bryozoans. Thus, molecular phylogenetic analysis has revealed contrasting patterns of divergence amongst bryozoan clades that vary in dispersal potential (Hartikainen et al., 2013). *Fredericella* exhibits a propensity for cryptic speciation and phylogeographical structure while *Plumatella* species exhibit low intraspecific divergence. Although sample sizes were small, these patterns are consistent with limited vs. widespread gene flow in fredericellids (which exclusively produce attached piptoblasts) and plumatellids (which produce both floatoblasts and sessoblasts), respectively (**Figure 1A**).

In a further relevant study we undertook matched sampling of *F. sultana* and *C. mucedo* populations in the UK and Switzerland (**Supplementary Tables 1, 2**). Contrasting patterns of genetic variation were in keeping with expected differences in dispersal potential. Analysis of molecular variance (AMOVA; **Figure 1B**) revealed greater genetic divergence between countries for *F. sultana* (which retains piptoblasts) compared to *C. mucedo* (which releases floatoblasts) (0.331 vs. −0.012, respectively). The lack of between country genetic divergence for *C. mucedo* populations suggests that dispersal over hundreds of km is more frequent than local dispersal. Accordingly, there is relatively high within country genetic divergence (0.429). These results implicate waterbirds as primary vehicles of *C. mucedo* dispersal. In addition, *F. sultana* exhibited a greater number of haplotypes than *C. mucedo* (**Figure 1C**). Analyses of data pooled across countries provided evidence for a greater number of haplotypes in *F. sultana* (32 vs. 17 for *F. sultana* and *C. mucedo*, respectively; $\chi^2 = 4.592$, $p = 0.032$). The proportion of unique haplotypes across all populations was not significantly different between the species (23/32 vs. 9/17 for *F. sultana* and *C. mucedo*, respectively; $Z$-test $= 1.325$, $p = 0.183$), but a significantly greater proportion of haplotypes was unique to populations of *F. sultana* (15/32 vs. 0/17 for *F. sultana* and *C. mucedo*, respectively; $Z$-test $= 3.388$, $p < 0.001$).

## POTENTIAL FOR PARASITE CO-DISPERSAL

Waterbirds carrying infections have been particularly implicated in dispersal of disease agents—examples being avian influenza virus (Lebarbenchon et al., 2009), schistosomes causing human cercarial dermatitis (Ebbs et al., 2016) and West Nile virus (Rappole et al., 2000). The potential for zoochory of infected hosts is, however, largely overlooked. Nevertheless, many parasites and pathogens may benefit from and be adapted to such dispersal. Thus, birds may facilitate dispersal of disease agents, although, unlike traditionally recognized disease vectors (e.g., mosquitoes), they do not effect transmission to new hosts. Dispersal of infected statoblasts by waterbirds is a salient example of parasites that hitchhike along with their host.

Myxozoans are endoparasitic cnidarians with complex life cycles, exploiting invertebrate and vertebrate hosts (Okamura et al., 2015). One myxozoan clade—the Malacosporea—uses freshwater bryozoans as hosts. The malacosporean *Tetracapsuloides bryosalmonae* is the causative agent of proliferative kidney disease (PKD) which results in serious economic loss to trout farms and is an emerging disease in wild salmon and trout populations (Okamura et al., 2011; Skovgaard and Buchmann, 2012; Dash and Vasemägi, 2014; Mo and Jørgensen, 2017; Vasemägi et al., 2017). The interactions of *T. bryosalmonae* with its bryozoan host, *F. sultana*, have thus received considerable investigation. *Fredericella sultana* statoblasts support cryptic *T. bryosalmonae* stages and colonies derived from these statoblasts develop infections (Abd-Elfattah et al., 2014, 2017; Fontes et al., 2017) that, in turn, transmit to fish (Abd-Elfattah et al., 2014). *Tetracapsuloides bryosalmonae* thus achieves vertical transmission (infection of new bryozoan colonies) via infection of statoblasts—a strategy that may introduce the parasite to new populations should infected statoblasts be transported by waterbirds.

Statoblast infection prevalences can be substantial. For example, 39% ($n = 54$) and 30% ($n = 165$) of statoblasts collected from *F. sultana* colonies in two river systems were infected with *T. bryosalmonae* and 95% ($n = 46$) and 100% ($n = 21$) of these infected statoblasts successfully hatched (Abd-Elfattah et al., 2014). Infection of statoblasts was similarly inferred for the malacosporean *Buddenbrockia allmani,* with infections detected in 9 of 10 statoblast-derived colonies of *Lophopus crystallinus* (Hill and Okamura, 2007). Although there is only a handful of described malacosporeans (Patra et al., 2017), molecular detection of unique isolates in both bryozoans (Hartikainen et al., 2014) and fish (Bartošová-Sojková et al., 2014) suggests that statoblasts may often carry malacosporean infections. Indeed, restriction fragment length polymorphisms and sequencing have revealed infections (including co-infections) of at least five malacosporean species in *C. mucedo* statoblasts (Ruggeri, Corbishley and Okamura, unpublished data). Survival of infected statoblasts following passage through waterbird digestive tracts has yet to be confirmed, however *T. bryosalmonae*-infected *F. sultana* statoblasts are viable after passing through carp digestive tracts (Abd-Elfattah et al., 2017).

In view of the evidence for substantial infection prevalences in statoblasts of a range of bryozoan hosts and infected statoblast viability (including following passage through vertebrate digestive tracts) we suggest that parasite co-dispersal may commonly be effected when transported statoblasts colonize new sites. Such co-dispersal requires further study and has been proposed to contribute to the distribution of PKD across Europe and North America (Henderson and Okamura, 2004).

### Impacts of Co-dispersal on Populations, Communities, and Biodiversity

Waterbird-mediated co-dispersal and establishment of parasites and hosts will almost certainly influence community interactions and food webs. Parasite biomass can be considerable (Kuris et al., 2008; Lambden and Johnson, 2013) and parasites contribute substantially to energy transfer and food web structure and stability (e.g., Dunne et al., 2013; Michalska-Smith et al., 2017). Parasites with complex life cycles may exploit hosts at different trophic levels. Parasites can also influence population and community dynamics by altering host behavior, determining host distributions and abundances, and mediating species interactions (e.g., competition, predation) (Hatcher et al., 2012).

Co-dispersal will also influence freshwater biodiversity and evolutionary dynamics. Because aquatic environments are fragmented and heterogeneous in space and time effective dispersal should continuously contribute to metacommunity dynamics. Local adaptation, host-parasite coevolution and disease dynamics will all be influenced by dispersal of parasites and hosts in interconnected networks (Parratt et al., 2016). The consequent mosaic pattern of selection regimes will fundamentally contribute to biodiversity (Thompson, 1999). For example, dispersal may introduce parasites that are particularly virulent if local hosts are not well-adapted to parasite strains (Laine, 2004) or when pathogens shift to new hosts (Longdon et al., 2015). This could result in host population declines that subsequently diminish as reciprocal host-parasite interactions progress through biological arms races. Alternatively, parasites may go extinct if host densities fall below a persistence threshold (Deredec and Courchamp, 2003).

The consequences of co-dispersal of parasites with different life histories across such landscapes are likely to vary. For example, parasites with complex life cycles or generalists infecting a diversity of hosts may particularly benefit. The former may persist indefinitely in populations of one host even if other hosts are unavailable. The latter may succeed when site conditions are unsuitable for co-dispersing hosts but alternate hosts are available. This scenario highlights potential links between host specificity and virulence evolution (Leggett et al., 2013). Lower virulence could facilitate utilization of dormant host propagules, enabling dispersal. Establishment likelihood would be further increased if the parasite had a broad host range. Co-dispersal could thus drive evolutionary trajectories, promoting the evolution of low virulence strategies in generalist parasites. The potential for parasites to actually manipulate host dispersal is supported by spatial epidemiological modeling (Lion et al., 2006). It would be of interest to explore whether host dispersal could be manipulated when hosts and parasites jointly rely on common dispersal vectors, such as waterbirds, with independent drivers for dispersal.

## DIRECTIONS FOR FUTURE STUDIES ON ZOOCHORY OF FRESHWATER INVERTEBRATES

The sedentary nature of plants has resulted in extensive study of how zoochory of seeds and fruit may explain plant distributions in terrestrial and aquatic environments (e.g., Green et al., 2016; Wenny et al., 2016; Lovas-Kiss et al., 2018b). This perspective has prompted exemplary modeling highlighting how traits such as seed size, survival, and retention time may influence seed dispersal across varying landscapes by local and migratory waterbird movements (Viana et al., 2013; Kleyheeg et al., 2017). Invertebrate dispersal is less well-understood but will critically contribute to patterns of distribution and abundance and organismal interactions. Bryozoans have served as model invertebrate systems for demonstrating zoochory by waterbirds and its corollaries, including codispersal of parasites, and consequences of dispersal variation. This body of work raises further general questions regarding zoochory of freshwater invertebrates including:

- Are migratory stop-overs zoochory hot spots?
- How important are other agents of zoochory (e.g., insects, mammals, fish, humans)?
- How do rates of zoochory vary over space and time?
- Is endozoochory more important than ectozochory?
- Can zoochory effect spillover of parasites?
- How does zoochory shape the metapopulation dynamics of hosts and parasites?
- How will climate change impact waterbird migrations and hence the distributions of invertebrates and co-dispersing parasites that undergo zoochory?
- Can zoochory select for low virulence strategies in generalist parasites?
- What is the relative importance of other forms of connectivity (e.g., hydrological, anthropogenic) in achieving dispersal?
- How was freshwater biodiversity partitioned before birds evolved?

## AUTHOR CONTRIBUTIONS

BO conceived and wrote the article. HH was fundamentally involved in many cited studies and contributed to the final development of the article. JT and HH conducted genetic analyses reported in **Figure 1** and **Supplementary Tables 1, 2**. HH developed **Figure 1**.

## ACKNOWLEDGMENTS

Many of the data and insights presented here result from studies funded by the Natural Environment Research Council (grants GR9/828; GR3/8961; GR9/04271; GR3/11068; NER/A/S/1999/00075; NER/B/S/2000/00336; NER/S/A/2004/12399; NE/019227/1; NE/N005902/1), the Biotechnology and Biological Sciences Research Council (BB/F003242/1), the Natural History Museum, London. Comments from two reviewers helped to improve our manuscript.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2019.00029/full#supplementary-material

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Ecology and Evolution

ORIGINAL RESEARCH
published: 20 February 2019
doi: 10.3389/fevo.2019.00039

# Unraveling the Seed Dispersal System of an Insular “Ghost” Dragon Tree (*Dracaena draco*) in the Wild

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Despite being abundant in urban gardens, the Canary Islands dragon tree *Dracaena draco* is close to extinction in the wild. It tends to produce relatively large fruits, which limits the pool of vertebrates that might disperse its seeds. We aimed to shed light on the seed dispersal system of this plant by studying its fruit size in relation to the feeding behavior of its present dispersers, and to discuss on possible differences with the past dispersal system, when large-sized dispersers were abundant. Besides fruit and seed characterization, we performed experiments on seedling emergence (using the characterized seeds), and field observations of the fruit handling behavior of frugivorous birds. Seed removal by granivores beneath and outside the dragon tree canopies was assessed through a field experiment. An additional seedling emergence experiment tested the effect of pulp removal from around the seed (using seeds contained within the fruits and manually depulped seeds). A feeding experiment was carried out with captive individuals of the Canary endemic white-tailed pigeon *Columba junoniae*—a large frugivore that occasionally consumes *D. draco* fruits—to test if its gut treatment influences seed viability. Small fruits produced seeds unable to germinate, while most seedling emergence was recorded only for seeds from large fruits. Our observations suggest that the only passerine species able to swallow large fruits is the medium-size passerine *Turdus merula*, whereas small passerines tended to pluck the pulp without aiding seed dispersal. Nonetheless, *Sylvia atricapilla*—the largest among the group of small passerines—occasionally transported fruits away from parent plants to consume the pulp, resulting in seed dispersal without any digestive treatment. This behavior indicates *S. atricapilla* might be occasionally a legitimate disperser of *D. draco*, since our experiments suggest that seed transport away from parent trees and pulp removal enhance both post-dispersal seed survival and seedling emergence. Lastly, the pigeons used in the experiment regurgitated mostly viable seeds, suggesting the legitimacy of *C. junoniae* as seed disperser for *D. draco*. Therefore, although *D. draco* likely had more seed dispersers in the past, we identified at least two bird species that can still disperse its seeds nowadays.

**Keywords: frugivorous birds, fruit size, legitimate seed dispersal, seed traits, seed predation, seedling emergence**

**Edited by:**
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**Specialty section:**
*This article was submitted to Biogeography and Macroecology, a section of the journal Frontiers in Ecology and Evolution*

***Received:*** *27 September 2018*
***Accepted:*** *01 February 2019*
***Published:*** *20 February 2019*

**Citation:**
*González-Castro A, Pérez-Pérez D, Romero J and Nogales M (2019) Unraveling the Seed Dispersal System of an Insular “Ghost” Dragon Tree (Dracaena draco) in the Wild. Front. Ecol. Evol. 7:39. doi: 10.3389/fevo.2019.00039*

## INTRODUCTION

Frugivory and seed dispersal is a crucial stage in the reproductive cycle of many fleshy-fruited plants because it allows them to colonize new territories, increase gene flow (Nathan, 2006) and move away from parent plants. These benefits also avoid competition and reduce exposure to natural enemies (Janzen, 1970; Connell, 1971). However, many plant species are currently facing an overwhelming loss of their seed dispersers due to factors like hunting and habitat destruction (e.g., Tilman et al., 1994; Sekercioglu et al., 2004; Tylianakis et al., 2008). Nonetheless, the dispersal service provided by an animal species may cease due to a decline in its population, before it becomes totally extinct (McConkey and Drake, 2006; McConkey and O'Farrill, 2016).

Loss of disperser animals has important demographic and evolutionary consequences for plants (Tilman et al., 1994; Traveset and Riera, 2005; Guimarães et al., 2008; Galetti et al., 2013; Säterberg et al., 2013; Pérez-Méndez et al., 2015, 2018). These consequences are especially important in isolated systems—like those on oceanic islands—because plant-disperser mutualisms may be more symmetric there (González-Castro et al., 2012) or plants might rely on just a few animals providing dispersal services (Schleuning et al., 2014). Also, it is worth remembering that although a plant species can still be dispersed by animals after the loss or decline of its main dispersers, its natural regeneration and demography can be compromised by the low efficiency of the remaining dispersers, leading to a suboptimal dispersal system (Valido, 1999; Cordeiro and Howe, 2002; Traveset and Riera, 2005; Pérez-Méndez et al., 2018). Therefore, it is important to know how and to what extent current seed dispersal systems may help threatened plant species to buffer the loss or decline of their main dispersal agents.

The fauna of the Canary archipelago has undergone a severe decline in large-sized frugivorous animals, like giant endemic lizards (*Gallotia* spp.) and birds like pigeons (*Columba bollii*, and *C. junoniae*), which might affect the natural regeneration and demography of plant species producing large-sized fruits (Hansen and Galetti, 2009; Wotton and Kelly, 2011; Pérez-Méndez et al., 2015). For instance, the maximum snout-vent length (SVL) of giant lizards in the past was 502 mm for the extinct *G. goliath,* whereas for *G. stehlini*, endemic to Gran Canaria, it is currently 280 mm (Pérez-Méndez et al., 2015). Nonetheless, it is also important to highlight that on the other islands, there are no giant lizards or their surviving natural populations are now restricted to small areas on isolated cliffs. Therefore, the largest non-giant lizard that could play a significant role as disperser of large-fruited plants on those islands is *G. galloti*, with a maximum SVL of 145 mm (Pérez-Méndez et al., 2015). In this contribution, we tried to shed light on the structure and functioning of the current seed dispersal system of the Canary Islands dragon tree *Dracaena draco* (Asparagaceae). It normally produces relatively large fruits, for which many frugivorous birds have size restrictions that prevent them acting as legitimate seed dispersers.

Specifically, this study pursued four main goals, to assess: (1) whether fruit size can affect seed traits, seedling emergence and growth, (2) how frugivorous birds of different sizes interact with fruits of *D. draco*, (3) if fruit carriage away from parent trees and pulp consumption by birds can have significant effects on seedling recruitment, and (4) the potential role of the white-tailed laurel pigeon *C. junoniae* as legitimate disperser of this tree.

Matching fruit size and gape width is a key factor determining the type of frugivory interaction (Wheelwright, 1985; Rey et al., 1997; Jordano and Schupp, 2000). Therefore, we expected that medium-sized passerines would swallow entire fruits containing seeds, hence acting as legitimate seed dispersers. On the other hand, small passerines, unable to swallow fruits whole, would act as pulp consumers without seed dispersal. Only small passerines able to carry fruits and consume them away from parent trees can be considered as occasional seed dispersers, but without any digestive treatment (**Figure 1**).

Such pulp consumers would be legitimate dispersers if the following needs are met: (1) pulp removal enhances seed germination (Samuels and Levey, 2005), and/or (2) seed predation is lower away from parent trees than beneath them (Janzen, 1970). However, the effect of pulp on seed germination is highly species-dependent (Robertson et al., 2006), and the vulnerability of *D. draco* to post-dispersal seed predators is unknown. Given the foregoing, we had no *a priori* expectations in this regard.

Lastly, the white-tailed laurel pigeon *C. junoniae* has been recorded as fruit consumer for *D. draco* (A. Valido, pers. comm.). However, both species (especially the plant) have become extremely rare in nature and their mutual interaction is difficult to observe (i.e., virtually extinct). For this reason, we performed a captivity experiment to find out if this pigeon's gut treatment has no detrimental effect on *D. draco* seeds, as would be expected for a legitimate disperser (see similar island-plant experiments in Culliney et al., 2012 for *Corvus hawaiiensis*, extinct in the wild).

## MATERIALS AND METHODS

### Natural History and Frugivory on *Dracaena draco*

*Dracaena draco* is a monocot tree endemic to Macaronesia and currently inhabits Madeira, the Canary Islands, Cape Verde and parts of North Africa (Marrero et al., 1998). In the case of the Canaries, this plant species was formerly well-distributed throughout the thermosclerophyllous woodland (100–700 m a.s.l.), along with Canary palm (*Phoenix canariensis*), Wild olive (*Olea cerasiformis*), Mastic trees (*Pistacia* spp.), etc (Fernández-Palacios et al., 2008). However, after colonization and settlement by pre-hispanic inhabitants and Europeans, both that vegetation habitat and *D. draco* have undergone an overwhelming decline, particularly on Tenerife. Moreover, its populations have been subject to anthropogenic pressure due to its resin—"dragon's blood"—being highly valued for medicinal and industrial purposes (Santos, 1979). Although this plant species is still cultivated in private and public gardens worldwide, its natural populations on Macaronesian islands are highly fragmented and show very low natural regeneration. This leads

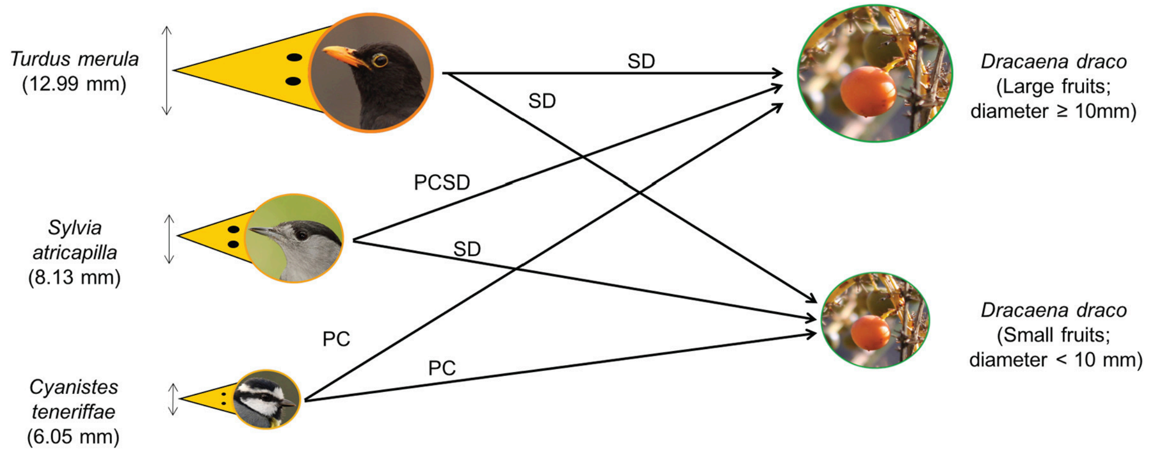

**FIGURE 1 |** Conceptual framework of our hypothesis. Medium size passerines, such as *Turdus merula*, can swallow *Dracaena draco* fruits whole and act as legitimate seed dispersers (SD), regardless of fruit width. Small passerines were expected to act mostly as pulp consumers (PC). Large small passerines (e.g., *Sylvia atricapilla*) may be able to swallow whole small-width fruits and legitimately disperse the seeds (SD), whereas they will only peck out the pulp of large fruits, acting as pulp consumers (PC). Nonetheless, small passerines may occasionally be able to transport large fruits to consume the pulp away from parent trees, leading to seed dispersal without digestive treatment (PCSD). For each bird, its mean beak gape width is shown between parentheses (AG-C, unpubl. data). The terms used to define the type of fruit-handling behavior follow Wheelwright (1985) and Jordano and Schupp (2000). Picture credits: birds by Beneharo Rodríguez and *D. draco* fruit by AG-C.

the species to be listed as "Vulnerable" in the IUCN Red List (2018) and considered a "ghost tree" in the wild.

Fruits of *D. draco* are orange-reddish globose berries, which can reach up to 13.59 ± 0.85 mm in diameter (Marrero and Pérez, 2012), whose seeds—between 1 and 3 per fruit—are dispersed by frugivorous animals (endozoochory). That fruit size is too large for most bird species to swallow. Consequently, any study addressing the seed dispersal system of this plant species should consider the variability of its fruit size. Currently, the bird species that have been reported to consume *D. draco* fruits are small passerines: blackcap *Sylvia atricapilla* and Sardinian warbler *S. melanocephala* (A. González-Castro unpublished data), the medium-sized passerine Eurasian blackbird *Turdus merula* (González-Castro et al., 2012), and the white-tailed laurel pigeon *Columba junoniae* (A. Valido, pers. comm.).

Several seeds of *D. draco* have been also found in feces from the endemic Canary lizard *Gallotia galloti* (Valido, 1999) and the Gran Canaria giant lizard *G. stehlini* (González-Castro et al., 2012). However, so far as we know, interactions of *D. draco* fruits with lizards are much less common than interactions with birds. Therefore, from a quantitative perspective, birds seem to play a more important role than lizards in the current seed dispersal system of *D. draco*.

## Categorizing Fruit Sizes

The frugivorous birds that currently consume fruits of *D. draco* can be split into two different functional groups: (1) small birds (encompassing small-sized passerines) and (2) large birds (including the medium-sized passerine *T. merula* and the pigeon *C. junoniae*). Consequently, fruits of *D. draco* were categorized according to their width into two different groups: "small" (< 10 mm) and "large" (≥ 10 mm). This threshold width was considered as the largest fruit that might be entirely swallowed by small passerines based on their beak gape width. Among the small passerines that consume *D. draco* fruits, the largest is *S. atricapilla*, whose gape width is 8.13 ± 0.72 mm (mean ± SD; A. González-Castro, unpubl. data). We selected 10 mm width instead of 8.13 mm as a conservative threshold because birds can usually swallow fleshy-fruits slightly wider than expected from their gape width. On the other hand, fruits considered "large" would only be swallowed by medium-sized passerines and frugivorous pigeons.

## Fruit-Seed-Seedling Relationships

To evaluate the relationship of fruit size with seed biometric features (diameter and weight) and seedling performance (emergence and growth), a total of 238 fruits were randomly collected from 11 parent plants (**Table 1**) located on the islands of Tenerife (individuals nos. 1–10, in the municipalities of La Laguna and Tegueste) and Gran Canaria (individual no. 11, in the municipality of Gáldar). Fruits were collected in September 2017, and the number of fruits and parent trees was constrained by their availability at time of collection. The fruits from each individual plant were stored separately to keep the source of seeds identified, allowing us to account for the mother tree effect.

The length and width of each fruit were measured. Based on the width, fruits were classified as "small" or "large" as previously explained. We chose to classify fruits into "small" and "large" rather than using the individual fruit width, so as to directly relate our results to what would occur in the wild in the interaction with small passerines (which can swallow only small fruits) or with medium passerines and pigeons (which can swallow both sizes of fruits). For each fruit, the number of seeds, as well as the seed width and weight were recorded. Width of

**TABLE 1 |** Summary of fruit width (mm) collected from individual trees.

| Plant ID | *n* | Fruit width (Mean ± SD) | Fruit width range (Min.–Max.) | Large fruits | Small fruits |
|---|---|---|---|---|---|
| Plant #1 | 20 | (9.55 ± 1.24) | (7.83–12.10) | 7 | 13 |
| Plant #2 | 20 | (10.43 ± 2.19) | (6.98–15.02) | 10 | 10 |
| Plant #3 | 20 | (10.35 ± 1.36) | (8.12–12.99) | 13 | 7 |
| Plant #4 | 30 | (8.73 ± 1.41) | (6.50–11.61) | 8 | 22 |
| Plant #5 | 25 | (11.84 ± 0.48) | (11.03–13.01) | 25 | 0 |
| Plant #6 | 12 | (11.78 ± 0.66) | (10.50–12.53) | 12 | 0 |
| Plant #7 | 20 | (11.73 ± 0.90) | (10.51–13.14) | 20 | 0 |
| Plant #8 | 31 | (9.97 ± 2.24) | (7.25–20.01) | 15 | 16 |
| Plant #9 | 20 | (10.44 ± 0.89) | (7.84–11.95) | 15 | 5 |
| Plant #10 | 20 | (9.55 ± 0.54) | (8.57–10.32) | 6 | 14 |
| Plant #11 | 20 | (11.26 ± 0.66) | (10.18–12.76) | 20 | 0 |

*Number of collected fruits (n), the mean (±SD) and range (min.–max.) of fruit width, as well as the most common fruit type produced by each.*

fruits and seeds were measured with a digital caliper ("Stainless hardened$^{TM}$" with a precision of ±0.01 mm) and seed weight with a digital balance ("Cassio Plus METTLER TOLEDO$^{TM}$" with a precision of ±0.1 mg).

A total of 210 measured and weighed seeds were used in a seedling emergence experiment. Seeds were individually sown in multi-pot trays filled with a standard substrate (50% peat and 50% agricultural soil). Trays were placed in a greenhouse and watered every 3 days between October 2017 and March 2018 (inclusive), according to the main rainy period in Canary Islands. The experiment was monitored every 5 days and seedling emergence was recorded when any part of the seedlings was visible. After emergence, seedling length was measured using a digital caliper every 5 days. At the end of the experiment, seedling growth rate was calculated as the difference between the final and the initial length divided by the days elapsed after its emergence.

## Frugivory Rate and Fruit-Handling Behavior

From October 2017 to March 2018, frugivory interactions were recorded at all six fruiting individuals at the study site. Due to the impracticability of access to a high-density natural population of dragon trees, a rural garden with planted individuals was chosen as study site for this purpose. The selected garden was in the municipality of Tacoronte (Tenerife; UTM: 28R 362650 m E/ 3154947 m N, 250 m a.s.l.), away from urban areas and surrounded by natural vegetation of the potential habitat of *D. draco* (i.e., thermosclerophyllous woodland). With the only exception of *C. junoniae*—whose interaction with dragon tree fruits has been seldom recorded in the wild (A. Valido, pers. comm.)—and *Erithacus rubecula*, the community of fruit-eating birds recorded at the study site was composed of the same species found in the wild (i.e., *S. atricapilla*, *S. melanocephala*, *S. conspicillata*, *T. merula,* and *Cyanistes teneriffae*). The gape width of *E. rubecula* is smaller than that of *S. atricapilla*. Therefore, if *E. rubecula* interacts with *D. draco* fruits in the wild, it is expected to act as a small passerine.

Frugivory censuses were performed on 3–5 days per week from 08:00 to 10:00 (local time) until the whole fruit crop was depleted, and a total of 67 h of observations were accumulated. We used binoculars at some distance from observed trees in order to not interfere with the normal activity of birds. Each census of each individual plant lasted for 15 min, and after each census, the next targeted plant was selected at random. During each census, we recorded the number of visits made by each type of frugivore (i.e., small and medium-sized passerines at species level) and the type of interaction, such as: pulp consumption (with no dispersal), fruit swallowing (which implies internal seed dispersal), and carriage of fruit away from the parent tree (which implies seed dispersal but without any digestive treatment).

We are aware of the need for frugivory censuses at wild trees. However, the last fruiting season was characterized by low fruit production. For this reason, in this study we have also included additional censuses performed from October 2010 to December 2010. These used the same methodology on two solitary individual plants growing in natural conditions: One in Bandama (UTM: 28R 455165 m E/ 3100775 m N, 235 m a.s.l.) and the other in El Palomar (UTM: 28R 454278 m E/ 3104512 m N, 250 m a.s.l.), both on Gran Canaria. With these censuses, we reached up to 7.92 additional hours of observations. However, these additional censuses lasted for a variable time (from 10 to 45 min). Therefore, to make all censuses comparable, the number of visits by birds was standardized by dividing it by the time in minutes the targeted plant was observed during each census. The goal of this part of the study was to record fruit handling by birds, and both observational periods (for Tenerife and for Gran Canaria, respectively) coincided with the natural fruiting phenology of *D. draco*. Also, during both periods, frugivory interactions were recorded for the same bird species (all of them resident in the Canary Islands). Therefore, the information recorded about fruit handling by birds is unlikely to be affected by censuses performed during two different periods.

## Consequences of Occasional Dispersal With No Digestive Treatment by Small Passerines

### Effect of Pulp Removal on Seedling Emergence

To test for the effect of pulp removal on the percentage and rate of seedling emergence, we performed a seedling emergence experiment. We sowed 496 seeds/fruits randomly collected from

13 parent trees: the 11 mentioned above (see the section "*Fruit-seed-seedling relationships*") plus another two parent trees located on Tenerife, whose fruits were also collected in September 2017. These emergence trials were carried out following the same protocol as above, to test the effect of seed size and weight on seedling emergence and growth. However, in this case, we only recorded the seedling emergence date.

This emergence experiment applied two treatments: the first consisted of 371 seeds whose pulp was manually removed (named "depulped" treatment), whereas the second treatment, named "with pulp," consisted of 125 entire fruits containing the seed(s) inside. Although most fruits contain one seed, some have two (more rarely three). For this reason, to be cautious regarding the differences in emergence time between treatments, we considered only the first seedling that emerged. Nonetheless, we never recorded more than one emerged seedling in pots where entire fruits were sown, so this precaution was no longer necessary.

### Seed Removal Beneath and Outside the Canopy of Parent Trees

If pulp consumption occurs at the fruiting tree, the seeds contained within fruits fall beneath parent trees. However, if the pulp consumer transports fruits before consuming them, the contained seeds are dispersed to areas away from parent trees. To assess if fruit carriage away from parent trees can help to reduce the probability of seed removal with respect to seeds falling beneath parent trees, we performed a post-frugivory seed removal experiment. This assessment was carried out in April, 2018. As replicates, we selected 12 parent trees that had just fruited or we were sure had fruited during the immediately previous fruiting season (i.e., that showed fallen fruits and seeds beneath their canopy).

The seed removal trial consisted of a Petri dish containing the seeds. For each parent tree (i.e., replicate), we set up two different trials with 10 seeds each: one trial beneath the canopy of the plant ("beneath" treatment), and the other at five meters away from the plant canopy ("outside" treatment). The choice of five meters for the "outside" treatment was based on the mean minimal distance that *S. atricapilla* flies from parent trees carrying *D. draco* fruits in its beak (pers. obs.).

These trials were left there for 15 days and were inspected every day and night to ensure they were not disturbed and to record the number of seeds remaining until the end of the experiment. Fifteen days was the minimal time that seedlings took to germinate in our experiment. It allowed us to simulate the time that a seed is naturally exposed to rodents and granivorous birds before germination.

## Recreating a "Ghost" Interaction

Due to the rarity of *D. draco* in the wild and the shy behavior of *C. junoniae*, it is very difficult to record this plant-animal interaction in the wild. Therefore, we performed a captivity experiment in aviaries at the *C. junoniae* breeding center (Gran Canaria). There were seven identical aviaries (8 × 4 × 3 m), each containing a pair of adult pigeons (one male and one female). Therefore, our seven feeding trials included 14 white-tailed laurel pigeons. This center was established for a project to reintroduce this pigeon on the island, and welfare of the animals is its priority. Therefore, access to aviaries was limited to once a week, which determined the frequency of offering fleshy-fruits of *D. draco* and collecting regurgitated/defecated seeds.

In addition to fleshy-fruits included in the feeding trials, all pigeons were maintained on a granivore diet composed of a commercial mixture of seeds (used to feed domestic pigeons, *C. livia*), complemented with freshly-cut branches of two Fabaceae plant species (*Chamaecytisus proliferus* and *Bituminaria bituminosa*). To facilitate treatment of seeds in the gizzard, birds had grit and water available *ad libitum*. Although the pigeons at the breeding center do not receive native fruits as part of their regular diet, all had been exposed to some species of native fruits occasionally as supplementary diet enrichment items.

In each of the seven trials, a total of 210 fruits from seven *D. draco* parent plants were offered to each pair of pigeons once a week. Fruits were offered naturally attached on the raceme immediately after collection. The foraging behavior of pigeons was observed during the first hour with binoculars from a hide to confirm that pigeons ate the available fruits and the type of interaction (swallowing the entire fruit or plucking the pulp). Fruits were offered for 7 days, and at the end of experiment we collected all these pigeons' regurgitations and defecations to look for *D. draco* seeds. Seeds found were exposed to the triphenyl tetrazolium chloride (TTC) test to check their viability, following the protocol described in Marrero et al. (2007). Whereas, most seeds required 48 h of incubation in tetrazolium, we left them for 96 h due to the low cell-permeability of *D. draco* embryos. As control group, we repeated this process with 30 manually depulped seeds from the same parent plants.

## Statistical Analyses

We performed a Generalized Linear Mixed Effect Model (GLMM) with a Poisson error distribution to test if the two types of fruits (large and small) had different numbers of seeds. To test if seed weight and width differed between types of fruits, a GLMM with a normal error distribution was used. To compare the percentage of emerged seedlings between large and small fruits, we used a GLMM with a binomial error distribution. In all GLMMs, the parent tree from which fruits were collected was included as a random effect factor.

To test if the seed weight and width influenced the vigor of emerged seedlings, we first performed a Principal Component Analysis (PCA) with both seed weight and width. The first component was a linear combination of seed weight and width, which accounted for 99.7% of the variance in seed biometry. Its loadings were used to create a predictor variable called "seed size." The growth rate was used as response variable in a linear regression.

To assess if the frugivory interaction rate was different between the two types of dispersers (small vs. medium-sized passerines), we performed a GLMM with a normal error distribution where the response variable was the standardized visitation rate (visits per minute of census). The individual plant observed at each census, nested within the census date,

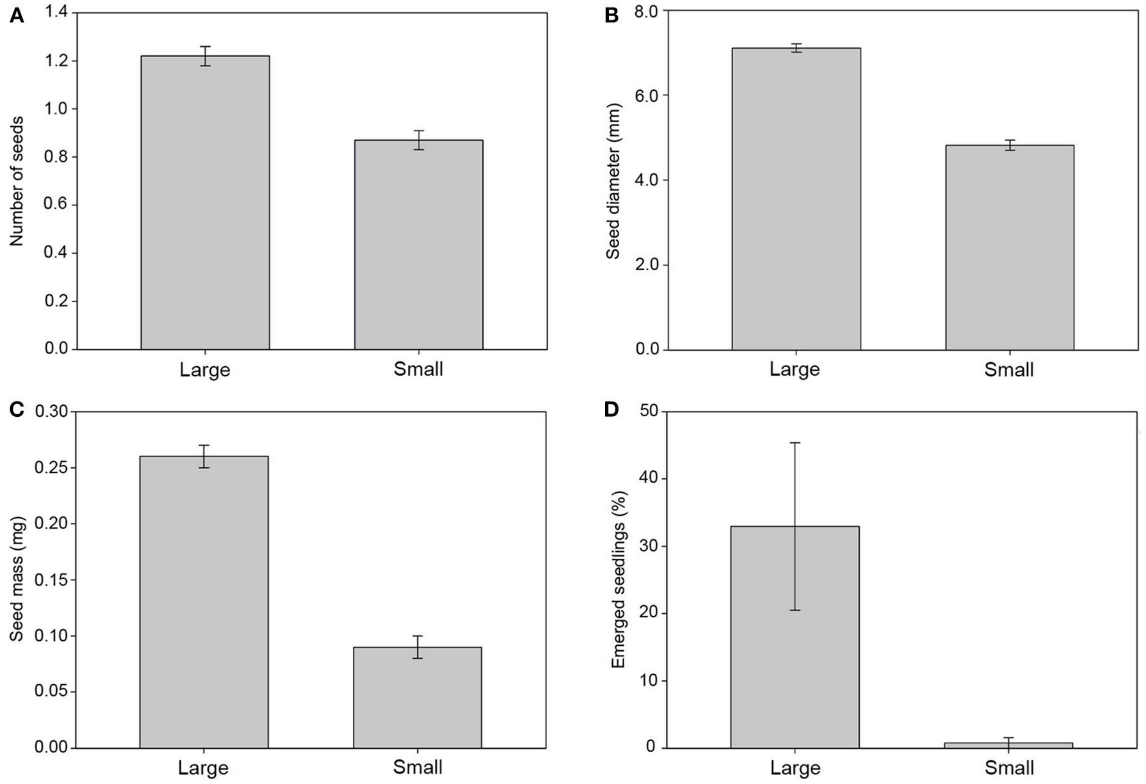

**FIGURE 2 |** Relationship of fruit type (large vs. small) to the number of seeds it contained **(A)**, seed width **(B)**, seed mass **(C)**, and seedling emergence success **(D)**.

was set as a random effect factor to account for pseudo-replication. To avoid zero inflation, all 15-min censuses within the same month were pooled. To compare the percentage of legitimate interactions (i.e., frugivory with actual seed dispersal) between the disperser types, we performed a *G*-test (Sokal and Rohlf, 1995).

To compare the percentages of seedlings that emerged from "depulped" seeds and those "with pulp" we performed a GLMM with binomial error distribution and set the parent tree as a random term. In the case of emergence rate, the cumulative number of seedlings that emerged during the experiment was recorded for both "depulped" and "with pulp" treatments. Comparison between treatments was made with a Kolmogorov-Smirnov test. The relative proportion of seed predation between seeds left beneath fruiting *D. draco* trees and seeds left away from them was tested with a Generalized Linear Model (GLM), with a binomial error distribution. Lastly, to test if ingestion of *D. draco* seeds by the pigeon *C. junoniae* significantly affected the viability of seeds with respect to non-ingested seeds we used a Likelihood Ratio test (i.e., *G*-test).

# RESULTS

## Fruit-Seed-Seedling Relationships

Only one of the eleven parent trees used for fruit characterization produced a similar number of small and large fruits, whereas the remaining parent trees produced mostly small or large fruits (**Table 1**). Large fruits significantly produced more seeds than small fruits ($\chi^2 = 5.23$; *d.f.*= 1; $P = 0.02$; **Figure 2A**). Regarding seed biometry, large fruits produced significantly larger ($\chi^2 = 122.76$; *d.f.*= 1; $P < 0.001$) and heavier ($\chi^2 = 33.60$; *d.f.*= 1; $P < 0.001$) seeds than small fruits (**Figures 2B,C**, respectively). Moreover, the proportion of seedling emergence was higher for seeds from large fruits than from small fruits ($\chi^2 = 8.21$; *d.f.* = 1; $P = 0.004$; **Figure 2D**). Lastly, the growth rate of seedlings was negatively related with seed size, although this trend was not significant [$F_{(1,58)} = 0.2$; $P = 0.65$].

## Frugivory Rate and Fruit-Handling Behavior

Five bird species and 156 frugivore interactions were observed during the 74.92 h of censuses. *T. merula* (the only medium-sized passerine) was by far the most recorded frugivore, encompassing 46.8% of interactions, followed by *Sylvia atricapilla* (32.7%), *S. melanocephala* (13.5%), *S. conspicillata* (3.2%), and *Cyanistes teneriffae* (3.9%), so that "small passerines" represented 53.2% of the total interactions observed. When the number of interactions was standardized accounting for observation time (**Figure 3A**), this difference between small and medium-sized passerines remained, but it was not statistically significant ($\chi^2 = 0.42$; *d.f.* = 1; $P = 0.51$).

*Turdus merula* showed a significantly higher proportion of legitimate interactions than small passerines ($G_1 = 170.26$; $P < 0.001$; **Figure 3B**). Among small passerines, *S. atricapilla* interacted with 51 fruits and was the only one able to disperse

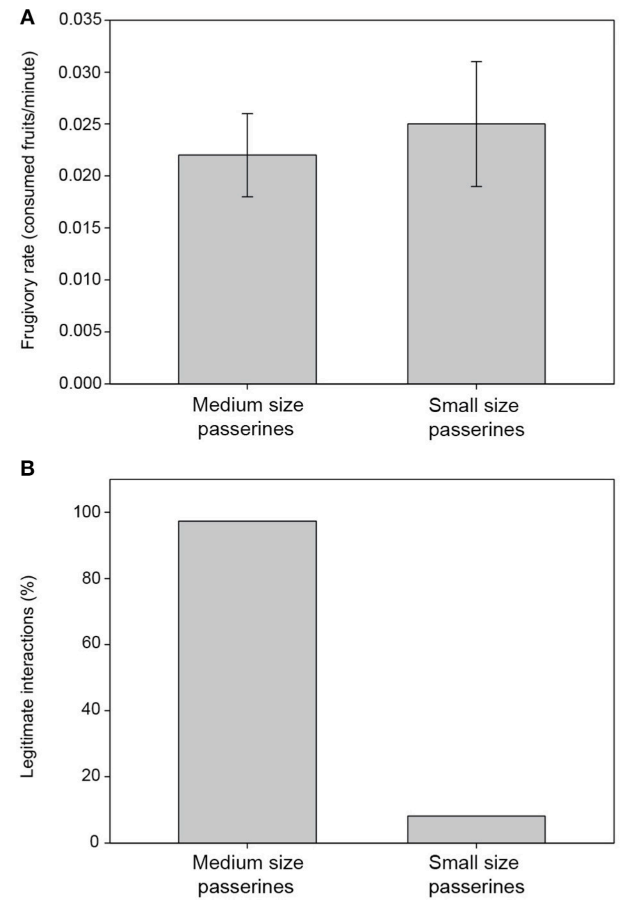

**FIGURE 3 |** Frugivory interactions involving passerine birds of different sizes were compared regarding interaction rate **(A)**, and their legitimacy as the proportion of interactions leading to actual seed dispersal **(B)**, regardless of whether digestive treatment occurred or not.

*D. draco* seeds by swallowing a few small fruits (1.96% of all its interactions with *D. draco*) or by carrying entire fruits away from parent trees to consume their pulp (11.8%). The remaining 86.3% of its interactions were as pulp consumer at the parent tree.

## Consequences of Occasional Dispersal With No Digestive Treatment by Small Passerines

- *Effect of pulp removal on seedling emergence–*

  The proportion of emerged seedlings (**Figure 4A**) was significantly higher for depulped seeds than for seeds sown still within the whole fruit ($\chi^2 = 20.44$; *d.f.* $= 1$; $P < 0.001$). The effect of pulp removal also produced a quicker seedling emergence (**Figure 4B**) than those seeds sown with pulp ($Z = 0.85$; $P < 0.001$).

- *Seed removal beneath and outside the canopy of parent trees–*

  The proportion of seeds damaged or removed by granivores (**Figure 4C**) was significantly higher beneath the canopy of fruiting *D. draco* trees than outside it ($\chi^2 = 9.44$; *d.f.* $= 1$; $P = 0.002$).

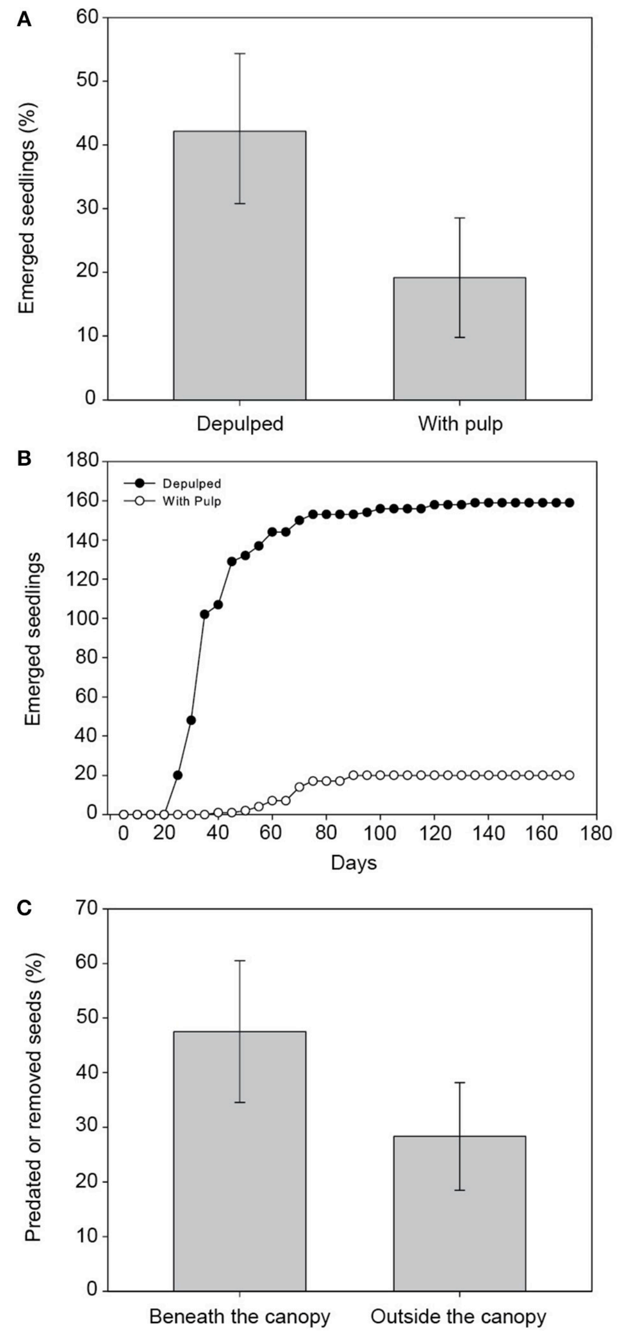

**FIGURE 4 |** Potential consequences of external seed dispersal for *Dracaena draco*. Depulped seeds lead to a higher proportion **(A)** and rate **(B)** of seedling emergence than seeds contained inside fruits. Also, seed removal **(C)** is higher beneath the canopy than outside the canopy of parent trees.

## Captivity Experiment With *Columba junoniae*

During the feeding trial, all observed fruit-pigeon interactions were legitimate, as individuals swallowed the entire fruits. Out

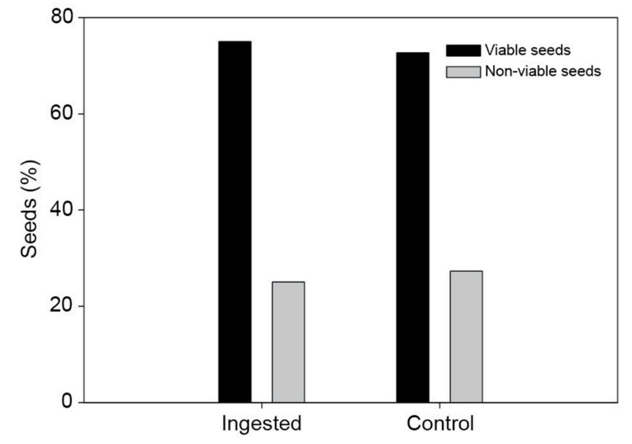

**FIGURE 5 |** Effect of *Columba junoniae* gut treatment on *Dracaena draco* seeds. Ingested and control seeds showed no statistical differences in viability.

of the 210 fruits offered, we recovered 17 seeds that were clearly regurgitated, whereas no seeds were found within feces or bearing fecal traces. Most of the fruits offered were not consumed and had fallen on the ground or were still attached to branches. We found no broken seed fragments in feces or regurgitations. The viability test (**Figure 5**) showed no significant differences between control seeds and seeds regurgitated by pigeons ($G_1 = 0.01$; $P = 0.91$).

## DISCUSSION

In this contribution, we have shed light onto intrinsic and extrinsic factors influencing the unknown seed dispersal system of an iconic "ghost" plant in the wild, the Canary Islands dragon tree *D. draco*. Our results show that fruit size is a clear constraint on its successful seed dispersal by birds. Although it is very common in public areas and private gardens, it seems this plant species mostly relies on just one abundant medium-sized passerine (*T. merula*) and one rare pigeon (*C. junoniae*) that might be its most important legitimate dispersers. Small passerines mostly acted as pulp consumers but not as seed dispersers. Therefore, given the generalized population decline among large lizards in the Canaries, we found that *D. draco* may still be dispersed by medium and large-sized birds. Its current vulnerability in the wild may be related to other potential problems, like habitat destruction and fragmentation and herbivory by introduced mammals.

### Intrinsic Aspects of the Seed Dispersal System of *D. draco*

Although *D. draco* can produce large and small fruits, most individual trees produced mostly large or small fruits, but rarely both fruit types (**Table 1**). Furthermore, large fruits produced more and better seeds—in terms of size and emergence capability—than small fruits (**Figure 2**). This suggests a strong maternal effect on fruit type, and hence on seed quality, for germination and seedling survival (Roach and Wulff, 1987). The low capability of some parent trees to produce viable seeds may be also related to the isolation of many of them, growing in urban gardens, leading to a deficit of natural pollinators to enhance the genetic flow among individuals (Wilcock and Neiland, 2002). Another possible (but not exclusive) reason that might explain the strong maternal effect is that the species could be undergoing a genetic bottle-neck effect (e.g., Gilpin, 1991), caused by its current isolation in urban gardens and/or the great population decline it has suffered in the wild.

Our results agree with previous studies showing a negative relationship between seed size and seedling growth rate, not only among different species but also individuals of the same plant species (Fenner and Thompson, 2005 and references therein). This negative trend might equalize the seedling vigor across individuals because, although seedlings from large-seeded individuals have an initial advantage, such a difference is offset by the quick growth rate of seedlings from small seeds (Fenner and Thompson, 2005). This could enhance survival probability in both shaded and open environments. Slow growth might allow individual plants to survive, taking advantage of endogenous resources until a canopy gap appears, whereas quick growth may help plants in open environments to take advantage from the rainy season and start using exogenous resources (Fenner and Thompson, 2005).

### Extrinsic Aspects of the Seed Dispersal System of *D. draco*

Our study shows that the legitimacy of frugivorous passerines as seed dispersers of *D. draco* is clearly constrained by the birds' gape width relative to fruit size, as demonstrated for other plant-bird interactions (Wheelwright, 1985; Rey et al., 1997; Jordano and Schupp, 2000). Indeed, warblers (*Sylvia* spp.), which are generally considered as seed dispersers (Herrera, 1995), mostly acted as pulp consumers of *D. draco* fruits in the census. Consequently, only half the interactions led to legitimate seed dispersal, mostly thanks to the medium-sized passerine *T. merula* and, to a lesser extent, a few legitimate interactions with *S. atricapilla*.

Nonetheless, *S. atricapilla* swallowing small fruits cannot lead to efficient seed dispersal, due to an already existing intrinsic limitation: small fruits—the only ones that can be swallowed by this small passerine—mostly produced non-viable seeds, unable to germinate (**Figure 2D**). On the other hand, viable seeds inside large fruits will not be internally dispersed by this passerine, since fruits will be mostly pecked instead of swallowed. This change in fruit handling by *S. atricapilla* (i.e., from seed disperser to pulp predator), depending on individual variations in fruit size seems to be a more general pattern than previously thought. Indeed, it has been demonstrated in other plant species with some intraspecific variability in fruit size (Rey et al., 1997). Therefore, the only way for *S. atricapilla* to be an efficient legitimate seed disperser for *D. draco* involves transporting the fruit outside the cover of the parent tree's canopy, for later pulp consumption.

Although the transport of large fruits by *S. atricapilla* does not imply digestive treatment, our experimental results suggest a potentially beneficial effect of this small passerine due to pulp removal enhancing seedling emergence (**Figures 4A,B**). Nonetheless, this effect of pulp removal is highly variable among plant species (Robertson et al., 2006), indeed the effectiveness of *S. atricapilla* as seed disperser when taking fruits away from parent plants will depend on the species under study. With respect to post-dispersal seed removal, granivorous birds able to predate upon large seeds like those of *D. draco* (i.e., *Fringilla coelebs*) were not recorded at the study site. The granivorous birds seen at the study site (e.g., *Serinus canarius* and *Carduelis cannabina*) usually fed upon small seeds of herbaceous plants. Therefore, seed removal detected during the experiment may likely be attributable to rodents (*Rattus* sp. and *Mus musculus*).

Although rodents may occasionally act as seed dispersers when storing seeds in caches (Forget and Milleron, 1991; Nyiramana et al., 2011), it has also been shown that seed caching mostly occurs outside tree canopies, whereas seeds under the canopies have a higher probability of being eaten (Muñoz and Bonal, 2011). Our experiment showed that the probability of rodents encountering and removing seeds (**Figure 4C**) is lower at a distance of five meters than beneath the parent tree. Despite such a short distance, the difference was significant. The reason may be the large uncountable amount of *D. draco* seeds clumped beneath parent trees, whereas at five meters the only available seeds were those used in the experiment. This result agrees with the Janzen-Connell hypothesis (Janzen, 1970; Connell, 1971), which states that the concentration of natural enemies for seeds, and hence seed mortality, is expected to be higher where the concentration of seeds is higher.

Given the foregoing, it seems that 11.8% of the interactions in which *S. atricapilla* transported fruits away from parent trees resulted in legitimate dispersal, since this helps seeds escape predation by rodents. Nonetheless, although seed density decreases with the distance from the parent tree (Clark et al., 1999; Nathan and Muller-Landau, 2000), we must point out that the seed dispersal tail is larger than the minimal dispersal distance in determining the density of dispersed seeds. In the case of fruit-eating animals, seed dispersal may be clearly directed to beneath other fruiting trees (Wenny, 2001; Russo and Augspurger, 2004; Carlo et al., 2007). Therefore, fruit carriage away from parent trees may lead to a higher probability of post-dispersal predation on *D. draco* seeds if the fruits are carried to other co-fruiting dragon trees, where probably the density of conspecific seeds will also be high. Nonetheless, during our observations, no *S. atricapilla* individual was recorded carrying fruits from one fruiting dragon tree to another.

Considering the role of *C. junoniae*, our experimental results suggest that this large-sized bird (mean weight ± SE; 395.86 ± 5.60 g, J. Romero. unpubl. data) is likely a legitimate seed disperser of *D. draco* as it swallowed the largest fruits whole, and regurgitated viable seeds in a similar proportion to control seeds (**Figure 5**). Although we recovered a small number of *D. draco* seeds in the aviaries, no fragments of its seeds were found in feces or regurgitations. This is contrary to what occurred with other large-seeded arboreal species such as *Apollonias barbujana* or *Persea indica*, whose seeds were predated. This suggests that the hard seeds of *D. draco* can survive the partial digestive treatment of *C. junoniae* before regurgitation. The small number of recovered seeds may be explained by the fact that the cleaning personnel at the aviaries (who entered more frequently) did not count the large amount of fallen fruits they cleaned away. However, beyond some isolated observations in wild *D. draco* trees (A. Valido, pers. comm.), its fruits have never been recorded as part of the diet of *C. junoniae* (see Martín et al., 2000; Marrero, 2009), although they share the same habitat. The lack of records for this interaction is probably caused by the rareness of the tree in the wild and constitutes an example of a virtually extinct interaction due to population decline of both interacting species (McConkey and Drake, 2006; McConkey and O'Farrill, 2016).

Although *D. draco* seeds may currently be dispersed by lizards of the genus *Gallotia* (Valido, 1999; González-Castro et al., 2012), these plant-lizard interactions do not seem to be very frequent. For example, from more than 190 *G. galloti* feces collected in the study area located on Tenerife, only one seed of *D. draco* was recovered (unpubl. data), whereas in the Gran Canaria study area only nine seeds were recovered from giant lizard *G. stehlini* feces (González-Castro et al., 2012). This small difference between *G. galloti* and *G. stehlini* may be caused by a gape-width restriction comparable to that observed in this study for birds. Furthermore, it is possible that interactions with lizards—especially giant lizards like the extinct *G. goliath*—were more frequent in the past, when both the plant and such lizards were more abundant. Indeed, if *G. stehlini* disperses seeds of *D. draco*, it is likely that other giant lizards did so in the past.

Beyond the loss of large lizards as plausible seed dispersers, another potential recruitment limitation of *D. draco* in the wild might be herbivory upon seedlings and juvenile plants by invasive mammals (goats, sheep, and rabbits). In the field study area, goats and sheep have been observed to seriously affect cultivated individuals 1.5–3 m tall. Furthermore, despite the effective dispersal of seeds, no self-seeded young plants have been observed in the surrounding areas after 15 years of experience. Additionally, it has been suggested that seedlings from small seeds may be more vulnerable to herbivores than those from large seeds, which clearly have greater uncommitted reserves available to compensate for various hazards, for example herbivory (Leishman et al., 2000). Therefore, the joint effect of herbivory by introduced mammals and the fact that only 50% of large seeds are legitimately dispersed may explain the common recruitment of *D. draco* in urban gardens and some rural areas where vertebrate herbivores are nowadays scarce or absent. These factors, in combination with others (e.g., climate change and habitat disappearance, livestock grazing and agriculture through nearly two thousand years since pre-Hispanic settlements), would be the main cause of disappearance of wild Canary dragon tree groves. This is already beginning to happen with the extant dragon tree populations (*D. cinnabari*) on Socotra (Attorre et al., 2007; Habrova et al., 2009).

## CONCLUDING REMARKS AND FUTURE AVENUES

Considering our results, the current vulnerable situation of *D. draco* as a ghost tree in the wild can be attributed to the synergistic effects of a partially eroded dispersal system and exposure to human exploitation and introduced herbivores. The probability of legitimate dispersal is inverse to the seed size and it strongly depends on the medium-size passerine *T. merula* and possibly on the pigeon *C. junoniae*, at least in the past, when both the plant and the pigeon were more abundant. Moreover, genetic drift in the widely separated relict wild populations might be acting as an additional vulnerability factor.

In future, to compare the functionality of the current dispersal system to that which could have existed in the past (likely including giant lizards), captivity-experimental studies would be necessary with endangered large-sized animals (mainly pigeons and giant lizards) to assess their level of frugivory on *D. draco* fruits and the consequences of their gut treatment on seeds, comparing it with the current dispersers. Also, further studies should explicitly address the net effect of introduced herbivores on the natural regeneration of this plant species. Lastly, it will be necessary to understand if the low quality of fruits and seeds produced by some individuals results from genetic drift and/or pollination limitations caused by the current small size and highly fragmented status of wild *D. draco* populations. These considerations must be taken into account to guide future studies and conservation actions on this iconic tree waiting to be revived from its present ghost status.

## DATA AVAILABILITY

The datasets analyzed for this study can be found in the figshare repository at doi: 10.6084/m9.figshare.c.4395602.v1.

## ETHICS STATEMENT

The fieldwork on Gran Canaria was carried out with permission provided by its Island Government (Cabildo de Gran Canaria). No pigeons used for the captivity experiment were harmed and procedures were according to the World Medical Assembly's Declaration of Helsinki of 1964 on human and animal rights, as well as the guidelines proposed by the Association for the Study of Animal Behavior. The captivity experiments were carried out with permission from the Cabildo de Gran Canaria and following the biosecurity protocols for management of captive birds "Plan de Confinamiento y de Bioseguridad de las aves de corral y otras aves cautivas" published by the Ministerio de Agricultura y Pesca, Alimentación y Medio Ambiente (Spanish Government).

## AUTHOR CONTRIBUTIONS

AG-C and MN conceived the idea. AG-C, DP-P, and JR performed the observational and experimental work. AG-C and DP-P performed statistical analyses. AG-C led the manuscript writing. JR significantly contributed to manuscript improvement. All authors revised manuscript drafts.

## FUNDING

Frugivory data on Gran Canaria were taken by AG-C during his doctoral fellowship (JAE-PRE from the Spanish National Research Council, CSIC). The fieldwork was financed by a project from The Spanish Ministry of Science and Education (CGL2007-61165/BOS), supported by FEDER funds from the European Union. AG-C is currently funded by the Cabildo de Tenerife, under the identification mark Tenerife 2030 (Program TF INNOVA 2016-2021) with MEDI and FDCAN funds. Pigeon feeding trials were carried out at the Cabildo de Gran Canaria's facilities funded by the LIFE+ Rabiche program (LIFE12 NAT/ES/000354).

## ACKNOWLEDGMENTS

The company Tagoro Medioambiente provided its greenhouse and access to its dragon tree plantations, to perform the seedling emergence experiments and frugivory censuses. The Cabildo de Gran Canaria provided AG-C with the necessary permissions to perform the fieldwork during his Ph.D. Thesis. We are especially grateful to Manuel Martín for his valuable help in the seedling emergence experiments, to Aurelio Martín for sharing his background knowledge of Canary Islands endemic pigeons, and to Alfredo Valido regarding the information about interactions. Guido Jones revised the English of this manuscript.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer, KM, declared her involvement in a large collaboration with two of the authors, AG-C and MN, to the handling editor.

frontiers in Ecology and Evolution

ORIGINAL RESEARCH
published: 26 February 2019
doi: 10.3389/fevo.2019.00040

# A Comprehensive Model for the Quantitative Estimation of Seed Dispersal by Migratory Mallards

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Long-distance seed dispersal is an important ecosystem service provided by migratory animals. Plants inhabiting discrete habitats, like lakes and wetlands, experience dispersal limitation, and rely heavily on zoochory for their spatial population dynamics. Granivorous waterbirds may disperse viable seeds of wetland plants over long distances during migration. The limited knowledge of waterbird migration has long hampered the evaluation of the importance of waterbirds in seed dispersal, requiring key metrics such as realistic dispersal distances. Using recent GPS tracking of mallards during spring migration, we built a mechanistic seed dispersal model to estimate realistic dispersal distances. Mallards are abundant, partially migratory ducks known to consume seeds of >300 European plant species. Based on the tracking data, we informed a mallard migration simulator to obtain a probabilistic spring migration model for the mallard population wintering at Lake Constance in Southern Germany. We combined the spring migration model with seed retention curves to develop seed dispersal kernels. We also assessed the effects of pre-migratory fasting and the availability of suitable deposition habitats for aquatic and wetland plants. Our results show that mallards at Lake Constance can disperse seeds in the northeastern direction over median distances of 293 and 413 km for seeds with short and long retention times, respectively, assuming a departure immediately after foraging. Pre-migratory fasting strongly affected the dispersal potential, with only 1–7% of ingested seeds left for dispersal after fasting for 12 h. Availability of a suitable deposition habitat was generally <5% along the migratory flyway. The high probability of seed deposition in a freshwater habitat during the first stopover, after the mallards completed the first migratory flight, makes successful dispersal most likely to happen at 204–322 km from Lake Constance. We concluded that the directed long-distance dispersal of plant seeds, realized by mallards on spring migration, may contribute significantly to large scale spatial plant population dynamics, including range expansion in response to shifting temperature and rainfall patterns under global warming. Our dispersal model is the first to incorporate detailed behavior of migratory waterbirds and can be readily adjusted to include other vector species when tracking data are available.

**Keywords:** ***Anas platyrhynchos***, **climate change, endozoochory, global warming, migration, range shift, seed dispersal, waterbirds**

**Edited by:**
*Casper H. A. Van Leeuwen,*
*Netherlands Institute of Ecology*
*(NIOO-KNAW), Netherlands*

**Reviewed by:**
*Duarte S. Viana,*
*German Center for Integrative*
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**Specialty section:**
*This article was submitted to*
*Biogeography and Macroecology,*
*a section of the journal*
*Frontiers in Ecology and Evolution*

***Received:*** *26 October 2018*
***Accepted:*** *04 February 2019*
***Published:*** *26 February 2019*

**Citation:**
*Kleyheeg E, Fiedler W, Safi K,*
*Waldenström J, Wikelski M and van*
*Toor ML (2019) A Comprehensive*
*Model for the Quantitative Estimation*
*of Seed Dispersal by Migratory*
*Mallards. Front. Ecol. Evol. 7:40.*
*doi: 10.3389/fevo.2019.00040*

# INTRODUCTION

Facilitation of long-distance seed dispersal is considered one of the many important ecosystem services provided by migratory animals across the globe (Bauer and Hoye, 2014; Green and Elmberg, 2014). Plant seeds may stick to the outside of animals (epi- or ectozoochory) or may be retained inside the digestive system (endozoochory) while these animals undertake their migrations (Costa et al., 2014; Albert et al., 2015; van Leeuwen, 2018). The distances covered by animal vectors often far exceed the dispersal distances of seeds transported by abiotic vectors (Vittoz and Engler, 2007). If seeds remain attached to an animal long enough to be transported over at least part of the migratory journey, they may be shed or excreted at locations hundreds of kilometers away from their origin (Nathan et al., 2008; Viana et al., 2016). Thereby, migratory animals do not only help seeds to avoid kin competition, high pathogen loads, and predation pressure near the parent population, they also allow them to colonize newly available habitat, thereby contributing to the spatial population dynamics of plants (Nathan et al., 2008). Moreover, animal migrations are characterized by strong directionality, resulting in non-random or directed seed dispersal by sequentially visiting similar habitat types along the migratory trajectory (Nathan and Muller-Landau, 2000). With anthropogenic and climatic pressures on plant populations building up in recent decades, a thorough understanding of the mechanisms underlying long-distance zoochorous dispersal can help prioritize conservation and management measures (Bauer and Hoye, 2014; Viana, 2017).

Due to their limited dispersal capacity, plants growing in fragmented or isolated habitats with low inter-patch connectivity are particularly sensitive to environmental changes (Pimm and Raven, 2000). Freshwater aquatic and riparian plants are classic examples of plants with a preference for an inherently discrete habitat. Many of these species are typically dispersed by water (hydrochory; Kleyer et al., 2008), making it challenging to reach other catchments or unconnected water bodies. However, in recent decades it has become increasingly evident that zoochory, and especially endo-zoochory (Figuerola and Green, 2002; van Leeuwen et al., 2012a) by waterbirds plays a key role in the long-distance dispersal of plants growing in a freshwater habitat (hereafter "wetland plants"; Amezaga et al., 2002; Santamaría, 2002). Many waterbird species are involved in endozoochory, including ducks, coots, waders, and gulls, of which individual birds may consume large quantities of seeds of a wide range of species (Figuerola and Green, 2002; Soons et al., 2016). While most seeds are destroyed and digested in the process, a proportion of seeds typically pass the digestive tract unharmed and remain viable (Figuerola and Green, 2002; van Leeuwen et al., 2012a). Given that many waterbirds in temperate and (sub)arctic regions are migratory, their seed consumption probably results in large-scale long-distance dispersal of wetland plants.

To understand the spatial population dynamics of wetland plants, it is important to estimate the scale and potential distances of waterbird-facilitated dispersal. Yet, field observations of long-distance dispersal by waterbirds are lacking due to practical challenges regarding seed collection from birds with a known movement history as well as determining the origin of the seeds. For this reason, mechanistic models are being used to estimate dispersal distances. Essentially, these models combine information on the digestive tract passage of seeds with information on bird movements. Gut retention time, the time between seed ingestion and excretion, can be identified in feeding experiments with captive birds (e.g., Charalambidou et al., 2005; Soons et al., 2008; van Leeuwen et al., 2012a for waterbirds) and linked to the movement of a bird in the field. Although increasing, the amount and detail of information on long-distance movements of waterbirds is still limited. Most previous studies have estimated long-distance dispersal by migratory birds mainly by multiplying retention time distributions with the known flight speed of a bird (Charalambidou et al., 2005; Soons et al., 2008; Kleyheeg et al., 2015). This provides maximum dispersal distances based on the assumption of unidirectional flight at a constant speed, initiated directly after seed ingestion. It is well-recognized that these are unrealistic assumptions for at least three reasons: (1) migratory movements are unlikely uninterrupted or in a straight line; (2) birds may not depart immediately after seed ingestion; and (3) seeds deposited during flight may not end up in a suitable habitat, leading to unsuccessful dispersal (Clausen et al., 2002).

Viana et al. (2013a,b) obtained more realistic estimates of migration speed and dispersal distances by using ring recovery data within one or a few days after ringing. Major limitations of this approach, however, are that the number of useful records is very low, information on bird behavior in the period between ringing and recovery is still lacking, and the temporal resolution is typically too coarse to match the process of endozoochory (seed retention times are in the order of hours rather than days; van Leeuwen et al., 2012a). Recent advances in the tracking of birds, using high-tech tracking devices such as GPS transmitters, removes most of the uncertainties about bird movement at scales relevant for endozoochorous dispersal. Movements of individual waterbirds recorded at a high spatial and temporal resolution have already been implemented in a mechanistic model for seed dispersal outside the migratory period (Kleyheeg et al., 2017a). However, due to the limited availability of high-resolution tracks of migrating waterbirds, it remains challenging to estimate seed dispersal distances resulting from their migratory movements.

Here, we present a novel approach to estimate realistic dispersal distances of seeds transported by mallards (*Anas platyrhynchos*) on spring migration. We used a set of GPS tracks of migrating mallards, combined with ring recovery data from the same population to inform a mallard migration simulation model with properties of the empirical migration trajectories, such as step lengths, turning angles, and timing of stopovers. This model allowed us to generate a large number of spring migration trajectories expected for the study population of mallards, which we converted into a spatially explicit probability map of mallard passage over time. We matched these predictions of mallard migrations with previously identified seed retention time curves, representative of small and large seeds, to model dispersal distances. To facilitate application of this novel approach to other systems of long-distance zoochorous dispersal, we present a detailed step-by-step description of the modeling procedure including R code in the **Supplementary Material** (**Data sheets 1, 2**).

We based our estimates of seed dispersal distances on the behavior of spring-migrating mallards, which typically follow a stepping stone migration strategy with migratory flights intermitted by staging at stopover locations (Krementz et al., 2011). Conceptually we distinguished between three relevant phases of mallard migration, each with implications for seed dispersal (**Figure 1**). Chiefly, the three phases were: the first migratory flight (phase I), the first stopover (phase II), and the second migratory flight (phase III). Note that phase I can be the first migratory flight after leaving the wintering site, or the first flight after stopping over at any location along the migratory journey. We expected that the time allocation of mallards into these different phases has major implications for seed dispersal, as the duration of phase I probably determines the dispersal distance of the majority of seeds, phase II provides an opportunity for seeds of aquatic and wetland plants to end up in a suitable habitat for germination and establishment, and those seeds that are retained until phase III are able to achieve extremely long dispersal distances. We further extended our mechanistic dispersal model to explore the effect of pre-migratory fasting (or departure lag time; Viana et al., 2013a) on the realized dispersal distances and explicitly accounted for availability of a suitable habitat to estimate the impact of stopover behavior on the probability of effective (successful) dispersal.

# METHODS

## Study System

We used GPS tracking data from mallards wintering at Lake Constance on the border between Germany, Switzerland, and Austria as the basis for this study. Mallards are abundant across the Northern hemisphere and have a winter diet of mainly seeds and other plant material (del Hoyo et al., 1992). A review of mallard diet studies revealed that they consume seeds of more than 300 different plant species in Europe alone (Soons et al., 2016). The mallard's digestive strategy is to maintain the throughput of large amounts of food rather than investing in the digestion of all food items, allowing many hard parts to rapidly pass the digestive tract unharmed (van Leeuwen et al., 2012a). Mallards are therefore considered a key seed dispersal vector in freshwater habitats of temperate regions (Kleyheeg et al., 2017a; Bartel et al., 2018). In the Lake Constance region, mallards are among the most numerous wintering waterbird species with numbers up to ∼15,000 individuals in December and January. A large proportion of mallards in the Lake Constance region are resident year-round, while a smaller proportion migrates over short to long distances (Werner et al., 2018).

We used the GPS tracking data of mallard spring migration from Lake Constance, to inform our mallard migration simulator. We extracted the data from Movebank.org (LifeTrack Ducks Lake Constance, Movebank ID 236953686). The entire tracking dataset included 51 individual mallards, but the vast majority of them were year-round residents. We used the tracks of eight mallards leaving the Lake Constance region on spring migration in a northern to eastern direction between March 08 and May 20, 2018. During the migratory period, the data for these eight individuals amounted to 36,331 relocations in total. These birds migrated toward locations in Germany, Austria, Czech Republic, Belarus, and Lithuania (**Figure 2**). Tracking data were collected by 25 g GPS-GSM transmitters with an elevated solar panel (OrniTrack E25 from Ornitela) that were mounted on the mallards' back using a Teflon ribbon harness as described by Roshier and Asmus (2009). As long as mallards stayed within a geofence around their wintering sites at Lake Constance (lat/lon coordinates NW corner: 47.78, 8.85; SE corner: 47.63, 9.16), the tags were programmed to record GPS-fixes with a 1-h interval. As soon as ducks left the geofenced area for migration, the tags instead recorded a burst of 15 fixes with a 1 Hz frequency at the same sampling interval. Trapping and GPS tracking of mallards was licensed by Regierungspräsidium Freiburg under registration number G-13/28.

## Mallard Migration Simulation

To obtain a general pattern of mallard spring migration from the Lake Constance region, we simulated a total of 50,000 mallard migrations using the empirical Random Trajectory Generator (eRTG; Technitis et al., 2016) in the R environment (R Core Team, 2018). This algorithm simulates the movement between two pre-defined locations with a fixed number of steps, based on a dynamic drift derived from a step-wise joint probability surface. The process is attracted to the destination location, and the strength of attraction is modulated by the time left to reach the target. The eRTG is thus similar to a biased correlated random walk and can be best described as a mean-reverting Ornstein-Uhlenbeck process (Smouse et al., 2010). One main advantage of the eRTG over traditional random trajectory generators is that the trajectories it simulates retain the geometric characteristics of the empirical tracking data, as it relies entirely on empirical distribution functions. Consequently, if a destination cannot be reached within the realms of the empirical distributions derived from the movement data, the simulation fails rather than forcing the last step toward the destination.

Simulating migratory trajectories of mallards using the eRTG requires: (i) empirical estimates of movement parameters (step length, turning angles, their autocorrelation at a lag of one step, and the covariance of step length and tuning angle) with a relevant sampling interval; and (ii) destination locations representing realistic breeding locations of mallards wintering at Lake Constance.

### Empirical Distributions for Step Length and Turning Angles

We first classified the mallard GPS tracking data with movement behavior using Hidden Markov models (HMMs) to derive empirical distributions of step lengths and turning angles for different states of movement behavior (i.e., migratory or local movements). We identified the behavioral state of each location using the R-package momentuHMM (version 1.4.2; McClintock and Michelot, 2018). HMMs expect regularly sampled data, so we initially calculated the average ground speed recorded in each burst, and then reduced the 15-s GPS-bursts to single locations (retaining only the first location of each burst),

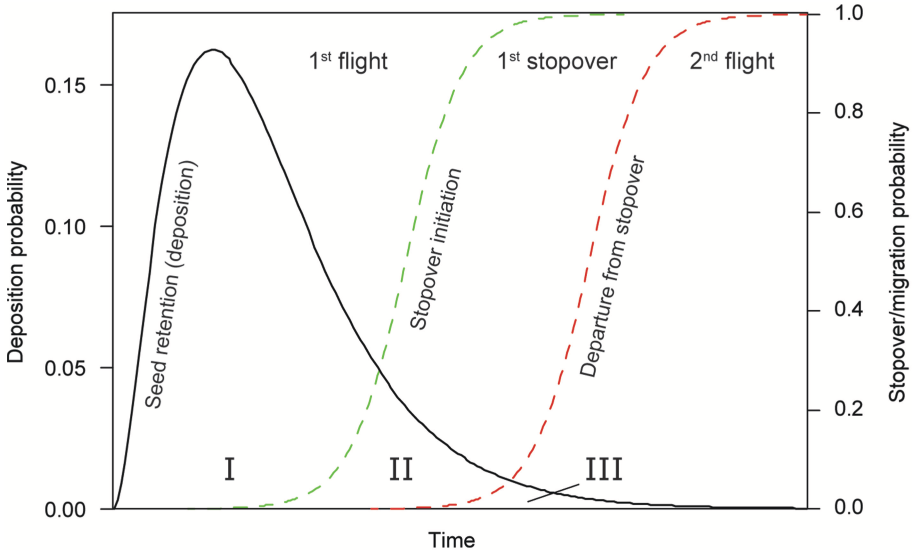

**FIGURE 1 |** Conceptual graph of seed dispersal during the migratory flight of a mallard. The seed retention time determines the excretion and therefore deposition probability, starting at t0 for seeds consumed just prior to departure. Seeds are deposited during the first migratory flight (phase I), the first stopover (phase II) or during the second migratory flight (phase III). The probability of arriving at (green dotted line) or departing from a stopover (red dotted line) is a function of time.

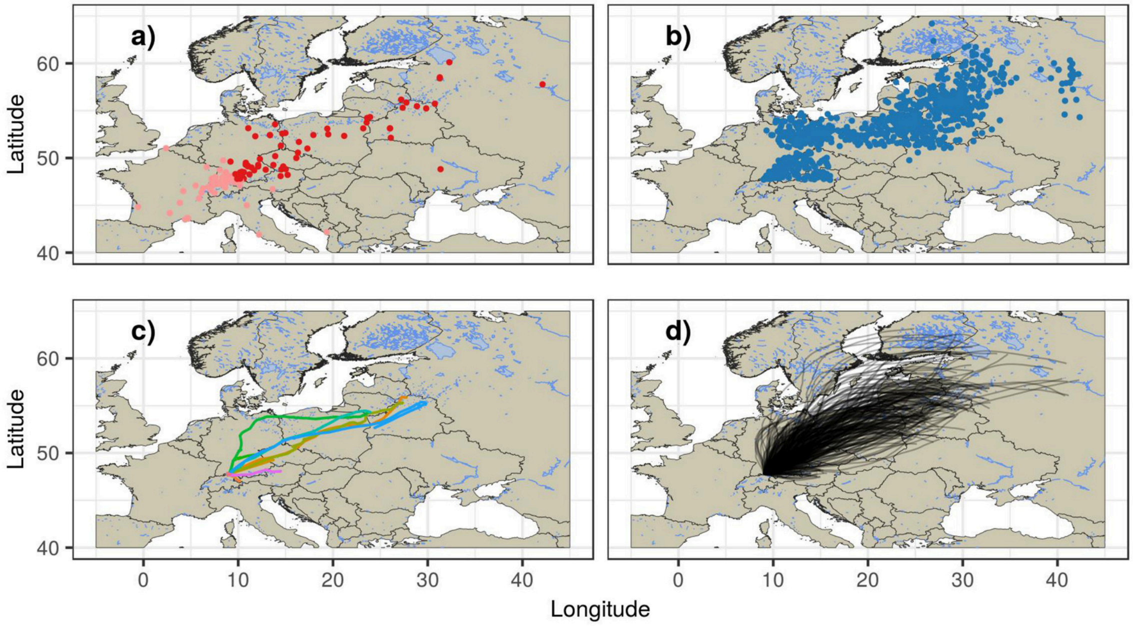

**FIGURE 2 |** Migration of mallards from the Lake Constance region based on empirical and simulated ring recoveries and migration trajectories. Panel **(a)** shows empirical ring recoveries with those relevant for spring migration in dark red, **(b)** shows 1,000 simulated destination locations for spring migrating mallards, **(c)** shows the empirical spring migration routes of mallards equipped with a GPS-GSM transmitter, and **(d)** shows 500 simulated spring migration trajectories.

and included missed fixes in the dataset, which occur when scheduled sampling does not take place, e.g., when the tag fails to find a GPS-signal. We further filtered the tracking data to only retain the migratory individuals during the migratory period. This filtered dataset contained a total of 8,817 locations at a sampling interval of 1 h. Missed fixes accounted for about 5% of the data (471 out of the final 8,817 relocations).

We applied a 3-state HMM with three data streams; specifically, the average ground speed of each burst (GPS-derived speed), step length (geodesic distances), and turning angle between subsequent locations. After visual inspection of the distribution of the data streams and exploratory runs using HMMs, we decided to assume a Gamma distribution for ground speed, a Weibull distribution for step length, and a wrapped Cauchy distribution for turning angles. The three states that were distinguished represent "resting" behavior, "local movement," and "flight." We listed the details for the final HMM, and the parameters estimated for the models for all three states in the **Supplementary Material** (**Data sheet 2**). The decision for the final model was informed by visual exploration and an AIC-guided model selection on an initial set of alternative HMMs with two, three, and four states, and different starting parameters and distributions for the different data streams. In the final HMM, we additionally included time of day as a predictor for state transitions. We calculated the time of day as fractional hours relative to the solar noon at the respective locations in the tracking dataset using the R-package maptools (version 0.9.2; Bivand and Lewin-Koh, 2017).

We applied the Viterbi-algorithm, an efficient algorithm to determine the most likely sequence of states provided in the momentuHMM package and the final HMM to annotate the locations of mallard migration trajectories with their corresponding state. For the subsequent calculations of step lengths and turning angles, we only used the locations classified as "flight." To inform our eRTG, subsequently termed Mallard Migration Simulator (MMS), we calculated step lengths, turning angles, the covariance of step lengths and turning angles, as well as their autocorrelation (the difference in step length and turning angles at a lag of one location) for each 15-s GPS-burst in "flight state." The final sample size for these parameters was $n = 535$.

This initial set of empirical distributions for the MMS describes mallard flight at a sampling interval of one second. Simulating mallard migrations with this high temporal resolution is however computationally demanding, and thus we decided to thin the MMS for a 300 s interval. To maintain realistic distributions of the movement parameters at this reduced sampling interval, we simulated a long unconditional trajectory with 3,000,000 steps using the sampling interval of 1 s. We subsequently thinned this long simulated trajectory to a sampling rate of 300 s, and calculated the step lengths, turning angles, the autocorrelation of both step lengths, and turning angles at a lag of one step, and the covariance of step lengths and turning angles for the new sampling rate (see also van Toor et al., 2018a).

### Sampling Destinations for Simulated Mallard Migrations

We used ring recoveries of mallards to derive a sampling tool for destination locations for the migration simulations (**Figures 2a,b**). A total of 696 ring recoveries from 494 individuals were available to us. The dataset contained data for mallards ringed between 1934 and 2016, and recoveries recorded between 1934 and 2017. We filtered the pairs of ringing and recovery events so the dataset would only contain individuals (i) ringed at a location within 50 km distance from Lake Constance, and recovered at a distance $>$ 50 km from Lake Constance, (ii) recoveries that were made within a year from the marking of the individual, and (iii) recoveries made between January and June to represent spring migratory movements. This restrictive filtering reduced the initial dataset to 28 ring recoveries. We supplemented these recoveries with locations from the migratory individuals in the tracking dataset, using only the location farthest away from Lake Constance for each individual. This supplemented dataset contained an additional eight destination locations, and thus 36 destination locations in total. Subsequently, we computed the distance (geodesic distance, in meters), and bearing (initial great circle bearing) between the ringing location and the recovery location. We then further restricted the recoveries to the supposed spring migratory flyway for these mallards, retaining only recoveries with a bearing between 0 and 90 degrees from Lake Constance, representing a north to east migratory direction (final sample size of 30 observations).

Our sampling tool for destination locations consisted of a 2D-kernel density estimator with bearing from Lake Constance on the x-axis, and distance to Lake Constance on the y-axis. We first determined the optimum kernel bandwidth, a parameter determining smoothness of the resulting estimate, for both bearing and distance using cross-validation (R-package sm, version 2.2–5.5; Bowman and Azzalini, 2014), which suggested a bandwidth of 19.32° for bearing, and 366.70 km for distance. We then computed the 2D-kernel density estimator using the suggested bandwidths with a resolution of 1 degree for bearing, and a resolution of 2.5 km for distance from the start location. We used this tool by sampling a unique bearing and distance from Lake Constance and calculated the corresponding location in space from the starting location using Great Circle methods.

### Simulating Mallard Migrations

Using the MMS, we simulated spring migration trajectories for mallards from Lake Constance (**Figures 2c,d**). We chose a shallow bay in the Lower Lake, the Markelfinger Winkel, as the starting location for each simulation (47.766931° N, 8.996157° E). We limited the simulations to this departure location for two reasons: firstly, because migration routes may vary with geography and the characteristics of the migratory trajectories are therefore likely different in other regions, and secondly because the ring recoveries we used provided destination locations specifically for mallards wintering at this site. We sampled destination locations for each simulation using the destination sampling tool described above. Next, we needed to determine the number of steps available for the simulated duck to reach its destination. This parameter determines how strongly the simulation is pulled to the target destination, and thus affects the ratio between total migration distance to displacement distance from the starting location. To pick a number of available steps that captured the ratio between migratory distance and migration duration observed in the tracking data we first divided the geographic distance between start and destination by the average step length represented in the MMS (6647.89 m per 300 s). However, it was evident from the tracking data that duck trajectories deviate from a straight line between start and destination, and so we determined the ratio between time spent on migration and migratory distance in the tracking data by calculating the distance between consecutive locations in mallard migratory flights and the expected flight time based on the average flight speed in the MMS. We used these metrics to calculate the ratio between observed and expected flight speed. We found that this mallard flight time, relative to expected flight time, followed a normal distribution with a mean of 0.982 and a standard deviation of 0.154. For each simulation, we randomly sampled a ratio for observed/expected flight time from the normal distribution and multiplied the number of steps with the sampled ratio to derive the number of steps available for the simulation.

We simulated 50,000 migratory simulations using the MMS ignoring potential stopovers along the way in this step, but accounting for staging behavior of migratory mallards during the seed dispersal simulations.

## Stopover Probability and Stopover Duration

We determined stopover periods from the tracking data to be able to account for the use of suitable staging locations by mallards during migration. We first determined the minimum expected flight speed from the HMM. We calculated the 0.1—percentile of the Gamma distribution for ground speed in the migratory state, corresponding to 4.36 m/s, or 15.68 km/h. Using this cut-off value for flight speed, we excluded every individual that had not moved at least 50 km from the location of deployment and retained only locations associated with a ground speed smaller than the minimum expected flight speed. We then applied a fuzzy clustering on the spatial location for each individual (R-package cluster, version 2.0.7–1; Maechler et al., 2018), assuming that locations collected during stopover periods would be clumped in space. First, we applied the clustering with the number of suggested clusters, or stopovers, ranging from 2 to 10. We then chose the optimal number of clusters based on the cluster silhouettes (Rousseeuw, 1987). The width of cluster silhouettes is an indication of separation between points in a cluster and can range from −1 (indicating that samples might be allocated to the wrong cluster) to +1 (samples are far away from neighboring clusters). The median silhouette width for the clustering applied in this study ranged from 0.87 to >0.99 for the different individuals.

We computed the centroid location for each stopover and calculated the geodesic distance between the deployment location and the first stopover location, as well as the geodesic distance between subsequent stopover locations. We used these distances to inform an empirical cumulative density function ($n = 27$), reflecting the probability of a migratory duck making the decision to end the leap of migration as a function of distance from the starting location. Similarly, we determined the duration of stopovers (in hours) and used these estimates to inform an empirical cumulative density function ($n = 27$) reflecting the proportion of ducks remaining at the stopover site as a function of time since beginning of the staging period (**Figure 3**).

## Seed Retention Parameters

We used gut retention time curves of seeds passing the digestive tract of mallards from a previous study by Kleyheeg et al. (2017a) to model the dispersal distances of mallard-dispersed seeds. The retention curves for different plant species were assumed to follow a Gamma distribution with a constant shape parameter ($k = 2.7$) and a rate parameter that depends on seed volume following a logarithmic function. This indicates that larger seeds typically have longer retention times than smaller seeds, with the survival rate of small seeds being generally higher (Soons et al., 2008). Here, we compared dispersal under a short retention time (small seeds) scenario and a long retention time (large seeds) scenario. The size of seeds consumed by mallards ranges from <0.001 to >1,000 mm$^3$, but typically lies around 1–10 mm$^3$ (Soons et al., 2016). For our two scenarios, we selected a seed volume of 0.2 and 20 mm$^3$, corresponding to a rate parameter of 0.63 (short retention time) and 0.44 (long retention time), respectively (Kleyheeg et al., 2017a).

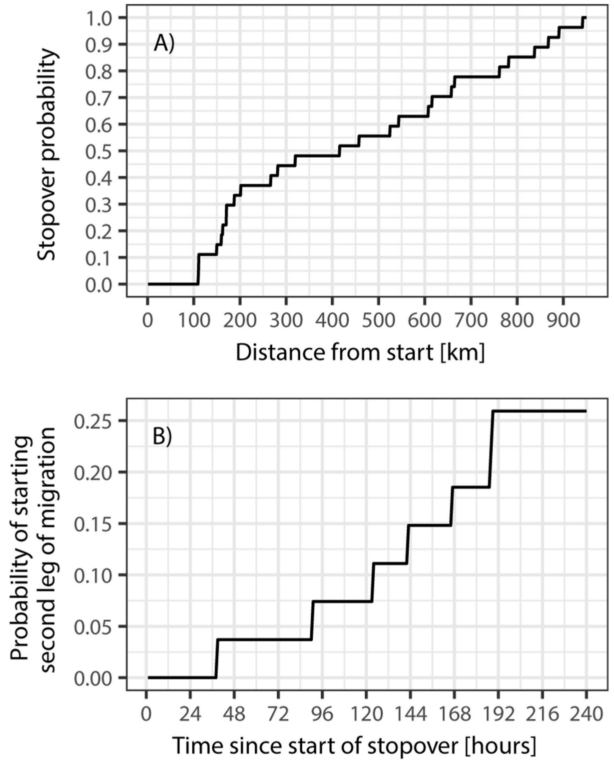

**FIGURE 3 |** Stopover probability over time for mallards on spring migration from the Lake Constance region **(A)** and probability of starting a second leg of migration as a function of time since arriving at the stopover **(B)**. Note that the y-axis of the latter does not reach 1 due to the very long stopover times for most mallards (exceeding 240 h). Furthermore, it should be noted that not every individual will embark on a second, or even third, leg of migration.

## Seed Deposition Habitat

We assessed the availability of wetlands in the mallard migration corridor to estimate the probability of seed dispersal into wetlands. We used the Global Lakes and Wetlands Database (GLWD; Lehner and Döll, 2004) to calculate the availability of wetlands. The GLWD contains worldwide information for 3,067 lakes with a surface area > 50 km² and 654 reservoirs with a storage capacity > 0.5 km$^3$, as well as detailed data on permanent open water bodies with a surface area > 0.1 km².

First, we computed a circle with a radius equivalent to the maximum expected dispersal distance $d_{max}$ around the migration starting location. We calculated $d_{max}$ as the maximum observed distance in the simulated migratory trajectories for an excretion probability greater than zero (from the gut retention curves) and a stopover probability smaller than 1, assuming long retention time scenario with no fasting. This resulted in a $d_{max}$ of 941 km, which we rounded to a circle radius of 950 km. This distance corresponds to the 98.5%-percentile of migratory distances in the ring recovery data set. We then computed circular sectors between 0 and 90 deg, representing the migratory corridor, in 5-degree increments, and subdivided the circular sectors at 50 km increments from the starting location.

We then computed the total wetland area (including lakes, large reservoirs, and permanent open waterbodies) in each of these segments of the circular sectors (hereafter called annulus section). We subsequently calculated the proportion of wetland area per annulus section by dividing the wetland area by the area of the annulus section.

## Effective Seed Dispersal Kernels

Finally, we combined the gut retention curves with pre-migratory fasting and staging behavior to determine general dispersal kernels for mallard migration, and added wetland availability to determine a specific dispersal kernel describing the probability of seeds being dispersed from Lake Constance to a potentially suitable habitat. For every location in a simulated trajectory, we calculated the geodesic distance from the starting location, hereafter *d*, and the time since start of migration, hereafter *t*. We then defined the stopover probability, derived from the empirical cumulative density function described above, as $\sigma(d)$. The inverse of $\sigma(d)$, $1\text{-}\sigma(d)$, consequently defines the probability of individuals being migratory for the same *d*. We first defined the proportion of seeds excreted during spring migration ($\psi_{\text{tot}}$), and not on the wintering site, as a function of the duration of pre-migratory fasting ($f$) using the gut retention curve for the respective seed size:

$$\psi_{\text{tot}}(f) = \int_{\text{f}}^{48\text{h}} p_{\text{excr}},$$

which is composed of the proportion of seeds excreted during migratory flight ($\psi_{\text{m}}$) and the proportion of seeds excreted at stopover sites ($\psi_{\text{s}}$):

$$\psi_{\text{tot}}(f) = \psi_m + \psi_s.$$

We could calculate $\psi_{\text{m}}$ directly for each simulated trajectory. We defined the probability of a seed being dispersed over any distance *d* at time $\tau$ by a migratory individual, $\gamma_m$, with $\tau = t + f$, as:

$$\gamma_m(\tau, \text{d}) = [1 - \sigma(d)] * \int_{(\tau-5\text{min})}^{(\tau)} p_{\text{excr}}.$$

By calculating the cumulative sum for all $\gamma_m(\tau, d)$ along a simulated trajectory, we obtained the proportion of seeds dispersed during flight:

$$\psi_m = \sum \gamma_m.$$

Similarly, the probability of a seed being dispersed over any distance *d* at time $\tau$ by a staging individual, $\gamma_s$, can be calculated as:

$$\gamma_s(\tau, \text{d}) = \sigma(d) * \int_{(\tau-5\text{min})}^{(48h)} p_{\text{excr}}.$$

Because the staging duration observed in the spring migration trajectories was $\geq$ the duration for which gut retention was estimated, we integrated excretion probability for the entire curve $> \tau$. This however leads to dispersal probabilities along a trajectory with a total sum $> 1$ for $\gamma_s$. We corrected for this using the total proportion of seeds dispersed at stopover sites, which we determined as:

$$\psi_s(f) = \psi_{\text{tot}}(f) - \psi_m(f).$$

We thus normalized $\gamma_s$, resulting in $\gamma'_s$, using:

$$\gamma'_s(\tau, d) = \frac{\sigma(d) * \int_{(\tau-5\text{min})}^{(48h)} p_{\text{excr}}}{\sum(\gamma_s * \sigma(d))} * \psi_s,$$

which ensures that $\sum(\gamma'_s) = \psi_s$.

Finally, we combined $\gamma_{\text{m}}(\tau, d)$ with $\gamma'_{\text{s}}(\tau, d)$ to determine the probability of a seed being dispersed from the starting location during both migratory flight and staging as:

$$\Gamma_{\text{tot}}(\tau, d) = \gamma_m(\tau, d) + \gamma'_s(\tau, \text{d}).$$

We further calculated the probability of seeds being dispersed into a suitable wetland habitat, again accounting for migratory and staging behavior. It was previously shown that during stopover, mallards spend about 95% of their time in wetland areas, and consequently the probability of seeds being dispersed into a suitable habitat during stopover is high (Jorde et al., 1984; Sauter et al., 2012; Kleyheeg et al., 2017b). During migration, however, the probability of a seed to be excreted above a suitable habitat is dependent on the availability of wetlands. Therefore, we defined the probability of seed excretion in a wetland as a function of mallard behavior, with the probability of a duck being in a wetland during stopover being $\beta_s = 0.95$. The probability of flying over a wetland during migration, in turn, was defined as $\beta_m(d) = u(d)$, where *u* is the average area percentage of wetlands of all annulus sections corresponding to *d*, weighted by the proportion of simulated tracks passing through the corresponding annulus section. Finally, we defined the probability of successful dispersal, i.e., the probability of a seed being dispersed into a potentially suitable wetland habitat, during mallard spring migration $\Gamma_{tot,wd}$ as:

$$\Gamma_{tot,wd}(\tau, d) = \gamma_m(\tau, d) * \beta_m(d) + \gamma'_s(\tau, d) * \beta_s.$$

We computed $\Gamma_{tot}$ and $\Gamma_{tot,wd}$ for both the small and large seed scenario, and for three different fasting durations ($f = \{0, 1, 12$ h$\}$), for all simulated trajectories.

# RESULTS

## Seed Dispersal Shadows

We initially considered the simplest case by describing the probability of a seed being dispersed by a migrating mallard over a certain distance. For this scenario, we assumed that mallards fly non-stop (without staging at stopover locations), do not undergo pre-migratory fasting, and that dispersal occurs at every location with a non-zero excretion probability. We then calculated the time after which 50 and 99% of the seeds had passed through the gut and calculated the mean geodesic distance from the corresponding location in the simulated tracks to the starting location (rounded down to the closest 5 min). We found that the resulting mean dispersal distance for small seeds was 294 km at the time that 50% of seeds had passed through the guts, and 970 km after 99% of seeds had passed through the gut. For large seeds, the mean expected dispersal distance was 417 km after 50% of seeds passed through the gut, and 1,369 km after 99% of the seeds had passed. These results are summarized in **Figure 4**, which additionally shows the density of simulated trajectories.

When correcting expected distances of the first migration leg for the mallards' stopover behavior, we found that the dispersal distances became substantially shorter. After accounting for the increasing probability of stopovers with increasing migratory distance, a mean distance of 254 km from the starting location would be expected after a passage of 50% of seeds (99% of seeds: 382 km) for small seeds. For large seeds with a longer retention time, 50% of the seeds were expected to have passed after ducks covered a mean distance of 332 km (99% of seeds: 379 km).

In the subsequent paragraphs, we extend from this simple scenario by accounting for the effects of pre-migratory fasting, the availability of suitable wetland habitat along the main migratory corridor and consider the time spent at the first stopover.

## Pre-migratory Fasting

As dispersal distance is a function of time since the ingestion of seeds, pre-migratory fasting strongly affects the expected seed dispersal distances, and the overall probability of dispersal events (**Figure 5**). We found that every hour of fasting reduced the expected median dispersal distance of small seeds by about 78 km for both small and large seeds. Consequently, whereas the median dispersal distance without accounting for fasting was about 294 km, a fasting duration of 1 h will result in a median dispersal distance of 215 km (99th percentile: 893 km), whereas a fasting duration of 12 h will result in a median dispersal distance of 0 km (99th percentile: 39 km). For large seeds, the corresponding median dispersal distance accounting for 1 h of fasting was 339 km (99th percentile: 1,296 km), and 0 km for 12 h of fasting (99th percentile: 462 km).

## Dispersal Into Wetlands

The availability of wetlands along the migratory flyway for mallards departing from Lake Constance is generally low

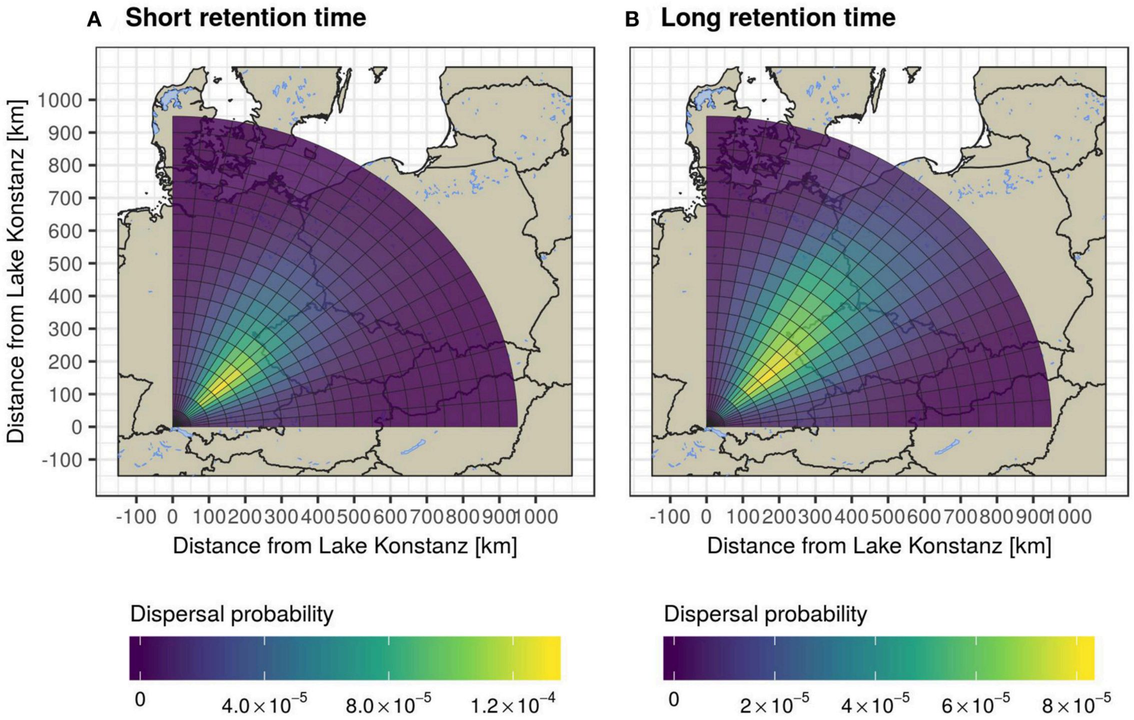

**FIGURE 4 |** Seed dispersal shadows for short **(a)** and long **(b)** retention time scenarios, resulting from endozoochorous dispersal of mallards on spring migration from the Lake Constance region. In this figure, mallards are assumed to ingest seeds just before departing on migration and to not use stopovers. Here, seed dispersal probability represents the product of excretion probability as suggested by gut retention curves and the proportion of simulated mallards passing through a given annulus section.

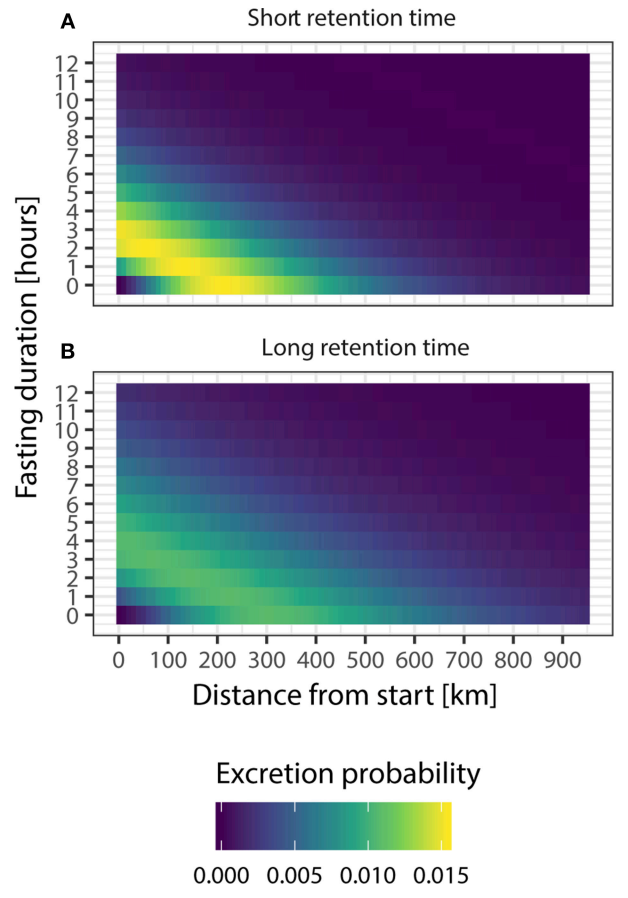

**FIGURE 5 |** The effect of the duration of pre-migratory fasting on seed dispersal kernels, expressed as the excretion probability over distance from the departure location, for the short **(a)** and long **(b)** retention time scenarios. Note that the probability of seeds to be dispersed away from the location where they are consumed drops to close to 0 if mallards would stop foraging 12 h prior to departure. In this figure, mallards are assumed to fly to their destination without using stopovers.

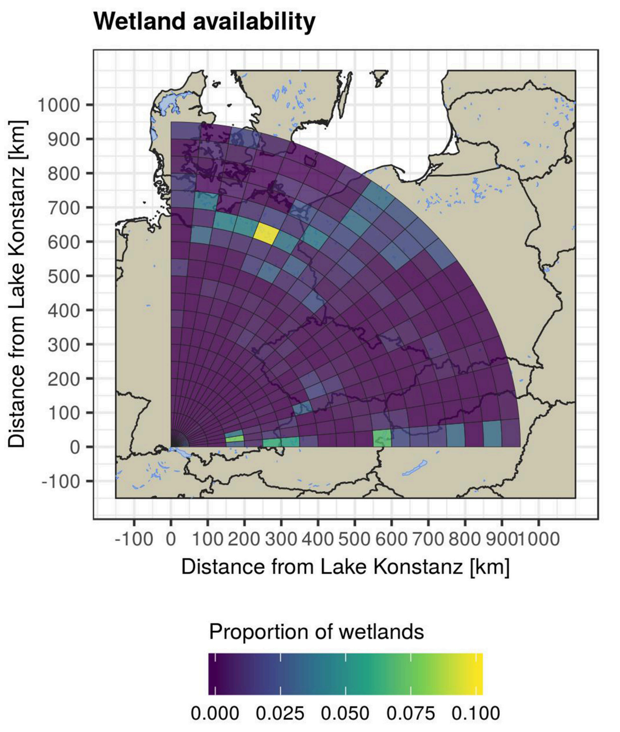

**FIGURE 6 |** Wetland availability within the reach of mallard spring migration from Lake Constance, expressed as the area-percentage of freshwater habitat within each annulus section. Note that the availability of wetlands is generally low, making it unlikely for seeds to end up in wetland habitat when excreted during the mallard migratory flight.

(**Figure 6**). The highest proportion of wetlands is found in the northeast of Germany, where a large lake and canal region known as the Mecklenburg Lake Plateau is located. Other areas with above-average wetland availability are located in Southern Bavaria, Germany (e.g., Lake Ammer, Lake Starnberg, and Lake Chiem), Lake Neusiedl in eastern Austria, and North-West Poland. However, with a maximum area percentage of wetlands below 10%, the probability of a seed ending up in a suitable habitat after excretion by a mallard in migratory flight was low.

## Effective Dispersal Kernels

Finally, we combined the gut retention curves, simulated mallard migration trajectories and detailed stopover behavior (**Figures 7a,b**), for which we specifically accounted for seed excretion at stopover locations. We found that in the absence of migratory fasting, 50% of small seeds would be transported over distances up to 293 km (99%: 675 km), and large seeds over distances up to 413 km (99%: 675 km). If ducks fasted for 1 h before migration, these distances are reduced to 215 km (99%: 675 km) for small seeds, and 338 km (99%: 675 km) for large seeds. A fasting duration of 12 h led to expected distances of 0 km (99%: 40 km) for small seeds, and 0 km (99%: 457 km) for large seeds.

However, these dispersal distances did not match the distances of peak dispersal probability (**Figures 7a,b**). Consequently, we calculated the distances over which the dispersal of seeds is most likely to occur. We found that for small seeds, the dispersal probability peaked at a distance of 203 km in the absence of fasting (1 h of fasting: 173 km; 12 h of fasting: 7 km). Large seeds were most likely transported over distances of 284 km (no fasting), 158 km (1 h of fasting), and 7 km (12 h of fasting).

Inversely, we also estimated the proportion of seeds ingested at the wintering site that are expected to be dispersed farther than 50 km from the site of ingestion. We found that 98.8% of all small seeds ingested prior to migration would be dispersed >50 km in the case of no pre-migratory fasting (fasting duration of 1 h: 88.2%, fasting duration of 12 h: 1.0%). For large seeds, we estimated that approximately 99.5% of the seeds would be transported over distances exceeding 50 km if ducks did not fast prior to migration (fasting duration of 1 h: 94.5%, fasting duration of 12 h: 6.3%). The proportion of seeds transported over distances > 50 km that also end up in a potentially suitable wetland habitat was much smaller. For small seeds, we estimated

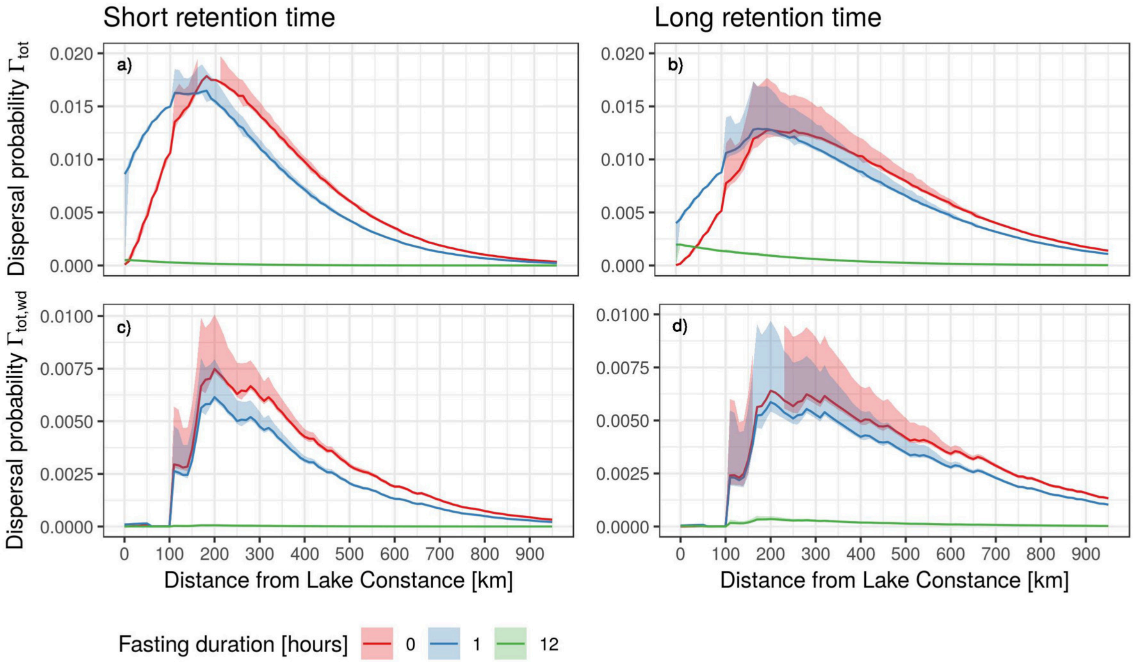

**FIGURE 7 |** Effective seed dispersal kernels accounting for staging behavior. The upper panels show $\Gamma_{tot}$ as a function of distance from Lake Constance for the short **(a)** and long **(b)** retention time scenarios. Lower panels show dispersal into suitable wetland habitat ($\Gamma_{tot,wd}$) for the short **(a)** and long **(b)** retention time scenarios. Line color shows dispersal kernels for different fasting durations. Note the different scaling of the y-axes of the lower panels.

that 39.9% would be dispersed into wetlands in the no-fasting scenario (fasting duration of 1 h: 30.3%, fasting duration of 12 h: 0.2%), whereas for large seeds, this estimate was 52.3% in the absence of fasting (fasting duration of 1 h: 44.3%, fasting duration of 12 h: 1.9%).

The probabilities of seed dispersal into wetlands, $\Gamma_{tot,wd}$, were ∼ 50–60% lower than the probabilities predicted by $\Gamma_{tot}$ without pre-migratory fasting (**Figures 7c,d**). Peak dispersal probabilities, however, were at relatively long distances compared to overall dispersal, with an estimated 204 km for small seeds, and 322 km for large seeds. Pre-migratory fasting of 1 h did not affect the peak of dispersal probability. For a longer fasting duration of 12 h, the most likely dispersal distances became only slightly shorter (174 km for small seeds and 282 km for large seeds).

# DISCUSSION

The fast movements by migrating birds contribute substantially to the long-distance dispersal and global distribution of plants. We used a novel approach to estimate realistic seed dispersal distances realized by an important dispersal agent on its northbound spring migration. Our mechanistic model shows that mallards wintering in central Europe may commonly disperse seeds over distances of 293–413 km when embarking on spring migration toward Northeast-Europe, with relatively high probabilities of deposition in freshwater habitat after a 204–322 km migratory flight.

## Mechanistic Model

The outcome of mechanistic dispersal models critically depends on the realistic parameter estimates of both seed and vector traits. The novel aspect of this study is that we, in contrast to earlier studies, used high-resolution tracking data for implementation of mallard movement in the model described here. Thereby, we considered the tortuous nature of migration trajectories as well as stopover behavior, which both strongly affect the dispersal distances and deposition habitat. Tracking data of mallards and other duck species in Europe are still scarce and in this study, we were able to use only eight GPS tracks, from a total of 51 tagged individuals of this partially migratory population. Although inclusion of more tracks likely would have reduced the confidence intervals around the estimated migration parameter values, the method relies more critically on a high sampling rate within tracks. Moreover, the simulation algorithm we used was designed specifically to overcome the limited availability of empirical tracks by generating trajectories with properties mimicking real trajectories from GPS tracking studies (Technitis et al., 2016), which has been applied successfully in earlier studies (van Toor et al., 2018a,b). It distinguishes itself from other random trajectory generation techniques in its reliance on parameter values from empirical tracking data. The specificity of these parameters by (sub)populations or species does require the availability of detailed tracking data from the animals of interest.

Seed retention curves were obtained from an earlier study and considered representative of relatively small and large seeds passing through the mallard digestive tract (Kleyheeg et al., 2017a). The shape of the retention curve is, however, affected by more seed traits than size alone and looks different for different plant species. Most importantly, seed survival (intact passage) interacts with retention time, whereby resistant seeds are able to survive sustained exposure to digestive processes, resulting in a fatter tail of the retention time distribution and longer dispersal distances (Kleyheeg et al., 2015). Resistance to digestion of seeds within the same size range depends among other factors on seed shape and seed coat traits (Wongsriphuek et al., 2008; Kleyheeg et al., 2018), which should be considered when estimating dispersal distances for specific plant species.

## Dispersal Distances

The integration of the detailed movement behavior of the dispersal agent into mechanistic models inevitably yields lower dispersal distance estimates than when assuming continuous unidirectional flight. Previous studies multiplying retention times with mallard flight speed estimated dispersal distances in the range of 300–1,400 km for mallards in North-America (Mueller and van der Valk, 2002), while others reported distances typically around 200 km and rarely exceeding 450 km (Kleyheeg et al., 2015), at least up to 312 km (Farmer et al., 2017), or a vast majority of dispersal events within 350 km (Soons et al., 2008). Viana et al. (2013b) compared these calculations with estimations based on ring recovery data during autumn migration and found median dispersal distances of 21–63 km based on ring recovery data vs. 148–840 km based on average flight speed of mallard and teal (*A. crecca*). The ring recovery data however did not only include birds on active migration.

Based on our estimates, mean dispersal distances resulting from transportation by migrating mallards after median seed retention times are 293–413 km. This corresponds with the higher range of distances reported by Clausen et al. (2002), who argue in a critical review of waterbird-mediated dispersal that the bulk of seeds will be dispersed over 100–500 km by birds on a long-distance flight. Although overall dispersal probabilities are strongly reduced when only dispersal into wetlands is considered potentially successful, the peaks of dispersal probability are at slightly longer distances for $\Gamma_{tot,wd}$ than for $\Gamma_{tot}$. Average distances of 204–322 km for a maximum $\Gamma_{tot,wd}$ match or exceed many estimates from mechanistic models assuming continuous flights, even though stopover behavior in wetlands is accounted for in the calculation of these distances. This is likely due to three factors: (1) for mallards departing from Lake Constance, the availability of freshwater habitat is higher at longer distances, (2) seeds are unlikely to end up in freshwater habitat during the migratory flight, i.e., within several hundreds of kilometers from the departure location, and (3) the flight speed of migrating mallards is often higher than the average flight speed used in earlier mechanistic models. Seeds with fat-tailed gut retention curves benefit in particular from these factors. Long dispersal distances are thus caused mainly by very low probabilities of effective dispersal at shorter distances.

Although rare, seeds retained long enough to be transported on a second leap of migration may reach extremely far dispersal distances. In the tracking data used here, the shortest stopover duration was recorded at 38 h, and some individuals never started a second leap of migration. Our analyses were cut off at 48 h, but longer retention times have been recorded in experimental feeding trials. Viable *Sparganium emersum* seeds were found at the end of a 60 h feeding trial with mallard and teal (Pollux et al., 2005), *Scirpus* seeds were found in blue-winged teal (*A. discors*) after 72 h (Swanson and Bartonek, 1970) and a maximum retention time of 93 h was found in mallard for an *Eleocharis quadrangulata* seed (de Vlaming and Proctor, 1968). It is unclear whether seeds were still viable after >72 h. Due to the long stopovers (**Figure 3**), the probability of mallards being in a migratory flight 72 h after departure is effectively zero in our study population.

## Pre-migratory Fasting

Pre-migratory fasting strongly affects the long-distance dispersal potential of plant seeds. It is poorly known whether waterbirds keep foraging until shortly before embarking on a migratory flight. Some bird species are known to fast before migration and fly with empty digestive tracts to minimize wing loading, while only few examples exist of birds being observed foraging until right before departure (Clausen et al., 2002). According to the mallard tracking data we used, mallards tended to depart on migration at dusk, the same time when mallards normally leave their day roost to fly to nocturnal foraging sites. Although daytime foraging also occurs, mallards forage primarily at night (Bengtsson et al., 2014; Kleyheeg et al., 2017b), suggesting that most food items would have left the digestive tract when mallards start their migratory flight in the evening. With a pre-migratory fasting time of 12 h, 93–99% of ingested seeds would already have been excreted by a mallard before the onset of migration, based on the retention curves used in this study. This drastically reduces the predicted dispersal distances for both small and large seeds. More research is needed to determine whether mallards and other waterbirds use a period of pre-migratory fasting.

## Seed Deposition Habitat

Seeds that are in the mallard digestive tract during the initiation of migration are likely excreted during the migratory flight or the first stopover. For aquatic and wetland plants, dispersal is successful only if their seeds are deposited in freshwater habitat, where conditions are suitable for germination and establishment. Seeds deposited during the migratory flight, i.e., those in phase I in **Figure 1**, have a very low probability of ending up in a wetland. Successful long-distance dispersal is approximately 20 times more likely if seeds are retained until mallards reach their first stopover site (phase II, **Figure 1**). Mallards typically spend >48 h at a stopover site (**Figure 3**), making it very unlikely that seeds remain inside the digestive system until the second leap of migration (phase III, **Figure 1**). To predict where seeds from a source population end up via endozoochorous dispersal, it is therefore critical to consider the first stopover sites for migratory birds departing from the area of interest.

## Implications for Spatial Plant Population Dynamics

At the local scale, resident and staging mallards disperse seeds in all directions over distances ranging from a few hundred meters up to several dozens of kilometers, depending on landscape use (Kleyheeg et al., 2017a; Bartel et al., 2018). Both the number of mallards and the number of seeds involved in local scale dispersal are much higher than the numbers involved in dispersal during migration. Yet, mallards on spring migration could contribute disproportionately to large-scale population dynamics, including poleward range expansion of plant populations, for example in response to climate change. Mallard spring migration is typically northward in the Northern Hemisphere and therefore in roughly the same direction as the moving climatic envelopes under global warming. The dispersal distances achieved by spring-migrating mallards far exceeded predicted annual rates of the climate envelope shift between 2,000 and 2,100, averaging 0.42 and 0.22 km $y^{-1}$ for mean annual temperature and precipitation, respectively (Loarie et al., 2009). This supports the conclusions of Viana (2017), who argued that aquatic plants may be able to keep pace with climate change when they are dispersed by waterfowl. It should be noted, however, that despite the long potential dispersal distances toward a potentially suitable habitat, successful establishment depends on a wide range of biotic and abiotic factors, including inter-specific competition and water chemistry, which may vary strongly from site to site (Peterson et al., 2011).

### Priorities for Future Research

An important question that remains is how many seeds of which species remain available and are consumed by mallards by the end of winter, when most seeds produced in summer and fall may be eaten or decomposed. The composition of seed species in the mallard diet was shown to vary considerably throughout the winter season in the Netherlands (Kleyheeg et al., 2016). Only those species still available to mallards in early spring are able to benefit from their northbound spring migration. Since mallards and many other waterbird species forage in the sediment, plant species forming a persistent seed bank may have a relatively high probability of being ingested during spring migration (Green et al., 2002).

A second long-standing question is how does the digestive tract behave during migration? Does increased physical activity speed up gut passage during the migratory flight (van Leeuwen et al., 2012b; Kleyheeg et al., 2015) or does the digestive system shut down during migration, only to pass food items again during a stopover? Either option would have major implications on the dispersal probability over distance. Wind tunnel experiments could shed more light on this.

Finally, as with all mechanistic dispersal models, field validation is required to confirm the dispersal phenomenon and verify the quantitative model outcomes. The large scale of animal-mediated long-distance dispersal and the multitude of other factors interfering with this process make validation in the field exceedingly challenging. Molecular analysis on seeds collected from feces of migrating birds or fecal analysis of birds with GPS tracking devices could provide field evidence for long-distance seed dispersal by waterbirds.

## CONCLUSIONS

In this study, we developed a novel method to estimate seed dispersal distances via endozoochory in a migratory population of mallards. With the rapidly increasing availability of high-resolution GPS tracks of migratory animals, further applications of our model are numerous. Applications are not limited to the field of seed dispersal by waterbirds, but also for dispersal by other migrating animals, as well as the spread of other organisms, including pathogens. Until the unlikely scenario where significant proportions of migratory animal populations are being tracked, modeling approaches such as described in this study will provide a useful tool for estimating the impact of animal migration on ecological processes. Improved understanding and appreciation of the ecological services provided by the billions of animals that undertake seasonal migrations may help secure the future of this important phenomenon.

## AUTHOR CONTRIBUTIONS

EK and MvT designed the study, performed the analyses and wrote the first version of the manuscript. WF, KS, JW, and MW provided guidance and assisted in the writing. EK and WF collected and administrated the tracking data.

## FUNDING

This project was supported by funding from the Netherlands Organization for Scientific Research under Rubicon grant No 019.162LW.009 and the European Union's Horizon 2020 research and innovation program under grant agreement No 727922 (Delta-Flu).

## ACKNOWLEDGMENTS

We thank Hans-Günther Bauer for kindly providing information on mallard numbers wintering in the Lake Constance region based on monthly waterbird census data.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2019.00040/full#supplementary-material

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Ecology and Evolution

REVIEW
published: 05 March 2019
doi: 10.3389/fevo.2019.00041

# Seeds and the City: The Interdependence of Zoochory and Ecosystem Dynamics in Urban Environments

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The increasing urban sprawl has contributed to the extensive fragmentation and reduction of natural habitat worldwide. Urbanization has a range of adverse effects on ecosystem functioning, including the disruption of plant dispersal processes across the landscape. Urban fragmentation can alter the distance and directionality of dispersal, leading to disrupted gene flow among populations. The dispersal processes of plants that rely on animal-mediated dispersal (zoochory) may be disproportionately affected by urbanization, as many animals avoid urban areas or restrict their movements within urban habitats. This could alter the efficiency of animal dispersal vectors and modify seed movements across urban habitats. While recent studies suggest that seed dispersal networks can be complex and dynamic even in highly managed green areas with relatively low biodiversity, zoochory in urban environments remains understudied. We synthesize the existing literature on zoochory in urban environments and place the findings in the context of ecosystem dynamics. We assess the ecological and evolutionary consequences for seed dispersal, following urbanization, by considering how zoochory is affected by specific features of urban environments. These include the complexity of habitats with varying continuity; high disturbance and intense management; a high proportion of alien species combined with low natural biodiversity; animal behavioral adjustments in different urban settings; and rapid evolutionary change due to urbanization. We conclude that (1) urbanization can disrupt and alter zoochory processes; and (2) successful zoochory can, in turn, alleviate or worsen the challenges to ecosystem dynamics originating from increased urbanization. The dynamic urban seed dispersal networks are emerging as useful models for the adaptability of seed dispersal communities. Their study can also shed light on eco-evolutionary processes under anthropogenic selective pressures, including species interactions. Finally, urban zoochory processes are critical to the functioning of urban ecosystems and as such, constitute an important ecosystem service with management implications. We propose directions for further research into urban zoochory processes to ensure the maintenance of ecosystem dynamics as urbanization continues.

**Keywords: urban seed dispersal, anthropogenic disturbance, animal behavior, local adaptation, altered communities, urban evolution, urbanization, urban animal communities**

**Edited by:**
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**Specialty section:**
*This article was submitted to*
*Biogeography and Macroecology,*
*a section of the journal*
*Frontiers in Ecology and Evolution*

***Received:*** *21 September 2018*
***Accepted:*** *04 February 2019*
***Published:*** *05 March 2019*

**Citation:**
*Gelmi-Candusso TA and Hämäläinen AM (2019) Seeds and the City: The Interdependence of Zoochory and Ecosystem Dynamics in Urban Environments.*
*Front. Ecol. Evol. 7:41.*
*doi: 10.3389/fevo.2019.00041*

## INTRODUCTION

*Anthropogenic disturbance* (see **Glossary**) is becoming the norm for most of the world's flora and fauna. Already half of the terrestrial surface has been altered by humans (Vitousek et al., 2008; Hooke and Martín-Duque, 2012). A major ongoing trend is increasing *urbanization* among human populations, with cities around the world growing in number and size (Grimm et al., 2008). The urban environment is a unique ecosystem characterized by fragmentation, patchiness, various forms and degrees of disturbance, and alien species (Rebele, 1994). The accompanying changes in community assemblages and increased selective pressures can have pivotal effects on mutualisms, such as animal-mediated seed dispersal, a key process in maintaining *ecosystem dynamics* and biodiversity (Richardson et al., 2000). To characterize and predict the consequences of urbanization on seed dispersal, it is necessary to examine the broad effects of urbanization on plant and animal communities and their interactions. We attempt to do so by focusing on the effects of three specific features of the urban environment: fragmented habitat mosaic, altered community composition, and the associated adaptations of urban populations.

Evidence from *natural environments* indicates that seed dispersal by animals (i.e., *zoochory*) is influenced by anthropogenic disturbances, such as habitat fragmentation, habitat loss and hunting. For example, a decline in animal disperser abundance can alter seed removal rates (Markl et al., 2012), and human activity and fragmentation can lead to changes in disperser movement patterns (Fernández-Juricic and Tellería, 2000; Ciuti et al., 2012; Leblond et al., 2013; Preisler et al., 2013; Cote et al., 2017; Emer et al., 2018; Gaynor et al., 2018). Such changes, in turn, influence seed dispersal patterns and scale (Westcott and Graham, 2000). In urban environments, disrupted seed dispersal may result in interrupted gene flow, and the isolation of small populations of zoochorous plants (Harris et al., 2016; Johnson and Munshi-South, 2017; Schilthuizen, 2018), with cascading consequences for the functioning of the ecosystem.

The types of anthropogenic disturbance in natural habitats (fragmentation, patchiness, and habitat degradation) are partially shared with urban environments. However, some disturbances are specific to urban environments. For example, as opposed to logging and hunting, urban disturbance includes direct management and arrangement of vegetation, and severe structural disturbance created by urban infrastructure. Thus, while many of the general effects of disturbance and fragmentation are expected to be similar between urban habitats and disturbed natural environments, the dispersal outcomes also depend on the distinct features of urban environments.

In the complex, fragmented, urban habitat mosaic, ecologically different green patches with their various taxa are exposed to each other in ways that would not occur in nature (Lepczyk et al., 2017). Urban communities typically differ from the surrounding natural habitats in species richness and abundance, community assemblages and species interactions (Rebele, 1994; Kowarik, 1995; Rolando et al., 1997; McKinney, 2006, 2008; Markl et al., 2012; Fontúrbel et al., 2015). While native species richness declines (Liang et al., 2008; McKinney, 2008; Faeth et al., 2011; Young et al., 2016), many alien species are introduced and interact with local communities (Rebele, 1994; Alberti et al., 2017). These patterns are characteristic enough that urban ecosystems are more similar to each other than to their surrounding natural landscapes (McKinney, 2006). Strong selective pressures generated by the same urban features frequently lead to rapid behavioral, phenotypic (Alberti et al., 2017) and genetic changes (Cheptou et al., 2008; Smith and Bernatchez, 2008; Harris et al., 2016; Gorton et al., 2018) in animal and plant species, reinforcing the unique ecosystem profile of urban environments. Seed dispersal has been indicated as a critical factor in driving assembly patterns of urban plant communities (Johnson et al., 2018), and is integral in connecting urban green habitats with one another and with the surrounding landscape (Culley et al., 2007). Seed dispersal by humans has been examined in detail (Wichmann et al., 2009; Auffret, 2011; Bullock et al., 2018) especially in urban habitats (von der Lippe and Kowarik, 2008, 2012; von der Lippe et al., 2013), however, animal seed dispersal in urban environments remains understudied.

To evaluate the overall effects of urbanization on zoochory, we synthesize the empirical research on urban zoochory and place the findings in the context of similar processes observed in natural and rural, human-altered environments. We discuss the ecological and evolutionary consequences for seed dispersal following urbanization by considering how zoochory is affected by three defining characteristics of urban environments: (1) a landscape mosaic characterized by habitat complexity, disturbance and fragmentation, (2) an altered plant and animal community composition; and (3) genetic and phenotypic adjustments of plants and animals to the urban environment. We then identify knowledge gaps in the study of urban zoochory. Finally, we propose ways in which knowledge of urban zoochory processes can be used in urban planning and as models for universal zoochory processes.

## SEED DISPERSAL IN THE URBAN MOSAIC

Nearly all urban areas are landscape mosaics containing patches of vegetation arranged with varying degrees of connectedness in a highly fragmented matrix of *built environment*. Patches of vegetation include a wide range of highly diverse habitat types with a diverse level of disturbance, ranging from nearly pristine urban forests to managed garden flowerbeds and including cracks in the walls of buildings (Francis and Chadwick, 2013). The habitat patches are connected within the urban matrix by corridors, such as railway networks, ravines, and small green areas acting as stepping stones. Together, diverse vegetation patches, corridors and the matrix of built environment form the urban mosaic (*sensu* the patch-corridor-matrix model; Forman, 1995). The consequences of the mosaic on the success of seed dispersal by animals are 2-fold: first, a

**TABLE 1 |** Effects of habitat complexity on urban zoochory and vice versa.

| Aspect of zoochory affected by altered urban habitat | Type of zoochory[a] | Animal focus | Plant focus | City size[b] | Country (continent[c]) | Region | References |
|---|---|---|---|---|---|---|---|
| Altered dispersal pattern/increased distance | E | Birds (various) | *Phoradendron affine* | L | Brazil (SA) | TR | Kiyoshi Maruyama et al., 2012 |
| Colonization/regeneration of disturbed habitat | E | Birds (various) | Various | XL | USA (NA) | TE | Robinson and Handel, 2000 |
| Disrupted dispersal across barrier (road) | S | Birds (*Garrulus glandarius*), terrestrial mammals (*Niviventer confucianus*) | *Quercus chenii* | XL | China (AS) | TE | Niu et al., 2018 |
| Effective removal/transportation of viable seeds of multiple species | E | Terrestrial mammals (*Vulpes vulpes*) | Various | XL | Belgium (EU) | TE | |
| | S | BIRDS (*Garrulus glandarius*), terrestrial mammals (*Niviventer confucianus*) | *Quercus chenii* | XL | China (AS) | TE | Niu et al., 2018 |
| Effective removal/transportation of viable seeds of multiple species in discontinuous habitat | E | Birds (*Turdus* spp.) | Various | S | Brazil (SA) | TR | Gasperin and Pizo, 2009 |
| | E, S | Bats (*Cynopterus brachiolis*) | Various | XL | Malaysia (AS) | TR | Tan et al., 2000 |
| Maintaining connectivity in discontinuous habitat | E | Terrestrial mammals (*Vulpes vulpes*) | Various | S | Spain (EU) | TE | Cancio et al., 2017 |
| | E | Birds (various) | *Cabralea canjerana* | L | Brazil (SA) | TR | Pizo, 1997 |
| | S | Birds (*Garrulus glandarius*) | *Quercus robur* and *Quercus petrea* | L | Sweden (EU) | TE | Hougner et al., 2006 |
| | S | Birds (*Garrulus glandarius*) | *Quercus robur* and *Quercus petrea* | L | Sweden (EU) | TE | Lundberg et al., 2008 |
| | S | Terrestrial mammals (*Sciurus carolinensis*) | *Quercus rubra* | S | USA (NA) | TE | Steele et al., 2011 |
| Maintaining seed removal/dispersal in fragmented habitat | assumed: E, S | Unknown | *Ferocactus wislizeni* | L | USA (NA) | TE | Ness et al., 2016 |

[a] *Type of zoochory: E, endozoochory; M, myrmecochory; S, synzoochory.*

[b] *City size: S, small urban area (population = 50,000–200,000); M, medium urban area (population = 200,000–500,000); L, metropolitan area (population = 0.5–1.5 million); XL, large metropolitan area (population ≥1.5 million) (OECD, 2018).*

[c] *Continent: AU, Australia, EU, Europe; NA, North America; SA, South America.*

higher diversity of habitats may increase the diversity of seed dispersers (Melles et al., 2003; Ossola et al., 2015). Second, the movement of seed dispersers within the matrix depends on the resource distribution among patches (Dunning et al., 1992) and their connectivity (Taylor et al., 1993). Empirical evidence on the effects of the urban mosaic on zoochory processes is summarized in **Tables 1**, **2** (see also sections Urban Communities and Species Interactions, and Adaptations of Animals to Urbanization and Consequences to Zoochory). In this section, we first describe how the features of the discontinuous habitat mosaic may disrupt seed disperser movements and further restrict seed movement between habitat patches. Then, we discuss evidence of the contrary, i.e., how animal dispersers may increase the connectivity of plant populations despite habitat discontinuity.

## Urban Disturbance and Fragmentation May Disrupt Seed Movement by Animal Dispersers

Empirical studies have found evidence that habitat degeneration, disturbance and fragmentation alter the seed movements of zoochorous plants. The changes appear to operate through shifts in the disperser community and behavior, with edge preference or avoidance playing a potentially important role (e.g., Levey et al., 2008; Warren et al., 2015; see sections Urban Communities, and Species Interactions and Adaptations of Animals to Urbanization and Consequences to Zoochory). For example, roads surrounding a forest patch were found to act as a movement barrier for scatter hoarders, effectively constraining seed movements to the fragment (Niu et al., 2018). This concurs with findings from non-urban (Asensio et al., 2017) and urban

**TABLE 2 |** Effects of altered communities on urban zoochory.

| Aspect of zoochory affected by altered urban community | Type of zoochory[a] | Animal focus | Plant focus | City size[b] | Country (continent[c]) | Region | Reference |
|---|---|---|---|---|---|---|---|
| Effective removal/transportation of viable seeds of multiple species | E | Birds (*Corvus frugilegus*) | Various | S/M | Poland/Romania (EU) | TE | Kitowski et al., 2017 |
| | E | Terrestrial mammals (*Vulpes vulpes*) | Various | SU | Spain (EU) | TE | Cancio et al., 2017 |
| | E | Terrestrial mammals (*Didelphis albiventris*) | Various | XL | Brazil (SA) | TR | Cáceres et al., 1999 |
| Higher effectiveness of removal/transportation of viable seeds by remaining species in community | M | Ants (various) | *Viola pubescens* | L | Canada (NA) | TE | Thompson and McLachlan, 2007 |
| Homogenization of biodiversity | E | Birds (*Corvus frugilegus*) | Various | S/M | Poland (EU) | TE | Czarnecka et al., 2013 |
| Replacement of primary vector/change in zoochory type | E | Terrestrial mammals (*Didelphis albiventris*) | Various | XL | Brazil (SA) | TR | Cáceres et al., 1999 |
| Secondary disperser effectively replaced primary vector/change in zoochory type | S | Terrestrial mammals (*Dasyprocta leporina*) | *Astrocaryum aculeatissimum* | XL | Brazil (SA) | TR | Zucaratto and dos Santos Pires, 2015 |
| Spread of alien plant species | E | Terrestrial mammals (*Vulpes vulpes*) | Various | XL | Belgium (EU) | TE | D'hondt, 2011 |
| | E | Birds (various) | Various | S | Portugal (EU) | TE | Cruz et al., 2013 |
| | M | Ants (*Linepithema humile*) | *Acacia sophorae, A. retinodes, Polygala myrtifolia, Dipogon lignosus* | XL | Australia (AU) | TE | Rowles and O'Dowd, 2009 |
| | S | Terrestrial mammals (*Sciurus carolinensis*) | Various | XL | Canada (NA) | TE | Thompson and Thompson, 1980 |

[a] *Type of zoochory: E, endozoochory; M, myrmecochory; S, synzoochory.*

[b] *City size: S, small urban area (population = 50,000–200,000); M, medium urban area (population = 200,000–500,000); L, metropolitan area (population = 0.5–1.5 million); XL, large metropolitan area (population ≥1.5 million) (OECD, 2018).*

[c] *Continent: AU, Australia; EU, Europe; NA, North America; SA, South America.*

areas (Fey et al., 2016; Johnson et al., 2017) that roads restrict animal movements enhancing the fragmentation and isolation of urban green patches, and further delineating the habitat mosaic. Smaller, more isolated, fragments tend to support fewer animal disperser species and smaller population sizes in urban areas (Srbek-Araujo et al., 2017), leading to lower fruit removal rates, as found for cactus fruit removal rates by mammals (Ness et al., 2016). If small, isolated fragments are avoided by animals, it is also likely that seed influx into isolated fragments may decline, eventually leading to local extirpation of certain plant species.

## The Role of Seed Dispersers in Maintaining the Integrity of the Urban Mosaic

Although animal dispersers are influenced by urban disturbance and fragmentation, an increasing number of studies suggest that dispersers play an important role in buffering the adverse effects of habitat alteration by increasing the regeneration and connectivity of urban green areas (**Table 1**).

### Seed Dispersers Can Influence Habitat Regeneration

Animal dispersers play a key role in the regeneration of urban degraded habitats (Robinson and Handel, 2000; Lundberg et al., 2008) by influencing the influx rate of seeds from source areas. For example, seed dispersal by jays is estimated to result in the recruitment of more than 33,000 saplings per year in a large urban park, representing a significant *ecosystem service* and reducing the level of habitat disturbance within the urban park (Hougner et al., 2006). Generally, however, it seems that animal seed dispersers contribute to the influx of seeds into disturbed areas mainly after regeneration has already started through other dispersal means (wind dispersal, seed banks). This was suggested by the finding that plants with predominantly *zoochorous* traits were found in larger and older urban forest fragments (Jesus et al., 2012) and older vacant lots (Knapp et al., 2016; Neuenkamp et al., 2016; Johnson et al., 2018), while *anemochorous* traits were predominant in smaller and recent fragments (see section Effects of Urbanization on the Community Composition of Plants for further details on plant community composition). Likely, as heterogeneity of a degenerated habitat (e.g., cleared lots) increases, areas become more suitable for animal movement, increasing the influx of animal-dispersed seeds and the incidence of zoochorous plants recruited. This is concordant with previous findings in tropical forest restoration where heterogeneity of a habitat attracted seed dispersers to sites in regeneration (Wunderle, 1997), and where tree density (Zapata et al., 2014) and availability of perching sites (González-Varo et al., 2017a) increased visitation rates of disturbed areas.

### Seed Dispersers May Maintain Habitat Connectivity and Reduce Habitat Disturbance

In addition to regeneration, animal dispersers can help maintain plant populations by facilitating seed exchange between habitat patches. Potential animal habitat connectors in the complex urban mosaic are either animals with differential habitat use for their daily activities (Lundberg et al., 2008; Gasperin and Pizo, 2009) or animals with long travel distances that can move through disturbed habitats (Cancio et al., 2017); see also section Consequences of Changes in Movement Patterns of Urban Dispersers on Seed Movement. An example of increased connectivity due to differential habitat use are urban thrushes, which eat fruits predominantly in open areas of cities (e.g., gardens and streets) but use forests for nesting and shelter, connecting these two differently disturbed green patches and increasing seed influx from forests into cities (Gasperin and Pizo, 2012; Da Silveira et al., 2016). These birds were also found to visit multiple forest fragments, showing these also play a role in the functional connectivity between forest fragments. Similarly, Eurasian Jays in Stockholm use different forest types for different purposes (i.e., oak stands for feeding/caching; coniferous stands for breeding), therefore coniferous forests in between oak stands function as stepping stones, enhancing connectivity between oak stands (Lundberg et al., 2008). Red foxes, prevalent and well-adapted in many urban areas, can move across disturbed areas, which can increase connectivity when dispersing in fragmented areas, as in the Mediterranean peninsula, where red foxes are able to move through differently disturbed areas, connecting remnants of the threatened keystone habitat. However, connectivity was limited to fragments separated by a distance smaller than the foxes' territory (Cancio et al., 2017). Therefore, we can expect connectivity by animal dispersers to be determined by the distance between urban fragments relative to the animals' movement ranges. Where the distances between fragments and remnant seed sources are beyond the usual seed dispersal distance of available seed dispersers, wildlife corridors can enhance connectivity (Beier and Noss, 1998; Chetkiewicz et al., 2006).

## URBAN COMMUNITIES AND SPECIES INTERACTIONS

Plant and animal communities in urban areas differ greatly from natural environments. The expansion of impervious surface in urban areas reduces habitable areas, while landscape management structurally simplifies the vegetation within these. A small number of habitable areas with simplified vegetation supports fewer species, reducing species richness (McKinney, 2008). However, other factors present in urban areas can instead increase species richness and population densities, such as high habitat heterogeneity, high primary productivity and food availability, and a high influx of alien species (Baker and Harris, 2007; McKinney, 2008; Šálek et al., 2015). Changes in species richness and abundance may considerably influence the ecosystem service provided by the local seed dispersal network. Therefore, it is critical to examine changes in communities to understand the effects of urbanization on the maintenance of plant population connectivity and extinction risk in urban areas. In this section, we describe how human activity within urban areas affects plant and animal communities, and how changes within these affect zoochory.

### Effects of Urbanization on the Community Composition of Plants

Plant species diversity increases with urbanization up to intermediate levels of urbanization but decreases again at very high levels of urbanization (at least in temperate regions; McKinney, 2008). Furthermore, studies of urban plant communities suggest a pattern of increasing proportions of primarily animal-dispersed plants in more urbanized areas (Knapp et al., 2008; Burton et al., 2009; Marco et al., 2010). The general prevalence of plants depending on zoochory could be partially explained by the finding that animal-dispersed plants can have a lower risk of local extinction at more urbanized sites (Williams et al., 2005). With a higher proportion of animal-dispersed plants, animal seed dispersers play an important role in maintaining or modifying plant communities in urban areas (see section Effects of Altered Communities on Urban Seed Dispersal). However, the pattern of increased presence of animal-dispersed plants in urbanized areas is not universal (Thompson and McCarthy, 2008). and fine-scale differences may exist in the predominant type of zoochory present along the rural-urban gradient. For example, Albrecht and Haider (2013) found a high prevalence of *synzoochorous* plant species in urban areas in contrast to a trend toward more *myrmecochorous* species in the surrounding rural areas. Deciphering why these systems differ from the general trend presents an interesting direction for future research.

Urban communities also differ from the surrounding environments through their higher proportion of alien plant species (Kowarik, 1995; Kühn and Klotz, 2006). A large number and abundance of alien plant species have been found in *soil seed banks* in urban forests (Overdyck and Clarkson, 2012) and in urban ornamental gardens (Marco et al., 2010; Reichard, 2010). While most alien plant species are introduced intentionally (e.g., in gardens), they spread unintentionally through urban areas, primarily via zoochory (Padayachee et al., 2017). These urban populations of alien plants (especially species dispersed by birds Gosper et al., 2005; Gaggini et al., 2017; can then act as seed sources for surrounding forests, leading to ecological consequences beyond the urban habitat. The spread of alien plant species to surrounding pristine environments can even be amplified by the interaction of multiple dispersers. In Poland, the increase in abundance of rooks made the alien Eurasian walnuts from human settlements available to natural forest dispersers, Jays (*Garrulus glandarius*). The jays removed the high number of acorns that rooks had cached in rural areas and further distributed them into the surrounding forests (Lenda et al., 2018). Therefore, understanding the complete dispersal network and multi-phase dispersal in urban areas is key in managing the expansion of zoochorous alien plant species.

## Effects of Urbanization on the Community Composition and Abundance of Animal Dispersers

Seed disperser abundance and species richness are both affected by urbanization through modification and reduction of available habitats (Larsen et al., 2005; Collen et al., 2014; Dirzo et al., 2014; Pimm et al., 2014); negative interactions with humans (Koch and Barnosky, 2006; Tregidgo et al., 2017); human infrastructure (Loss et al., 2015); and vehicles (Laurance et al., 2009). Animal species richness tends to decline with increasing urbanization level (McKinney, 2008) independently of the type of vegetation present (Faeth et al., 2011). The total abundance of dispersers, on the other hand, depends on the population dynamics of each disperser species and the surrounding urban environment. Population size can be strongly reduced in urban habitats as seen for birds in an urban forest fragment, where only half as many birds were present relative to pristine areas (Pizo, 1997). Disperser abundance may also be higher in urban areas if disperser species or populations are especially successful, as seen for opportunistic, urban-adapted species, such as Eastern gray squirrels (Steele et al., 2011) and red foxes (Šálek et al., 2015). The number and species array of the animals that are available as dispersers thus result from the composition of the urban animal community and population processes of those species.

## Effects of Altered Communities on Urban Seed Dispersal

Empirical studies have found evidence of successful maintenance of urban seed dispersal in spite of altered species composition. However, we can expect a diverse array of effects on seed dispersal after community alterations, depending on the composition of the plant and animal communities, and the interactions between them (**Table 2**).

### Consequences of Defaunation and Functional Replacement of Seed Dispersers on Zoochory

The effectiveness of zoochory is influenced by the local abundance of animal individuals capable of dispersing seeds, as well as the assemblage of disperser species. Disperser abundance and assembly may affect the number of seeds dispersed, the diversity of seed species dispersed, their specific deposition sites, and whether they germinate. Defaunation combined with the higher plant diversity in urban areas (McKinney, 2008) can lead to a situation where a larger number of plant species is dispersed by a smaller number of animal species, with potential consequences in terms of competition between dispersers.

Defaunation will specifically influence disperser abundance, with consequences for the number of seeds dispersed. However, changes in population densities can also influence intra- and inter-specific interactions between dispersers, ultimately influencing the dispersers' feeding and post-feeding behavior (see section Behavioral Responses of Urban Dispersers to Humans and Other Animals Have Consequences for Seed Dispersal). These behaviors, in turn, result in altered seed dispersal distances and patterns, as commonly happens in natural habitats (McConkey and O'Farrill, 2016).

Following defaunation, *functional replacement* of lost animals may occur, where a new species takes on the functional role (e.g., as seed disperser) of a species lost from the area. When the exclusive seed disperser is extirpated and not replaced functionally by other species, then the recruitment of plant species that rely on that disperser will completely subside. The absence of animal-dispersed plant species from otherwise suitable urban habitats (i.e., secondary urban forests) would suggest such seed dispersal failure (Tsang and Corlett, 2005). The local extinction of a seed disperser might be particularly disruptive in fragmented landscapes (Rodríguez-Cabal et al., 2007), such as urban areas, since animal species richness may be reduced and fragment isolation may limit disperser replacement by animals from other fragments. Nonetheless, very few plant-disperser associations are strictly obligate (Richardson et al., 2000), hence dynamic changes in dispersal networks rather than extinctions are generally predicted.

The significance of the changes in the community assemblage on dispersal effectiveness depends on the degree of functional equivalence of the primary and novel species as dispersers. When functional replacement is incomplete, i.e., seed dispersal by the animals that take the place of a lost *primary disperser* differs from the original dispersal service, the strongest effects on zoochory may be seen. Such effects in urban environments are poorly understood, but evidence from natural areas suggest community changes can lead to differences in dispersal effectiveness (Schupp, 1993; Uriarte et al., 2011; Schupp et al., 2017). This is because seed dispersal distance, removal rate, and seed processing are all taxon-dependent, ultimately altering the total *seed shadow* and recruitment success of dispersed plants (Martínez et al., 2008). First, novel disperser species may have different morphological characteristics than the primary disperser, such as gape constraints (Pizo, 1997) or body size (Ness et al., 2004; Carbone et al., 2005; Young et al., 2016), that limit their feeding or movement ranges, ultimately influencing seed dispersal distance. Second, disperser species consume different food resources leading to differences in the *seed rain* they contribute to in terms of species diversity and richness. For example, the recent higher abundance of rooks in urban areas of Poland transport a high number of seeds with low species diversity (primarily of weeds and ruderal species), from rural areas to urban areas, contributing to the homogenization of plant biodiversity in urban areas (Czarnecka et al., 2013). Novel dispersers may also have different feeding behavior. For example, novel dispersers in an urban forest fragment differed from the primary disperser community by feeding more on the forest ground but regurgitating seeds also in urban areas. The consequences of this were that despite the loss of some seeds from the forest area, an influx of natural seed species into urban green patches was created (Pizo, 1997). Third, plant recruitment may depend on the disperser assemblage when germination success differs among disperser species, such as for urban mistletoes (Kiyoshi Maruyama et al., 2012). Germination success may decline if seeds that are originally adapted to be dispersed by other taxa are damaged by gut passage through novel or secondary disperser species, such as bird- and lizard-dispersed seeds when processed by mammals (Nogales et al., 2005). Finally, the loss of a specific seed disperser species may even increase seed dispersal effectiveness by relieving interspecific resource competition, potentially allowing dispersal by other, competitively weaker, urban species. When those species are more efficient foragers, the loss of a primary disperser may lead to increased seed removal rates (Thompson and McLachlan, 2007; see also Mahandran et al., 2018). However, the impact of such changes on plant recruitment success remains poorly studied.

When the novel dispersers fully functionally replace a lost local disperser, seed movements are unaffected. For example, the loss of agoutis in an urban forest fragment showed no effects on seed dispersal distance, likely due to a fully functional replacement by other rodents (Zucaratto and dos Santos Pires, 2015).

### Consequences of Alien Animal Species on Native Plant Dispersal Success

Introduced animal species necessarily lead to novel species interactions, which may alter seed dispersal and recruitment of native plant species by displacing native dispersers or by predating on native seeds. Displacement of native dispersers has been seen in the coastal shrub along Melbourne, Australia, by the invasive argentine ants (*Linepithema humile*). These removed fewer seeds of the native plant species *Acacia retinoide* and buried seeds of another native plant *Acacia sophorae* closer to the source plant, reducing seed dispersal distance (Rowles and O'Dowd, 2009). Predation of native seeds by introduced animal dispersers has been seen for mammals and ants. For example, introduced mammals compromise the success of artificially planted native seeds in regeneration procedures (Overdyck et al., 2013). Similarly, introduced ant species that are common in urban areas commonly act as predators of native seeds species, leaving seeds unburied or consuming *elaiosomes* but discarding seeds beneath the source (Ness et al., 2004). Therefore, the outcome for the plant populations depends on how these processes by alien animals compare to the functions of the native dispersers, and the overall effects of the novel animal community on seed dispersal and seed predation.

### Consequences of Alien Plant Species on Urban Dispersal Networks

Introduced plants may alter seed dispersal networks in urban areas by competing with native plants for seed dispersal services. The effects of alien competition with native plants for seed dispersal services in urban areas will depend on the attractiveness of their *diaspores* to seed dispersers (Aslan and Rejmanek, 2012; see also section Consequences of Dietary Changes of Urban Seed Dispersers on Seed Rain). For example, the invasive ant-dispersed South African shrub *Polygala myrtifolia* outcompeted native plant species for seed dispersal services by ants (Rowles and O'Dowd, 2009). However, competition for seed dispersal services may not necessarily result in negative outcomes for native species if these benefit from the additional dispersers brought into the area by the resources provided by alien plant species (van Leeuwen, 2018).

Alien plant species can also have cascading effects on urban seed dispersers by modifying the habitat. For example, weed invasions may lead to alterations in the ant disperser community, potentially changing the seed removal rates of both alien and native species (Grimbacher and Hughes, 2002). The overall effects of alien plant species on the ecosystem thus depend on the direct competition with native species for dispersal services and the indirect effects on local dispersers through modification of the environment. However, their success in competition with local species will influence their invasive potential within urban areas and beyond.

## ADAPTATIONS OF ANIMALS TO URBANIZATION AND CONSEQUENCES TO ZOOCHORY

There is increasing evidence that features of the urban environment cause rapid evolutionary change in urban plants and animals (Alberti et al., 2017; Rivkin et al., 2018). Selective pressures in urban areas include human presence, the *heat island effect* (Brans et al., 2017, 2018), changes in food and material resources, higher toxin concentrations than in natural environments (Johnson and Munshi-South, 2017), and the already discussed habitat disturbance and fragmentation (section Seed Dispersal in the Urban Mosaic). Furthermore, changes in the community and the associated mutualistic species interactions could also act as a selective pressure for species within the network. Evolutionary change results from the general effects of such selective pressures, combined with genetic drift, reduced gene flow, and bottleneck effects due to small, isolated populations (Alberti et al., 2017). Rapid adaptations, as well as non-adaptive changes, lead to declining genetic diversity *within* urban populations, while genetic differentiation *between* urban populations increases (Johnson and Munshi-South, 2017). The consequences for zoochory processes depend on whether the plant and disperser coevolve in a way that mutualistic relationships are retained. Nonetheless, even when mutualistic relationships are retained, seed dispersal efficacy of animals—dispersal distance, microhabitat of deposition sites, and germination success—can all be affected when animals adjust their behavioral, movement and dietary patterns in response to urbanization.

### Behavioral Responses of Urban Dispersers to Humans and Other Animals Have Consequences for Seed Dispersal

Urban vertebrates tend to express more bold and explorative behaviors (Prosser et al., 2006; Martin and Réale, 2008; Evans et al., 2010; Uchida et al., 2016), less vigilance (Chapman et al., 2012), and even lower stress response to human proximity (Sol et al., 2018; Weaver et al., 2018) than conspecifics in rural environments. Evidence from urban and rural blackbird populations suggest changes in behavior could be genetically determined and heritable, as genetic divergence was found for a gene associated with harm avoidance (Mueller et al., 2013). Despite such evidence of increased tolerance to human presence, many studies have also observed increased avoidance-related behaviors. Diurnal animals might become more active at night (Gaynor et al., 2018) or animals might alter their movement ranges (Tracey et al., 2013; Tucker et al., 2018) and feeding patterns (Fernández-Juricic and Tellería, 2000) when exposed to human activity. Changes in intra- and inter-specific interactions, and predation risk created by urbanization may change disperser behavior, e.g. foraging behavior (McConkey and O'Farrill, 2016) altering seed dispersal processes within the urban environments.

Intra- and interspecific competition can specifically influence behaviors with consequences for seed dispersal effectiveness. For example, the population density of flying foxes on small islands in Tonga was positively associated with dispersal distances due to changes in intra-specific competition (McConkey and Drake, 2006). At low densities, the bats appear to feed *in situ* and drop seeds under the parent tree, whereas at high densities, to avoid aggressive interactions in the fruiting trees, they transported the fruit to feed elsewhere, increasing seed dispersal distances (Richards, 1990). A similar phenomenon was also observed in three bat species of a suburban habitat in India (Mahandran et al., 2018). The largest bat species, which consumed overall more fruits, often consumed fruit *in situ* leading to short seed dispersal distances, and when intraspecific aggressions arose, they had longer seed dispersal distances. Dispersal effectiveness by smaller bat species was also apparently influenced by the avoidance of interspecific competition, as they typically carried fruit away to a feeding roost at varying distances from the parental tree. Another example of the effect of interspecific interactions on seed dispersal patterns is *peri-urban* eastern gray squirrels (*Sciurus carolinensis*), which modified their choice of caching sites in response to the presence of potential competitors (Steele et al., 2015).

## Consequences of Changes in Movement Patterns of Urban Dispersers on Seed Movement

Urban areas have been associated with changes in average animal movement distances, with potential consequences for seed dispersal distances. Shorter movements are generally observed in areas affected by humans, likely due to reduced habitat availability or higher local food availability that reduces the need to travel long distances to forage (Tucker et al., 2018). However, the effect of urban areas on movement patterns varies according to the scale of measurement, as urbanization affects animal movement patterns on multiple scales within and beyond urban areas.

At the scale of habitat patches, habitat preference and avoidance of open areas may influence seed movement. For example, preference for nesting in forest interiors by ants in *exurban* areas, changed ant movement away from forest edges, changing seed dispersal directionality and leading to a reduced presence of ant-dispersed woodland herbs on forest edges (Warren et al., 2015). Scatter hoarders had faster seed removal rates and shorter dispersal distances along forest edges due to edge avoidance (Niu et al., 2018). Seed movement at the scale of habitat patches may be affected more at a higher urbanization degree with stronger fragmentation, smaller fragment sizes and greater edge proportion (see also section Seed Dispersal in the Urban Mosaic).

At an inter-patch level, the discontinuity of suitable habitat in the urban mosaic could also lead to increased seed dispersal distances in species with home ranges larger than the typical patch size. For example, reduced availability of foraging and roosting areas increased the distance between core areas used by mallards, resulting in longer-distance seed dispersal (Kleyheeg et al., 2017). The study was not restricted to urban sites, but the processes likely apply also to urban areas as mallards are common inhabitants of urban parks. Dispersal between patches may be facilitated by corridors with vegetation favoring disperser movement. For example, the linear distribution of host trees of bird-dispersed mistletoe *Phoradendron affine*, artificially planted along roadsides, may change directionality of seed dispersers (e.g., *Euphonia chlorotica*) and increase seed dispersal distance as opposed to rural areas, where host trees were naturally distributed in clumps (Kiyoshi Maruyama et al., 2012). Research from agricultural areas shows the clearance of vegetation along roads may also result in longer seed dispersal distances by ants (Palfi et al., 2017). Therefore, management of vegetation along roadsides may be key in managing urban seed dispersal.

At a larger scale, some migratory urban birds showed a decline in migratory tendencies (Partecke and Gwinner, 2007) and others showed anticipation of migratory movements in urban areas due to the heat island effect (Tryjanowski et al., 2013). As migration is one of the mechanisms for long-distance dispersal, shifts in migration could substantially influence the "long tail" of seed shadows for dispersed plant species.

## Consequences of Dietary Changes of Urban Seed Dispersers on Seed Rain

The altered community structures, including the high abundance of alien plant species, and the availability of anthropogenic food items may induce shifts in the diets of urban dispersers, with potential effects on the diversity and richness of seeds dispersed. A preference for alien species has been observed in red foxes in certain areas (Cancio et al., 2017); in gray squirrels dispersing seeds at an urban cemetery (Thompson and Thompson, 1980); and in the argentine ant (*Linepithema humile*), which preferred seeds of the invasive South African shrub *Polygala myrtifolia* (Rowles and O'Dowd, 2009). In this case, body size of ant dispersers in relationship to diaspore size may be an important determinant of their differential preference between diaspores of native and alien plants (Gorb and Gorb, 1995). In fact, in Sydney, Australia, invasive fruit species with similar seed size as native species were removed by ants at a similar rate (Grimbacher and Hughes, 2002). Seeds of a large number of native and alien species have been observed in the scats of urban canids, viverrids, and mustelids (Tsang and Corlett, 2005; Corlett, 2011; D'hondt, 2011; Tsuji et al., 2011; Cancio et al., 2017), suggesting that omnivorous predators are also important dispersers in urban areas. A disperser preference for alien plants may also disrupt the dispersal of some native species (Bermejo and Guitian, 2000; López-Bao and González-Varo, 2011; Rost et al., 2012), by dispersing alien seeds instead of native seeds.

The abundance of food items from garbage in urban areas and other artificial sources of food, such as bird feeders, can also modify the diets of seed dispersers, in particular for opportunistic frugivores, ultimately influencing seed rain in terms of species richness and abundance. For example, regurgitated pellets of urban rooks contained mostly seeds from fleshy fruits, thought to result from higher consumption of garbage in urban areas, since pellets of rural rooks contained mostly seeds from dry-fruits (Kitowski et al., 2017). The diet of red foxes contained more fruits and berries from anthropogenic sources (i.e., garbage and orchards) in urban areas than in peri-urban areas (Contesse et al., 2004) potentially influencing dispersed species richness in different areas. Finally, evidence of a preference for bird feeders can be seen by changes in beak morphology of an urban population of finches in Tucson, USA, directionally selected toward larger sizes as a result of the advantage provided by bird feeders which contain larger and harder seeds than natural seeds in the surrounding habitat (Badyaev et al., 2008). Bird feeders may therefore potentially influence seed shadows created by birds. When behavioral and morphological changes in animals lead to a more frequent dispersal of some types of seeds at the expense of others, it may, in turn, serve as a selective pressure for the plants that rely on zoochory for their dispersal.

## POTENTIAL EVOLUTIONARY CHANGES IN PLANTS IN RESPONSE TO ALTERED SEED DISPERSAL

Plant species might adapt to the novel dispersal challenges via changes to their seed or fruit morphology or even phenology. Diaspore characteristics, including dispersal mode, appears to be a relatively flexible construct that represents a continuum rather than a discrete trait, and as such, is amenable to relatively rapid evolutionary change (Vander Wall and Longland, 2004; Forget et al., 2005; Cheptou et al., 2008; Galetti et al., 2013). In evidence of such processes, a gradual reduction in palm seed size has been observed in fragmented tropical forests where large-gaped frugivores have been lost (Galetti et al., 2013), and similar changes may be predicted in urban areas where body size of birds has been reduced (Battisti and Dodaro, 2016). We are aware of no research demonstrating evolutionary responses of urban plants to changes in animal-mediated dispersal. The potential for rapid evolutionary change due to urbanization has, however, been demonstrated in wind-dispersing plants. Cheptou et al. (2008) found that seed size of holy hawkbeard (*Crepis sancta*) evolved in urban areas to favor a morph adapted to short-distance dispersal over a wind-dispersed morph that disperses over longer distances. Later research suggested the differentiation in the life history of the plant may be due to higher isolation of subpopulations within the urban environment (Dubois and Cheptou, 2017).

Other studies have identified relevant changes in plant life history in urban areas, such as changes in fecundity, growth rate and phenology (Brans et al., 2018). In common-garden experiments, seeds of Virginia pepperweed (*Lepidium virginicum*) from urban populations produced more seeds than those collected from rural populations. Furthermore, plants from urban populations were phenotypically and genetically more homogenous than those from rural populations (Yakub and Tiffin, 2017). More general evidence of the adaptive potential of fecundity was demonstrated by Thompson and McCarthy (2008) who found heavier seeds to predict higher success in native plants within urban habitats. Interestingly, this relationship was the opposite in non-native plants within urban habitats. Plant phenology may also change (possibly due to the "heat island effect"), as suggested by the accelerated germination time and thus earlier flowering of urban common ragweed (*Ambrosia artemisiifolia*) relative to rural conspecifics (Gorton et al., 2018). The phenology and fecundity of certain species within urban environments can alter resource availability and distribution for seed dispersers, which in turn might influence the abundance and effectiveness of those animals as dispersers. Together, these findings suggest a strong potential for plants to adapt to challenges posed by features of the urban environment, possibly including adaptive responses to the changes in the communities and behaviors of their dispersers.

## DISCUSSION

The reviewed literature shows strong evidence of interdependence between urban zoochory and broader ecosystem dynamics. Given the predominance of animal-dispersed plants in urban areas (Knapp et al., 2008; Burton et al., 2009; Marco et al., 2010). zoochory is integral in sustaining urban vegetation patches. As such, zoochory represents an important ecosystem function in urban areas, but its success relies on the adaptability and effectiveness of the animal dispersers that themselves inhabit a disturbed, fragmented environment. The literature we reviewed highlighted a range of consequences, limitations, and side effects of the disperser community composition and behavior on seed dispersal in the urban mosaic, as well as their interdependence with the surrounding environment (**Figure 1**):

1. Urbanization alters various aspects of zoochory (**Figure 1**, black continuous arrows).
    a. Fragmentation and degradation lead to changes in the abundance, movement, and diets of dispersers, with concurrent changes in inter-specific interactions and seed predation All of these have consequent effects on seed dispersal patterns (see sections Seed Dispersal in the Urban Mosaic, and Adaptations of Animals to Urbanization and Consequences to Zoochory).

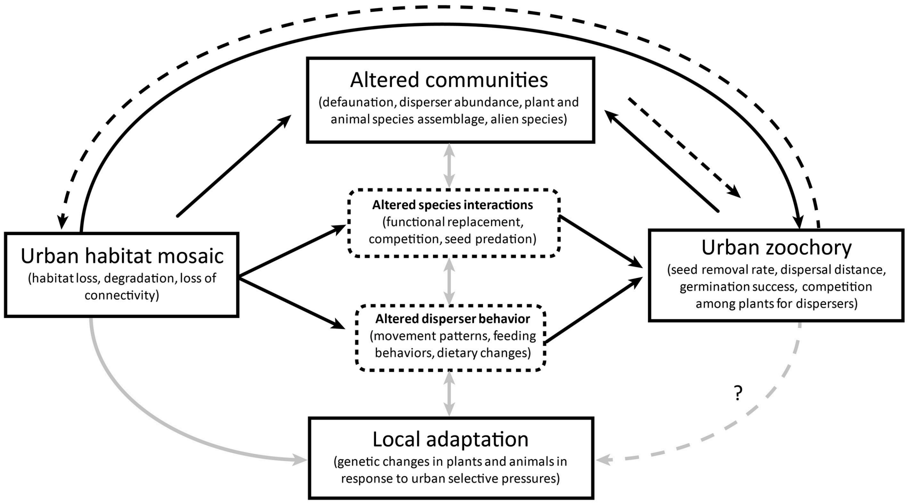

**FIGURE 1 |** Interdependence between zoochory processes and the urban ecosystems. The urban effects of an altered habitat on zoochory may be direct (e.g., by influencing the abundance of dispersers or their success) or mediated through changes in seed disperser behaviors and novel species interactions (black continuous lines). In turn, seed dispersers shape urban vegetation within the mosaic, strengthening or weakening the processes within the urban habitat (black dashed lines). Complex interactions exist between the urban plant and animal networks, disperser behaviors, and local adaptations of populations in the urban habitat mosaic (gray continuous lines). Zoochory also has the potential to directly influence local adaptations if it acts as a selective pressure. Plant dispersal outcomes and ecosystem dynamics are thus linked to zoochory processes in urban areas.

   b. Dispersed plant communities and disperser communities are altered through habitat loss, defaunation, and a high presence of alien species. These changes influence the abundance of individuals and assemblage of species available to form mutualistic relationships and maintain functioning dispersal networks. Presence of alien animal species may also influence levels of seed predation, influencing germination success. In addition, the availability and potentially higher attractiveness of alien plants to dispersers may increase the competition for dispersal services between native and alien plants, leading to further selective pressures for plant dispersal (see section Urban Communities and Species Interactions).
   c. Strong selective pressures and small, fractioned populations, lead to urban evolution and phenotypic adjustments in both dispersed plants and animal disperser species. If such changes are adaptive, they can improve the persistence of a species in the urban environment but could disrupt pre-existing seed dispersal processes if the plants and their dispersers do not co-adapt successfully (**Figure 1**, gray arrows) (see sections Adaptations of Animals to Urbanization and Consequences to Zoochory, and Potential Evolutionary Changes in Plants in Response to Altered Seed Dispersal).

2. Animal seed dispersal feeds back into the ecosystem dynamics by shaping the urban environment (**Figure 1**, black dashed arrows).
   a. Animal seed dispersal in urban areas plays a fundamental role in connecting habitat patches, contributing to the regeneration of degraded habitats, and maintaining the renewal of plant populations within green areas. These may even sustain directional dispersal into suitable habitats within the degraded and patchy urban mosaic (see section Seed Dispersal in the Urban Mosaic).
   b. Zoochory can also be detrimental for local communities, promoting the spread of potentially invasive alien species through urban areas and even toward surrounding natural habitats (see sections Urban Communities and Species Interactions, and Adaptations of Animals to Urbanization and Consequences to Zoochory).

Because of the complex interrelationships between urban plants, dispersers, and their surrounding landscape, the molding of urban dispersal networks and zoochory processes in cities can vary among systems. However, research on urban zoochory to date has focused on a fairly small number of study systems (**Tables 1**, **2**). Most of the reviewed research focused on seed dispersal by birds and terrestrial mammals in big cities (>0.5 million inhabitants) in the temperate region of the Northern Hemisphere, therefore potentially representing relatively simple seed dispersal networks. Despite the complexity of seed dispersal systems, the patterns of interdependence between zoochory and ecosystem dynamics we have discussed should apply across systems. The relative strength and importance of the different associations may vary. The effects of urbanization on seed dispersal depend on how the local dispersers react to urbanization, but also on whether changes in one dispersal interaction are compensated for or further disrupted by other actors in the urban species community.

Research into these complexities has only begun but presents unsurpassed opportunities for better understanding fundamental zoochory processes, examining changes in species interactions in anthropogenically altered environments; and maintaining essential urban ecosystem services on an increasingly urbanized planet. Below, we will identify the current most significant gaps in the research, propose actions to address these open questions, and present evidence for the potential gains from investing in systematic research on urban zoochory.

## Future Directions

So far, empirical investigations of urban zoochory have primarily been restricted to observational studies of seed dispersal processes and seed removal experiments. While such studies can provide a useful starting point, they alone cannot address the more complex mechanisms operating in urban zoochory networks. We identify some of the most pressing open questions in the field and suggest approaches to proceed in addressing them.

### From Focusing on Single Interactions Toward Understanding Networks

Understanding plant-animal seed dispersal interactions of urban areas in a community context is necessary to draw valid conclusions about evolutionary and ecological processes (Vázquez et al., 2009). However, future studies should move beyond the examination of specific interactions between a small number of plants and their dispersers and aim to examine entire seed dispersal networks. So far, only one study has comprehensively analyzed seed dispersal networks of birds in an urban park (Cruz et al., 2013). Since different dispersers in the network can affect overall seed dispersal effectiveness differently according to their diet and movement patterns (Mello et al., 2011; Correa et al., 2016), analyzing seed dispersal networks can give a sense of the robustness of the community. Also, seed dispersal networks can describe seed dispersal connectivity within the landscape mosaic better than single animal-plant interactions given the versatility of seed dispersal interactions (Timóteo et al., 2018). Network nestedness and modularity (i.e., number of interactions and distribution of links), level of redundancy of the links, and dietary specialization of dispersers can all provide information on the resilience and robustness of the community (Burgos et al., 2007; Bastolla et al., 2009; McConkey and Drake, 2015). In tropical seed dispersal networks, higher plant diversity and lower fruit abundance (in fragmented landscapes) have been related to less specialized seed dispersal networks (Schleuning et al., 2012; Chama et al., 2013), and we would expect a similar pattern in urban areas. Furthermore, urbanization could be affecting the redundancy of interactions, by altering disperser abundance and habitat use (Blendinger, 2017).

The study of seed dispersal networks in urban areas would benefit from methods commonly used to track seed movements in natural areas, such as genetic barcoding of disperser species from fecal matter remaining on dispersed seeds (González-Varo et al., 2014), color-coding for identification of seeds in fecal samples (González-Varo et al., 2017b), or maternal identification of seed coats and seedlings through microsatellite genotyping (Godoy and Jordano, 2001; Smouse et al., 2012).

### From Urban Forests Toward Understanding Connectivity Within Cities and Beyond

To complement the existing research, more information is needed on the functional connection through seed dispersal between urban areas and the surrounding natural areas, and between different habitats within the urban mosaic. Urban areas can be major barriers for plant dispersal at the landscape level, disrupting gene flow from one side of an urban area to the other. However, most studies to date have focused on seed dispersal within urban forests, leaving out seed dispersal across the urban matrix. Therefore, little is still known about the movement of seeds and dispersers among the different habitats within the urban mosaic, or about the magnitude of dispersal disruption across the wider landscape due to urban barriers. Furthermore, the proximity and quality of the surrounding environment probably influence biodiversity within green urban patches, but this possibility has yet to be examined. A better understanding of seed flow between urban habitats and across urbanized areas can inform management measures on how to better sustain natural seed dispersal processes, to reduce the spread of invasive species, or increase the regeneration rate of degraded areas and vacant lots.

To analyze connectivity within and across cities, plant movements and community changes across large distances should be examined. A possible approach would be the combined use of landscape genetics methods (Machon et al., 2003; Manel and Holderegger, 2013; Miles et al., 2018) and extensive observational or experimental approaches estimating seed dispersal patterns (Kiyoshi Maruyama et al., 2012; Kleyheeg et al., 2017). To analyze regeneration of degraded habitats, useful approaches include examining spatio-temporal succession or recolonization of severely degraded areas, such as vacant lots (Johnson, 2015; Johnson et al., 2018) or of former landfills (Robinson and Handel, 2000).

### From Observing Urban Fragmentation Toward Understanding Its Evolutionary Consequences

The consequences of isolation of populations within fragmented urban landscapes on urban zoochory processes have barely been examined. However, the extreme fragmentation of urban habitats is one of the reasons to expect genetic changes in urban populations, and thus also the potential for plant-disperser co-adaptations. Further research on the effects of isolation in urban communities is needed to understand whether urban movement barriers can influence adaptation rates of plants, dispersers, and plant-disperser co-adaptations. On one hand, isolation can enhance the rate of local adaptation by disrupting gene flow, and thus may also allow for more rapid co-adaptations of plants and their short-distance dispersers. On the other hand, isolated populations are more susceptible to local extirpation, and any long-distance dispersal could even have negative effects through outbreeding depression (Johnson and Munshi-South, 2017). Most of what we know so far in terms of local adaptation in urban areas is for wind-dispersed species. Wind–dispersed plants with alternative dispersal morphs can show a tendency toward one morph in fragmented urban environments vs. continuous populations, leading to adaptation over generations (Cheptou et al., 2008). Furthermore, in such dispersal mode, roads can counteract isolation by acting as corridors for long dispersal events (Kowarik and von der Lippe, 2011). We are not aware of any studies demonstrating local adaptation to animal–mediated dispersal due to urbanization, despite its high representation in urban areas. If animal movement is restricted, local adaptation should be enhanced (Arendt, 2015), as evidence shows limited seed exchange can create high population substructure and provide the opportunity for divergence through genetic drift (Twyford et al., 2014). Furthermore, changes in inter-specific competition between dispersers, created by urbanization, may also promote rapid evolution (Hart et al., 2018). However, pollen dispersal may compensate for gene flow restriction by seed dispersal (Scheepens et al., 2012) and should not be overlooked when estimating genetic connectivity.

Local adaptation has been successfully examined with common-garden experiments (Kawecki and Ebert, 2004; Cheptou et al., 2008) and landscape community genomics (Hand et al., 2015). Prospective methods to analyze genetic differentiation in isolated areas within cities include next–generation genomic tools (Sork, 2016) combined with improved statistical approaches examining spatial distributions (Safner et al., 2011; Renner et al., 2016).

### From Patterns of Seed Rain Toward Patterns of Regeneration

Finally, animal-mediated seed dispersal dynamics were often conjectured from plant distributions or seed banks in combination with the most likely primary dispersal mode of the species present. However, the final location of most seeds is a result of more than one dispersal mode or vector (Ozinga et al., 2004). In the fine-scale mosaic of microhabitat suitability of highly managed and disturbed sites, recruitment success may be significantly influenced by the fine-scale movements of seeds by invertebrates. So far, few studies have analyzed microscale processes by invertebrates and of other *secondary seed dispersal* vectors influencing recruitment success. In the seed dispersal literature in general, and in urban areas in particular, there is a pressing need to better account for seed fates and the contribution of different dispersal vectors to recruitment success (Vander Wall and Longland, 2004; Forget et al., 2005; Hämäläinen et al., 2017). The estimation of seed dispersal effectiveness (Schupp, 1993; Schupp et al., 2017) without knowledge of plant recruitment is of limited use for predicting plant distributions and evolutionary responses.

Models that incorporate all relevant aspects of the dispersal process from seed removal to post-dispersal events across different landscape features would be valuable for the analysis of effective seed dispersal networks, by-passing shortcomings of interpretations created by excluding recruitment success (Kleyheeg et al., 2017). Recruitment success may also be included into the study of seed dispersal networks by analyzing seedling distribution (Donoso et al., 2016) or parentage analysis of seedlings (Ismail et al., 2017).

## Managing Urban Zoochory as an Ecosystem Service

Given the integral role of seed dispersal in maintaining ecosystem dynamics in cities and functional connectivity within and across cities, understanding urban seed dispersal should become a priority for urban management strategies. Connectivity between urban green areas could be improved by retaining pockets of natural vegetation (Lepczyk et al., 2017), developing a greenway network of backyards habitats and planted boulevards (Rudd et al., 2002), and creating wildlife corridors, such as linear transportation structures, e.g., railroads (Penone et al., 2012), soft linear developments (Suárez-Esteban et al., 2013), forest-like environments (Da Silveira et al., 2016) and high canopy areas (González-Varo et al., 2017a). Minimally managed parks that foster various stages of ecological progression, permit animal movements and maintain long-distance dispersal, could additionally help maintain diversity within the plant communities. Even small protected areas within cities can serve as seed sources of a large number of plant species for surrounding areas (Dyderski et al., 2017). Studying the influence of urbanization on seed dispersal networks of keystone native species, such as the significant role of Eurasian jays in the dispersal of oaks, and that of red foxes in the dispersal of Jujube (*Ziziphus lotus*) (Lundberg et al., 2008; Cancio et al., 2017), would be of particular interest in terms of the maintenance of remnant vegetation. The manipulation of seed disperser movement patterns (Gosper et al., 2005), for example by enhancing existing infrastructures or improving habitat structural connectivity (Beninde et al., 2015), could prove effective in achieving these management goals.

Efforts to sustain seed dispersal networks are valuable not only because they sustain ecosystems, but also because the maintenance of ecosystem services, such as seed dispersal can have substantial economic value. For example, the sustained oak regeneration by Eurasian jays in Stockholm city has been valued at $2,100–$9,400 per hectare annually, based on the estimated cost of manually planting an equal number of oaks (Hougner et al., 2006). Combined with the benefits of urban biodiversity for human health and quality of life (Jackson, 2003; Tzoulas et al., 2007), conserving urban seed dispersal networks should be considered a key component in the planning of sustainable cities and surrounding environments. The value of sustaining functional ecosystems is critical to biodiversity and human wellbeing, and its value increases in significance as habitat alterations and climate change place pressure on diaspore movement across the landscape.

## Urban Dispersal Networks as a Model for Seed Dispersal Dynamics

The complexity of urban communities and habitats, their novel interspecific interactions and phenotypic changes of plants and animals can lead to very dynamic seed dispersal networks. Common characteristics of urban environments, such as increased anthropogenic pressure, fragmentation and edge effects, prevalence of invasive organisms, and commonality of local extinctions, mirror some of the most widespread threats to ecosystems across landscapes (Sundriyal and Sharma, 1996; Wright, 2005; Portugal et al., 2016; Grizzetti et al., 2017). Therefore, urban development serves as an exceptional, easily accessible, and replicated experiment that can yield important insights into eco-evolutionary processes under such threats (McDonnell and Pickett, 1990; Johnson and Munshi-South, 2017; Rivkin et al., 2018).

First, the highly variable urban environment, with its "unnatural" species assemblages and altered communities, can create novel dispersal interactions via replacement of lost primary dispersers with functionally similar native or alien species that are more tolerant of the urban environment (Blendinger, 2017; section Effects of Altered Communities on Urban Seed Dispersal). The study of such functional replacements can provide ways to examine the evolution of species interactions and the adaptability of communities to changing environments.

Second, an improved understanding of seed dispersal processes in fragmented and degraded urban settings could be further used to understand the movement of zoochorous plants across diverse landscapes under similar degradation conditions at different spatial scales, for example under loss of natural habitat or climate change. Such information can be used to predict the range expansion of animal-dispersed plants, with particular relevance for distribution modeling under climate change scenarios.

Third, urbanization has been referred to as "the best and largest-scale unintended evolution experiment" (Johnson and Munshi-South, 2017) because of the rapid evolutionary change observed in numerous organisms across thousands of cities around the world. This replicated experiment offers opportunities for large-scale, coordinated comparative studies. Such goals might be accomplished by making use of unexplored methods in zoochory research, such as standardization of data collection protocols and citizen science. A promising example of such large-scale efforts is the ongoing Global Urban Evolution Project on the evolution of white clover (*Trifolium repens*) (Thompson et al., 2016). While the study of species coevolution due to urbanization is only beginning, seed dispersal interactions are a promising target for increasing our understanding of urban coevolution and the consequent adaptability of communities.

## CONCLUSIONS

Animal-mediated seed dispersal in urban environments is, unquestionably, understudied. Given the explosive growth of urban habitat worldwide, the urgency of facilitating vital ecosystem processes in increasingly anthropogenic environments, and the central role of zoochory in maintaining such processes in cities, there is much to be gained from more systemic research on urban zoochory. The easy accessibility of cities, the improving techniques that facilitate increasingly elegant designs, and the availability of testable hypotheses based on theoretical foundations, should make urban zoochory an increasingly valuable field of research.

## AUTHOR CONTRIBUTIONS

TAG-C and AMH contributed equally to the conception of the presented idea, the analysis of the literature, and the writing of the manuscript. Both authors revised and approved the final version.

## ACKNOWLEDGMENTS

We thank Casper Van Leeuwen for the invitation to contribute to this research topic, Omer Nevo, and the two reviewers for the insightful comments that helped improve the manuscript, and the Leibniz-Institut and Deutsches Primatenzentrum for financing the open-access publication through the Leibniz Association's Open Access Publishing Fund.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

# GLOSSARY

| | |
|---|---|
| Anemochory, anemochorous | Wind-mediated dispersal. Plants that are primarily dispersed by wind are referred to as anemochorous plants |
| Anthropogenic disturbance | Human impact on the environment, such as vegetation clearing, fragmentation through construction, human presence, or the introduction of invasive species |
| Built environment | Aspects of the surroundings that are built by humans, including all forms of buildings (housing, industrial, commercial) and infrastructure that supports human activity (e.g., transportation networks) |
| Diaspore | The dispersing unit of a plant, consisting of the seed and associated tissues that facilitate dispersal, such as the elaiosome |
| Ecosystem service | Functions performed by ecosystems, that are beneficial for humans in some way |
| Ecosystem dynamics | Ecosystem processes that change over time, such as plant regeneration and turnover, gene flow, resource abundance and distribution |
| Elaiosome | Nutrient-rich structure attached to a seed that attracts seed-dispersing animals. The tissues are typically oily and protein-rich and attract ants |
| Endozoochory, endozoochorous | Seed dispersal through ingestion by animals, followed by regurgitation or defecation of viable seeds. Plants that are primarily dispersed by animal ingestion and defecation are referred to as endozochorous plants |
| Exurban | Commuter towns or neighborhoods located in the outskirts of cities, typically beyond the suburbs immediately connected to the city |
| Functional replacement | Replacement of the ecosystem function of one species by another species in a way that maintains the ecosystem function |
| Heat island effect | Urban areas form heat islands because heat is generated and trapped in the physical urban environment, making urban areas warmer than the surrounding landscape |
| Myrmecochory, myrmecochorous | Ant-mediated dispersal |
| Natural environment | Ecological units without much human interference, considered natural systems. |
| Peri-urban | Areas immediately surrounding urban areas, urban periphery |
| Seed shadow | "the spatial pattern of seed distribution relative to parent trees and other conspecifics; it results from the process of seed dispersal and represents the starting template for plant regeneration" (Jordano and Godoy, 2002) |
| Soil seed bank | The quantity of dormant but viable seeds found in the soil or substrate |
| Synzoochory, synzoochorous | Seed dispersal through deliberate transportation of seeds by animals, such as for the purpose of caching |
| Urbanization | The process of an area becoming urbanized, taking characteristics of a city, an inhabited place of greater size, population, or importance than a town or village. |

frontiers
in Ecology and Evolution

ORIGINAL RESEARCH
published: 05 March 2019
doi: 10.3389/fevo.2019.00049

# Frugivory and Seed Dispersal by Lizards: A Global Review

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Birds and mammals are the only vertebrates which receive comprehensive attention in studies of dispersal of fleshy-fruited plant species. However, recently the importance of fleshy fruit in the diet of lizards (order Squamata: suborder Sauria), and their role as seed dispersers have been recognized in a number of studies, especially in studies from arthropod-poor habitats, such as oceanic islands. Here, we revisit the evidence of fruit-eating lizards on a global scale in order to test if fruit consumption is more common on islands than expected by chance. We constructed a database of 470 lizard species (from a global count of 6,515 species), that have been reported to consume fleshy fruits. This set of lizards belong to 27 families with Scincidae ($N = 78$ species), Gekkonidae (69), and Dactyloidae (55) having more frugivorous species than other lizard families. We found that 62.4% of these lizards inhabit islands, whereas only one third (35.3%) of all lizard species inhabit islands. These values support the presence of an "insular phenomenon," however; we also tested if this biogeographical pattern might be driven by body size and evolutionary history of lizards. Thus, we looked for any phylogenetic signals in the distributions of lizard body size, island-presence, and frugivory and calculated phylogenetically corrected correlations among the three variables on a global subset of 2,417 lizard species for which we had detailed phylogenetic information. Both lizard body size and island-presence were weakly influenced by phylogeny; whereas, frugivory was not. In addition, we found that (1) body size and frugivory were weakly positively correlated; (2) body size and island-presence were uncorrelated; and (3) island-presence and frugivory were strongly positively correlated. Thus, we conclude that the main driver of frugivory on islands is the specific island environment and not lizard body size *per se*. Islands are said to be poor in arthropods and predators, and this may force/allow island lizards to forage for additional food sources, such as fleshy fruits. We also suggest that modern lizards as well as their ancestors may potentially play an important role to many plants as seed dispersers. However, we do not known how tight the correlation is between frugivory and seed dispersal. Thus, lizards repeatedly inspire us to ask new ecological and evolutionary questions.

**Keywords: frugivory, lizard, mutualism, plant-animal interaction, saurochory, seed dispersal, island, body size**

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**Specialty section:**
*This article was submitted to*
*Biogeography and Macroecology,*
*a section of the journal*
*Frontiers in Ecology and Evolution*

**Received:** *22 November 2018*
**Accepted:** *08 February 2019*
**Published:** *05 March 2019*

**Citation:**
*Valido A and Olesen JM (2019)*
*Frugivory and Seed Dispersal by*
*Lizards: A Global Review.*
*Front. Ecol. Evol. 7:49.*
*doi: 10.3389/fevo.2019.00049*

## INTRODUCTION

Birds and mammals are the principal seed dispersers of fleshy-fruited plant species (e.g., van der Pijl, 1982; Jordano, 2013). Fruit pulp is an important resource to these animals, which in return may promote an important ecosystem service, the dispersal of their seeds (e.g., Herrera and Pelmyr, 2002; Forget et al., 2011; Bascompte and Jordano, 2014; Wandrag et al., 2018). By dispersing seeds further away from the mother plants, seed mortality gets reduced and survival is favored if colonization happens to new microhabitats (e.g., Janzen, 1970; Howe and Smallwood, 1982; Cousens et al., 2008). In addition, seed dispersal promotes gene flow within and among plant populations (e.g., Rousset, 2004; Pérez-Méndez et al., 2018; Wandrag et al., 2018). Until recently, however, few studies have considered the role of other vertebrates as seed dispersers of fleshy-fruited plant species, e.g., fish, chelonians, crocodilians, and lizards (e.g., Olesen and Valido, 2003, 2004; Liu et al., 2004; Correa et al., 2007; Valido and Olesen, 2007; Platt et al., 2013; Falcón et al., 2018).

Here, we focus on lizards (order Squamata: suborder Sauria), because these animals are usually overlooked or dismissed as frugivores and seed dispersers. The main reason is that most are regarded as primarily feeding on small invertebrates (e.g., Greene, 1982; Cooper and Vitt, 2002; Pianka and Vitt, 2003), whereas only a few species bigger in size are herbivorous, i.e., folivorous (**Supplementary Material:** Herbivorous lizards). Thus, since Pough (1973) a relation between lizard body size and herbivory (specifically "folivory") has been noted. In this respect, larger lizards would present some digestive and physiological modifications which favor an herbivorous diet (e.g., Iverson, 1982; Zimmerman and Tracy, 1989). This idea has also been extended to lizards with frugivorous habits, such as *Anolis*-like species whereas fruit intake has been related to large body size in some species (Herrel et al., 2004a). However, fruit pulp is an easily metabolized plant part, being low in fibers and proteins, and high in soluble carbohydrates (e.g., Valido et al., 2011; Jordano, 2013). Thus, lizards do not require large gape, strong bite or a large digestive system to process these items. In this respect, several studies have shown that small arthropod-eating lizards do consume fruit if their favorite arthropod food is scarce. This is most often observed on islands (e.g., Patterson, 1928; Vinson and Vinson, 1969; Schoener et al., 1982; Cheke, 1984; Whitaker, 1987; Pérez-Mellado and Corti, 1993; Valido and Nogales, 1994; Wotton, 2002; Valido et al., 2003; Hare et al., 2016; Wotton et al., 2016; Melzer et al., 2017; Parejo et al., 2018), where arthropods might be in short supply, but also in arthropod-poor habitats on mainland, such as high mountains and deserts (e.g., Fuentes, 1976; Mautz and Lopez-Forment, 1978; Hódar et al., 1996; Whiting and Greeff, 1997; Kiefer and Sazima, 2002; Espinoza et al., 2004; Valdecantos et al., 2012). By feeding from at least two trophic levels, these lizards become omnivorous. Generally, they do not have any morphological, digestive or physiological modifications related to their frugivorous diet (e.g., Valido and Nogales, 2003; Herrel et al., 2004b; Vidal and Sabat, 2010; but see Sagonas et al., 2015). Alongside their fruit diet choice, they may act as seed dispersers (e.g., Pérez-Mellado and Traveset, 1999; Olesen and Valido, 2003; Godínez-Álvarez, 2004; Valido and Olesen, 2007, 2010).

The full extent of this mutualistic plant-lizard interaction is unknown, because fruit rarely is scored as a separate component in diet studies. In the literature, plant material consumed by lizards is often pooled into one diet component, notwithstanding the wide range in energy content and digestability of various plant parts, e.g., foliage, which is difficult to digest because of the presence of cellulose. In the past, however, mutualistic reptiles were also present in the past (**Supplementary Material**: Mutualistic reptiles in the past), and the natural history literature is rich in stories about fruit-eating lizards (**Supplementary Material**: Early records of lizard-fruit interactions), but this knowledge is still not integrated into general ecology and evolutionary biology (see Miranda, 2017). Consequently, most reviews about seed dispersal focus on birds and mammals (Traveset et al., 2013; e.g., Jordano, 2013).

Here, our aim is (1) to present a detailed global overview of the extent of fleshy fruit consumption and seed dispersal in lizards, by mapping their geographical and taxonomical distribution, and (2) to test if insularity favors plant-lizard mutualism, if we control for lizard body size and phylogenetic relationships.

## MATERIALS AND METHODS

### Collection Methods

Variation in collecting efforts and nomenclature changes make the global number of lizard species very dynamic, but on the 29th of August 2018, the number was 6,512 species (Uetz et al., 2018; **Table 1**). The taxonomy of lizards used here follows the *European Molecular Biology Laboratory Reptile Database*, EMBL. Two species were excluded from our analysis because their geographic range were missing in the EMBL, and, additionally, we included four new species from New Zealand and one from the Canary Islands (Hare et al., 2016; **Table S1**). Thus, our database included a total of 6,515 species sorted into 38 families, which further belonged to the infraorders Iguania, Gekkota, Scincomorpha, Diploglossa, Dibamia, and Platynota (Squamata: Sauria). Using this database, we classified lizards according to their geographical distribution, i.e., only island (*I*), only mainland (*M*), and both island and mainland (*IM*). We classified Australia as a mainland together with the other continents, whereas New Zealand, Madagascar, New Guinea, Borneo, Japan, and Taiwan were analyzed as islands. The borderline between island and mainland is obviously subjective. However, in island literature, Australia is most often regarded as a mainland, and here, we prefer to keep it that way. Several *IM*-species are introductions from their native islands to a mainland, e.g., from the Caribbean Islands to USA (e.g., *Anolis cristatellus,* Dactyloideae), from the Balearic Archipelago to the Iberian Peninsula (*Podarcis pityusensis*, Lacertidae), or *vice versa*, from USA to Japanese Ogasawara Islands (*A. carolinensis*), from North Africa to Menorca Island (*Scelarcis perspicillata*). Such species were here categorized as *IM.* Some lizard species are also island-to-island introductions (e.g., the lacertid *Teira dugesii* from Madeira to the Azores, and *Gallotia stehlini* from Gran Canaria to Fuerteventura). Finally, near-shore islets were regarded as part of their adjacent mainland or island. We also included the continuous variable lizard body size (maximum snout-vent length "max SVL"; Meiri, 2008, 2018), and the binary trait presence of fruits: 0 (absence) and 1 (presence) (see **Supplementary Material**: Quality of the data).

First, we compared species frequencies of fruit-eating lizards on islands and mainland. Our null hypothesis was that the frugivorous lizards had a geographical distribution similar to lizards in general. This was tested with a Chi squared

**TABLE 1 |** Number of lizard species (Sauria) in the families of the infraorders Iguania, Gekkota, Scincomorpha, Diploglossa, Dibamia, and Platynota, sorted according to their geographic distribution: *I*, island-only species; *M*, mainland-only species; and *IM*, species present on both island and mainland.

| Family | Total no. spp. | I-spp (%) | M-spp. (%) | IM-spp (%) |
|---|---|---|---|---|
| **IGUANIA** | | | | |
| Agamidae | 489 | 106 (21.7) | 340 (69.5) | 43 (8.8) |
| Chamaeleonidae | 210 | 94 (44.8) | 113 (53.8) | 3 (1.4) |
| Corytophanidae | 9 | 0 | 9 (100) | 0 |
| Crotaphytidae | 12 | 0 | 11 (91.7) | 1 (8.3) |
| Dactyloidae | 426 | 187 (43.9) | 230 (54.0) | 9 (2.1) |
| Hoplocercidae | 19 | 0 | 19 (100) | 0 |
| Iguanidae | 44 | 27 (61.4) | 9 (20.5) | 8 (18.2) |
| Leiocephalidae | 31 | 31 (100) | 0 | 0 |
| Leiosauridae | 33 | 0 | 33 (100) | 0 |
| Liolaemidae | 307 | 0 | 304 (99.0) | 3 (0.98) |
| Opluridae | 8 | 8 (100) | 0 | 0 |
| Phrynosomatidae | 156 | 12 (7.7) | 133 (85.3) | 11 (7.1) |
| Polychrotidae | 8 | 0 | 6 (75.0) | 2 (25.0) |
| Tropiduridae | 137 | 11 (8.0) | 125 (91.2) | 1 (0.7) |
| **GEKKOTA** | | | | |
| Gekkonidae | 1181 | 388 (32.9) | 720 (61.0) | 73 (6.2) |
| Carphodactylidae | 30 | 0 | 30 (100) | 0 |
| Diplodactylidae | 153 | 58 (37.9) | 92 (60.1) | 3 (2.0) |
| Eublepharidae | 38 | 8 (21.1) | 25 (65.8) | 5 (13.2) |
| Phyllodactylidae | 146 | 44 (30.1) | 96 (65.8) | 6 (4.1) |
| Sphaerodactylidae | 218 | 104 (47.7) | 89 (40.8) | 25 (11.5) |
| Pygopodidae | 46 | 1 (2.17) | 43 (93.5) | 2 (4.4) |
| **SCINCOMORPHA** | | | | |
| Cordylidae | 68 | 0 | 68 (100) | 0 |
| Gerrhosauridae | 37 | 19 (51.4) | 17 (45.9) | 1 (2.7) |
| Lacertidae | 335 | 34 (10.2) | 273 (81.5) | 28 (8.4) |
| Scincidae | 1,656 | 702 (42.4) | 864 (52.2) | 90 (5.4) |
| Xantusiidae | 34 | 1 (2.9) | 32 (94.1) | 1 (2.9) |
| Alopoglossidae | 23 | 0 | 22 (95.6) | 1 (4.4) |
| Gymnophthalmidae | 246 | 2 (0.8) | 236 (95.9) | 8 (3.3) |
| Teiidae | 160 | 26 (16.3) | 118 (73.8) | 16 (10.0) |
| **DIPLOGLOSSA** | | | | |
| Anguidae | 78 | 2 (2.6) | 69 (88.5) | 7 (9.0) |
| Diploglossidae | 51 | 26 (50.9) | 25 (49.0) | 0 |
| Anniellidae | 6 | 0 | 5 (83.3) | 1 (16.7) |
| Xenosauridae | 12 | 0 | 12 (100) | 0 |
| **DIBAMIA** | | | | |
| Dibamidae | 24 | 10 (41.7) | 11 (45.8) | 3 (12.5) |
| **PLATYNOTA** | | | | |
| Helodermatidae | 2 | 0 | 2 (100) | 0 |
| Lanthanotidae | 1 | 1 (100) | 0 | 0 |
| Varanidae | 80 | 36 (45.0) | 30 (37.5) | 14 (17.5) |
| Shinisauridae | 1 | 0 | 1 (100) | 0 |
| **TOTAL** | **6515** | **1938** | **4212** | **365** |
| **(%)** | | **(29.7)** | **(64.6)** | **(5.6)** |

*Number of species and their geographic range follow Uetz et al. (2018), update at 29 August 2018.*

*This table includes 6,515 species. In the analysis, we excluded two species with no information about their geographic range in the Reptile Database: Diploglossus microlepis (Diploglossidae) and Leiolopisma fasciolare (Scincidae), but we have included four new species from New Zealand, which were not in the Reptile Database: Oligosoma aff. polychroma (Scincidae), Dactylocnemis "Poor Knights," Woodworthia aff. brunnea, and W. "Southern Alps" (Diplodactylidae) (Hare et al., 2016). The extinct Gallotia goliath (Lacertidae) from Canary Islands was also included, because of available diet information.*

test of data from each family separately and for the global count of lizards. Globally, 29.7% of all lizard species are *I*-species, 64.6% only *M*-species, and 5.6% are *IM*-species (**Table 1**; **Figure 1**).

We also tested if the variables body size, frugivory, and island-presence in their distribution among lizards had a phylogenetic signal (see below). As backbone lizard phylogeny, we used the one published by Pyron et al. (2013). This phylogeny only included 2,847 lizard species. We pruned the phylogeny for those lizard species without any information about their max SVL or geographic distribution, resulting in 2,417 lizard species, which were included in our phylogenetic analysis.

## Phylogenetic Signal

The phylogenetic signal of a trait is a measure of the statistical dependency among values of this trait on the phylogenetic relationships among species in the study sample (e.g., Blomberg and Garland, 2002). If other factors than phylogenetical relatedness influence trait variation, for example convergence due to related ecology, this will reduce the phylogenetic signal.

The phylogenetic signal of a continuous trait (max SVL) can be measured by Blomberg's $K$ and Pagel's $\lambda$ (Pagel, 1999; Blomberg et al., 2003). Both measures react slightly different to number of species included in the phylogeny, amount of information about branch length and number of polytomies (Münkemüller et al., 2012). For both measures, the actual observed value is compared to a null model of trait evolution, a Brownian motion (*BM*) evolutionary model (Blomberg et al., 2003), which is based upon either pure genetic drift, randomly varying selection, or varying stabilizing selection, but not on directional selection. According to this *BM* model, trait evolution follows a random walk along the branches of the phylogenetic tree. To test this null hypothesis of no phylogenetic signal, the observed values of the focal trait was compared with values expected under random (1,000 permutations) value distribution by using a likelihood ratio test (Münkemüller et al., 2012).

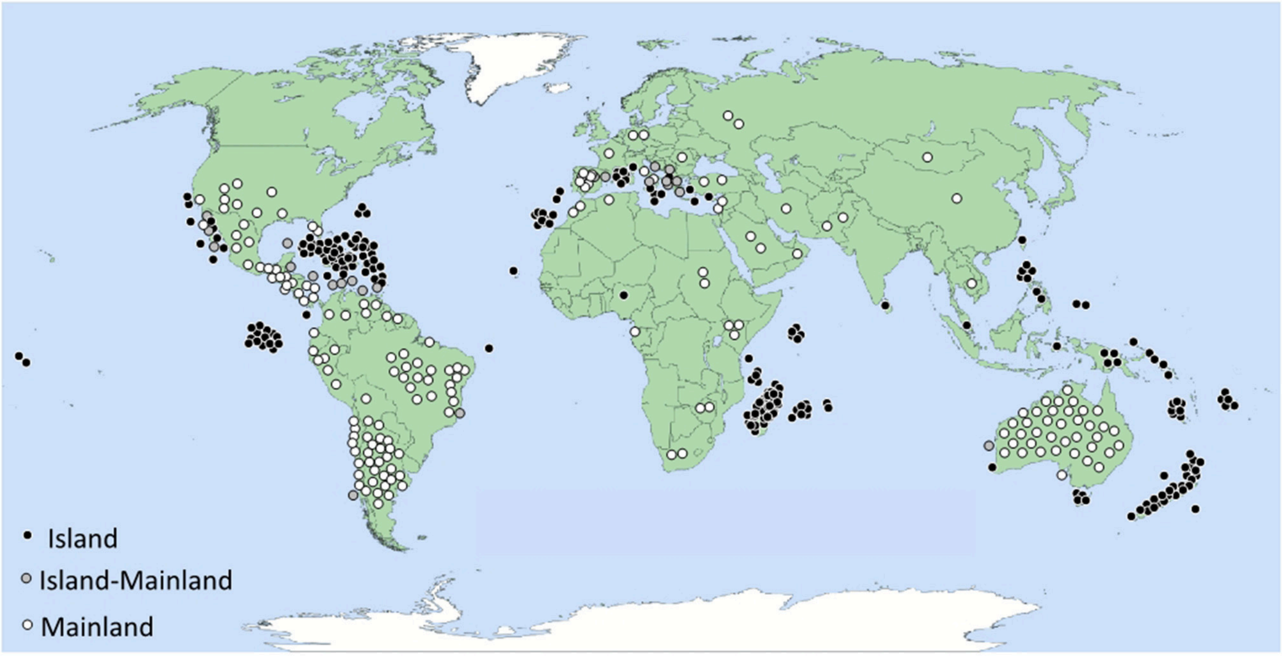

**FIGURE 1 |** Geographic distribution of 470 fruit-eating lizard species sorted as island-only species (*I*), mainland-only species (*M*) and species present on both island and mainland (*IM*). See also **Table S1** in Supplementary Material for a full list of frugivorous lizard species. The map shows not just the distribution of lizard frugivory, but also the heterogeneous sampling efforts. Note, for example, the absence of dots in Japan and Indonesia, which most likely is caused by insufficient field observation.

Both measures ($K$, $\lambda$) vary from 0 to 1, with values close to zero indicating lack of phylogenetic dependency, i.e., the trait has evolved in response to local selective processes. Whereas, a value close to 1 indicates an evolution according to the *BM*, i.e., a gradual accumulation of changes over time. $K$ may also be larger than one, which indicates a strong phylogenetic signal. At least theoretically, $\lambda$ might also become slightly larger than one (Münkemüller et al., 2012). The lower and upper bounds of $K$ and $\lambda$ indicate which of the two scenarios is the most likely.

The phylogenetic signal for binary traits (frugivory and island-presence) can be measured by $D$ (phylogenetic dispersion) (Fritz and Purvis, 2010). $D = (d_{\mathrm{obs}} - \text{mean } d_{\mathrm{b}})/(\text{mean } d_{\mathrm{r}} - \text{mean } d_{\mathrm{b}})$, where $d_{\mathrm{obs}}$ is the number of trait state changes needed to get the observed trait state distribution in our phylogeny, $d_{\mathrm{b}}$ is the expected distribution of $d$ under a *BM* model (1,000 permutations) and $d_{\mathrm{r}}$ is the expected distribution of $d$, if trait states are randomly distributed among species. $D$ typically varies from 0 to 1. $D = 0$ indicates that the trait evolves according to the Brownian model, *BM* (i.e., phylogenetic signal). $D = 1$ indicates that the trait evolves according to a random model (i.e., independent of the phylogeny), $D > 1$, if the trait is phylogenetically overdispersed, and $D < 0$, if the trait is more phylogenetically clustered than expected according to a *BM* model (Nunn, 2011).

The finding of a significant phylogenetic signal in some of these variables requires the use of comparative phylogenetic analysis to test for correlation between traits.

## Phylogenetically Corrected Correlations

We tested for correlations between max SVL, island-presence and frugivory, using phylogenetically independent contrasts (*PIC*), i.e., any influence of statistical dependency among trait values was removed before the correlation analysis. The lizard body size (max SVL) data were log-transformed.

By using the lizard phylogeny (Pyron et al., 2013), and our compiled database including max SVL of lizards (Meiri, 2008, 2018), island-presence and frugivory (**Table S1**), we answered: 1. Is there, globally, any phylogenetic signal in the distribution of frugivory, body size, and island-presence in lizards; and 2. to what extent do island-presence and max SVL influence frugivory after correcting for any phylogenetical influence. For these phylogenetical analyses we used *picante* (Kembel et al., 2018), *geiger* (Harmon et al., 2016), *caper* (Orme et al., 2018), and *ape* (Paradis et al., 2018) R packages (R Core Team, 2014).

# RESULTS

## Fruit-Eating Lizards

We found reports of 470 lizard species from 27 families and 128 genera consuming fleshy fruits (**Tables 2**, **S1**). Thus, 7.2% of all lizards use fleshy fruits to some extent in their diet. These species are widely distributed taxonomically, since 71% of all lizard families included some frugivorous members; Scincidae ($N = 78$ species), Gekkonidae (69), and Dactyloidae (55) being most frugivorous. Other lizard families with a high percentage of frugivorous species are Iguanidae (54.5%), Corytophanidae

**TABLE 2 |** Taxonomic and geographical distribution of fruit-eating lizard families.

| Family | Total no. spp. | No. fruit-eating lizards | I (%) | M (%) | IM (%) | *P* | % |
|---|---|---|---|---|---|---|---|
| **IGUANIA** | | | | | | | |
| Agamidae | 489 | 19 | 7 (36.8) | 12 (63.2) | 0 | ns | 3.9 |
| Chamaeleonidae | 210 | 2 | 1 (50.0) | 1 (50.0) | 0 | ns | 0.9 |
| Corytophanidae | 9 | 4 | 0 | 4 (100.0) | 0 | – | 44.4 |
| Crotaphytidae | 12 | 2 | 0 | 1 (50.0) | 1 (50.0) | – | 16.7 |
| Dactyloidae | 426 | 55 | 47 (85.5) | 5 (9.1) | 3 (5.5) | *** | 12.9 |
| Iguanidae | 44 | 24 | 16 (66.7) | 3 (12.5) | 5 (20.8) | ns | 54.5 |
| Leiocephalidae | 31 | 13 | 12 (92.3) | 0 | 1 (7.7) | – | 41.9 |
| Leiosauridae | 33 | 1 | 0 | 1 (100.0) | 0 | – | 3.0 |
| Liolaemidae | 307 | 34 | 0 | 34 (100.0) | 0 | – | 11.1 |
| Opluridae | 8 | 2 | 2 (100.0) | 0 | 0 | – | 25.0 |
| Phrynosomatidae | 156 | 8 | 1 (12.5) | 6 (75.0) | 1 (12.5) | ns | 5.1 |
| Polychrotidae | 8 | 4 | 0 | 4 (100.0) | 0 | – | 50.0 |
| Tropiduridae | 137 | 24 | 9 (37.5) | 14 (58.3) | 1 (4.2) | * | 17.5 |
| **GEKKOTA** | | | | | | | |
| Gekkonidae | 1,181 | 69 | 66 (95.7) | 3 (4.3) | 0 | *** | 5.8 |
| Diplodactylidae | 153 | 17 | 17 (100.0) | 0 | 0 | *** | 11.1 |
| Phyllodactylidae | 146 | 2 | 1 (50.0) | 1 (50.0) | 0 | – | 1.4 |
| Sphaerodactylidae | 218 | 2 | 1 (50.0) | 1 (50.0) | 0 | – | 0.9 |
| Pygopodidae | 46 | 17 | 0 | 17 (100.0) | 0 | – | 37.0 |
| **SCINCOMORPHA** | | | | | | | |
| Cordylidae | 68 | 3 | 0 | 3 (100.0) | 0 | – | 4.4 |
| Gerrhosauridae | 37 | 7 | 4 (57.1) | 3 (42.9) | 0 | ns | 18.9 |
| Lacertidae | 335 | 38 | 21 (55.3) | 11 (28.9) | 6 (15.8) | *** | 11.3 |
| Scincidae | 1,656 | 78 | 45 (57.7) | 32 (41.0) | 1 1.3) | ns | 4.7 |
| Xantusiidae | 34 | 3 | 2 (66.7) | 1 (33.3) | 0 | ns | 8.8 |
| Teiidae | 160 | 36 | 15 (41.7) | 19 (52.8) | 2 (5.6) | * | 22.5 |
| **DIPLOGLOSSA** | | | | | | | |
| Anguidae | 78 | 1 | 0 | 1 (100.0) | 0 | – | 1.3 |
| Diploglossidae | 51 | 2 | 2 (100.0) | 0 | 0 | ns | 3.9 |
| **PLATYNOTA** | | | | | | | |
| Varanidae | 80 | 3 | 3 | 0 | 0 | ns | 3.7 |
| **TOTAL**<br>**(%)** | **6113** | **470** | **272**<br>**(57.9)** | **177**<br>**(37.6)** | **21**<br>**(4.5)** | *** | **7.7** |

*I, island-only species; M, mainland-only species; and IM, species present on both island and mainland. P, probability that the geographical distribution of the fruit-eating lizards in a given family differed from a random draw of the same number of species from the world pool of lizards (see Methods). %, percent fruit-eating lizard species out of total count. Number of species and their geographical range follow Uetz et al. (2018), update at the 29 August 2018 (**Table 1**). See also **Table S1** in Supplementary Material for a full list of frugivorous species.* $^{*}P < 0.05$; $^{***}P < 0.001$.

(44.4%), and Leiocephalidae (41.9%) (**Tables 2**, **S1**, Importance and examples of fleshy fruit to the diet of lizards).

## Geographic Distribution of Frugivorous Lizards

64.6%, 29.7% and 5.6% of the 6,515 lizard species in the world inhabit mainlands, islands, and both, respectively (**Table 1**). However, frugivory in lizards were much more frequent among only island species (57.9% of all frugivorous lizards) than among only mainland species (37.6%). Only 4.5% of all frugivorous lizards were found on both island and mainland (**Tables 2**, **S1**). First, we tested the null-hypothesis $H_0$: the frequencies of fruit-consuming lizard species on islands, mainland and both could be explained by the global geographical distribution of lizards. In this analysis, only the 27 lizard families with reported frugivory were included ($N = 6{,}113$ species). $H_0$ was rejected ($P << 0.001$; **Table 2**). Thus, the high frequency of fruit-consuming lizards on islands could not be explained by the general biogeography of the group. This analysis was repeated at family level. Island species were significantly overrepresented as fruit consumers in Dactyloidae, Gekkonidae, Diplodactylidae, and Lacertidae (**Table 2**). In contrast, in the Tropiduridae, frugivory was significantly more frequent on mainland than on islands (**Table 2**). Thus, worldwide fruit-consumption among lizards is almost twice as common on islands than on mainland, but on the family level there is some variation.

## Phylogenetic Correlations

The distribution of max SVL showed a significant, but weak phylogenetical signal ($K = 0.291$; **Table 3**). Pagel's λ was closer to 1 than 0, but it was still significantly different from both values (95% confidence interval = [0.911; 0.941], and both the lower and upper bound were strongly unlikely; **Table 3**). Thus, $K$ and λ showed that phylogeny, but also other factors, were important as drivers of body size variation in lizards. That lizard body size, in general, is influenced by both phylogenetic relationships and other factors (ecology) is certainly also what one would expect.

Our null hypothesis, stating that any presence or absence of lizards on islands is influenced of phylogeny, was not rejected ($P = 0.80$; **Table 3**), and this binary trait was distributed according to a Brownian motion model ($D \approx 0$; **Table 3**). We would also expect evolution to have played a role here, especially because many lizard radiations are endemic to islands.

For the binary trait frugivory, the null model was rejected ($P = 0.003$). Thus, the trait was not distributed according to a Brownian motion model of evolution ($D \approx 1$). However, $D \approx 1$ (no influence of phylogeny) was also rejected ($P < 0.001$). Thus, both phylogeny and other factors (ecological) play a role in the distribution of frugivory among lizards.

After correcting for any influence of phylogeny (*PIC*, phylogenetically independent contrasts; **Table 4**), we found (1) that body size (max SVL) and frugivory were positively, but weakly correlated; (2) that body size and island-presence were uncorrelated; and (3) that island-presence and frugivory were strongly positively correlated. Thus, in our database, frugivory was significantly associated with insular species after correcting for any influence of evolutionary relationships.

## DISCUSSION

In this review, we reported that 470 lizard species consume fleshy fruit, many more than previously thought (Cooper and Vitt, 2002; Olesen and Valido, 2003; Godínez-Álvarez, 2004; Valido and Olesen, 2007; Meiri, 2018). However, it is still low compared to equivalent estimates for birds (around 4,000 bird species consume fleshy fruit, Wenny et al., 2016), but similar to mammals (460 species are primarily frugivorous, Fleming and Sosa, 1994). In addition, 182 Neotropical freshwater fish are fruit eaters (Correa et al., 2007), and recently Falcón et al. (2018) reported that up to 72 species of turtles include fleshy fruits in their diet.

However, for several reasons our number of frugivorous lizards is clearly an under-estimate: For example: (1) modern researchers have just recently begun to pay more attention to lizards as frugivores/plant mutualists; (2) many omnivorous (i.e., with 10–90%volume (V) plant matter in their diet) and herbivorous (>90%V) lizard examples reported in e.g., Cooper and Vitt (2002) were not incorporated in our database since the original reports did not specify which vegetative parts were consumed, and these species may use fleshy fruit as well; (3) lizard diet sampling conducted outside the fruiting period will not detect frugivory, and (4) for most lizards we did not find any data about their diet, particularly for endemics inhabiting remote islands, although some of their congenerics are in our database. Thus, we believe that many more fruit-eating and seed-dispersing lizards are waiting to be discovered.

## Evolution of Frugivorous Lizards

Irrespectively of recent finding of an herbivorous lizard from early Cretaceous (Evans and Manabe, 2008), carnivory is the ancestral feeding mode in modern lizard species and most species are still exclusively or mainly carnivorous (e.g., Cooper and Vitt, 2002). However, trends toward true herbivory are seen repeatedly (Iverson, 1982; King, 1996; Cooper and Vitt, 2002), e.g., ∼18 herbivory origins within the South American

**TABLE 4 |** Phylogenetic independent contrasts (*PIC*) correlations between "max SVL," "frugivory," and "island-presence" of 2,417 lizard species, based on the phylogeny of Pyron et al. (2013).

| Correlation | $F_{phy\ 1,2414}$ | $P_{phy}$ |
|---|---|---|
| Max SVL × island-presence | 0.029 | 0.86 |
| Max SVL × frugivory | 8.793 | 0.004 |
| Island-presence × frugivory | 52.31 | 0.001 |

*"Island-presence" and "frugivory" were categorized as binary variables (0, 1) (see Methods for details). $P_{phy}$ is the probability that there is no correlation between two variables.*

**TABLE 3 |** Size of phylogenetic signal in the distribution of the continuous trait "max SVL" (*K*, λ), and the binary traits "island presence" and "frugivory" (*D*) of 2,417 lizard species, according to the phylogeny of Pyron et al. (2013).

| Trait | *K* | *P*(*K*) | λ | Confidence interval of λ | *P*(λ) (lower bound) | *P*(λ) (upper bound) |
|---|---|---|---|---|---|---|
| Max SVL | 0.291 | <0.001 | 0.927 | [0.911; 0.941] | 0.001 | 0.001 |
| | ***D*** | | | | ***P* (*D* ≈ 0)** | ***P* (*D* ≈ 1)** |
| Island presence | −0.080 | | | | 0.80 | 0.001 |
| Frugivory | 0.314 | | | | 0.003 | 0.001 |

*P of null model of Brownian motion (BM) (see Methods for details).*
*$K \approx 0$ no influence of phylogeny; $K \approx 1$ trait evolution according to BM model; $K > 1$ trait is phylogenetically clustered; $\lambda \approx 0$ no influence of phylogeny; $\lambda \approx 1$ trait evolution according to BM model; $D < 0$ trait values are phylogenetically clustered; $D \approx 0$ trait evolution according to BM model; $D \approx 1$ no influence of phylogeny.*

*Liolaemus* (Espinoza et al., 2004). These lizards are smaller (max. SVL <100 mm) than other herbivorous species, and also inhabit mainland cold areas. Thus, small montane lizards may keep a higher body temperature (Vitt, 2004), being necessary for microbial hindgut endosymbionts, by digesting plant fibers. Cooler habitats have fewer insects and selection may favor a switch to herbivory (Pearson, 1954; Fuentes and Di Castri, 1975; Jaksic, 1978). However, the rarity of plant-eating lizards also suggests that herbivory in lizards is constrained (Szarski, 1962; Ostrom, 1963; Sokol, 1967; Pough, 1973; Espinoza et al., 2004). Here, we demonstrate that the use of easily digestible plant matter (i.e., fleshy pulp) is relatively frequent, occurring in several lizard lineages. However, the extent of this shift to frugivory in lizards is affected by insularity and body size.

## Insularity

One third of all lizard species live on islands, but two thirds of all the fruit-consuming lizards are reported from islands. This geographical pattern is not caused by a higher number of scientists working both on seed dispersal (e.g., Estrada and Fleming, 1986; Fleming and Estrada, 1993; Levey et al., 2002; Dennis et al., 2007; and references therein) or diet of lizards on islands compared to mainland habitats (Meiri, 2018). In addition, the pattern is not caused by a presence of more fleshy-fruited plant species on islands compared to mainland habitats; on the contrary, islands have fewer fleshy-fruited plant species than comparable mainland habitats (e.g., Heleno and Vargas, 2015). Herrel et al. (2004a) compared the geographic distribution of frugivory among 45 island and 16 mainland *Anolis* species and also demonstrated that fruit in the diet of lizards is more common on islands (90% of all cases) than on mainland (10%). Analyzing many lizard species, but pooling all dietary plant parts, earlier studies also found this relationship (e.g., van Damme, 1999; Cooper and Vitt, 2002). However, island lizards include fleshy fruit, but also other vegetable food items, into their diet because they may be food limited (Janzen, 1973; Andrews, 1979; Schluter, 1984; Pérez-Mellado and Corti, 1993; van Damme, 1999; Cooper and Vitt, 2002; Polis et al., 2002; Olesen and Valido, 2003; Barret et al., 2005). This recurrent island phenomenon is an example of "niche expansion" or interaction release (*sensu* Traveset et al., 2015) and was first demonstrated for island birds (Grant, 1966; MacArthur et al., 1972). This pattern is possibly caused by a three-step process: 1) Compared to mainland, islands usually support fewer insect taxa (Gulick, 1932; MacArthur and Wilson, 1967) of a lower overall abundance (Allan et al., 1973; Janzen, 1973); 2) island lizards are less exposed to interspecific competition and predation because of a general low species density (MacArthur et al., 1972; Case, 1975; Andrews, 1979), and 3) some island lizard species respond to 2) by density compensation (MacArthur et al., 1972; Case, 1975; Rodda et al., 2001), resulting in intense intraspecific competition and consequently an expansion of their feeding niche toward the use of alternative resources, such as fleshy fruits (Olesen and Valido, 2003). However, only scant information is available on island arthropod abundance (Allan et al., 1973; Janzen, 1973; Andrews, 1979; Case, 1982; Faeth, 1984), but the impression from the general island biology literature is that arthropods are less abundant on islands and that they do not density compensate. On tiny islets, however, where coastline habitats dominate, flies functioning as decomposers of algae may be so abundant (Polis et al., 2002), that, at least theoretically, arthropod-eating lizards may be less inclined to shift to fruit.

On many islands, lizards have higher densities than related groups from adjacent mainland (Rodda and Dean-Bradley, 2002; Buckley and Jetz, 2007). Exceptionally high densities of lizards on islands have attracted considerable attention (Case, 1975; Case and Bolger, 1991; Rodda et al., 2001; Buckley and Jetz, 2007). The world record seems to be >5 *Sphaerodactylus macrolepis* individuals/m$^2$ in the Virgin Islands (Rodda et al., 2001). Insular founders may reach such high densities in just 3 years (Schoener, 1989). Density compensation in island lizards is a ubiquitous and global phenomenon, and total island lizard density is an order of magnitude higher than on mainland (128 *vs.* 1,920 individuals/ha) (Rodda et al., 2001; Buckley and Jetz, 2007). Many of these density-compensating island lizards are also present in our database (genera *Anolis*, *Gallotia*, *Gehyra*, *Hemidactylus*, *Lepidodactylus*, *Oligosoma*, *Phelsuma*, *Podarcis*, *Xantusia*, etc.). Reduction in species richness of predators (e.g., birds of prey) and number of competitors (insectivorous birds) may be the dominant drivers of lizard abundance on islands (Schoener and Schoener, 1978; Andrews, 1979; Wright, 1979; Buckley and Jetz, 2007). Density compensation in fruit-consuming lizards may thus be of high importance to seed dispersal in many plant species, influencing their chance of colonization and establishment on small islands, and their general population structure. Besides, the poor ability of mammals to reach remote islands will leave part of the diet niche dimension empty for other animal groups to explore (Whittaker and Fernández-Palacios, 2007).

Fruit -eating lizards are also observed in mainland habitats poor in arthropods. Frugivory in lizards inhabiting desert-like habitats (e.g., Clark and Comanor, 1976; Vitt et al., 1981; Hódar et al., 1996; Whiting and Greeff, 1997; Belver and Avila, 2002), high mountains (e.g., Hurtubia and Di Castri, 1973; Fuentes, 1976), cerrado habitats (e.g., Vitt, 1993; Mesquita and Colli, 2003) and caves (Mautz and Lopez-Forment, 1978) make up most of the mainland observations. Seasonal scarcity of arthropods and frugivory in lizards are also reported (e.g., Schleich et al., 1996; Duffield and Bull, 1998; Fialho et al., 2000). Thus, the arthropod-scarcity hypothesis can also explain mainland observations of lizards as fruit eaters.

## Lizard Body Size

Since the review by Pough (1973) about lizard body size and herbivory diet, the generalization that only lizards >300 g (>217 mm SVL) are truly herbivorous and that medium-sized lizards, 100–300 g (150–217 mm SVL) are omnivorous have become a dogma in lizard ecology. It has been used to explain the paucity of modern, truly herbivorous lizards. However, a debate about this has also been running for more than 50 years (Szarski, 1962; Ostrom, 1963; Sokol, 1967; Pough, 1973; Iverson, 1982; van Devender, 1982; Auffenberg, 1988; King, 1996; van Damme, 1999; Cooper and Vitt, 2002; Cooper, 2003; Espinoza et al., 2004; Herrel et al., 2004a,b, 2008), including issues about predation risk, insularity, lizard body size, and digestive and physiological adaptative modifications. However, Espinoza et al. (2004), and more recently Vervust et al. (2010) demonstrated true herbivory in smaller species (max. SVL <100 mm) than in other known herbivorous species. These species were inhabiting insular (-like) habitats and true islands.

Many small (<150 mm SVL) lizards are being classified as frugivorous, e.g., species of *Gallotia*, *Podarcis* (Lacertidae), *Anolis* (Dactyloidae), *Carinascincus, Trachylepis* (Scincidae), *Gehyra*, *Phelsuma* (Gekkonidae), *Platysaurus* (Cordylidae), *Ameiva, Cnemidophorus* (Teiidae), *Leiocephalus* (Leiocephalidae), *Microlophus* (Tropiduridae), and *Lepidophyma smithii* (Xantusidae). Large lizards have large gapes and a strong bite (e.g., Herrel et al., 1999, 2004a,b), and this may be advantageous if the diet is vegetarian, because it allows the lizards to crush the material efficiently (Szarski, 1962; Sokol, 1967). Fleshy fruits, on the other hand, do not require this.

## Frugivorous Lizards as Legitimate Seed Dispersers

Lizards do not fully chew their food and seeds passing through their gut may remain intact. Reviewing experimental studies of 40 plant species and 17 lizard species, Traveset (1998) and A. Valido (*unpublished*) concluded that seeds dispersed by lizards germinated just as well as seeds dispersed by frugivorous birds and mammals. The proportions of experiments in which germination of seeds was enhanced (25%), unaffected (57%) and inhibited (18%) after lizard gut passage were similar to figures for seed-dispersing birds (36, 48, and 16%, respectively), non-flying mammals (39%, 42%, 19%), and bats (25%, 67%, 8%). Here, we summarize some of these results.

Iverson (1985) detected a 6% increase in seed germination of *Coccoloba uvifera* (Polygonaceae) after passage through the gut of *Cyclura* compared to controls. Studies of other rock iguanas gave similar results. Within Lacertidae, a significant increase in germination in *Gallotia*-consumed seeds of *Withania aristata* (Solanaceae) has been reported (Valido and Nogales, 1994). Other reports from the Canaries and Balearics show similar results (Nogales et al., 1998; Castilla, 2000; Pérez-Mellado and Riera, 2004; Pérez-Mellado et al., 2005; Rodríguez-Pérez et al., 2005). Thus, many of these island lizards may contribute to plant fitness. However, for *Cnemidophorus murinus* from Bonaire Island, Lesser Antilles, Schall (1996) did not find any germination response for *Erithalis fruticosa* (Rubiaceae).

In Brazilean *Melocactus violaceus* (Cactaceae), 36% of the seeds passing through *Tropidurus torquatus* germinated compared to no germination at all for controls (Côrtes-Figueira et al., 1994). Fruits of *Melocactus* species appear to be consumed by lizards only (Dearing and Schall, 1992). On Chiloé Island, Chile, Rubiaceae seeds defecated by *Liolaemus pictus* germinated better or to the same extent as controls (Willson et al., 1996). Seeds of *Genipa americana* (Rubiaceae) and *Cereus peruvianus* (Cactaceae) consumed by *Salvator merianae* germinated significantly better and or to the same extent as controls, respectively (De Castro and Galetti, 2004). Vasconcellos-Neto et al. (2009) showed that seeds of *Solanum thomasiifolium* (Solanaceae) were dispersed less frequently by lizards (4%) than by birds (77%) and foxes (19%), but with higher germination rate (80%) compared to birds (64%) and foxes (53%).

The digestive treatment of seeds seems to be quite similar among different frugivore groups (Valido and Olesen, 2007). Although examples are still scarce, lizards seem to be in the same seed disperser league as the classical and better studied frugivorous birds and mammals. However, the series of seed germination studies mentioned above suggests that frugivory may not just lead to dispersal of seeds, but also to dispersal of viable seeds, which may germinate and increase plant fitness. However, we do not know how strong frugivory is as a proxy for plant fitness, and we also lack an experimental comparison of seed germination after passage through sympatric native lizards, birds and mammals, with the control treatment of intact fruits (Samuels and Levey, 2005).

## CONCLUSIONS

Many lizard species are potential seed dispersers, and fruit feeding among lizards is taxonomically and geographically widespread, including 7.2% of all lizard species. It is especially important in species and populations inhabiting islands (62.4% of all reported cases). Finally, compared to other, more classical vertebrate mutualists (birds, mammals) seed dispersal by lizards is relatively poorly documented, but may be just as effective in terms of the quantitative and qualitative component of the seed dispersal effectiveness (*sensu* Schupp et al., 2010).

We believe that the results presented here are only the tip of the "lizard-plant seed dispersal iceberg." Thus, we find that fruit is an important supplementary diet component for island lizards. Other easily digestable plant material, like nectar and pollen, may show the same difference between islands and mainland, whereas we expect consumption of fiber-rich plant parts to show a deviating pattern. In general, much about lizard diet and its ecological and evolutionary consequences remain unknown. Our review may encourage ecologists, herpetologists, island biologists and natural historians in general to pay more attention to this type of plant-animal interaction, which may expand our general understanding of the ecology and evolution of mutualisms. For several decades, lizards have offered us outstanding opportunities for many kinds of ecological and evolutionary study (e.g., Pianka and Vitt, 2003; Losos, 2009), and here a new research line about lizard-plant mutualisms is suggested.

## DATA AVAILABILITY

The datasets analyzed for this study are available in Supporting Information (**Table S1**) and the lizard phylogeny in Pyron et al. (2013).

## AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

## FUNDING

This research was supported by the Ministerio de Economía y Competitividad (CGL2L013-47429-P), Severo Ochoa Excellence Award (SEV-2012-0262), and by a Ramón y Cajal research program (RYC-20077-00620) to AV, and the Danish Science Research Council and Carlsberg Foundation to JO.

## ACKNOWLEDGMENTS

We are grateful to many researchers around the world, contributing with original data, references, and personal communications about lizards feeding on fleshy fruits (see **Table S1** for a complete list of personal communications). We also thank Casper H. Van Leeuwen, Jose Luis Tella, and Andy J. Green for the invitation to participate in this issue. The manuscript benefited from constructive comments by Andy J. Green, Sean T. Giery, and Daniel Wenny.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2019.00049/full#supplementary-material

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The handling editor declared a shared affiliation, though no other collaboration, with one of the authors, AV, at time of review.

frontiers
in Ecology and Evolution

REVIEW
published: 07 March 2019
doi: 10.3389/fevo.2019.00038

# Plants on the Move: Hitch-Hiking With Ungulates Distributes Diaspores Across Landscapes

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We here describe the multiple mechanisms by which ungulates distribute diaspores across landscapes. There are three primary and three secondary seed dispersal mechanisms by which ungulate dispersal agents contribute to the spread of plant diaspores, both with and without the intervention of other biotic and abiotic agents. These dispersal mechanisms may be combined in successive inter-dependent steps. Native, introduced and domestic ungulates co-occur in many ecosystems and frequently interact with numerous plant species, which facilitates long-distance dispersal of both native and exotic plants. However, ungulate taxonomic diversity conceals a much higher diversity in terms of the functional traits involved in ungulate-mediated dispersal (e.g., feeding regime, fur morphology). These traits may strongly affect emigration, transfer and immigration in the animal-mediated plant dispersal, and consequently; they may also impact overall seed dispersal effectiveness, both quantitatively and qualitatively. In this review, we compare internal mechanisms, where seeds must survive digestive treatments (regurgitation, endozoochory), with external mechanisms, where diaspores are carried on the outside of the vectors (epizoochory). We include both primary epizoochory (direct adhesion to fur essentially) and secondary epizoochory (diaspore-laden mud adhering to hooves or the body and, transfer through contact with a conspecific). We addressed the overlap/complementarity of ungulates for the plant species they disperse through a systematic literature review. When two ungulate species co-occur, there is always an overlap in the plant species dispersed by endozoochory or by fur-epizoochory. Further, when we consider the proportion of plant species dispersed both internally and externally by an ungulate, the overlap is higher for grazing than browsing ungulates. We identify two challenges for the field of dispersal ecology: the proportion of all diaspores produced that are carried over long distances by ungulates, and the relative importance of ungulates on the whole as the main dispersal agent for plants. Furthermore, the fact that numerous plants dispersed by fur-epizoochory do not feature any specific adaptations is intriguing. We discuss unsolved methodological challenges and stress research perspectives related to ungulate-mediated dispersal: for example, taking animal behavior and cognition into account and studying how ungulates contribute to the spread of invasive exotic plants and altitudinal plant dispersal.

**Keywords: epizoochory, endozoochory, long distance dispersal (LDD), functional diversity index, plant-animal interactions**

**Edited by:**
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**Specialty section:**
*This article was submitted to*
*Biogeography and Macroecology,*
*a section of the journal*
*Frontiers in Ecology and Evolution*

**Received:** *28 September 2018*
**Accepted:** *01 February 2019*
**Published:** *07 March 2019*

**Citation:**
*Baltzinger C, Karimi S and Shukla U*
*(2019) Plants on the Move:*
*Hitch-Hiking With Ungulates*
*Distributes Diaspores Across*
*Landscapes. Front. Ecol. Evol. 7:38.*
*doi: 10.3389/fevo.2019.00038*

## INTRODUCTION

Contrasting with the defaunation process currently impacting large mammals in tropical forest ecosystems (Galetti and Dirzo, 2013), ungulate populations in temperate forests are rapidly increasing, sometimes locally reaching higher populations than their historic records. This phenomenon concerns overabundant native deer populations (Côté et al., 2004) and introduced species thriving in different parts of the world (e.g., Canada, Japan, Australia, New Zealand).

They are mostly large mammals, their body mass ranges from kilograms to hundreds of kilograms, that inhabit open, semi-open and closed habitats (Loison et al., 1999). These animals explore large home ranges and cover long daily distances across composite landscapes, along more or less sinuous paths (e.g., the gradient in path tortuosity from roe deer–*Capreolus capreolus*, to red deer–*Cervus elaphus*, to wild pig–*Sus scrofa*, in Pellerin et al., 2016). Since they are mainly herbivores, they process and transport plant materials when roaming their home ranges, and are thus involved in ecosystem engineering by chemical transport (Wilby et al., 2001) through nutrient fluxes and contribute to soil chemical content heterogeneity (e.g., by feeding in nutrient-rich areas and randomly releasing feces in forest-poor areas, Abbas et al., 2012; Murray et al., 2013). Albert et al. (2015a) demonstrated that temperate ungulates dispersed 44% of the regional pool of plants. Ungulate-mediated plant dispersal mainly occurs in the summer-fall seasons when most diaspores shed (Malo and Suárez, 1995). Dispersal also depends on ungulate feeding regime and other specific traits (Albert et al., 2015b).

Endozoochory, including frugivory, is the most widely studied ungulate-mediated plant dispersal mechanism (e.g., hoof- and fur-epizoochory concerns <12% of the samples, see Table 3 in Albert et al., 2015a). However, Albert et al. (2015a) also stressed that, even though they are comparatively understudied, hoof- and fur-epizoochory were more selective processes than endozoochory and ungulates are involved in many different dispersal processes, both internal and external. Ungulate regurgitation, for instance, has been studied even less than hoof- and fur-epizoochory. This research gap seems logical since seed dispersal for fleshy-fruited plants occurs mainly in the tropics where birds, bats, primates and rodents are the main dispersal vectors (Jordano, 2000). Recently, however, more emphasis has been placed on other taxa, for instance reptiles (Sobral-Souza et al., 2017). Ungulates are important in plant dispersal for different reasons. First, they may play a role in long-distance plant dispersal (with maximal endozoochorous dispersal distances varying from 2.0 km for roe deer to 3.5 km for red deer, Pellerin et al., 2016). Second, they are present worldwide (except for Antarctica), either as native or introduced species and they have great taxonomic diversity (240 and 17 species within the Artiodactyla and Perissodactyla orders, respectively, Wilson and Reeder, 2005). Their communities are diverse (e.g., 5 species in remnant old-growth forests in Poland–Jaroszewicz et al., 2013; 10 species in Renosterveld, South Africa–Shiponeni and Milton, 2006; see **Table 3**), and they occur in a variety of ecosystems (e.g., forests, tree savannahs, grasslands). Finally, they have high functional diversity in plant-dispersal related traits (Albert et al., 2015b), e.g., various feeding regimes (Hofmann, 1989) and a wide range of body sizes (Clauss et al., 2007).

Mc Alpine et al. (2016) called for the integration of plant- and animal- based approaches for biodiversity conservation actions and restoration efforts. Emphasis should be on key biotic interactions, for example how both plants and animals are involved in pollination and plant dispersal. Recent studies in various ecosystems suggest that many ungulates–native (e.g., white-tailed deer–*Odocoileus virginianus*, Connecticut, Williams and Ward, 2006), domestic (e.g., cattle–*Bos taurus*, California, Chuong et al., 2016) and introduced (e.g., Philippine deer–*Rusa marianna* and wild pig, Mariana Islands, Gawel et al., 2018) are involved in the spread of exotic plants, questioning their potential to help restore degraded habitat. Human-modified ecosystems and plant communities can also be affected by the presence of these large ungulates.

In this review, we aim to shed new light on the specific role of ungulates in long-distance plant dispersal, and to better understand how they have contributed to past plant distribution patterns, how they shape present plant communities and how they might help future plant communities cope with rapid and drastic human-induced changes (e.g., land use modifications, biological invasions, global warming, habitat loss and fragmentation, Mc Conkey et al., 2012).

We have specifically addressed the following four objectives. In the first part, we sum-up the primary and secondary dispersal mechanisms through which ungulates distribute diaspores across landscapes and describe how each of these processes influences the fate of the diaspores carried by the vectors. We highlight diplochorous sequences where ungulates are involved in at least one of the dispersal steps. In the second part, we propose to adapt the seminal conceptual framework of seed dispersal effectiveness for frugivory and endozoochory proposed by Schupp (1993) and revisited by Schupp et al. (2010) to the two other ungulate-mediated primary processes: regurgitation and fur-epizoochory. In the third part, we discuss the functional diversity of ungulates, how it might affect the fate of the seeds dispersed, and how this should be used to build further research. In the fourth part, we performed a systematic literature review to assess the overlap and complementarity of sympatric ungulates in plant dispersal first and then of different ungulate-mediated plant dispersal mechanisms. Finally, we discuss unsolved methodological challenges, potential ungulate-mediated habitat restoration options and suggest research perspectives.

## DIVERSITY OF UNGULATE-MEDIATED DISPERSAL MECHANISMS

No review to date has systematically addressed all the dispersal mechanisms through which ungulates convey diaspores across the landscapes. These mechanisms comprise both internal and external dispersal, primary and secondary dispersal events (i.e., diplochory, Vander Wall and Longland, 2004). They involve either biotic vectors alone (ungulates, ungulates and coprophagous beetles–D'hondt et al., 2008), or more complex systems involving primates or birds, ungulates and coprophagous beetles (Newton, 1989) or both abiotic vectors (wind, water, gravity) and ungulates. These different mechanisms move diaspores from the parent plant to different releasing locations. Some primates or birds feeding on fruits in the tree canopy can make them fall to the ground where they become accessible to forest-dwelling ungulates. These interactions were reviewed by Newton (1989) and have more recently been assessed for the langur-chital association in India (Ramesh et al., 2012).

Internal mechanisms (left side of **Figure 1**) concern consumed diaspores, which must withstand digestion (mechanical, thermal and chemical treatments). They include two specific processes: regurgitation, or partial endozoochory (where diaspores are ingested and regurgitated, Prasad et al., 2006), and full endozoochory (where diaspores are ingested and defecated). External mechanisms (right shaded side of **Figure 1**), where diaspores are carried on the outside of the vectors on various body parts, include primary fur-epizoochory (direct adhesion to fur essentially) and secondary epizoochorous processes: transfer through contact with a conspecific (Liehrmann et al., 2018) and diaspore-laden mud adhering to hooves (Schulze et al., 2014) or the body (Heinken and Raudnitschka, 2002). We have used a model ungulate to depict primary and secondary dispersal mechanisms of diaspores from a parent plant present in its home range (**Figure 1**).

## Regurgitation or Partial Endozoochory

First, let us look at one of the most understudied primary internal processes, regurgitation or partial endozoochory (described by scenario In1a in **Figure 1**). Here, fruit is ingested, then the endocarp is regurgitated after a lapse of time in the rumen. Regurgitation has been documented all around the world: e.g., in India for the different fruits consumed by the chital (*Axis axis*, Prasad et al., 2006), in Mexico for the white-tailed deer (Mandujano et al., 1994), in western Africa for the duikers (*Cephalophus sp.*, Feer, 1995), in southern Morocco for goats (*Capra aegagrus hircus*, Delibes et al., 2017), and more recently, in Spain for red deer (Castañeda et al., 2017). Some fleshy-fruited plants known to be consumed by European roe deer (Cornelis et al., 1999; Cransac et al., 2001) for example, dogwood (*Cornus sanguinea*) and ivy (*Hedera helix*) share similar characteristics (fruit, endocarp and seed size) with the plants mentioned in these studies. Dogwood and ivy do not germinate from roe deer dung samples (Heinken et al., 2001; Picard et al., 2016); we therefore suppose that the endocarps are regurgitated and not defecated. Clean regurgitated endocarps were found at specific and predictable resting/ruminating sites (i.e., directed dispersal, Wenny, 2001) whereas defecated seeds were more or less randomly deposited within the home range.

## Endozoochory and Secondary Dispersal by Dung Beetles

Now let us look at the most studied primary internal process: endozoochory (Picard et al., 2016, described by scenario In1b in **Figure 1**). Here diaspores are consumed, undergo complete gut passage and are defecated. Releasing sites are much less predictable than for regurgitation as ungulates can defecate away from their resting/ruminating site, while walking or feeding. Feces and their diaspore content can then be mobilized by other biotic vectors in a secondary step as depicted with coprophagous beetles (scenario Bi2, **Figure 1**). Depending on their functional group (Milotić et al., 2018, 2019), dung beetles move diaspores horizontally (small and large rollers), bury them more or less deeply (small and large tunnellers and rollers, D'hondt et al., 2008) or leave them roughly in the same place (dwellers). This case is known as diplochory, or secondary dispersal.

## Fur-Epizoochory Including Transfer-Epizoochory

The right side of **Figure 1** represents external processes, which have been much less studied than endozoochory (Albert et al., 2015a). One primary external process is fur-epizoochory (described by scenario Ex1, **Figure 1**). Here diaspores become attached to the fur of passing ungulates. Diaspores carried in the fur of ungulates generally present a high turn-over–most of them will fall off during the first few hours (Bullock et al., 2011) - though attachment time does depend on the characteristics of the fur (e.g., hair curliness, hair length, Liehrmann et al., 2018). Diaspores can drop accidentally or the animals can detach them during specific grooming sessions with teeth, by scratching or by rubbing against tree trunks (Heinken et al., 2006). Allo-grooming events or games between conspecifics in social ungulates can also lead to secondary external dispersal (described by scenario Ex2a, **Figure 1**). Liehrmann et al. (2018) have recently documented this mechanism, called transfer-epizoochory for dwarf goats, Poitou donkeys and red deer hinds. Diaspore transfers might occur more frequently during the reproduction period and while rearing young. We also suppose that transferred diaspores move from the home range of the first vector to the neighboring home range of its conspecific (**Figure 1**).

## Hoof-Epizoochory

When diaspores are not dispersed by ungulates and other biotic or abiotic vectors, they simply fall to the ground when the parent plant withers, this is called barochory (described by scenario Ab1, **Figure 1**). These diaspores therefore have a maximal distance equivalent to the diaspore releasing height. All the diaspores released on the ground within the ungulate's home range, whatever the dispersal process, constitute the soil surface pool (**Box 1**). These diaspores may either germinate and take root, enter the soil seed bank or be dispersed again by ungulates through hoof-epizoochory (described by scenario Ex2b, **Figure 1**), as often occurs on loose soils when diaspore-laden mud sticks to the hooves (roe deer, red deer and wild pig in Picard and Baltzinger, 2012; European bison–*Bison bonasus* in Schulze et al., 2014) or to other body parts. Secondary epizoochory may also happen when ungulates like wild pig wallow for thermal comfort or to get rid of ectoparasites (Heinken and Raudnitschka, 2002). These diaspores can drop off further along trails (e.g., white-tailed deer in Lefcort and Pettoello, 2012; and horse, *Equus caballus* in Wells and Lauenroth, 2007) where germinating conditions may be more

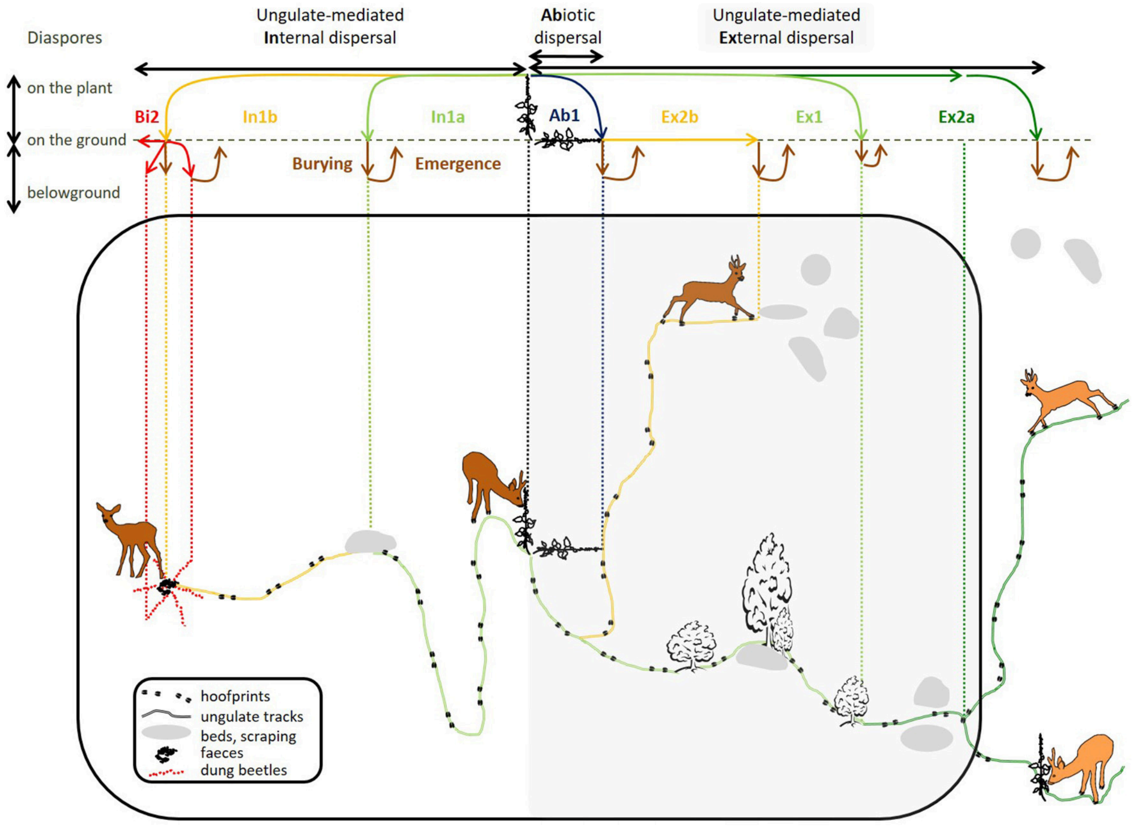

**FIGURE 1 |** General spatial representation of primary internal ungulate-mediated plant dispersal mechanisms (indicated by 1: **In1a** regurgitation, **In1b** endozoochory) and primary (**Ex1** fur-epizoochory) and secondary external ungulate-mediated plant dispersal mechanisms (indicated by 2: **Ex2a** transfer-epizoochory, **Ex2b** hoof-epizoochory) and the associated horizontal and vertical trajectories of the carried diaspores. To the left side of the parent plant, the diagram describes internal mechanisms; the shaded part to the right represents external mechanisms. **Ab**iotic dispersal by gravity (**Ab1** barochory) can be considered as a first step of dispersal. And secondary dispersal by other **bi**otic vectors like coprophagous beetles (**Bi2**) is considered as a second step following endozoochory (**In1b**). The top part of the diagram shows the fate of a diaspore for each dispersal mechanism from the parent plant to its final destination (moving from the diaspore regional pool to the transferred pool, the soil surface pool and finally, the soil seed bank, **Box 1**). The large black rectangular shape delimits the home range of the vector.

favorable, for example in microhabitats like hoof prints of ungulates (**Figure 1**) where rainwater can stand longer. Diaspores can also drop off nearby rubbing trees (Welander, 2000).

## Spatial Trajectory of Conveyed Diaspores

The top part of **Figure 1** shows the fate, spatial trajectory and associated successive movements (from release by the parent plant to deposition on the ground) of the conveyed diaspores for each dispersal mechanism considered (the color code corresponds to the associated internal or external mechanism). Some of the diaspores will contribute to the build-up of the soil seed bank (Jaroszewicz, 2013) through gradual burying (Burying, **Figure 1**) or thanks to the tunneling activities of paracoprid and telecoprid dung-beetles and can further reemerge (Emergence, **Figure 1**) on the soil surface through physical changes in the soil, with or without mediation by ungulates (Jones et al., 1994) to find propitious germination conditions (e.g., light and humidity).

## SEED DISPERSAL EFFECTIVENESS REVISITED FOR UNGULATE-MEDIATED PROCESSES INCLUDING REGURGITATION AND FUR-EPIZOOCHORY

Schupp (1993) defined a conceptual framework to study seed dispersal effectiveness (SDE), defined as the result of a quantitative component, the **emigration phase**, and a qualitative component, the **transfer** and **immigration phases** (**Table 1**). The plant dispersal process is therefore composed of three distinct phases, namely emigration, transfer, and immigration (**Table 1**). The **emigration phase** for ungulate-mediated dispersal relies on the spatial and temporal availability of diaspores in the vegetation physically accessible within the home range of a given dispersal agent. This diaspore load depends on the interaction between the traits of the plant and of its diaspores and the traits of the dispersal agent (Albert et al., 2015b). The **transfer phase** determines the trajectory covered by the diaspores transported by the dispersal agent during gut retention and

**Box 1** | Spatial and temporal diaspore availability within ungulate home ranges and populations.

The **regional plant pool** corresponds to all the plants in their adult reproductive stage accessible to the ungulate, within its home range or within the geographic area occupied by the population of ungulates. The **local plant pool** corresponds to the plants members of the plant community at the local scale.

At the diaspore stage (e.g., seed, fruit) and within an ungulate's home range, four pools of diaspores differ in their spatial and temporal availability: the **regional diaspore pool**, the **transferred diaspore pool**, the **soil surface diaspore pool,** and the **soil seed bank**.

- The **regional diaspore pool** defines all the diaspores available on the parent plants at diaspore releasing height during the seed shedding period. Some plants maintain a dead erect stem and therefore lengthen temporal availability for potential dispersal agents.
- The **transferred diaspore pool** defines diaspores when they have left the mother plant via biotic or abiotic dispersal. The duration and associated distance of the ungulate-mediated transfer phase depend on internal or external retention time (**Figure 2**).
- The **soil surface** diaspore **pool** combines diaspores released on the ground by biotic or abiotic vectors with diaspores falling on the ground when the plant withers. In that case, maximal dispersal distance equals diaspore releasing height. Released diaspores can germinate, be moved by dung beetles or between the hooves of ungulates during secondary dispersal, or build-up the **soil seed bank**.
- The **soil seed bank** includes diaspores with varying longevity, from transient (<1 year), short-term (<5 years) to longer term (over 5 years, as for soft rush, *Juncus effusus*). These diaspores can encounter favorable germination conditions following soil disturbance and/or improved light conditions at different time scales.

regurgitation time or the time elapsed between the attachment of the diaspore to the dispersal agent and its detachment. The **immigration phase** concerns the germination of the released diaspores, their establishment as seedlings, their growth to adult plants able to reproduce. Thus ungulate-mediated seed dispersal is potentially important for plant demography from one generation to the next (Wang and Smith, 2002; Vellend et al., 2006) and plays a role in metapopulation dynamics (e.g., Figure 6 in Jabot et al., 2008, and the link between migration and the proportion of mammal-dispersed trees). In an updated version of this conceptual framework, Schupp et al. (2010) suggested that their framework should not be restricted to the sole study of frugivory and endozoochory, but that it could be adapted to other dispersal processes like fur-epizoochory. In **Table 1**, we follow this suggestion by comparing the three primary ungulate-mediated dispersal processes: endozoochory and regurgitation (internal) and fur-epizoochory (external). We will sequentially treat the different components and sub-components involved in SDE and highlight the ungulate characteristics that affect each dispersal phase.

**The quantitative component** (emigration phase) corresponds to the product of the number of visits to the plant and the number of diaspores loaded per visit (**Table 1**).

## Number of Visits

Whatever the dispersal process considered, the number of visits to the parent plant will depend on three parameters: the local ungulate abundance, a degree of selectivity and the frequency and length of contacts with the parent plant. For internal dispersal processes (endozoochory and regurgitation), feeding selectivity will determine where, when and which plant will be consumed (Boulanger et al., 2009) and will depend on the feeding regime of the ungulate considered. For instance, Intermediate Mixed Feeders like the red deer (Latham et al., 1999; Gebert and Verheyden-Tixier, 2001) are less selective and consume a higher diversity of plants than sympatric Concentrate Selectors like roe deer (Cornelis et al., 1999; Cransac et al., 2001). The frequency and the length of the feeding bouts will also determine the occurrence of contacts with the selected feeding items. The number of active bouts (including feeding bouts) varies across the year and is generally higher during summer (e.g., 12 for red deer–Pépin et al., 2006; 16 for moose, *Alces alces* and 26 for roe deer in Cederlund, 1989). By comparison with external processes (fur-epizoochory), daily home range fidelity (Richard et al., 2014), how regularly ungulates use the same paths (Wells and Lauenroth, 2007; Torn et al., 2010; Lefcort and Pettoello, 2012) or how frequently they use specific parts (e.g., core areas in Le Corre et al., 2009) of their home range should determine the number of visits. The frequency of the active bouts and their length during each visit will lead to the passive attachment of some diaspores to different body parts of the ungulate (Fischer et al., 1996), more specifically to the head and/or the breast of the animal while feeding on specific plants ("foliage is the fruit" hypothesis extended to fur-epizoochory–Janzen, 1984; Couvreur et al., 2005).

## Number of Diaspores Loaded Per Visit

The number of diaspores consumed during each visit will depend on the feeding regime. More diaspores are consumed by generalist herbivores like Grass and Roughage Eaters (e.g., European bison, cattle) or Intermediate Mixed Feeders (e.g., red deer, chamois–*Rupicapra rupicapra*) than by more selective herbivores like Concentrate Selectors (e.g., roe deer, moose in Hofmann, 1989). It will also depend on the body mass, as heavier species and heavier individuals will eat more plant material than lighter ones to meet energy requirements (e.g., the difference between two concentrate selectors: roe deer, <30 kg vs. moose, >300 kg, Loison et al., 1999). Finally, diaspore availability on the plant and its accessibility to the ungulates also have an influence (**Box 1**). Albert et al. (2015b) showed that Concentrate Selectors consumed diaspores at a specific diaspore releasing height. Some of the diaspores detached from the parent plant might be lost before ingestion, especially when the fruits and diaspores are not the main focus of the feeding bouts and are accidentally consumed (Janzen, 1984). For fur-epizoochory, the number of diaspores loaded during each visit will depend on the number of diaspores attached per contact, resulting from the interaction between diaspore releasing height and ungulate body height (Fischer et al., 1996; Albert et al., 2015b). The number of diaspores attached to the ungulate body also depends on fur characteristics like hair length and curliness (Albert et al., 2015b)

**TABLE 1 |** Components of ungulate-mediated seed dispersal effectiveness comparing three primary processes: endozoochory, regurgitation and fur-epizoochory [modified from Table 1 in (Schupp, 1993), and Figure 2 in Schupp et al., 2010].

| Plant dispersal | Component | Sub-component | Endozoochory | Regurgitation | Fur-epizoochory |
|---|---|---|---|---|---|
| Emigration phase | Quantitative<br>How many diaspores are loaded by the vector? | Number of visits | - Local ungulate abundance<br>- Selective feeding (feeding regime)<br>- Frequency and length of feeding bouts | | - Local ungulate abundance<br>- Within home range fidelity (used trails, core areas)<br>- Frequency and length of active, including feeding, bouts |
| | | Number of diaspores loaded per visit | - Number of diaspores consumed per visit<br>- Loss before and during ingestion<br>- Physical accessibility<br>- Body mass | | - Number of diaspores hung per contact<br>- Loss by grooming<br>- Height of contact<br>- Body surface, body part and fur characteristics |
| Transfer phase | Qualitative<br>What is the chance for a loaded diaspore to become an adult plant? | Treatment quality by the vector | - Mechanical (mastication), thermal and chemical (digestive strategy)<br>- Gut passage time (**Figure 2**) | - Mechanical (mastication), thermal and chemical (rumination)<br>- Regurgitation time | - Mechanical (rubbing), thermal (body temperature buffer) and climatic (humidity)<br>- External retention time (**Figure 2**) |
| | | Deposit quality of the released diaspores | - Random or directed defecation<br>- Fecal matrix<br>- Diverse and numerous diaspores | - Regurgitation at ruminating/resting site<br>- No matrix<br>- Assumed few diaspores at a time | - Random or directed to rubbing structures (trees, rocks or the ground)<br>- No matrix<br>- Weakly diverse and isolated diaspores |
| Immigration phase | | Quality of the deposition site for germination and growth | - Environmental filter (abiotic conditions)<br>- Strong biotic interactions with predators, decomposers, herbivores or among plants | - Environmental filter (abiotic conditions)<br>- Weak biotic interactions with predators, herbivores or among plants | |

and on which body surface area is exposed (Bohême, 2012). Wild pig dispersed more diaspores of more plants than did red deer, and red deer more than did roe deer (see Figure 2 in Picard and Baltzinger, 2012). Bohême (2012) assessed the body surface exposed per individual for wild pig, red deer and roe deer and no longer found any significant difference in seed load per surface unit between red and roe deer. Liehrmann et al. (2018) confirmed the interspecific effects of fur characteristics (by comparing red deer, dwarf goat and Poitou donkey, *Equus asinus*) and extended that pattern to different body parts (head, flanks or rump) of a given individual. They also showed that some diaspores may be lost during auto-grooming events.

**The qualitative component** (transfer and immigration phases) combines the quality of the treatment exerted by the vector, the deposit quality of the released diaspores and finally the quality of the deposition site, i.e., both abiotic conditions and biotic interactions encountered at the release site (**Table 1**).

## Treatment Quality by the Vector

Diaspores consumed by an ungulate undergo different treatments of variable duration: physical (mastication and rumination), and thermal and chemical (digestive process). Mastication, i.e., the chewing process, may lead to the destruction of the diaspores consumed; especially concerning large seeds (e.g., acorns). Indeed, most of the studies on ungulate endozoochory highlight preferential dispersal of small-sized (Janzen, 1984; Heinken et al., 2002; Pakeman et al., 2002; Picard et al., 2016) and rounded seeds (Mouissie et al., 2005a), which germinate in higher proportions in ungulate dung, though Bruun and Poschlod (2006) showed that this pattern might be linked to the greater overall availability of small seeds (i.e., reproductive trade-off). The digestive process itself adds thermal and chemical treatments in the gut (Milotić and Hoffmann, 2016b), which differentially affect the seed coat, its permeability and subsequent ability to germinate once released in the fecal matrix. Picard et al. (2015) showed, for instance that bramble (*Rubus fruticosus*) seeds germinated when consumed by wild pig whereas unconsumed control seeds and those consumed by ruminant deer species did not. Gut passage time is a function of ungulate body mass (Illius and Gordon, 1992) for both ruminants and non-ruminants: the heavier the animals the longer the transit (Clauss et al., 2007; and e.g., the shift between the roe deer and wild pig in Figure 2, Picard et al., 2015). Digestive systems opposing ruminants to non-ruminants will also have differing effects on the fate of the seeds. For ruminants, larger seeds will be processed longer and smaller ones will pass the gut more rapidly (Picard et al., 2015). Above a given size, endocarps from fleshy fruits will be regurgitated (Sridhara et al., 2016). Empirical experimental

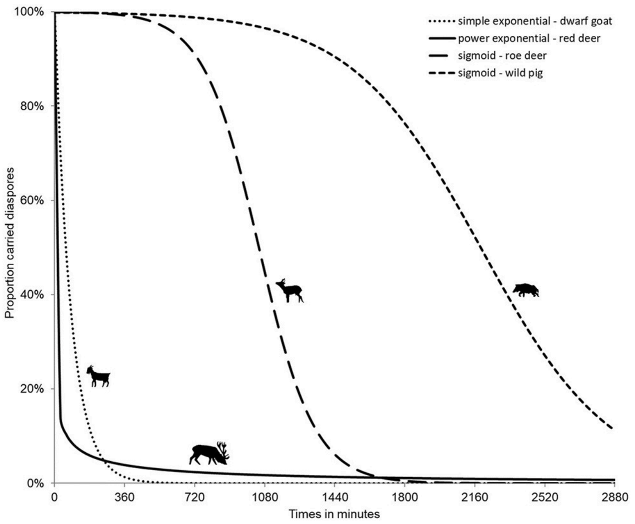

**FIGURE 2 |** Internal (endozoochory, right curves for roe deer, *Capreolus capreolus* and wild pig, *Sus scrofa*) and external (fur-epizoochory, left curves for dwarf goat, *Capra aegagrus hircus* and red deer, *Cervus elaphus*) diaspore retention time (adapted from Picard et al., 2015; Liehrmann et al., 2018).

data on gut passage time vary from 1 to 3 days for ungulates (e.g., Table 2 in Pakeman, 2001) for temperate forest ungulates ranging in body mass between <30 kg (roe deer) and more than 300 kg (moose, Loison et al., 1999), though Illius and Gordon's equations have been questioned (Clauss et al., 2007). Internal retention time requires a minimal time for the first diaspores to be released, and non-ruminant species release different-sized diaspores simultaneously (Picard et al., 2015). Data on regurgitation times are scarce: in India for chital, they range from 7 to 27 h (Prasad et al., 2006) and for red deer in Spain from 1 to 4 days after ingestion (Castañeda et al., 2017).

The treatment effect for externally attached diaspores is much weaker. This effect could be linked to rubbing (against trees or through grooming) or to weather (body vs. air temperature and humidity–effect of precipitation). Whereas in internal processes, all diaspores are released after a specific gut passage time, in external processes like fur-epizoochory, most of the diaspores drop off very quickly, though but a few can be retained much longer (**Figure 2**, for red deer), thus contributing to very long-distance dispersal (Bullock et al., 2011; Liehrmann et al., 2018).

## Deposit Quality of the Released Diaspores

As ungulates defecate either after leaving resting or ruminating sites, during walking, or feeding events, we can consider defecation sites to be randomly distributed in comparison to regurgitation sites, where endocarps are released only at ruminating sites (Prasad et al., 2006). However, Picard et al. (2016) suggest that internally-dispersed plants are typically selected in open feeding habitats and are then released when ungulates rest under forest cover (Abbas et al., 2012); this could be considered a non-random directional movement. Diaspores can detach accidentally and randomly from the fur, however it could also be considered as a non-random process of dispersal as evidenced by soil seed bank of diaspores near rubbing trees, where more viable diaspores are found than nearby non-rubbed trees (Welander, 2000; Heinken et al., 2006). The deposit quality of the released diaspores is linked to the presence of a fecal matrix. In experiments done with 15 grassland plants, Milotić and Hoffmann (2016c) showed that sowing seeds in ungulate dung reduced germination rate and lengthened germination time; this pattern was even stronger for cattle compared to horse dung. These findings highlight the significance of dung material characteristics (ruminant vs. non-ruminant) in deposit quality. The diversity of the feeding regime will determine the diversity of the seeds present in the dung (higher for Intermediate Mixed Feeder than for Concentrate Selector, e.g., Picard et al., 2016) and the body mass will determine the abundance of seeds, as heavier ungulates or individuals will ingest more plant material (red deer vs. roe deer, Picard et al., 2016). We assume that regurgitated endocarps might be less diverse as they generally concern one specific nutrient-rich resource at a time (Prasad et al., 2006). As externally conveyed diaspores are not released in a fecal matrix, their chances to be released as isolated and undetectable diaspores in the field are high (e.g., through experimental assessment in Liehrmann et al., 2018).

## Quality of the Deposition Site for Germination and Growth

The quality of the deposition site will, of course, depend on the local abiotic conditions (i.e., environmental filter, Kraft et al., 2015) including light, temperature and humidity. Ungulates leave hoofprints while walking on loose soils, and they also create specific microhabitats while scraping (e.g., roe deer in Johansson, 2000) or digging (e.g., wild pig in Welander, 2000) the ground. Acting as ecosystem engineers (Jones et al., 1994), they modulate the resources available to other taxa, including diaspores. The quality of the deposition site will also depend on biotic factors. The presence of the fecal matrix, which differentiates full endozoochory from both regurgitation and fur-epizoochory, will favor biotic interactions with different functional and taxonomic groups. Decomposers (e.g., soil macroinvertebrates, different types of dung beetles, Milotić et al., 2018, 2019) will move diaspores toward specific microhabitats. D'hondt et al. (2008) showed that dung beetles had a negative effect on short-term seedling establishment, probably due to the deep burial of diaspores by large tunnellers. Fungi frequently develop on feces and may affect the tegument of the dispersed seeds. Small rodents, attracted by the clumped seeds in the feces, may also predate on the seeds dispersed. Other plants may benefit from the nutrients released and compete for resources with establishing seedlings. Milotić and Hoffmann (2016a) showed that the effect of the fecal matrix was beneficial for post-germination stages of the plant development. Large herbivores that feed selectively on nitrophilous plants (Janzen, 1984; Albert et al., 2015a) may be attracted by nutrient-rich vegetation patches, and also interact at the deposition site with establishing seedlings.

**TABLE 2 |** Effects of ungulate functional diversity gradients on the different phases of internal (endo: endozoochory and regurgitation) and external (epi: fur-epizoochory) plant dispersal (CS: Concentrate Selector, IMF: Intermediate Mixed Feeder, GRE: Grass and Roughage Eater and OM: Omnivore).

| Socio-spatial scale | Ungulate characteristics | Emigration | Transfer | Immigration |
|---|---|---|---|---|
| Individual level (physiology and morphology) | Body mass | endo [a,b] | endo [c] | |
| | Feeding regime (CS, IMF, GRE, and OM) | endo [b]/epi[d] | | |
| | Digestive strategy (ruminant or not) | | endo[c,e] | |
| | Body size (shoulder height) | endo[f]/epi[f,g] | | |
| | Body surface area | epi[h] | epi[h] | |
| | Fur characteristics (hair length and curliness, fur thickness) | epi[i,j] | epi[i,j] | |
| | Auto-grooming (wallowing, rubbing against structures) | epi[j] | epi[j,k,l] | epi[k,l] |
| Population level | Sociality/hierarchy (from pairs to herds) | epi[m]/endo[m] | epi[j] | |
| | Allo-grooming | epi[j] | epi[j] | |
| Landscape level | Habitat use (home range fidelity, activity rhythm) | endo[n,o,p]/epi[n,o,p] | endo[n,o,p]/epi[n,o,p] | endo[n,o,p]/epi[n,o,p] |
| | Movement (home range size, daily distance, tortuosity) | | endo[q,r]/epi[q,r] | |

*[a] Illius and Gordon, 1992; [b] Picard et al., 2016; [c] Picard et al., 2015; [d] Couvreur et al., 2005; [e] Schwarm et al., 2008; [f] Albert et al., 2015b; [g] Fischer et al., 1996; [h] Bohême, 2012; [i] Picard and Baltzinger, 2012; [j] Liehrmann et al., 2018; [k] Heinken et al., 2006; [l] Welander, 2000; [m] Sarasa et al., 2009; [n] Richard et al., 2014; [°] Keuling et al., 2008; [p] Le Corre et al., 2009; [q] Pakeman, 2001; [r] Pellerin et al., 2016.*

*Upper letters refer to citations listed below the table.*

## THE FUNCTIONAL DIVERSITY OF UNGULATES AND ASSOCIATED EFFECTS ON THE FATE OF DIASPORES

The ungulates are numerous and taxonomically and functionally diverse (Groves and Grubb, 2011). This diversity may intervene at different steps in ungulate-mediated diaspore dispersal processes, from the scale of the individual vector to groups of individuals, populations and communities (**Table 2**). At the scale of the individual, mostly physiological and morphological traits will be concerned, and are depicted in the two following sub-sections. The third sub-section reports to higher scales of organization (i.e, from pairs to groups of individuals).

### Body Mass, Feeding Regime and Digestive Strategy

Concerning endozoochory, large body mass increases the amount of diaspores consumed (Picard et al., 2016), and once consumed, body mass will affect gut retention time (Picard et al., 2015 but see Steuer et al., 2011 for a comprehensive review). Furthermore, gut retention time varies with diaspore size (Clauss et al., 2009; Picard et al., 2015). Digestive strategy will also affect endozoochory, with ruminants sorting food items according to their size (Schwarm et al., 2008). Picard et al. (2015) showed that smaller rounded diaspores were retained for shorter times in the gut of red and roe deer than were larger diaspores, whereas in wild pig all types of diaspores were generally released at the same time (see also differences between banteng, *Bos javanicus* and pygmy hippopotamus, *Hexaprotodon liberiensis* in Schwarm et al., 2008). Feeding regime will determine the growth form, diversity and amount of the plants consumed (European bison, a Grass and Roughage Eater, Kowalczyk et al., 2011; red deer, an Intermediate Mixed Feeder, Gebert and Verheyden-Tixier, 2001; roe deer, a Concentrate Selectors, Cornelis et al., 1999 and wild pig, an Omnivore, Schley and Roper, 2003), but also which part of the plant is consumed, with browsers being much more selective than grazers. Feeding regime will affect the emigration phase for both endo- and fur-epizoochory, because by feeding and spending time in open areas, herbivores will enhance chances for diaspores to attach to different parts of their body (e.g., head, belly, flanks).

### Body Size, Body Surface, Fur Characteristics and Grooming

Body size, with respect to plant-animal interactions, relates to the height at which vegetation is encountered in the area explored by the ungulates. This affects both endo- and fur-epizoochory as it determines which plants are accessible, or reachable, for feeding (Fischer et al., 1996; Albert et al., 2015b). Albert et al. (2015b) showed that body size approximated by shoulder height interacted with plant diaspore releasing height for both endozoochory (i.e., which vegetation layers are consumed) and fur-epizoochory (i.e., which vegetation can touch the animal's body). Eventhough, ungulates may stand on their hind legs or take advantage of snow cover to access vegetation above their head height, shoulder height remain a good predictor that can be used for comparative approaches. Body surface area is another important characteristic with regard to external dispersal. Bohême (2012) revealed that the abundance of diaspores on different individuals with similar fur characteristics (red deer and roe deer) was directly related to the total surface area made accessible to the plants to attach. Liehrmann et al. (2018) further showed that hair length and curliness were also factors of diaspore attachment and detachment. Ungulates such as the Poitou donkey (undercoat and long hairs) or the wild pig stock the diaspores in their fur, while others like the dwarf goat (short and wavy hairs) or the red and roe deer showed a rapid turnover of diaspores. Fur thickness can affect diaspore attachment and detachment and thus directly determine the time the diaspores stay attached to the different parts of the animal's body. Finally, single individuals will groom themselves with their teeth or legs (Liehrmann et al., 2018), rub against trees or wallow to get rid of parasites (Welander, 2000; Heinken et al., 2006). These grooming events will affect the loss, the gain and also the transfer phase of different diaspores.

### Sociality, Habitat Use and Movement Patterns

At the population scale, or at least for individuals living in pairs, interactions among individuals (e.g., playing games, mother-young relationships, resting in groups, allo-grooming) can lead to both diaspore detachments and transfers from one individual to another (Liehrmann et al., 2018). These interactions will mainly affect external seed dispersal. Sarasa et al. (2009) showed that the access by Iberian ibex to feeding stations was dependent on the sex and age of the individuals, which conditioned the access to the vegetation but also the infestation by pseudoectoparasites, and potentially the attachment of diaspores adapted to fur-epizoochory. At larger scales, the way ungulates use the different habitats that compose their home range will affect the fate of seed dispersal (e.g., Keuling et al., 2008 for the wild pig; Le Corre et al., 2009 for the roe deer). Picard et al. (2016) suggested that feeding habitat preferences filtered out some of the potential diaspores which could be conveyed by ungulates. Directed dispersal might occur if animals regularly return to the same sites and use the same trails between feeding and resting/ruminating sites. Home range fidelity at different temporal scales (e.g., day, season, year, Richard et al., 2014) also means that ungulates might move diaspores to very predictable places associated to routine movements (Riotte-Lambert et al., 2017). The alternation of active and passive bouts of interaction with vegetation also determines when diaspores are attached and when they can be released. The extent (see definition in Pakeman, 2001) of the home range, which is closely related to animal body mass and energy requirements, constrains daily movements and determines how animals explore the space available. For instance, roe deer describe more tortuous trajectories than do red deer or even wild pig (Pellerin et al., 2016), leading to shorter dispersal distances for a given walked distance.

## OVERLAP AND COMPLEMENTARITY OF UNGULATE-MEDIATED DISPERSAL

In plant dispersal networks, diaspores produced by the parent plant can be dispersed through endozoochory by different co-occurring vectors. These networks have been established principally for frugivory and endozoochory (Dugger et al., 2018; Miguel et al., 2018). Fedriani and Delibes (2009) studied the role of different mammals (e.g., wild pig; red deer; badger, *Meles meles* and red fox, *Vulpes vulpes*) dispersing the Iberian pear (*Pyrus bourgaeana*). Jaroszewicz et al. (2013) showed that numerous plant species were dispersed by a guild of dispersal agents (**Table 3**). On the other hand, situations also occur where one specific ungulate vector disperses the same plant through different mechanisms, i.e., endozoochory, fur- and hoof-epizoochory. Birch (*Betula pendula*) was dispersed between the hooves and on the fur of wild pig, red deer and roe deer (Picard and Baltzinger, 2012). Both the characteristics of the dispersal vector and the considered mechanism will ultimately affect seed dispersal effectiveness (**Table 1**) and may generate complex and unpredictable dispersal kernels.

In this section, we look at plant species that can potentially germinate after long-distance ungulate-mediated dispersal; however, without considering how the transfer phase occurred. We emphasize the overlap and complementarity resulting from co-occurring ungulates, which disperse plants through either endozoochory or fur-epizoochory, and from a single ungulate on a specific site dispersing the same plants through both endo- and fur-epizoochory. Here, overlap and complementarity are understood in terms of plant species diversity dispersed between ungulates and between dispersal mechanisms, though other components of the SDE (**Table 1**) such as seed load and distances traveled are also relevant. We carried out a systematic literature review on internal and external ungulate-mediated dispersal processes across worldwide with the following search string in ISI Web of Science (July 9th, 2018).

*TS = (Ungulate* OR Artiodactyl* OR Perissodactyl* OR Ruminant* OR Antilocapridae OR Bovidae OR Camelidae OR Cervidae OR Equidae OR Giraffidae OR Hippopotamidae OR Moschidae OR Rhinocerotidae OR Suidae OR Tapiridae OR Tayassuidae OR Tragulidae OR [*Genus of all different ungulate species*]) AND TS = (Seed* OR endo*zoochor* OR ecto*zoochor* OR epi*zoochor* OR exo*zoochor* OR regurgitation OR frugivor* OR zoochor*) AND TS = (Plant* OR invasive* OR exotic* or introduced or non-native*).*

The list of all different ungulate genera was retrieved from www.ultimateungulate.com.

We then used basic functional traits (feeding regime and fur characteristics, Albert et al., 2015a,b) to check if we can predict how sympatric ungulates provide overlapping or complementary endozoochorous or epizoochorous dispersal services. We proceeded similarly to predict the overlap and complementarity between endozoochory and fur-epizoochory.

This search provided 22 studies (corresponding to 27 datasets) for endozoochory where at least two ungulate vectors were considered on the same site (i.e., some studies referred to multiple sites and different ungulate communities and were handled as distinct datasets, **Table 3**). For fur-epizoochory, we retrieved only six studies (corresponding to 7 datasets, **Table 4**), mainly in Europe. For both endo- and fur-epizoochory combined, we retrieved 17 datasets from 12 studies (i.e., studies including endo- and fur-epizoochory for two ungulate vectors were considered as distinct datasets, **Table 5**). All the retrieved studies and extracted data are included in **Tables 3–5**.

Most of the studies on ungulate-mediated dispersal retrieved from this search by ungulate community mentioned two or three co-occurring ungulates–studies with 2 ungulates: 11 and 5 for endozoochory and fur-epizoochory, respectively; with 3 ungulates: 12 and 2, respectively (**Tables 3**, **4**). Studies on ungulate endozoochory (**Table 3**) involving more than three ungulates were rare (e.g., Sigwela, 2004; Young, 2012; Jaroszewicz

**TABLE 3 |** Studies on ungulate endozoochory included in the overlap and complementarity of ungulate-mediated plant dispersal: ungulate sample size (given as weight in grams or as number of fecal samples), publication, location (site, country), total number of plant species dispersed, % of plant species dispersed by 2 vectors or more, and germination period (* indicates studies where diaspores were identified under a magnifying glass and not after seedling emergence).

| Ungulate vectors (sample size) | Publication | Site, country | Total number of plant species dispersed | % of plant species dispersed by 2 vectors (number of plant species) | % of plant species dispersed by >2 vectors (number of plant species) | Germination period (in months) |
|---|---|---|---|---|---|---|
| European bison (46), elk (35), red deer (80), roe deer (33), wild pig (90) | Jaroszewicz et al., 2013 | Białowieza Forest, Poland | 191 | 28.80% (55) | 25.13% (48) | 36 |
| Fallow deer (104), red deer (103), cattle (104) | Malo and Suárez, 1995 | Castillo de Vinuelas estate, Spain | 102 | 30.39% (31) | 38.24% (39) | 10 |
| Red/fallow deer (235), muntjac (296), roe deer (225) | Eycott et al., 2007 | Thetfort Forest, England, UK | 100 | 25.00% (25) | 21% (21) | 10 |
| Red deer (105), roe deer (48), wild pig (77) | Karimi et al., 2018 | Hyrcanian forest, Golestan NP, Iran | 86 | 19.77% (17) | 13.95% (12) | 15 |
| Red deer (77), wild pig (72), wild goat (70) | Karimi et al., 2018 | Scrub & woodland, Golestan NP, Iran | 81 | 29.63% (24) | 7.41% (6) | 15 |
| Cattle (20), elk (20), mule/white-tailed deer (10) | Bartuszevige and Endress, 2008 | Oregon, USA | 52 | 40.38% (21) | 7.69% (4) | 3 |
| Cattle (10), horse (10), sheep (10) | Mouissie et al., 2005b | Oosterwalde, Netherlands | 49 | 30.61% (15) | 36.73% (18) | 9 |
| Camel (6), cattle (1,143), goat (19), sheep (49), donkey (1), eland (200 g), gemsbok (100 g), giraffe (400 g), duiker (159), rhebuck (100 g), kudu (225), springbok (1), wildebeest (100 g) | Milton and Dean, 2001 | North & West provinces, South Africa | 48 | 27.08% (13) | 14.58% (7) | na |
| Red deer (60), roe deer (60), wild pig (60) | Picard et al., 2016 | Lorris, France | 46 | 21.74% (10) | 8.70% (4) | 12 |
| Zebra (na), eland (na), wildebeest (na) | Shiponeni and Milton, 2006 | EPNR, South Africa | 43 | 25.58% (11) | 20.93% (9) | 12 |
| Muntjac (173), roe deer (126), red/fallow deer (54) | Panter and Dolman, 2012 | England, UK | 41 | 12.20% (5) | 29.27% (12) | >2 |
| Chamois (61), red deer (106), wild pig (45), sheep (12,966) | Young, 2012 | Arthurs Pass NP, New Zealand | 34 | 11.76% (4) | 8.82% (3) | 36 |
| Nilgai (100), cattle (100), wild pig (100) | Middleton and Mason, 1992 | Keoladeo NP, Rajasthan, India | 25 | 16.00% (4) | 36.00% (9) | 12 |
| Rhinoceros (na), eland (na), kudu (na), bushbuck (na), goat (na), duiker (na), grysbok (na) | Sigwela, 2004 | Eastern Cape, South Africa | 23 | 26.09% (6) | 21.74% (5) | 0* |
| Gazelle (100), oryx (194), wild ass (84) | Polak et al., 2014 | Negev desert, Israel | 22 | 22.73% (5) | 4.55% (1) | 13 |
| Bushpig (119), bushbuck (103), grysbok (19) | Castley et al., 2001 | ACD, South Africa | 16 | 12.50% (2) | 6.25% (1) | 0* |
| Donkey (87), goat (88) | Treitler et al., 2017 | Sardinia, Italy | 113 | 43.36% (49) | – | 6 |
| Sheep (10), goat (6) | Benthien et al., 2016 | Luebeck, Germany | 97 | 17.53% (17) | – | 0* |
| Red deer (190), wild pig (87) | Lepková et al. (2018) | Bohemia, Czech Republic | 80 | 35.00% (28) | – | 12 |
| Cattle (4), konik horse (7) | Cosyns et al., 2005 | Westhoek North, Belgium | 67 | 79.10% (53) | – | 6 |
| Cattle (4), Shetland horse (19) | Cosyns et al., 2005 | Westhoek South, Belgium | 63 | 87.30% (55) | – | 6 |

*(Continued)*

**TABLE 3 |** Continued

| Ungulate vectors (sample size) | Publication | Site, country | Total number of plant species dispersed | % of plant species dispersed by 2 vectors (number of plant species) | % of plant species dispersed by >2 vectors (number of plant species) | Germination period (in months) |
|---|---|---|---|---|---|---|
| Fallow deer (3,728 g), wild pig (3,942 g) | Heinken et al., 2001 | Kraemer, Germany | 50 | 36.00% (18) | – | 6 |
| Cattle (14), sheep (15) | Mitlacher et al., 2002 | Öland, Sweden | 45 | 46.67% (21) | – | 4 |
| Urial (70), gazelle (70) | Karimi et al., 2018 | Steppe, Golestan NP, Iran | 32 | 31.25% (10) | – | 15 |
| Roe deer (152 g), wild pig (2,448 g) | Heinken et al., 2001 | Breiselang, Germany | 25 | 12.00% (3) | – | 6 |
| Roe deer (60), wild pig (60) | Picard et al., 2016 | Montargis, France | 15 | 20.00% (3) | – | 12 |
| Philippine deer (20), feral pig (31) | Gawel et al., 2018 | Guam, Mariana Islands, USA | 10 | 30% (3) | – | 15 |

*Na: unavailable information.*

**TABLE 4 |** Studies on ungulate fur-epizoochory included in the overlap and complementarity of ungulate-mediated plant dispersal: ungulate sample size (given as number of brushed individuals), publication, location (site, country), total number of plant species dispersed, % of plant species dispersed by 2 vectors or more.

| Ungulate vectors (sample size) | Publication | Site, country | Total number of plant species dispersed | % of plant species dispersed by 2 vectors (number of plant species) | % of plant species dispersed by >2 vectors (number of plant species) |
|---|---|---|---|---|---|
| Cattle (125), donkey (46), horse (30) | Couvreur et al., 2004 | Flanders, Belgium | 75 | 25.33% (19) | 14.67% (11) |
| Red deer (5), roe deer(16), wild pig (6) | Picard and Baltzinger, 2012 | Lorris, France | 18 | 5.56% (1) | 5.56% (1) |
| Goat (17), sheep (3) | Shmida and Ellner, 1983 | Har Gilo, Israel | 57 | 38.60% (22) | – |
| Roe deer (25), wild pig (9) | Heinken and Raudnitschka, 2002 | Breiselang, Germany | 55 | 40.00% (22) | – |
| Roe deer (41), wild pig (25) | Schmidt et al., 2004 | Herzogtum Lauenburg and Luechow-Dannenberg, Germany | 42 | 30.95% (13) | – |
| Sheep (10), goat (6) | Benthien et al., 2016 | Luebeck, Germany | 38 | 28.95% (11) | – |
| Roe deer (7), wild pig (11) | Picard and Baltzinger, 2012 | Montargis, France | 29 | 6.90% (2) | – |

et al., 2013), though one study mentioned up to 13 ungulates in South Africa (Milton and Dean, 2001). Second, most of the studies involved wild ungulates; and domestic ungulates were mentioned more rarely (Mitlacher et al., 2002; Cosyns et al., 2005; Mouissie et al., 2005b; Benthien et al., 2016; and Treitler et al., 2017, **Table 3**).

## Endozoochory by at Least Two Ungulates

In its "foliage is the fruit hypothesis," Janzen (1984) proposed different predictions. The first one states that *[...] herbaceous plant vegetation is edible to several large herbivores [...]*. The review we made confirms this first prediction as we showed that co-occurring ungulates dispersed at least two similar plant species in each considered study. Indeed, we revealed a systematic overlap when two ungulates are present at a site, with both ungulate species dispersing between 11.76% (Young, 2012) and 87.30% (Cosyns et al., 2005) of the total number of plant species dispersed (**Table 3**). These proportions correspond to two (Castley et al., 2001) to a maximum of 55 plant species (Cosyns et al., 2005; Jaroszewicz et al., 2013). This pattern is reinforced when we consider studies where at least three ungulates are present. In these cases, again a significant proportion of all the plant species dispersed are dispersed by three ungulates or more. This proportion ranges from 4.55% (Polak et al., 2014) to 38.24% (Malo and Suárez, 1995) of the total number of plants dispersed (**Table 3**) and corresponds to one (Castley et al., 2001; Polak et al., 2014) to a maximum of 48 plant species (Jaroszewicz et al., 2013). These results demonstrate

**TABLE 5 |** Studies with both fur-epizoochory and endozoochory on the same site included in the overlap and complementarity of ungulate-mediated plant dispersal: ungulate sample size (given as number of brushed individuals for fur-epizoochory and weight in grams or number of fecal samples for endozoochory); feeding regime (CS: Concentrate Selector, IMF: Intermediate Mixed Feeder, GRE: Grass and Roughage Eater and OM: Omnivore), publication, location (site, country), total number of plant species dispersed, % of plant species dispersed by both mechanisms, % of plant species strictly dispersed by fur-epizoochory and % of plant species strictly dispersed by endozoochory.

| Ungulate vectors (sample size for fur-epizoochory/ for endozoochory; feeding regime) | Publication | Site, country | Total number of plant species dispersed | % of plant species dispersed by both mechanisms (number of plant species) | % of plant species strictly dispersed by fur-epizoochory (number of plant species) | % of plant species strictly dispersed by endozoochory (number of plant species) |
|---|---|---|---|---|---|---|
| Bison (557/1,131; GRE) | Eyheralde, 2015 | Iowa, USA | 65 | 55.38% (36) | **29.23% (19)** | 15.38% (10) |
| Bison (111/144; GRE) | Rosas et al., 2008 | Oklahama, USA | 53 | 43.40% (23) | **30.19% (16)** | 26.42% (14) |
| Sheep (6/10; GRE) | Benthien et al., 2016 | Luebeck, Germany | 132 | 11.36% (15) | 35.61% (47) | **53.03% (70)** |
| Sheep (60/8; GRE) | Schoenbaum et al., 2009 | Migda, Israel | 45 | 20.00% (9) | 22.22% (10) | **57.78% (26)** |
| Donkey (41/28; GRE) | Couvreur et al., 2005 | Flanders, Belgium | 66 | 24.24% (16) | 19.70% (13) | **56.06% (37)** |
| Cattle (40/45; GRE) | Chuong et al., 2016 | California, USA | 37 | 21.62% (8) | 27.03% (10) | **51.35% (19)** |
| Red deer (5/60; IMF) | Picard and Baltzinger, 2012; Picard et al., 2016 | Lorris, France | 40 | 0% (0) | 7.50% (3) | **92.50% (37)** |
| Goat (6/6; IMF) | Benthien et al., 2016 | Luebeck, Germany | 49 | 4.08% (2) | 40.82% (20) | **55.10% (27)** |
| Roe deer (25/152 g; CS) | Heinken et al., 2001; Heinken and Raudnitschka, 2002 | Breiselang, Germany | 41 | 9.76% (4) | **82.93% (34)** | 7.32% (3) |
| Roe deer (41/805.9 g; CS) | Schmidt et al., 2004 | Herzogtum Lauenburg and Luechow-Dannenberg, Germany | 41 | 29.27% (12) | 12.20% (5) | **58.54% (24)** |
| Roe deer (16/60; CS) | Picard and Baltzinger, 2012; Picard et al., 2016 | Lorris, France | 11 | 0% (0) | 27.27% (3) | **72.73% (8)** |
| Roe deer (11/60; CS) | Picard and Baltzinger, 2012; Picard et al., 2016 | Montargis, France | 8 | 0% (0) | 37.50% (3) | **62.50% (5)** |
| Wild pig (25/2,513 g; OM) | Schmidt et al., 2004 | Herzogtum Lauenburg and Luechow-Dannenberg, Germany | 71 | 25.35% (18) | 28.17% (20) | **46.48% (33)** |
| Wild pig (15/136; OM) | Dovrat et al., 2012 | Ramat-Hanadiv, Israel | 47 | 14.89% (7) | 34.04% (16) | **51.06% (24)** |
| Wild pig (9/3,942 g; OM) | Heinken et al., 2001; Heinken and Raudnitschka, 2002 | Breiselang, Germany | 42 | 38.10% (16) | **50.00% (21)** | 11.90% (5) |
| Wild pig (11/60; OM) | Picard and Baltzinger, 2012; Picard et al., 2016 | Montargis, France | 40 | 2.50% (1) | **67.50% (27)** | 30.00% (12) |
| Wild pig (6/60; OM) | Picard and Baltzinger, 2012; Picard et al., 2016 | Lorris, France | 33 | 3.03% (1) | 42.42% (14) | **54.55% (18)** |

*Bold figures in one of the last two columns indicate the highest value per study case between fur-epizoochory and endozoochory.*

that some plant species can rely on different co-occurring ungulates. At the same time, other plant species are solely dispersed by a single vector species. In this case, ungulates provide complementary dispersal services at the scale of the plant community.

Further, we assessed all combinations of two ungulates ($n = 98$ cases, **Table 6**) from the datasets retrieved (ungulate endozoochory, **Table 3**) and we summarized the proportion of plant species dispersed, taking into account the feeding regime of each ungulate (Hofmann, 1989; Hempson et al., 2015). The sample size of each combination varies between four (a Grass and Roughage Eater with an Omnivore) and eighteen (a Grass and Roughage Eater with an Intermediate Mixed Feeder, **Table 6**). Combinations of two Grass and Roughage Eaters ($n = 9$) shared the highest number of plant species dispersed, nearly 50%, whereas for all other combinations of different feeding regimes, this proportion ranged from 10% (two Concentrate Selectors) to 25% (a Grass and Roughage Eater with an Omnivore) and showed high variability (**Table 6**).

## Fur-Epizoochory by at Least Two Ungulates

We used the same approach to evaluate the six studies retrieved on fur-epizoochory (**Table 4**). Couvreur et al. (2004) mentioned a maximum of 75 plant species dispersed by three domestic ungulates (cattle, donkey, and horse) whereas Picard and Baltzinger (2012) found 18 plant species dispersed by three wild ungulates: red deer, roe deer and wild pig. Here again, we revealed a systematic overlap when two ungulates are present at a site, with both ungulate species dispersing between 5.56% (one plant species, Picard and Baltzinger, 2012) and 40 % (22 plant species, Heinken and Raudnitschka, 2002) of the total number of plant species dispersed (**Table 4**). When we consider studies where at least three ungulates are present, again a significant proportion of all the plant species dispersed are dispersed by three ungulates. This proportion ranges from 5.56% (one plant species, Picard and Baltzinger, 2012) to 14.67% (11 plant species, Couvreur et al., 2004) of the total number of plants dispersed (**Table 4**).

Further, we assessed all combinations of two ungulates from the datasets retrieved (ungulate fur-epizoochory, **Table 4**) and we summarized the proportion of plant species dispersed, taking into account two fur characteristics (hair length and curliness, as described by Albert et al., 2015b). Fur-epizoochory provided many fewer comparisons ($n = 11$ cases for each characteristic, **Table 6**); therefore, our results must be carefully interpreted and considered to be mere trends. The highest shared number of plant species dispersed systematically involved a curled-haired ungulate (between 26 and 34% of shared plant species dispersed) or the presence of one long-haired ungulate (between 21 and 30% of shared plant species dispersed, **Table 6**).

## Complementarity of Fur-Epizoochory and Endozoochory

The last part of this section is dedicated to the complementarity of the two main ungulate-mediated dispersal mechanisms, fur-epizoochory and endozoochory. We retrieved 17 cases where both mechanisms were studied for at least one ungulate on a specific site, 12 cases involving different wild ungulates (roe deer, red deer, American bison, *Bison bison,* and wild pig) and 5 cases involving domestic ungulates (goat, sheep, *Ovis aries*, donkey and cattle) (**Table 5**; **Figure 3**). We have seen that the temporal dynamics of the seed release varies with the ungulate species and the dispersal mechanisms (**Figure 2**) with implications for plant dispersal distances. The extreme number of plants dispersed by a domestic ungulate vary between 37 (cattle, Chuong et al., 2016) and 132 (sheep, Benthien et al., 2016) different plant species dispersed through endozoochory and/or fur-epizoochory (**Table 5**). If we consider wild ungulates, the total number of plant species dispersed ranges between 8 (roe deer, Picard and Baltzinger, 2012; Picard et al., 2016) and 71 (wild pig, Schmidt et al., 2004, **Table 5**). No plant species was dispersed through both mechanisms by roe deer or red deer in France (Picard and Baltzinger, 2012; Picard et al., 2016), whereas American bison displayed the highest number of plant species dispersed through both endo- and fur-epizoochory with 36 different plant species, representing more than 55% of the total number of plant species dispersed (Eyheralde, 2015). Roe deer ($n = 4$), the single Concentrate Selector and wild pig ($n = 5$), the single Omnivore, are the most frequently studied ungulates. They disperse variable numbers of plant species, ranging between 8 and 41 for roe deer, and between 33 and 71 for wild pig (Schmidt et al., 2004; Picard and Baltzinger, 2012; Picard et al., 2016). We can take a similar picture if we consider the proportion of plant species dispersed by endo- and fur-epizoochory, ranging between 0% and nearly 30% for Concentrate Selectors (here, roe deer), and between 2.5 to 38% for Omnivores (here, wild pig, **Table 5**). Concerning Grass and Roughage Eaters ($n = 6$, including American bison, cattle, donkey and sheep), and especially American bison, a significant proportion (nearly 30%) of the dispersed plant species are dispersed both externally and internally. Intermediate Mixed Feeders ($n = 2$, red deer and goat) dispersed both externally and internally the lowest proportion plant species (**Figure 3**). Finally, among the 17 study cases reviewed and whatever the ungulate species concerned, 12 study cases report higher number of plant species strictly dispersed by endozoochory in comparison with 5 study cases by fur-epizoochory.

# PERSPECTIVES

## Methodological Challenges

First, we would like to again underline the limitations involved in comparing the available studies on ungulate-mediated endozoochory in the literature. There are still no standardized criteria applied to the samples for either germination conditions (e.g., closed vs. open greenhouses; greenhouse vs. natural conditions) or abiotic conditions (e.g., controlled vs. fluctuating temperatures; with or without irrigation). Even the length of the germination experiments differed greatly amongst the studies (see **Table 3**). Generally, at least one full year is advised to assess germination success and identify the plant species, but Young (2012) and Jaroszewicz et al. (2013) prolonged that period for up to 3 years. Notably, Jaroszewicz et al. (2013) showed that Yellow Star of Bethlehem (*Gagea lutea*), an ancient forest species,

**TABLE 6 |** Proportion of shared dispersed plant species for different combinations of feeding regimes (CS: Concentrate Selector, IMF: Intermediate Mixed Feeder, GRE: Grass and Roughage Eater and OM: Omnivore) for endozoochory (left columns) and hair characteristics (hair curliness: curly, wavy and straight; hair length: short, medium and long as described in Albert et al., 2015b) for fur-epizoochory (right columns).

| **Endozoochory** | | **Fur-epizoochory** | |
|---|---|---|---|
| **Feeding regimes (sample size)** | **% of shared dispersed plant species (mean ± 95% CI)** | **Hair characteristics (sample size)** | **% of shared dispersed plant species (mean ± 95% CI)** |
| GRE–GRE (*n* = 9) | **47.44 ± 10%** | curly–wavy (*n* = 2) | **33.77 ± 10%** |
| GRE–IMF (*n* = 18) | 17.43 ± 6% | curly–straight (*n* = 2) | **26.67 ± 10%** |
| GRE–CS (*n* = 16) | 12.89 ± 6% | wavy–wavy (*n* = 1) | 5.56% |
| GRE–OM (*n* = 4) | 25.34 ± 19% | wavy–straight (*n* = 5) | 18.9 ± 13% |
| IMF–IMF (*n* = 6) | 12.93 ± 14% | straight–straight (*n* = 1) | 16.00% |
| IMF–CS (*n* = 17) | 16.05 ± 7% | long–medium (*n* = 6) | **21.09 ± 12%** |
| IMF–OM (*n* = 10) | 16.15 ± 11% | long–short (*n* = 3) | **29.63 ± 10%** |
| CS–CS (*n* = 9) | 10.26 ± 9% | medium–medium (*n* = 1) | 5.56% |
| CS–OM (*n* = 9) | 19.64 ± 12% | medium–short (*n* = 1) | 16.00% |

*Bold figures show highest values.*

only germinated in European bison dungs during the third year of the experiment. They further observed that some seedlings emerged up to 7 years after the beginning of the experiment. Time for germination of ungulate-dispersed seeds is rarely reported (but see Milotić and Hoffmann, 2016c). The application of average germination conditions might not fit the germination requirements of each of the diaspores present in the feces. One solution might be to check each seed for viability (tetrazolium test). However, this method only indicates the intrinsic ability of a seed to germinate while the abiotic conditions at the release site might not fit its germination requirements; such a viability test could easily lead to an over-estimation of germination success. Pakeman and Small (2009) showed that the germination success under natural conditions was lower than in greenhouses. In three of the retrieved studies on endozoochory (**Table 3**), dispersed seeds were morphologically identified and submitted neither to seedling emergence nor to viability test (Castley et al., 2001; Sigwela, 2004 and Benthien et al., 2016).

Fur-epizoochory and regurgitation need to be studied in much greater depth, and in association with endozoochory on the same sites and within communities of ungulates (**Table 3**). Future research should also focus on plant dispersal networks involving ungulates and other taxa of dispersal agents to help addressing the relative importance of ungulates as plant dispersal agents (e.g., Fedriani and Delibes, 2009).

## Overlap and Complementarity of Ungulate-Mediated Dispersal Services for Habitat Restoration

The results from our systematic literature review enable us to provide preliminary recommendations concerning ungulates as potential tools for habitat restoration, thanks to their dispersal services. At the landscape scale, dispersal agents with the highest overlap in dispersed plant species between them might replace one another to some extent, while those with the least overlap provide a complementary service. When choosing appropriate dispersal vectors to be included in the management or restoration of a landscape (i.e., rewilding concept), the total amount of plant species and diaspores dispersed by a given ungulate should be a selection criterion for consideration (e.g., sheep, Rico et al., 2014). Grass and Roughage Eaters, thanks to their diversified feeding regime, disperse a large amount of different plant species. They are also the most similar vectors when more than one ungulate species of this same feeding regime co-occur; this is even true when we compare different dispersal processes, like endozoochory and fur-epizoochory. Consequently, if the aim is to restore degraded habitats, managers of natural areas should consider introducing or re-introducing complementary ungulates and at least one of the following species: sheep, cattle or bison. Associating a Grass and Roughage Eater, efficient for quantitative dispersal, with an ungulate from a different feeding regime (Concentrate Selector or Omnivore) for qualitative dispersal would create a beneficial complementarity in the restoration program.

Fur-epizoochory highlights the overlapping plant dispersal services of long- and curly-haired ungulates with other ungulates. Sheep would again offer effective dispersal services. Rico et al. (2014) demonstrated that rotational shepherding might be useful in restoring plant communities. Wild pigs are likely to offer contradictory services, being an effective epizoochorous dispersal agent but also a potential consumer of the seeds.

## Research Perspectives

In a recent paper, John et al. (2016) have called for researchers to include the role of animal cognition on plant-animal interactions such as seed dispersal, herbivory and pollination. Animal memory can lead to directed dispersal: animals may select specific plants at specific places and release them at predictable safe resting places. Richard et al. (2014) provided quantitative proof of temporal home range fidelity for ungulates like red and roe deer and, Riotte-Lambert et al. (2017) developed a framework for the study of routine movement behavior. Similarly, taking animal behavior (Russo et al., 2006), and animal sociality (Sarasa et al., 2009; Liehrmann et al., 2018) into account will open new research

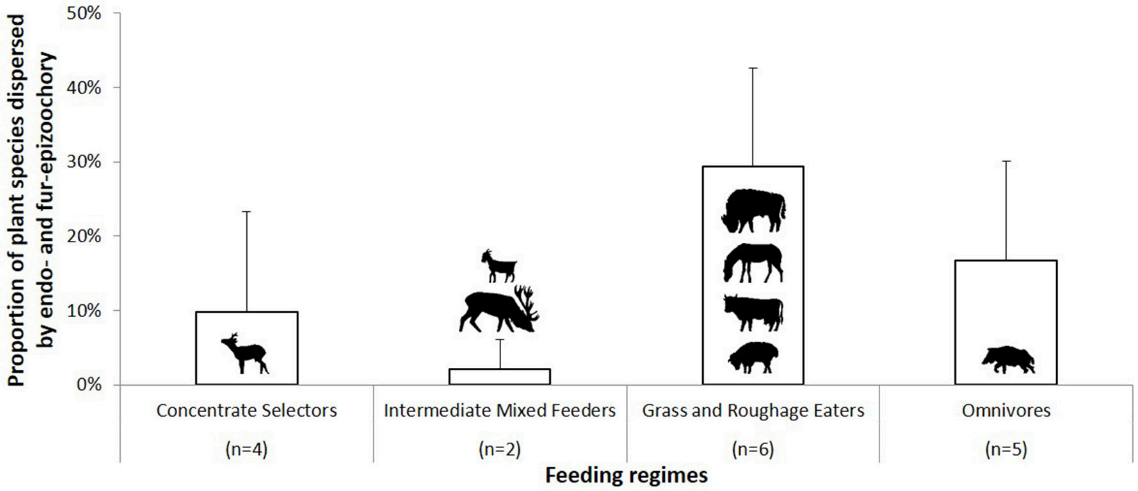

**FIGURE 3 |** Proportion of plant species dispersed through endozoochory and fur-epizoochory by ungulates according to their feeding regime (**Table 5**). Concentrate Selectors are represented by roe deer (*Capreolus capreolus*); Intermediate Mixed Feeders by goat (*Capra aegagrus hircus*) and red deer (*Cervus elaphus*); Grass and Roughage Eaters by American bison (*Bison bison*), donkey (*Equus asinus*), cattle (*Bos taurus*), and sheep (*Ovis aries*) and Omnivores by wild pig (*Sus scrofa*).

opportunities for the further investigation of ungulate-mediated plant dispersal. Though this may be challenging in closed forest environments, one could start by matching behavioral observations of ungulates mainly dwelling in open areas, for instance reindeer in mountainous areas (Mårell et al., 2002) or mountain ibex in alpine grasslands, with the study of plant dispersal. The use of acceleration sensors (Nams, 2014; Kröschel et al., 2017) and its calibration with control animals will help determine activity (active vs. resting) and specific behaviors (e.g., lying, feeding, walking, trotting) of the equipped animals together with its location in open or closed habitats. This could render more realistic the study of the transfer phase of ungulate-mediated dispersal that generally combines retention times and associated distances traveled (Westcott et al., 2005; Pellerin et al., 2016). Wang and Smith (2002) proposed new techniques to the study of seed dispersal among which stable isotope ratios and molecular genetic markers to link dispersed seeds to parent plants. More recent applications of genetic tools to seed dispersal allow the identification of the disperser (DNA barcoding) and relate dispersed seeds to parent plants (DNA microsatellites, González-Varo et al., 2017). They could be used for guild of ungulate dispersers.

We found very few studies on mountain ungulate communities (but see Young, 2012 for New Zealand alpine ecosystems and Karimi et al., 2018, for North-Eastern Iran), whereas ungulate contribution to altitudinal plant dispersal should be investigated. Bertrand et al. (2011) showed that mountain plant communities coped with climatic changes better than did lowland communities. Rumpf et al. (2018) also used vegetation resampling to assess temporal changes of lower and upper range limits of a set of plants of the European Alps, but dispersal-related traits failed to explain the upward movement of the plants. However, these traits were computed from data obtained on lowland ungulates (Mouissie et al., 2005a; Römermann et al., 2005) probably inappropriate in mountainous areas. Following this, further studies are required to ascertain and quantify the role of mountain ungulates like chamois, mouflon or mountain ibex, especially in relation to plant response to climate change in alpine ecosystems.

Recent studies have stressed the implication of native, domestic and introduced ungulates in the dispersal of exotic plants. Some researchers have found that native ungulates aid the spread of exotic invasive plants (Myers et al., 2004; Vavra et al., 2007; Eschtruth and Battles, 2009) as Schiffman (1997) suggested. But, how do the traits of these exotic invasives compared to those of native plants in terms of ungulate-mediated dispersal? Preliminary observations tend to show that some exotic plants occupy a complementary feeding niche (i.e., phenological shift) for large herbivores by offering green edible material when the rest of the vegetation is dry. This is the case for instance for the leaves and fruits of tickberry (*Lantana camara*) consumed by giraffes (*Giraffa camelopardalis*), nyalas (*Tragelaphus angasii*), and zebras (*Equus burchelli*) in South Africa during austral winter.

In addition to being dispersal agents, these large herbivores mediate plant-plant interactions and modify the local abiotic conditions where diaspores are released, through nutrient fluxes (white-tailed deer; Seagle, 2003) and physical disturbance (repeated rooting or scraping). They thus create windows of opportunity for plants to establish (Myster, 1993). Ungulates also interact with other taxa, both animal (earthworms, Dávalos et al., 2015) and plant (shrubs, Boulanger et al., 2018), for the recruitment of forest plants. Finally, few studies have formally demonstrated the role ungulates in long distance plant dispersal (Vickery et al., 1986); most studies use indirect approaches (Boulanger et al., 2011; Milotić et al., 2017). To conclude, large strides have been made despite methodological constraints in the direct measurement of dispersal by large ungulates. However, one fundamental question still remains, i.e., what is the proportion of diaspores produced by a given plant that are carried over long distances by large ungulates? Addressing this question will help us to gain a deeper understanding of the full range of effects ungulates have in an ecosystem.

## AUTHOR CONTRIBUTIONS

CB proposed the idea and led the writing. SK provided data and commented on the content of the manuscript. US and CB equally contributed to the systematic literature review. CB, SK, and US all contributed to the re-reading of the manuscript.

## FUNDING

US is currently working under a PhD grant from Irstea and Center-Val de Loire Region (2015-00099650, Costaud project). SK benefitted from a 3-month exchange grant at Irstea in spring 2017 from the University of Isfahan, Iran. CB benefitted from a 2-month research fellowship funded by the Center of Excellence for Invasion Biology in Stellenbosch, the French Embassy in Pretoria and the MiDi Thematic Research Network in Center-Val de Loire Region.

## ACKNOWLEDGMENTS

Final discussions on this review project occurred during a workshop organized in July 2018 (Pietermaritzburg, South Africa) involving Stellenbosch University, the University of KwaZulu-Natal in Pietermaritzburg and SAN Parks. We also thank our European colleagues (listed in Albert et al., 2015a) from the informal network on ungulate-mediated seed dispersal for discussions at different steps that fed the content of this manuscript. We are grateful to Marie Baltzinger for her drawings. We would like to thank the two reviewers and the associate editor who helped us improve the first version of this manuscript, Victoria Moore and US for proofreading the English manuscript.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Ecology and Evolution

ORIGINAL RESEARCH
published: 13 March 2019
doi: 10.3389/fevo.2019.00054

# Plant Dispersal in a Temperate Stream by Fish Species With Contrasting Feeding Habits: The Role of Plant Traits, Fish Diet, Season, and Propagule Availability

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Whether fish mediate plant dispersal in temperate freshwaters is largely unknown. A prerequisite for successful dispersal is ingestion and surviving the journey in the intestinal tract. This study asks whether plant propagules are being ingested under field conditions and what factors shape dispersal potential, focusing on differences across plant species and propagule form (seed or fragment), seasonal differences and plant and fish traits that facilitate dispersal. We focused on three common fish species reported to differ in foraging strategy. Fish were caught monthly over a 1-year period in a Dutch lowland stream. Before they were returned to the stream, fish were kept in water for 26 h and their feces were collected, resulting in 150 fecal samples. Excreted animal remains and plant propagules were identified and enumerated. Plant propagules were tested for viability. In total, 88,579 vegetative fragments of vascular plants, 316 of mosses and 14 of charophytes were identified. Viability was low (<<1%) except for mosses (53.5%). Roach (*Rutilis rutilis*) and Rudd (*Scardinius erythrophthalmus*) displayed a preference for filamentous algae and certain plant species (i.e., *Elodea nuttallii* and *Lemna* sp.), likely because they were more palatable. Of the 1,787 generative propagules of vascular plants that were identified, 120 germinated (6.7%), representing 15 species. *Betula pendula*, *Juncus effusus,* and *Poa trivialis* were most abundant. Tench (*Tinca tinca*) egested most seeds, despite being the least herbivorous species. Particularly, germination was high for seeds that were light (<1.07 mg) and that floated for a long time. Our results show that fish do ingest plant propagules under field conditions and that fish can contribute to vegetative dispersal of vascular plants and several aquatic and riparian moss species. Ingestion of propagules is affected by water temperature and season, their availability in the propagule bank, and their palatability. Both seed traits (related to buoyancy, size and

**Edited by:**
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**Specialty section:**
*This article was submitted to*
*Biogeography and Macroecology,*
*a section of the journal*
*Frontiers in Ecology and Evolution*

**Received:** *30 October 2018*
**Accepted:** *15 February 2019*
**Published:** *13 March 2019*

**Citation:**
*Boedeltje G, Klutman B, Schaap M, Sollman P, de Vos M, Lenssen JPM and Verberk WCEP (2019) Plant Dispersal in a Temperate Stream by Fish Species With Contrasting Feeding Habits: The Role of Plant Traits, Fish Diet, Season, and Propagule Availability. Front. Ecol. Evol. 7:54. doi: 10.3389/fevo.2019.00054*

hardness) and fish traits (related to size and identity) were important. Despite substantial dietary overlap, the three fish species displayed subtle differences in their diet, and together can act as vectors for the dispersal of a range of plant and moss species of freshwater systems.

**Keywords: bryophyte, endozoochory, ichthyochory, seed dispersal, *Rutilus rutilus*, *Scardinius erythrophthalmus*, *Tinca tinca*, vegetative dispersal**

## INTRODUCTION

Streams are important vectors for plant propagules facilitating dispersal between habitats, communities, and populations (Honnay et al., 2010; Nilsson et al., 2010; Fraaije et al., 2017). Many plant species have evolved seeds or vegetative propagules that are well equipped to "go with the flow" and thus achieve long-distance dispersal via hydrochory (Boedeltje et al., 2003, 2004; Sarneel, 2012; Favre-Bac et al., 2017). However, hydrochory in flowing waters is only in one direction, and going against the flow requires different vectors such as ducks or fish (Wubs et al., 2016). Fish may be particularly important vectors for upstream dispersal (Horn et al., 2011).

Knowledge of ichthyochory, i.e., seed dispersal by fish, is largely derived from Neotropical studies (Kubitzki and Ziburski, 1994; Galetti et al., 2008; Anderson et al., 2011; Correa et al., 2015, 2016), but a few studies also addressed the role of fish-mediated plant dispersal in temperate freshwater systems (Chick et al., 2003; VonBank et al., 2018). Experiments under controlled conditions have shown that seeds of several plant species from temperate regions are capable of germination following ingestion and egestion by fish (Pollux et al., 2006a, 2007; Sumoski and Orth, 2012; Boedeltje et al., 2015, 2016). However, to ensure seed ingestion in these experiments, fish were offered pellets of fish food in which plant seeds were enclosed. It is therefore not clear whether native fish of temperate fresh waters actually ingest seeds under natural conditions (but see Chick et al., 2003), and which factors influence seed consumption. Horn et al. (2011) have suggested that temperate fish ingest seeds unintentionally when foraging in the sediment. Seed availability in the sediment seed bank might therefore be a determining factor for seed ingestion. In addition, the availability of macro-invertebrates as an alternative food source may also play a role (Garvey and Chipps, 2012). The availability and need for macro-invertebrates or plants as food sources may vary throughout the year, with the possible consequence that fish-mediated dispersal may be determined by seasonal diet shifts of the fish as well as dietary differences across fish species.

Plant propagule uptake, egestion and dispersal may also depend on plant traits such as seed size (Pollux et al., 2007), seed hardness and seed shape (Boedeltje et al., 2015). However, the abundance of plant species in the local vegetation might also be important, as was demonstrated for hydrochorous plant dispersal (Boedeltje et al., 2003, 2019).

This study addressed the potential for plant dispersal in a temperate lowland stream by three native fish species: Roach (*Rutilis rutilis*), Rudd (*Scardinius erythrophthalmus*) and Tench (*Tinca tinca*). Tench is reported to primarily feed on benthos (Perrow et al., 1996). Roach and Rudd additionally forage throughout the water column, with Rudd being best adapted to feeding on prey at the water surface (Kennedy and Fitzmaurice, 1974; García-Berthou and Moreno-Amich, 2000). Their feeding behavior may vary in relation to temperature and according to the seasonal and spatial availability of food (e.g., Brabrand, 1985; Jamet, 1994; Michel and Oberdorff, 1995; Guinan et al., 2015). Roach and especially Rudd are considered to be the most herbivorous native fish in the European region (Prejs, 1984; Dorenbosch and Bakker, 2012), but Tench may also occasionally consume vegetation (Michel and Oberdorff, 1995), making them all potential plant dispersers.

Over a 1-year period, in this study we quantified each month the amount and viability of generative and vegetative plant parts as well as macro-invertebrate remains in the feces of the three fish species in a vegetated lowland stream. We first related the diet of the three fish species to season and temperature and the abundance of plant species in the vegetation and in the propagule bank. We next addressed the question whether these factors, in addition to plant specific traits, affected the probability of being successfully dispersed (i.e., viably egested) by the fish.

## MATERIALS AND METHODS

### Study Area

The study was conducted in the Groenlose Slinge (52°116′ N, 6°494′ E), a channelized lowland stream in the eastern part of the Netherlands (**Figure S1**). The stream is a tributary of the small river *Berkel*, which in turn flows into the river *IJssel*, one of the lower reaches of the river *Rhine*. Thanks to fish passages (**Figure S1**), fish can move freely from the IJssel to the upstream areas of the tributaries and vice versa. The Groenlose Slinge is c. 25 km long and its slope at the research stretch is ~0.4 m/km. Its width ranges from 1 m in the upper course up to 10 m downstream. At the research stretch, discharge varies from 16 $m^3.s^{-1}$ at yearly peak discharges to 0.8 $m^3.s^{-1}$ at base flow, with a corresponding flow velocity of $\sim$0.04 $m.s^{-1}$. Fish were caught in a downstream stretch of 1.9 km length. The east bank of this stretch is steep with a narrow zone of helophytes bordering the water, whereas the west bank consists of 3 m wide, shallow zone (**Figure S1**). This is overgrown with helophytes of which *Glyceria maxima* is the most abundant plant species (**Table S4**). The higher parts of the west bank are forested (**Figure S1**) with *Alnus glutinosa* and willows (*Salix* spec.) as the dominant species. The eutrophic water is densely vegetated with submerged vascular plants, filamentous algae, mosses, and charophytes. Duckweeds (*Lemna* spec.) and floating-leaved species such as *Nuphar lutea* frequently occur (**Table S4**). To ensure unimpeded water discharge, the floating, submerged, and emergent vegetation is cut in mid-June and September. The riparian helophyte vegetation is not mown.

## Measuring Plant Abundance in the Vegetation

In July 2017, when vegetation was at its peak of development, the presence of vascular plant species, filamentous algae, and charophytes was investigated. The 1.9 km research stretch was divided into sections of 100 m, each comprising the aquatic and bank zone. An aquatic plot included the entire stream width; a bank plot covered the 1–4 m wide helophyte zone of the bank, assuming that diaspores of species from this area could potentially reach the water. In addition, riparian species outside this helophyte zone were included if their height was such that seeds were likely to be able to reach the water. Species were recorded within the plots whilst wading through the water. The abundance was estimated using a scale of nine classes: 1 = 1–4, 2 = 5–20, 3 = 20–100, 4 = >100 individual(s) or tiller(s) in a plot and covering <5%; 5 = covering 5–12.5%, 6 = 12.5–25%, 7 = 25–50%, 8 = 50–75%, 9 = 75–100%. As mosses occurred on solid substrates (mostly stones) only, moss diversity was recorded separately in July 2018. Moss abundance was established in 8 sampling plots of 0.25 $m^2$ (0.5 × 0.5 m), from 20 cm below to 30 cm above mean water level using the scale mentioned above. Nomenclature of vascular plants and mosses is, respectively according to van der Meijden (1996) and Siebel and During (2006).

## Sampling the Propagule Bank

Propagule bank samples were taken in March 2017, assuming that natural stratification of seeds had occurred during the previous winter. In eight 4 $m^2$-plots, evenly distributed over the research stretch, a sample was taken from the surface sediment, comprising eight cores each, using a transparent PVC-tube (Ø 6 cm). Four cores were taken in the shallow bank zone and four in the bordering aquatic zone. Sampling was restricted to the upper layer (5 cm) of the sediment, assuming that only this (organic) material might be foraged by fish. The cores of a plot were pooled and mixed, transferred to the laboratory and processed immediately.

The samples were treated according to the seedling emergence technique of Boedeltje et al. (2002). First, they were sieved (mesh width 200 μm) to remove fine soil material and dead organic parts. Potentially viable vegetative parts however, were kept in the samples. The remaining seeds and vegetative fragments of each sample were spread out in a thin layer (<5 mm) in one or more trays filled with a mixture of equal parts of sterilized sand and potting soil and set to germinate in a greenhouse under submerged conditions (water level 2 cm above soil surface) for 2 weeks and next under waterlogged conditions (water level 4 cm below soil surface) for 10 weeks (**Figure S3**). Air temperature in the greenhouse was at least 22°C between 06:00 and 21:00 h and 15°C between 21:00 and 06:00 h. A photoperiod between 06:00 and 21:00 h was maintained throughout the germination period. Seedlings and regenerated vegetative propagules were identified, counted, and removed from the trays.

## Sampling and Housing the Fish

Between August 2016 to July 2017, the three fish species were captured monthly using a control box "TENCH 20" electro-fishing unit (Fishtronics.nl), connected to a standard gasoline generating set (230 V), mounted on a drift boat (**Figure S1**). The box was adjusted exactly so that the direct current of 3,000 W at 10 A in the water was strong enough to bring about a forced swimming movement to the anode-net, and causing minimum cramping of the fish. While actively electrofishing, a close proximity to the riparian vegetation was maintained, enabling fishing in both the aquatic and riparian vegetation. Both sides of the stream were sampled. Sampling continued until the end of the research section or until the minimum number of each species (50 individuals monthly) was reached. All fish were quickly put in an aerated tank on board and transported to the field station within half an hour after being caught. The field station was located 15 m away from the stream (**Figure S1**). For capture and subsequent release of fish, ethical approval was not required as per the local legislation.

In the field station, fish were sorted by species and size (estimated in "small": <15 cm and "large": ≥15 cm) so that conspecifics with similar size ended up in the same aerated 100 L tank. The (visually) estimated mean size of fish in each tank was recorded to be used as a covariate in statistical analysis. We choose not to measure fish more precisely as this might have caused too much stress and physical damage. To prevent propagules from being inadvertedly introduced into the tanks where fish were kept for defecation, the fish were rinsed in a bucket of tap water before being introduced to the tank. In addition, these tanks contained stream water that was filtered over a 200-μm sieve to prevent potential input of plant propagules from the stream. To prevent fish from leaping out, tanks were covered with fine-meshed nets (**Figure S2**). For each species we used five tanks, each containing small or large fishes. The actual number of fish per tank depended on the catch success and varied from 1 to 20 individuals (mean 10, median 10; **Table S3**). Fishes were then left undisturbed for 26 h, at which time they were transferred to the stream and released. The temperature in the field station was approximately equal to the ambient temperature and varied from 5°C in winter to 24°C in summer. No mortality was observed in response to electrofishing and housing the fish in aerated tanks.

Over the research year, 150 samples were taken, in which 1,467 fish individuals were caught: 41 samples (with 330 individuals) of Roach, 50 (461 individuals) of Rudd, and 59 (676 individuals) of Tench (**Table 1**). Roach and Tench could be caught every month, Rudd in 11 months (no catches in May).

Water temperature data were obtained from data collected at a gauge station, 500 m downstream from the study site. From these measurements, we selected the temperature data coinciding with the time we caught fish.

**TABLE 1 |** Frequency and abundance of plant species in the vegetation and propagule bank, the number of generative (gen.) and vegetative (veg.) propagules found in the feces and the number of propagules found that proved to be viable (germinated or regenerated) for each of the three fish species.

| **Fish species** | | | | | **Roach** | | **Rudd** | | **Tench** | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Total number of fish** | | | | | **333** | | **461** | | **673** | |
| | **Vegetation** | | **Propagule bank** | | **Found** | **Viable** | **Found** | **Viable** | **Found** | **Viable** |
| | **Freq.** | **Abund.** | **Freq.** | **Abund.** | **No.** | **No.** | **No.** | **No.** | **No.** | **No.** |
| **VASCULAR PLANTS (gen.)** | | | | | | | | | | |
| *Betula pendula* | 0.26 | 3.80 | 0.75 | 6.83 | 298 | 5 | 172 | 7 | 169 | 6 |
| *Callitriche obtusangula* | 0.95 | 3.67 | 0.88 | 3.00 | 19 | 0 | 16 | 0 | 49 | 0 |
| *Carex acuta* | 0.11 | 1.50 | 0.00 | 0.00 | 0 | 0 | 1 | 1 | 0 | 0 |
| *Epilobium hirsutum* | 0.84 | 3.81 | 0.38 | 2.00 | 2 | 2 | 0 | 0 | 1 | 1 |
| *Epilobium tetragonum* | 0.21 | 1.00 | 0.38 | 5.33 | 2 | 2 | 2 | 2 | 2 | 2 |
| *Juncus effusus* | 0.79 | 2.73 | 1.00 | 123.25 | 16 | 5 | 18 | 5 | 105 | 30 |
| *Mentha aquatica* | 0.89 | 4.24 | 0.88 | 2.29 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Myosotis scorpioides* | 0.79 | 3.20 | 0.38 | 3.00 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Plantago major* | 0.00 | 0.00 | 0.25 | 1.50 | 0 | 0 | 0 | 0 | 1 | 1 |
| *Poaceae* (all seeds) | | | | | 185 | 2 | 38 | 5 | 656 | 9 |
| *Agrostis stolonifera* | 0.79 | 3.07 | 0.63 | 1.20 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Glyceria maxima* | 0.95 | 5.28 | 0.75 | 2.83 | 0 | 0 | 0 | 0 | 2 | 2 |
| *Phalaris arundinacea* | 0.89 | 4.24 | 0.50 | 6.00 | 0 | 0 | 3 | 1 | 0 | 0 |
| *Poa trivialis* | 0.53 | 3.00 | 0.75 | 2.00 | 2 | 2 | 4 | 4 | 7 | 7 |
| *Sagittaria sagittifolia* | 0.74 | 4.79 | 0.38 | 1.33 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Salix cinerea* | 0.58 | 2.36 | 0.88 | 1.86 | 1 | 1 | 0 | 0 | 0 | 0 |
| *Salix fragilis* | 0.47 | 1.78 | 0.88 | 1.29 | 3 | 3 | 3 | 3 | 5 | 5 |
| *Scirpus sylvaticus* | 0.74 | 3.36 | 0.25 | 1.50 | 1 | 1 | 0 | 0 | 3 | 3 |
| *Sparganium emersum* | 0.68 | 4.38 | 0.63 | 4.80 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Sparganium erectum* | 0.79 | 4.47 | 0.63 | 1.60 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Stachys palustris* | 0.79 | 4.00 | 0.00 | 0.00 | 0 | 0 | 6 | 6 | 11 | 11 |
| *Urtica dioica* | 0.63 | 3.25 | 0.88 | 3.71 | 1 | 1 | 0 | 0 | 0 | 0 |
| *Typha latifolia* | 0.58 | 3.45 | 0.25 | 1.00 | 0 | 0 | 1 | 1 | 0 | 0 |
| *Veronica catenata* | 0.05 | 1.00 | 0.50 | 4.50 | 0 | 0 | 0 | 0 | 0 | 0 |
| **VASCULAR PLANTS (veg.)** | | | | | | | | | | |
| *Callitriche obtusangula* | 0.95 | 3.67 | 0.88 | 3.00 | 4 | 0 | 8 | 0 | 5 | 0 |
| *Ceratophyllum demersum* | 0.53 | 3.10 | 0.00 | 0.00 | 1 | 0 | 9 | 0 | 4 | 0 |
| *Elodea nuttallii* | 0.89 | 6.65 | 0.00 | 0.00 | 10163 | 0 | 68628 | 2 | 1615 | 0 |
| *Lemna minor + L. minuta* | 0.89 | 3.88 | 0.75 | 2.83 | 1037 | 1 | 7065 | 8 | 27 | 1 |
| *Lemna trisulca* | 0.84 | 3.88 | 0.13 | 1.00 | 3 | 0 | 8 | 2 | 0 | 0 |
| *Spirodela polyrhiza* | 0.63 | 3.33 | 0.00 | 0.00 | 0 | 0 | 2 | 1 | 0 | 0 |
| **MOSSES (veg.)** | | | | | | | | | | |
| All fragments | | | | | 76 | 40 | 122 | 55 | 118 | 74 |
| *Brachythecium rutabulum* | 0.38 | 4.00 | 0.00 | 0.00 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Bryum argenteum* | 0.13 | 4.00 | 0.38 | 2.00 | 27 | 27 | 32 | 32 | 50 | 50 |
| *Bryum barnesii* | 0.13 | 4.00 | 0.13 | 2.00 | 0 | 0 | 5 | 5 | 4 | 4 |
| *Bryum dichotomum* | 0.00 | 0.00 | 0.38 | 2.33 | 6 | 6 | 10 | 10 | 9 | 9 |
| *Bryum gemmiferum* | 0.00 | 0.00 | 0.00 | 0.00 | 0 | 0 | 0 | 0 | 1 | 1 |
| *Bryum pseudotriquetrum* | 0.25 | 4.00 | 0.00 | 0.00 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Ceratodon purpureus* | 0.13 | 4.00 | 0.00 | 0.00 | 4 | 4 | 0 | 0 | 2 | 2 |
| *Fissidens fontanus* | 0.00 | 0.00 | 0.00 | 0.00 | 0 | 0 | 1 | 1 | 0 | 0 |
| *Hypnum cupressiforme* | 0.25 | 4.50 | 0.00 | 0.00 | 1 | 1 | 3 | 3 | 4 | 4 |

*(Continued)*

**TABLE 1** | Continued

| **Fish species** | | | | | **Roach** | | **Rudd** | | **Tench** | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Total number of fish** | | | | | **333** | | **461** | | **673** | |
| | **Vegetation** | | **Propagule bank** | | **Found** | **Viable** | **Found** | **Viable** | **Found** | **Viable** |
| | **Freq.** | **Abund.** | **Freq.** | **Abund.** | **No.** | **No.** | **No.** | **No.** | **No.** | **No.** |
| *Leptodictyum riparium* | 0.88 | 6.86 | 0.63 | 1.60 | 1 | 1 | 0 | 0 | 0 | 0 |
| *Oxyrrhynchium speciosum* | 0.00 | 0.00 | 0.00 | 0.00 | 0 | 0 | 0 | 0 | 1 | 1 |
| *Pohlia wahlenbergii* | 0.00 | 0.00 | 0.50 | 3.00 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Schistidium crassipilum* | 0.00 | 0.00 | 0.00 | 0.00 | 1 | 1 | 1 | 1 | 1 | 1 |
| *Tortula muralis* | 0.13 | 2.00 | 0.00 | 0.00 | 0 | 0 | 3 | 3 | 2 | 2 |
| **FILAMENTOUS ALGAE** | | | | | | | | | | |
| All fragments | 0.83 | 6.00 | ND | ND | 210710 | ND | 78720 | ND | 6751 | ND |
| **CHAROPHYTA** | | | | | | | | | | |
| *Nitella mucronata* (gen.) | 0.11 | 4.00 | 0.00 | 0.00 | 3 | 2 | 0 | 0 | 5 | 5 |
| *Nitella mucronata* (veg.) | 0.11 | 4.00 | 0.00 | 0.00 | 0 | 0 | 10 | 0 | 4 | 0 |

## Sorting and Testing the Viability of Plant Parts

After the 26th hour of fish captivity, feces were collected by filtering the water of each tank over a 200-μm sieve (**Figure S2**), and next stored in tap water in 250 mL vials. By using a dissecting microscope, potentially viable seeds and vegetative plant parts were sorted out, identified and counted. The amount of vegetative filamentous algae and Characeae was determined by counting the number of fragments. When their quantities were large, the amount was estimated by counting fragments in 1/8 part of the microscope image field and then multiplying the number by 8. Since fragments differed in length, this is a proxy of their presence in the feces samples. Viability of algal fragments was not determined. Seeds were identified at least to the genus level using the Digital Seed Atlas of Cappers et al. (2006). Animal remains were transferred into alcohol (30%) and stored until identification. To test whether feces contained viable seeds and vegetative propagules, samples were next transferred to the greenhouse and handled according to the propagule bank protocol.

Seeds separated and identified were set to germinate in petri dishes (Ø 9 cm) on a double layer of Whatman No. 1 filter paper (**Figure S2**). The paper was kept water-saturated with tap water, as earlier experiments (Boedeltje et al., 2002, 2003) indicated that this was the optimal hydrological condition for both aquatic and riparian species. For 60 days, the dishes were placed in a climate-controlled room over 15-h light at 24°C and a night temperature of 15°C. Seedling emergence was assessed weekly.

After the germination and regeneration period, the non-germinated, but viable seeds in petri dishes and trays were placed water-saturated and waterlogged, respectively, in a dark room at 5°C for 10 weeks to promote loss of dormancy (Baskin and Baskin, 2014). After this cold treatment, petri dishes and trays were set to germinate again, as above.

Wet filter papers in petri dishes also were used as regeneration substrate for vegetative parts of bryophytes (Boedeltje et al., 2019; **Figure S3**). The closed dishes were exposed to natural day light conditions (without direct sunlight) in an unheated room (mean temperature 18°C) and watered with tap water when necessary. After 6 weeks, regeneration was recorded under a dissecting microscope and fragments were considered viable when they had produced rhizoids, shoots or green leaves (**Figure S3**). Hereafter, the term "viability" for a propagule is used for generative plant parts i.e., seeds or oospores that germinated or vegetative parts that grew into a new plant.

## Aquatic Macroinvertebrate Identification

Remains of invertebrates were identified at least to the family level and, if possible, to the genus level using the literature cited in Bijkerk (2010). Hard chitinous remains allowed identification of trichopterans, chironomids, coleopterans, and heteropterans (e.g., elytra, hemielytra, abdominal claws, frotoclypeus, labium, and pronotum). Molluscs were identified from their shell remains. Taxa with less sclerotized body parts or with softer exoskeletons (e.g., *Malacostraca* and *Ephemeroptera*) could still be identified, but could not be accurately enumerated. Cladocerans were also counted, but not further identified.

## Data Analyses

To visualize patterns of taxa (hereafter "species") abundance (plants and invertebrates) in the feces samples across the 12 months surveyed, principal coordinate analysis (PCoA) was applied using Canoco 5 (ter Braak and Smilauer, 2012). We focused on assessing differences in diet, and therefore centered and standardized by plant and animal species represented in the diet. Abundance of plant parts and invertebrates was calculated by dividing the number of items per taxon over the number of fish per tank. Redundancy analysis (RDA) was applied to determine the significance of the variables fish species, fish size, number of fish in a sample, and month of feces collection in explaining differences in the abundance of plant and animal species in the samples. To rank the explanatory variables, forward selection of the variables was performed (ter Braak and Smilauer, 2012). To meet the assumptions of PCoA and RDA, species abundances were $\log_{10}$ - transformed. We also used PCoA to visualize differences in plant species abundance between the vegetation and the propagule bank. We also summed the total number of plant parts found in the feces of each fish species and included these as additional samples to illustrate how the vegetable diet of the fish compared to the plant species abundances of the vegetation and the propagule bank.

To relate abundance of plant propagules and faunal remains in each of the fish species to time of year and temperature, we summed the abundance for each of six food categories: (1) aquatic invertebrate remains; (2) Bryophyta; (3) Characeae; (4) filamentous algae; (5) seeds of vascular plants, and (6) vegetative parts of vascular plants. This resulted in 6 observations for each of the 150 samples. Next, we ran a linear model with log(x+1)-transformed abundance as the dependent variable, and fish species, the number of fish in the tank and fish size as the independent factors. As water temperature and month covaried, we ran two separate models including either a 2nd order polynomial for temperature or the 3rd order polynomial for month. Polynomials were used to account for non-linearity and models with polynomials had much improved model fits (lower AIC values) compared to linear models. Next, we also included food category and the interaction between fish species, food category, and either temperature or month. The model summaries for each of the two models are given in **Tables S1**,**S2**.

To test whether the three fish species foraged in different microhabitats, we tested for difference in the habitat preference of egested invertebrates. Substrate preference of the prey groups is derived from information in Verdonschot (1990) on habitat and mode of locomotion and checked with information in Verberk et al. (2012). We first summed the abundance of invertebrates for each of three habitat preference categories: (1) sediment; (2) open water, and (3) plants. For taxa which fell into two habitat preference categories, their abundance was equally divided among both categories. Next, we ran three separate linear models, one for each category, with log(x+1)-transformed abundance as the dependent variable and fish species as the independent variable. In these comparisons a significance level of $0.05/3 = 0.0167$ was used to account for multiple comparisons.

To unravel the relative impact of plant traits, fish-related traits and abiotic factors on the egestion of viable seeds we used a General Linear Mixed Model (GLMM) approach. As response variable we used the presence or absence of a plant species' seed in the feces. For each sample, i.e., each unique combination of fish species, month of sampling and replicate, a plant species was considered present if one or more viable seeds of that species were found. Note that for most samples there was either no viable seed or only one seed of a particular plant species. Therefore, rather than analyzing abundance, a response variable in terms of presence/absence was deemed more appropriate and this was analyzed using GLMM with a binomial distribution. Initially we also tried to model number of viable seeds with either negative binomial or poisson distribution, including zero-inflated versions, but all these models failed to converge. Only plant species with at least one viable seed in either combination of fish species, month and replicate were included in this analysis. Thirteen plant species met this criterium.

The plant species-specific traits included were seed mass and floating capacity (buoyancy), obtained from the D3 (Hintze et al., 2013) and LEDA trait base (Kleyer et al., 2008). In addition to these functional traits, we included species abundance in the standing vegetation (mean value of the abundance measure in each trajectory) and abundance in the sediment propagule bank (mean number of propagules in the nine samples) as explanatory variables. Fish-related "traits" were number, estimated size and species-identity of the fish in each sample. Water temperature was included as environmental parameter. A check on multicollinearity by visual inspection of plots and calculated correlation coefficients ($<0.5$ considered as acceptable) indicated no objections against this selection of variables. Prior to analyses, mean abundance in the propagule bank was log (x+1)-transformed. All continuous explanatory variables were standardized to zero mean and unit variance prior to analysis.

In our GLMM, we followed the approach of Jamil et al. (2013). In this approach, significance of trait-environment relationships is addressed while simultaneously including plant species and samples as random factors to avoid the problem of pseudo replication and heteroscedastic variance. In model selection, we followed the tiered forward selection procedure as recommended by Jamil et al. (2013). We started with a simple model that only included a fixed intercept and random intercepts for species and sample (i.e., a unique combination of replicate, fish species and sampling date); we subsequently added a new term, as a main term and in interaction with fish species. Interaction of size-class and number of fish with fish species could not be included since these were non-orthogonal. Before a new term was added, we removed the non-significant terms, but kept a non-significant main term in the model if its interaction was significant. Significance of an additional term was assessed by a likelihood ratio test and difference in Akaikes Information Criterium. Models were run and compared with the package lme4 (Bates et al., 2015) in the open source statistical software R (R Development Core Team, 2017).

# RESULTS

## Dietary Composition of Fish

Based on the fecal samples, fish consumed filamentous algae, vegetative parts of vascular plants, mosses and Characeae, generative parts of vascular plants and Characeae, and invertebrates (**Figure 1**, **Table S3**, **Figure S2**). Filamentous algae were found in large numbers. Fragments of vascular plants and mosses comprised 7 and 11 species, respectively; leaves and stems of *Elodea nuttallii* were most abundant (**Figure S2**). Seeds were found from 16 vascular plant species (**Table 1**). In total, 108 taxa of invertebrates were detected of which planktonic crustaceans (*Daphnia* and *Cyclops* species), larvae of midges (*Chironomidae*), and caddisflies (*Trichoptera*), amphipod crustaceans (*Gammarus* sp.) and *Mollusca* occurred in high numbers (**Table S3**).

Redundancy analysis revealed that month (5%, $F = 7.7$, $P = 0.008$), fish species (4.5%, $F = 7.1$, $P = 0.008$), and fish size (1.4%, $F = 2.9$, $P = 0.048$) were the most significant variables to

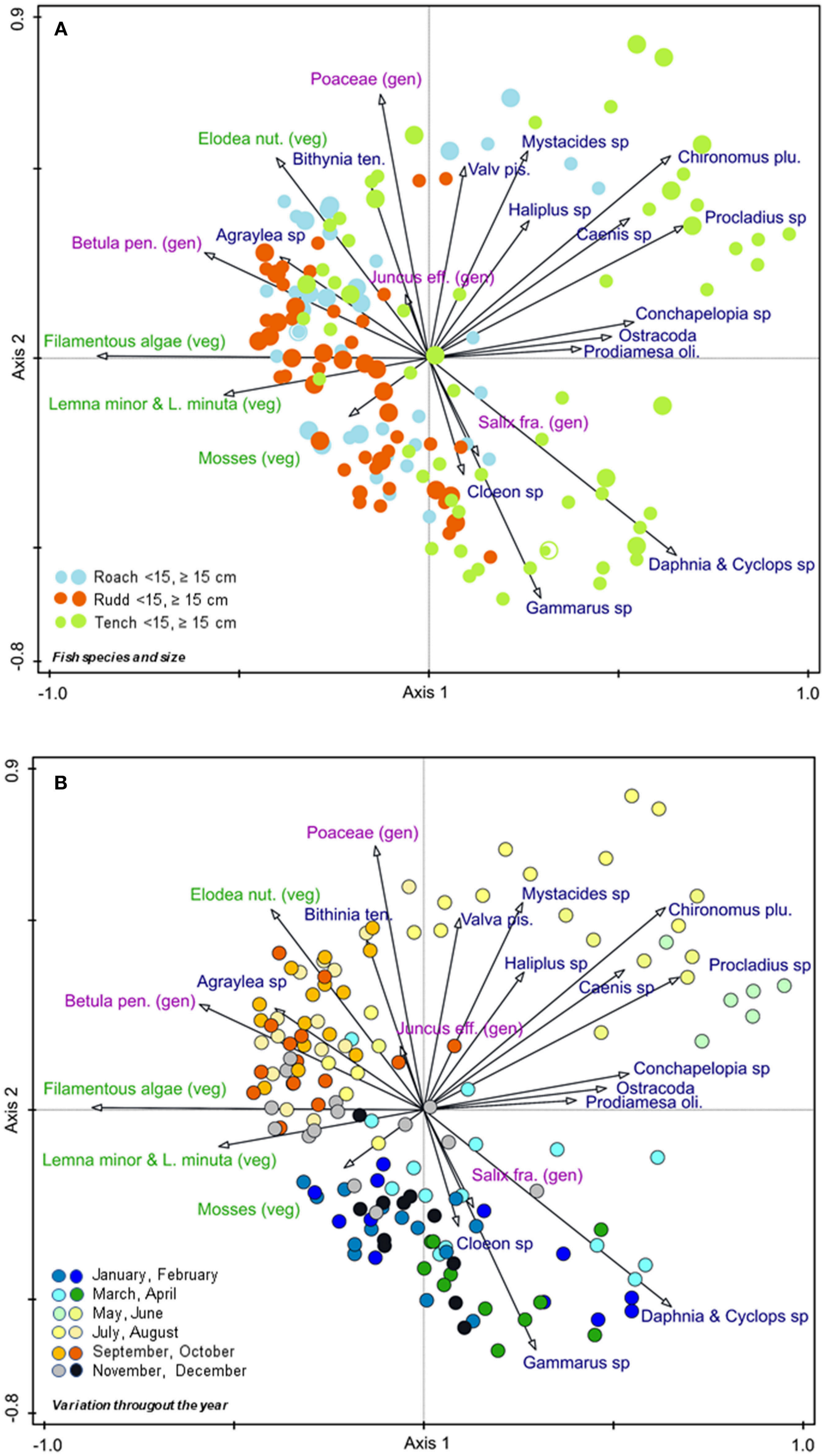

**FIGURE 1 |** PCoA-diagrams for the first two axes, showing the relative positions of samples and taxa. In **(A)** samples are classified by fish species and fish size, in **(B)** by month. Eigenvalues and explained variation of axis 1 and 2 are 0.178 and 17.8%, and 0.155 and 15.5%, respectively. For full names of taxa, see **Table S1**. Gen, generative propagules (pink); veg, vegetative propagules (green), items in blue are invertebrates.

explain variation in dominance of egested species across our 150 fecal samples.

Vegetative plant parts were mainly eaten from July to October, seeds of grasses (*Poaceae*) in summer, larvae of *Chironomidae* in May and June, and *Gammarus* sp. and planktonic crustaceans in early spring (**Figure 1B**).

Numbers of egested items for the different food categories showed seasonal differences that were distinct for the three fish species (**Figure 2A**, **Table S1**). Invertebrates were egested most in spring and early summer, especially by Tench. In summer and autumn, vegetable food categories were eaten most, especially filamentous algae, but also plant seeds. Toward winter, consumption and egestion was generally lower. When plotting the same data against temperature (**Figure 2B**), fish generally started egesting more food items with increasing water temperature, although this varied both with fish species and species-group being consumed (**Tables S1**, **S2**). Rudd and Roach were clearly the two most herbivorous species, with Rudd becoming more herbivorous with increasing water temperature, egesting many vegetative parts of vascular plants. For Tench and Rudd, a higher consumption of invertebrates was accompanied by an increase of seed uptake, but no such relationship was

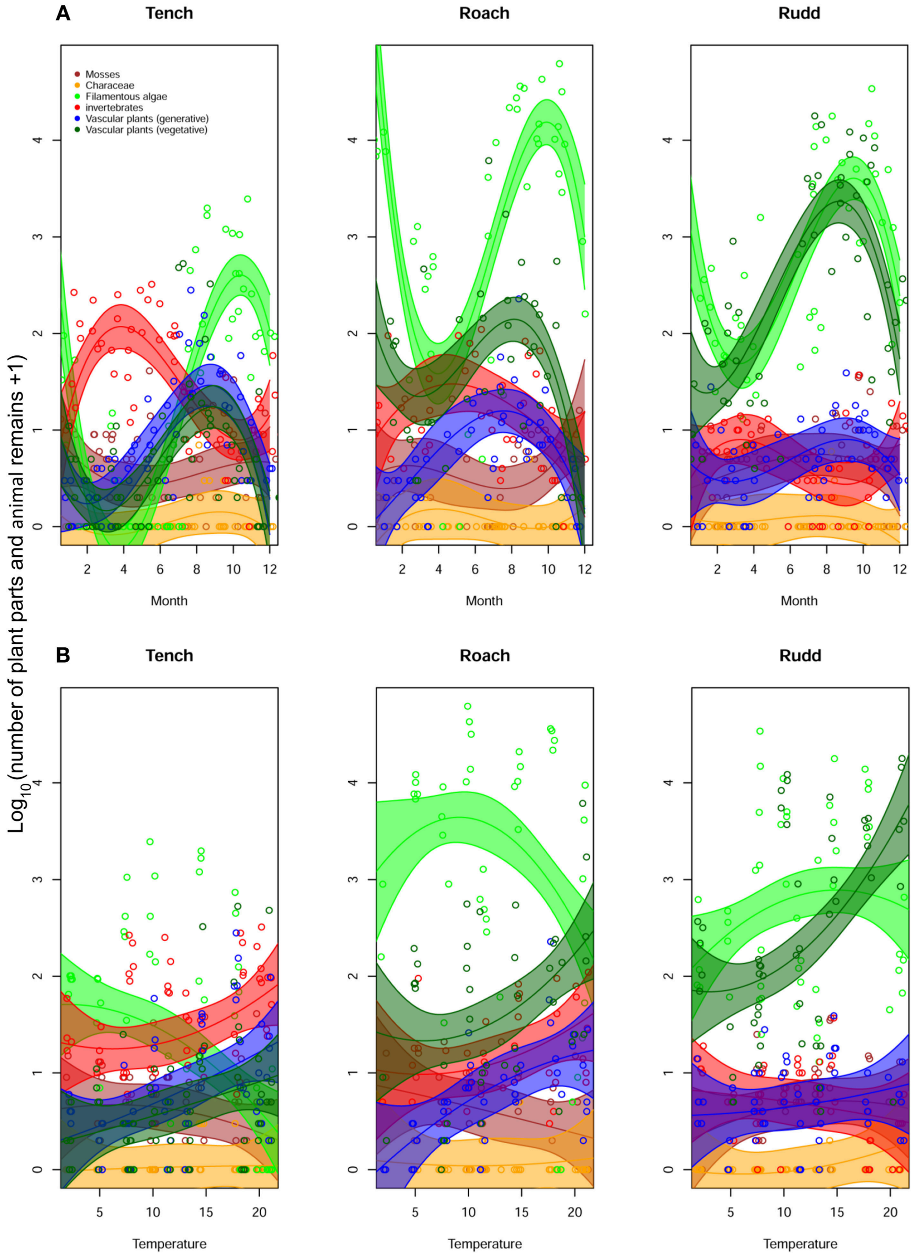

**FIGURE 2 |** Variation in the number of plant parts and animal remains of taxonomic groups throughout the year **(A)**, and in relation to water temperature **(B)** for each of the three fish species.

apparent for Roach (**Figure 2**). Characeae appeared to be low in abundance, irrespective of water temperature or fish species, whereas Bryophyta were egested less with increasing water temperature.

## Relation Between Vegetable Items and Their Abundance in the Vegetation, Propagule Bank and Fish Feces

Plant species that were egested by fish, originated both from the standing vegetation and from the sediment propagule bank. The vegetation comprised 111 vascular plant species, 1 Charophyte and 12 mosses; the propagule bank comprised propagules from 37 vascular plants and 5 mosses (**Table S4**).

In total, 22 vascular plant species, 1 Charophyte and 12 mosses were retrieved and identified from the fish feces (**Table 1**, **Table S3**). All vascular plant species and the charophyte *Nitella mucronata* found were present in the vegetation, whereas four moss species (*Bryum gemmiferum*, *Fissidens fontanus*, *Oxyrrhynchium speciosum,* and *Schistidium crassipilum*) were found in the feces, but were not detected in the vegetation (**Table 1**).

Plant species composition and abundances differed between the vegetation and the propagule bank (**Figure 3**) and this difference was captured by the 2nd ordination axis. The three fish species were also differentiated along this 2nd ordination axis, and Tench showed the highest similarity between egested generative propagules (i.e., seeds of vascular plants) and species abundances in the benthic propagule bank, whereas Rudd showed the highest similarity between egested vegetative plant parts and species abundances in the vegetation (**Table 3**). The main difference (i.e., the 1st ordination axis) reflected that only a small subset of the plant, algae and moss species that were egested by fish had propagules present in feces in large numbers (**Figure 3**).

Leaves of *E. nuttallii* and threads of filamentous algae growing in the water column dominated the fecal samples, especially for Roach and Rudd (**Figure 3**). Other common species growing in the water column such as *Callitriche obtusangula*, *Ceratophyllum demersum*, and *Sagittaria sagittifolia*, were absent or occurred less frequently in the feces (**Table 1**, **Figure 3**). Free floating duckweeds (*Lemna minor* and *L. minuta*) were egested most by Rudd and Roach (**Figure 3**). Other common floating plants such as *Lemna trisulca* and *Spirodela polyrhiza* were found only infrequently (**Table 1**, **Table S3**).

In the sediment layer, the propagule bank is dominated by seeds of *Juncus effusus*, *Betula pendula*, several grasses (*Phalaris arundinacea* and *G. maxima*), *Urtica dioica* and *Sparganium emersum*. Seeds of *Veronica catenata* were also frequently present (**Figure 3**, **Table S4**, **Figure S3**). Reflecting their high abundance in the propagule bank, seeds of *J. effusus*, *B. pendula,* and grasses were most frequently retrieved from the feces of the fish (**Table 1**, **Table S3**). Significantly more seeds from *J. effusus* and grasses were retrieved from the feces of Tench than those of Rudd (**Figure 3**; ANOVA, $P < 0.05$).

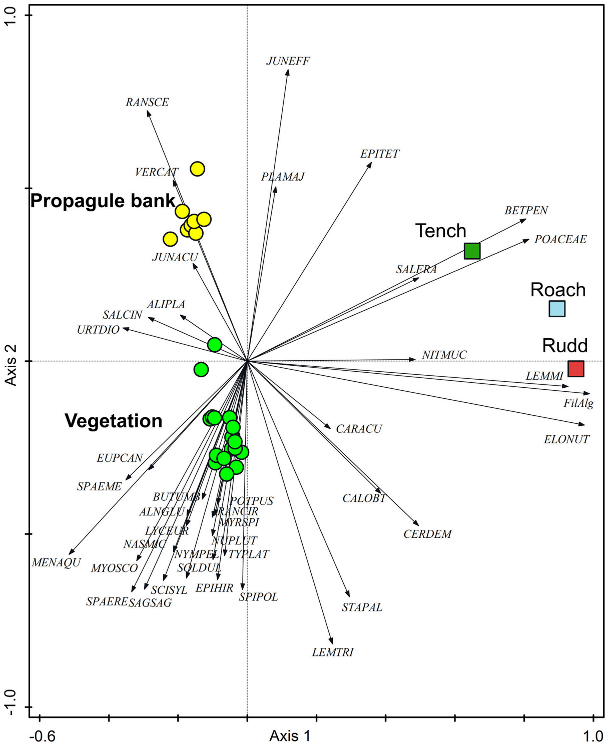

**FIGURE 3 |** PCoA-diagram for the first two axes, showing the relative positions of vegetation (green) and propagule bank (yellow) samples, of the main plant species (arrows) and of the samples with total number of plant parts found in feces of each fish species (squares). Eigenvalues and explained variation of axis 1 and 2 are 0.357 and 35.7%, and 19.5 and 19.5%, respectively. Abbreviations of species names: first three letters of the genus name, followed by the first three letters of the species name (for full names see **Table S2**).

## Viable Plant Parts Found

In total, 88,579 vegetative fragments of vascular plants, 316 fragments of mosses and 14 fragments of Charophytes were identified of which <<1, 53.5, and 0% remained viable, respectively (**Table 1**). Viability of filamentous algae was not established. Thirteen vascular plant fragments, representing 4 species (*E. nuttallii*- **Figure S5**, *Lemna minor*, *L. trisulca*, *S. polyrhiza*) regenerated from the feces of Rudd, whereas in Roach and Tench only a single vegetative propagule appeared viable. For mosses, 52.6% fragments, representing 6 species, regenerated from feces of Roach, 45.1% fragments (7 species) from feces of Rudd, and 62.7% (9 species) from feces of Tench (**Figure S3**). *Bryum argenteum* was the most common moss species and constituted 85% of the viable moss fragments in fish feces (**Table 1**).

In total, 1,787 generative propagules of vascular plants were identified of which 6.7% germinated, representing 15 species (**Table 1**). For Roach, these numbers were 528 (identified), 4.2% (germinated), and 9 (species), respectively; for Rudd: 257 (identified), 11.7% (germinated) and 9 (species); for Tench: 1003 (identified), 6.8% (germinated), 10 (species). Seeds of *J. effusus* germinated in highest numbers. From the Charophyte *N. mucronata* viable oospores were found in feces of Roach and

Rudd (**Table 1**). We detected seasonal patterns in egestion of grasses, *J. effusus* and *B. pendula*, but not of Bryophyta (**Figure 4**).

## Factors Related to Egestion of Viable Seeds

Plant species were more likely to be dispersed, or at least egested as viable seeds, if fish were bigger and water temperature was higher (**Table 2**). The temperature effect was similar for all three species, i.e., we found no significant fish-water temperature interaction. Plant specific factors that increased the probability of fish-mediated dispersal were abundance in the seed bank and buoyancy of seeds. The beneficial effect of seed buoyancy was fish species-dependent (**Table 2**). As indicated by the coefficients of the interaction terms, seeds that float for a longer time had a higher change of being dispersed in case of Rudd, but buoyancy seemed to have no such beneficial effect with either Roach or Tench as seed vectors. However, mean probability of being dispersed was rather low, with modeled estimates never exceeding a 10% probability (**Table 2**).

Compared to the regional species pool, i.e., all the different plant species present in either the water or shoreline vegetation (**Table S4**), ichthyochory seemed to be prevalent for a subset only, comprising especially plant species with seeds that have both a relatively high buoyancy and a relatively small size (**Figure 5**). As indicated by high similarity in trait distribution between the benthic seed bank and fish feces (**Figure 5**), traits that facilitate incorporation in the seed bank may also facilitate dispersal by the fish.

## DISCUSSION

Successful dispersal of plants requires that seeds are ingested and survive their journey in the intestinal tract before being egested. In our previous work (Boedeltje et al., 2015), we addressed how seeds differ in their ability to survive the process of ingestion, mastication and digestion, showing that survival depended both on traits of the plant seed (e.g., related to size and seed hardness) and traits of the fish (e.g., related to mechanical or chemical digestion). Since most studies employ pellets of fish food in which plant seeds were enclosed, an open question is whether fish actually ingest seeds under natural conditions in temperate waters. Here, we have demonstrated that a range of generative and vegetative propagules are being consumed under natural conditions by temperate freshwater fish (**Table 1**). The quantity and type of propagules consumed differs seasonally and may partly depend on their availability in the field and the temperature of the stream water. Moreover, there were differences across the three fish species with respect to the strata in which they foraged (water layer, sediment or vegetation) and with respect to their preference for either plants or invertebrates (**Figures 1**, **2**). A caveat of the current method is that diet is inferred from egested plant and animal remains. The food items digested beyond recognition were, therefore, missed in our counts, especially in the case of prey without bones or chitin (Garvey and Chipps, 2012). While flushing of the stomach could have given a more complete view of what food items are being ingested by the fish, we opted for fecal collection as this provides unambiguous evidence for the ability of plant propagules to survive ingestion and digestion.

For Rudd and to a lesser extent for Roach and Tench, vegetative and generative plant parts were mainly retrieved from July to October, whereas in winter and spring the feces were dominated by animal remains. This indicates that fish shifted their feeding strategy from being mostly carnivorous to being mostly herbivorous in a seasonal basis, corroborating previous observations in both Roach (Brabrand, 1985) and Rudd (Guinan

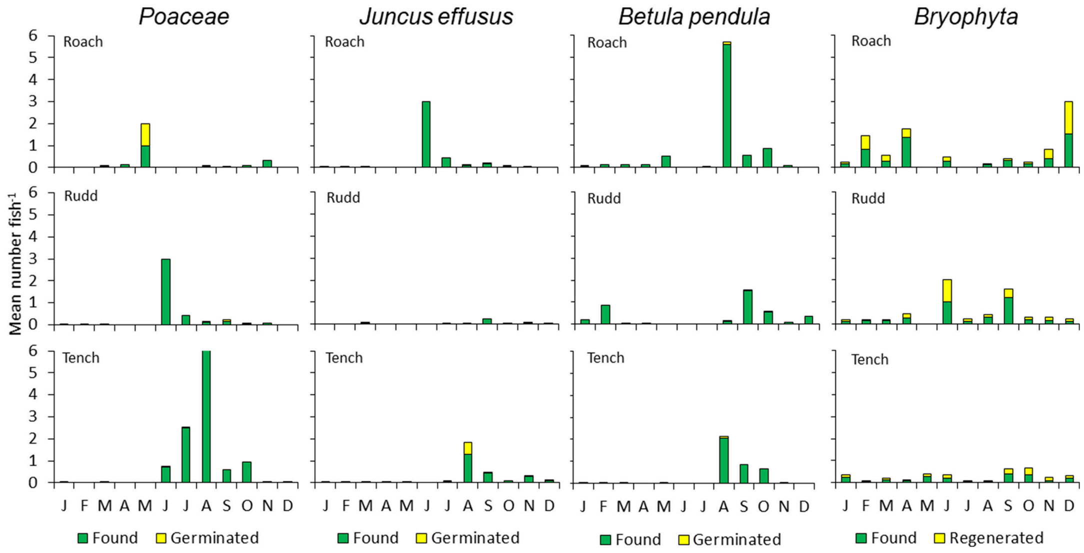

**FIGURE 4 |** Monthly mean number of seeds and bryophyte fragments per fish for Roach, Rudd, and Tench. The proportions of germinated seeds or regenerated fragments are indicated for seeds or fragments of grasses (*Poaceae*), *Juncus effusus*, *Betula pendula* and *Bryophyta*. J–D (January–December, months during the research year).

**TABLE 2 |** Coefficients and their significance of the binomial GLMM-model best explaining the presence of plant species (as a viable seed) in feces of Rudd, Roach, and Tench.

| **EFFECT** | | |
|---|---|---|
| ***Random*** | **Variance** | **SD** |
| (1\|sample) | 0.16 | 0.40 |
| (Water temperature \| plant species)-intercept | 0.55 | 0.75 |
| (Water temperature \| plant species)-slope | 0.03 | 0.16 |
| ***Fixed*** | **Estimate** | **SE** |
| Intercept | −4.17 | 0.39*** |
| Water temperature | 0.51 | 0.20* |
| Number of fish | n.i. | |
| Fish size | 0.26 | 0.11* |
| Abundance in the sediment seed bank | 0.58 | 0.22** |
| Abundance in the vegetation | n.i. | |
| Seed mass (mg) | n.i. | |
| Seed buoyancy (days) | −0.17 | 0.43 |
| Fish species-Rudd | 0.14 | 0.36 |
| Fish species-Tench | 0.48 | 0.33 |
| Seed buoyancy * Fish species-Rudd | 1.00 | 0.42* |
| Seed buoyancy * Fish species-Tench | 0.73 | 0.41† |

*All random and fixed main effects included in the model selection with tiered forward selection are indicated. For fixed effects, estimates refer to estimated regression coefficients, their standard error and significance. Except size and number of fish, all the other main terms were also tested in interaction with fish species, but only coefficients for significant interaction terms, and their constituent main terms, are indicated here. Main and interaction terms for Rudd and Tench are relative to Roach, therefore no separate coefficients for Roach are indicated. Coefficients for mean abundance in the seed bank are based on natural log-transformed values. All the tested models included "plant species" and "samples" as random intercepts. Total variance explained by this model ($R^2$-conditional) was 36 %. Levels of significance: † -marginally significant $P < 0.10$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; n.i., not included in the optimal model due to lack of significance.*

et al., 2015). Even Tench, the fish which was least herbivorous, consumed more food items of plant origin during the warm summer and autumn months. Increasing water temperature and more abundant growth of aquatic plants (**Figure 2**) are the likely mechanisms underlying this transition to herbivory (cf. Guinan et al., 2015). As *Cyprinidae*, including Rudd, Roach, and Tench, lack organs for the fermentation of cellulose by microorganisms, herbivory may only be energetically beneficial at elevated water temperatures when cellulolytic activity is higher in aquatic ecosystems (Niederholzer and Hofer, 1979; Behrens and Lafferty, 2007; Guinan et al., 2015). Plants also differ from each other in stoichiometry, having different carbon to nitrogen ratios. Stoichiometric considerations would predict that the higher energetic requirements in warmer water allows for use of food sources that are low in metabolizable nitrogen content relative to carbon-rich compounds like fat or carbohydrates which can be metabolized to generate energy (see Klaassen and Nolet, 2008). It would therefore be interesting to consider plant stoichiometry in future dispersal studies.

Our results indicate that all three fish species may contribute to plant dispersal, but may differ in their dispersal propensity across plant species. Differences in the consumption of either vegetative or generative propagules could be partly related to differences in foraging strategy. We could broadly confirm the different foraging strategies reported in the literature: Tench foraged most on benthos, consuming more animals that preferred the sediment (**Figure 2**, **Figure S6**) and showing a greater propensity to eat seeds present in the propagule bank (**Figure 3**, **Tables 1**, **3**), although the propagule bank was sampled in March when Tench fed most on animal prey. Roach and Rudd foraged most in the water column, consuming mainly vegetative plant parts and Rudd appeared to forage more on the water surface, eating more floating seeds (**Table 2**) and free-floating duckweeds. Still, there appears to be substantial overlap in diet across fish species, especially when all seasons are considered, suggesting that they are rather opportunistic feeders and therefore all three of them can contribute to dispersal of a broad range of plant species.

With regards to vegetative parts, *E. nuttallii* and filamentous algae were dominant in the feces of Roach and Rudd while other plant species such as *C. demersum* and *L. trisulca* were largely absent from their feces, despite being common in the vegetation. The absence of these latter plants from the feces could, in theory, be explained by being fully digested. However, it is more likely that certain plant species are more palatable and therefore preferentially consumed. As experiments with Rudd (Lake et al., 2002; Kapuscinski et al., 2014) and generalist snail herbivores (Grutters et al., 2017) have shown, the consumption rate of aquatic plants (e.g., *Ceratophyllum demersum*) with high chemical defense (e.g., phenolic content) and low nutritional quality is much lower than those of plant species with low defense and high nutritional quality (e.g., *Nitella flexilis*, *Elodea canadensis, E. nuttallii*). This implies that plant palatability increases the probability of being vegetatively dispersed, provided that propagules remain viable after gut passage. In our study, the survival of vegetative vascular plant parts after gut passage was extremely low (<<1%). We observed the regeneration (regrowth into viable parts) from two stem fragments of *E. nuttallii* (**Figure S4**), whereas regeneration from leaves of this species (which were by far the dominant organs in the samples) did not occur. Furthermore, only 11 duckweed plants with roots, stems and leaves, present in 5 samples with a total of 46 fish, were found to be viably egested. Although regeneration of egested vascular plant fragments was very low, certain palatable vascular plants were eaten frequently and could therefore be dispersed via ichthyochory.

In contrast to vascular plants, 53.5% of the vegetative bryophyte fragments regenerated. Nurminen et al. (2003) has previously shown bryophytes to be part of the diet of Rudd, but these authors did not establish viability of egested fragments. Our study thus presents the first evidence that ichthyochory may significantly contribute to vegetative dispersal of several aquatic and riparian moss species, confirming earlier assumptions by Glime (2017) and complementing dispersal by hydrochory (Boedeltje et al., 2019). Given the small size (0.2–0.4 mm) of egested moss fragments, and that they were found throughout the year, we hypothesize that bryophytes were ingested when fish were foraging in the soft sediment (including the propagule bank with moss fragments) or feeding on invertebrates living in moss cushions on submerged stones in the stream (*cf.* Glime, 2017).

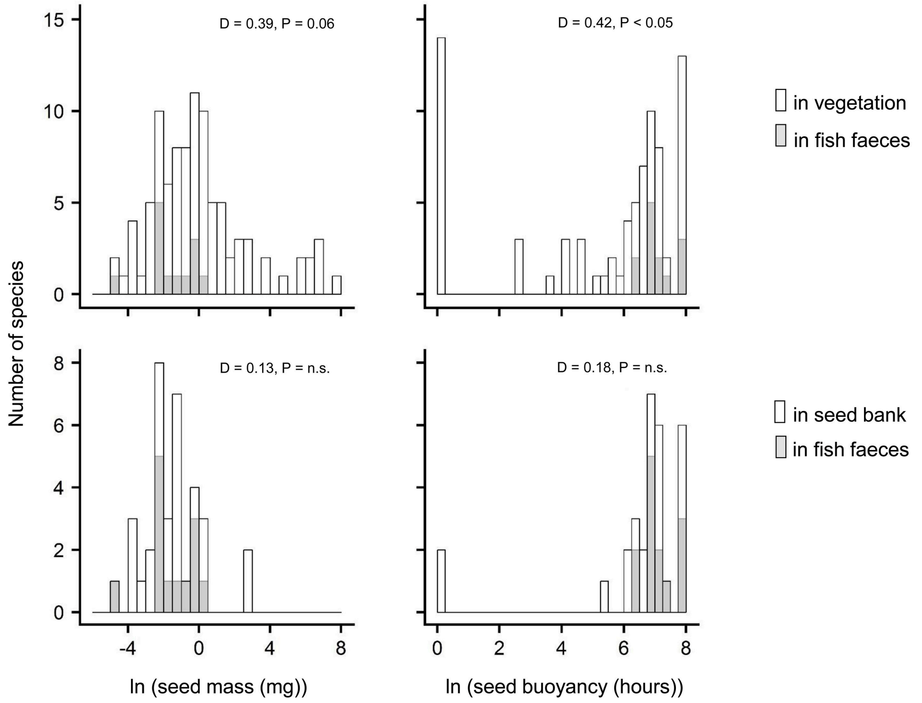

**FIGURE 5 |** Frequency distribution of seed mass and seed buoyancy for number of vascular plant species in fish feces, vegetation, and the seed bank. Distributions were compared with Kolmogorov's two sample test; outcomes are indicated by the *D*-values and their significance in each panel. n.s. = not significant.

Vegetative dispersal is also likely for filamentous algae. Although we did not test the viability of egested filamentous algae, it is likely that at least some algal threads remained viable. Filamentous algae retained their green color and relatively firm structure after gut passage, corroborating the assertion that they are not affected by mechanical or chemical processing in *Cyprinidae* (Sibbing, 1991). Vermeij et al. (2013) also demonstrated that red and green macroalgae remained viable after gut passage in herbivorous coral reef fishes.

Previous studies have highlighted bird mediated vegetative dispersal of aquatic ferns and vascular plants (Lovas-Kiss et al., 2018; Silva et al., 2018) and bryophytes (Wilkinson et al., 2017) complementing our findings that fish may also be vectors for vegetative dispersal of aquatic plant species. This suggests that endozoochory of plant fragments is a widespread but overlooked dispersal pathway.

With regard to generative propagules (i.e., seeds of vascular plants), we observed a clear peak in summer for egested seeds from grass species (mainly *Poa trivialis* and *G. maxima)*. This peak can be related to the period of seed shedding and hydrochorous dispersal of *P. trivialis* and *G. maxima* (Boedeltje et al., 2004). Fish likely have actively foraged on the seeds of these grasses, as they are rich in nutrients (Hintze et al., 2013). Moreover, we found that buoyancy positively affected the probability of occurrence in the feces (**Table 2**), suggesting that fish may have preferentially foraged upon seeds floating at the water surface or in the water column, which seems most likely for Rudd. Tench likely consumed seeds of grasses and *B. pendula* after they were incorporated into the sediment propagule bank. This also matches with grass seeds being retrieved early in the season for Rudd, and later in the season for Tench (**Figure 4**).

**TABLE 3 |** Similarity percentages for egested vegetative and generative propagules and the species pool of the vegetation and propagule bank, respectively.

| | Similarity percentages | | | |
|---|---|---|---|---|
| | **Vegetation** | | **Propagule bank** | |
| | **veg. prop.** | **gen. prop.** | **veg. prop.** | **gen. prop.** |
| Roach | 13.4 | 30.5 | 10.2 | 16.2 |
| Rudd | 22.2 | 30.9 | 9.5 | 19.9 |
| Tench | 20.1 | 31.5 | 11.2 | 22.9 |

In contrast to the active foraging on grass seeds, seeds of *J. effusus* where abundantly present in the sediment propagule bank and numerous small (0.5 mm) seeds of this species were retrieved from the feces of Tench throughout the year. The egested seeds germinated to a large extent, indicating that they were not crushed and digested. Cyprinid fish have pharyngeal teeth to crush and grind food but lack chemical digestion (Sibbing and Witte, 2005), making seed hardness an important trait to survive gut passage (Boedeltje et al., 2015). While seeds of grasses such as *G. maxima* are relatively soft and comparable with those of the soft-seeded *Carex pseudocyperus* (Boedeltje et al., 2015), they were likely crushed and digested, but the small and somewhat harder seeds of *J. effusus* could have slipped intact through the pharyngeal teeth. Germination percentages observed in this study for *J. effusus* were comparable to those observed after gut passage in common carp (Boedeltje et al., 2016).

The probability of finding a viable seed in the feces of fish was higher in large than in small fishes, indicating that the potential for effective seed dispersal might increase with body size. This may reflect ontogenetic differences in diet as small juvenile cyprinids feed mostly on invertebrates and switch to a more herbivorous diet as they grow (e.g., Nurminen et al., 2003). This result is in accordance with the findings of Galetti et al. (2008) and Costa-Pereira et al. (2017) for tropical fish. However, in temperate streams a larger dispersal potential of larger fish might apply only to small, relatively hard seeds. As bite force of *Cyprinidae* increases with age, dispersal of large, relatively soft seeds becomes increasingly unlikely in larger fish (Boedeltje et al., 2015).

Whether fish are effective vectors for long-distance plant dispersal depends also on the retention time in the digestive tract of the fish (Pollux et al., 2006a) and the distances it can travel during that time. Migration in fish may occur at a range of spatial and temporal scales, from diel migration among habitats to seasonal migrations on a landscape level (Lucas and Baras, 2001). All three species studied here have been reported to migrate seasonally between a tributary to the main river (Hohausová et al., 2003; Pollux et al., 2006b; Nunn et al., 2010). During winter, diurnal migration between a floodplain lake and a connected channel was observed for Roach (Heermann and Borcherding, 2006). Dispersal by fish may therefore enable upstream dispersal. This could explain why population genetic diversity, which is expected to increase downstream as a result of hydrochorous dispersal without upstream compensation, was not necessarily smaller upstream in a Belgium river (Honnay et al., 2010; Wubs et al., 2016). In addition, larger distances between stream networks may be mediated by secondary dispersal where fish and the plant seeds therein are consumed by piscivorous birds (van Leeuwen et al., 2017).

The first step to successful dispersal is ingestion by fish. Our results show ingestion is affected by water temperature and season, palatability of plant vegetative parts, fish species and fish size, and seed traits such as buoyancy, size, and seed hardness. Even the most carnivorous species included in our study (Tench) may contribute to plant dispersal as it forages preferentially on seeds in the sediment, and seeds are more likely than vegetative propagules to survive the journey in the intestinal tract of fish. Despite substantial dietary overlap, three different fish species can overlap in their roles as vectors for the dispersal of a range of moss species and vascular plants with palatable shoots and with small-sized, floating, hard seeds.

## ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the research organization Reptiles, Amphibians, and Fish Research of the Netherlands, RAVON, Nijmegen, The Netherlands.

## AUTHOR CONTRIBUTIONS

GB conceived and designed the research, collected and identified seeds and vegetative vascular plant parts, performed the germination and regeneration experiments, sampled the vegetation and propagule bank, and processed and partly analyzed and interpreted data. BK identified invertebrates and analyzed part of these data. MS and MdV collected fishes by electrofishing (**Figure S1**). PS identified the mosses. JL and WV analyzed and interpreted data. GB led writing the manuscript. BK, PS, JL, and WV contributed to drafts. All authors gave approval for submission.

## FUNDING

The authors acknowledge the *Prins Bernhard Cultuurfonds*, section *Meester Prikkebeenfonds*, for supporting this research through a grant to GB.

## ACKNOWLEDGMENTS

We wish to thank Arthur de Bruin for his support during the initial phase of the research work and the tug of war club *Heure* for making a part of their clubhouse available as a field station for housing the fish. We thank André van Aalst for his expert help in housing the fish, Bastiaan van Zuidam for his help during sampling of the propagule bank, and the colleagues of the greenhouse of Radboud University for assistance. The suggestions for improvement, given by two reviewers and Andy Green, are highly appreciated.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2019.00054/full#supplementary-material

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Ecology and Evolution

HYPOTHESIS AND THEORY
published: 19 March 2019
doi: 10.3389/fevo.2019.00070

# Driver's Seat: Understanding Divergent Zoochorous Dispersal of Propagules

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The functional role, relative importance, and the spatial and temporal parameters of different vector species, which underpin the passive dispersal (zoochory) of organisms (or their propagules), are frequently poorly understood. Accordingly, a conceptual framework capable of providing a rigorous and unified assessment for the dispersal capacity of vector species is required. Here, we propose and apply a series of novel metrics, the Dispersal Potential (DP), the Relative Dispersal Potential (RDP), and the Combined Dispersal Potential (CDP), to predict and classify likely dispersal and vector importance. In essence, DP = Np × Tv, whereby Np is the *per capita* propagule load (e.g., mean, minimum, or maximum abundance) or species richness of propagules carried per individual vector species, while Tv is the total number of possible vectors (e.g., individuals of a single species at a source site, local scale abundances, or entire continental populations). Further, the ratio based metric RDP allows for DP comparison between species, while the CDP accumulates the DP of a variety of vector species. An additional Relative CDP (RCDP) metric facilitates comparison between the CDP for multiple vectors to that of one or more additional vectors. The proposed metrics can also be used to assess intraspecific differences (e.g., ontogeny). Accordingly, we examine a variety of case studies and present calculations to ascertain the usefulness of our proposed metrics. Overall, the metrics can be used to quantify and rank the prominence of different dispersers that facilitate biological connectivity. Finally, we argue that adoption of these metrics and variants thereof, will provide a more realistic measure of species' functional roles than examination of interaction intensities alone, which will enhance understanding of zoochory within and across dispersal networks.

**Keywords: ectozoochory, endozoochory, frugivory, propagules, dispersal networks, secondary dispersal, vector**

**Edited by:**
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**Specialty section:**
This article was submitted to
Biogeography and Macroecology,
a section of the journal
Frontiers in Ecology and Evolution

**Received:** 30 September 2018
**Accepted:** 25 February 2019
**Published:** 19 March 2019

**Citation:**
Coughlan NE, Dickey JWE,
Cuthbert RN, Kelly TC, Jansen MAK
and Dick JTA (2019) Driver's Seat:
Understanding Divergent Zoochorous
Dispersal of Propagules.
Front. Ecol. Evol. 7:70.
doi: 10.3389/fevo.2019.00070

## INTRODUCTION

Dispersal is an essential ecological process which impacts biodiversity through metacommunity and population dynamics across multiple spatial scales (Leibold et al., 2004; Shurin et al., 2009; Moritz et al., 2013). In particular, dispersal events can impact species establishment, demographic viability, and eco-evolutionary dynamics (Trakhtenbrot et al., 2005; García et al., 2017). Moreover, assisted dispersal away from the natal site can increase species distribution, aid enemy-release, and reduce both inter- and intraspecific competition for resources (Corlett, 2017). The assisted transport of plants and invertebrates by more mobile organisms (zoochory) is considered to be a key mechanism for both long- and short-distance dispersal, and can be essential to the maintenance of connectivity between isolated habitat patches on a metacommunity scale (Nathan, 2006; Viana et al., 2016; van Leeuwen, 2018). However, despite the ecological importance of assisted dispersal, current knowledge of zoochorous interactions within terrestrial (Corlett, 2017; Hämäläinen et al., 2017; Castañeda et al., 2018; Steyaert et al., 2018), and aquatic ecosystems (Tesson et al., 2015; Coughlan et al., 2017a,b; Lovas-Kiss et al., 2018a,b,c), frequently remains limited.

To date, the majority of studies examining animal-mediated dispersal have focused on the transport of fleshy fruits in terrestrial systems (**Figure 1**; Czarnecka and Kitowski, 2013; Coughlan et al., 2017a; Bartel et al., 2018; Lovas-Kiss et al., 2018c). Despite this, evidence suggests that the assisted dispersal of whole organisms or propagule stages that lack a fleshy fruit, e.g., seeds, spores, eggs, ephippia, gemmules, statoblasts, or cysts, frequently occurs through either endozoochory (internal transport within the digestive system: Pellerin et al., 2016; Lovas-Kiss et al., 2018b,c) or ectozoochory (external adherence; synonyms epizoochorous, exozoochorous: Coughlan et al., 2017a; Lovas-Kiss et al., 2018a). However, whilst frugivory-based dispersal networks have received substantial scientific attention, many basic questions concerning the relative importance of individual frugivorous vector species, propagule survival, and likely dispersal distances, frequently remain unanswered (Corlett, 2017). For example, due to the destruction of seeds or lack of post consumption movements, consumers of fruits may not necessarily act as viable seed dispersers (Simmons et al., 2018). These issues are further magnified in non-frugivorous dispersal networks, such as those operating between isolated aquatic habitats, which have been considerably less studied (**Figure 1**; Coughlan et al., 2017a,b; Lovas-Kiss et al., 2018a,b,c; van Leeuwen, 2018).

Although an array of valuable ecosystem services provided by dispersers have been repeatedly documented, the functional role of vector species, even possible keystone dispersers in mutualistic systems, remains poorly studied (Farwig and Berens, 2012; Mello et al., 2015). Moreover, research has revealed that vectors not traditionally associated with the dispersal of certain propagule taxa, frequently facilitate substantial dispersal events (Vanschoenwinkel et al., 2011; Farmer et al., 2017; Hämäläinen et al., 2017; Bartel et al., 2018). Dispersal events can be considered "primary dispersal" when organisms adhere to the external surfaces of vector species, or when dispersers feed directly on seeds, fruits, invertebrates or other propagule structures but fail to digest all of them (Coughlan et al., 2017b; Lovas-Kiss et al., 2018b,c). Additionally "secondary dispersal" can occur when a predator consumes a prey item that has itself already ingested a propagule, resulting in the predator facilitating a transport event (Hämäläinen et al., 2017; van Leeuwen et al., 2017). A predator may also secondarily disperse through the ingestion of propagules found adhering to the external surfaces of prey (Lovas-Kiss et al., 2018a). Thus, animal-mediated dispersal is underpinned by a broad range of specific interactions between disperser and propagule.

Although the number of studies examining zoochory continues to increase (**Figure 1**), in many cases only the abundance, diversity and subsequent viability of propagules recovered from vectors, i.e., propagule load, are reported (Coughlan et al., 2017a). In a more advanced examination of assisted dispersal, particularly in relation to frugivory research, construction, and assessment of dispersal networks has highlighted a complex web of interactions between propagules and potential dispersers (Simmons et al., 2018; Timóteo et al., 2018). In essence, networks are simplified representations of reality which can be used to assess the overall patterns across a complex web of interaction frequencies (Heleno et al., 2014). Although the proportional interactions of both different vector species and propagules are frequently examined, greater consideration of the relationship between assisted dispersal and the temporal or spatial variation of vectors species abundance is required (Gleditsch et al., 2017; Kleyheeg et al., 2017; Andresen et al., 2018; Steyaert et al., 2018). In particular, taxon-specific dispersal and interaction networks should not be considered in isolation. For example, a poor vector species may have a high proportion of interactions with a specific taxon, yet these interactions may lead to almost zero dispersal (Simmons et al., 2018). The extensiveness and success of dispersal events will depend on both the identities and abundance of vector species (Peredo et al., 2013; Rumeu et al., 2017). Moreover, even poor or infrequent facilitators of assisted dispersal may make substantial contributions to the transport of propagules at high vector densities. Therefore, the number of propagules dispersed by each individual is an important component of the dispersal process (Schupp et al., 2010). In particular, even at low densities, large bodied dispersers can have a profound impact on dispersal networks, which is in part due to either their high *per capita* ingestion of propagules (Vidal et al., 2013), or the increased surface area for external propagule attachment. Accordingly, as the number of studies of animal-mediated dispersal continue to increase (**Figure 1**), there is a clear and timely need to begin analyzing dispersal data in a more quantitative manner.

Basic ecological networks facilitate mapping of observed links between vector species and propagule sources. Further development of these networks through the inclusion of a comparable link weight (e.g., interaction frequency or vector biomass) across network matrices has greatly enhanced understandings of dispersal (Vidal et al., 2013; Heleno et al., 2014). However, ecological networks are often devoid of species abundance estimates, combined functional role, vector intraspecific differences, and spatially and temporally resolved network data (Heleno et al., 2014). Despite this, changes in vector species abundance or richness can have a substantial effect on the assisted dispersal of propagules (Pérez-Méndez et al., 2016; Rumeu et al., 2017).

Recently, Comparative Functional Response (CFR) has been proposed as a concept capable of unifying the fragmented discipline of invasion ecology (Dick et al., 2017a,b; Laverty et al., 2017). In essence, the Functional Response (FR; Holling, 1959) can be defined as the relationship between resource

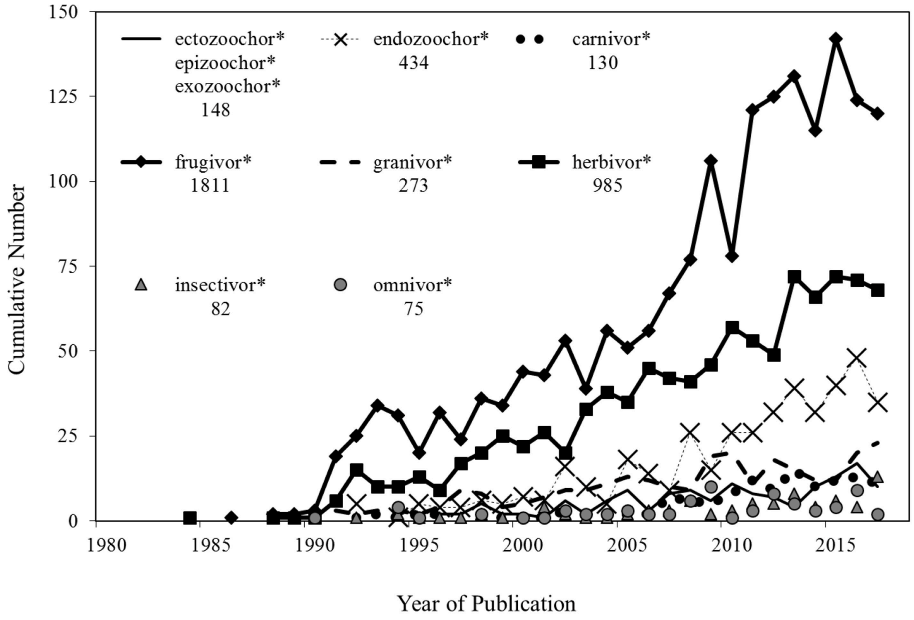

**FIGURE 1 |** The annual cumulative number of zoochory-related publications cataloged within the on-line scientific database Thomson-Reuters *Web of Science*, 1st January 1980–31st December 2017. Data were extracted through a Topic Search using the zoochorous and dietary terminologies shown, which were combined with the additional search terms of "seed," "propagule," "dispersal," and "dispersion" to create a search string. For example: (endozoochor*) AND (seed OR propagule) AND (dispersal OR dispersion). The total number of publications for each examined search string are shown. Ectozoochor* was combined with frequently used synonyms, epizoochor*, and exozoochor*.

availability (e.g., prey density) and *per capita* consumer uptake (e.g., prey consumption rate; Dick et al., 2014, 2017a,b). Further, in developing this classical ecological metric, to improve understanding of resource and consumer uptake relationships, Dick et al. (2017c) proposed a series of novel metrics known as the Impact Potential (IP) and the Relative Impact Potential (RIP), whereby species CFR data is combined with known abundance data to better estimate likely ecological impact. Abundance data is used as a readily measurable proxy for Numerical Response (NR), which describes the population state of an organism in relation to that of a resource (e.g., prey). Ultimately, the ecological impact of an organism on a resource may be best described as the product of the consumer FR and NR see Dick et al. (2017c). Although originally designed to assess and predict the ecological impacts of existing, emerging, and future invasive species, the concepts of IP and RIP are transferable to other ecological scenarios where taxa utilize available resources in a different manner. For instance, the rate at which propagules are taken-up (e.g., ingested) can vary widely with different potential vector species.

To date, many basic questions concerning the extent to which different vectors facilitate dispersal often remain unanswered. However, if propagules capable of surviving vector-mediated dispersal are considered as an acquirable resource, then the ecological concepts of IP and RIP can be further developed to decipher, compare and contrast the functional role, relative importance, and the spatial and temporal dispersal parameters of different vector species. Here, based on the premise of the IP and RIP metrics, we propose an analogous sequence of conceptual metrics: (1) the Dispersal Potential (DP); (2) the Relative Dispersal Potential (RDP); (3) the Combined Dispersal Potential (CDP), and (4), the Relative Combined Dispersal Potential (RCDP). The usefulness of the proposed metrics is that calculation is straightforward and can be based on readily obtainable data. Furthermore, the calculated metrics will enable improved assessment, prediction and classification for likely propagule dispersal and vector importance, at species, community and population levels.

## METRIC ONE: THE DISPERSAL POTENTIAL (DP)

If classical FR is considered in the context of assisted dispersal, the relationship between "resource availability" and "*per capita* consumer uptake" could be considered as a function of the available propagule density and the *per capita* propagule load carried by a disperser. However, many studies only report data concerning the number of propagules recovered from dispersers. Therefore, firstly, we propose that the basic DP of any vector species can be calculated as:

$$DP = Np \times Tv \tag{1}$$

whereby, Np is the *per capita* propagule load recovered from a disperser, such as the mean, minimum, or maximum number propagules carried per individual vector species. However, the *per capita* propagule load (Np) can be used to assess various stages of the dispersal process, such as propagule uptake rates, recovery rates, survival rates (e.g., germination or hatching), or viability rates in relation to post-dispersal growth and establishment. For example, with an uptake rate of 100 seeds by mallard duck, *Anas platyrhynchos*, Soons et al. (2008) documented maximum recovery and subsequent germination rates of 54 and 78%, respectively. Ultimately, however, only comparison of post-dispersal propagule viability data will provide the most informative assessment. Notably, Np can represent the *per capita* propagule load in relation to a single taxon or multiple taxa. Moreover, the *per capita* propagule load can also be substituted with *per capita* propagule species richness, if assessment of the number of different species being transported by a disperser is required.

As a concept, Tv is considered the total number of possible available vectors, such as the number of individuals of a single species at a source site, their local scale abundance, or an entire continental population. Vector abundance data could be further refined in the context of proportional "seed shadows," whereby the number of dispersers which relocate prior to or beyond selected distances, e.g., 10 km, are considered for metric compassions. Additionally, in response to data availability, or temporal and spatial changes in vector abundance, Tv can be further presented as the mean (Mv) or minimum (Minv) number of vectors. Although dependent on data availability, estimates of minimum or mean vector abundances may be more attainable than confirmation of the total number of dispersers utilizing an area.

As a worked example of the DP, we will consider the data presented by Reynolds and Cumming (2015), who sampled a variety of waterbird species from two different freshwater sites in South Africa. Not knowing the actual species counts for these sites, we will arbitrarily assume that Reynolds and Cumming (2015) sampled a fifth of the population in all cases. This assumption is used to allow us to present the metric. However, the metric is a "snap-shot" and will always be subject to change in relation to fluctuations of propagule and vector abundances. In their study, 12 resting eggs of *Daphnia* sp. were recovered from the examination of 60 Egyptian goose, *Alopochen aegyptiaca*, fecal samples obtained from the first study site, "Barberspan." Each sample was recovered from a different individual. Accordingly, the DP of any vector species can be calculated as:

$$DP = \left(\frac{Total\ no.\ of\ resting\ eggs}{Total\ no.\ of\ birds\ sampled}\right) \times Tv$$

$$DP = \left(\frac{12}{60}\right) \times 300$$

$$DP = 0.2 \times 300$$

$$DP = 60$$

In contrast, five resting eggs of *Daphnia* sp. were recovered from across 55 *A. aegyptiaca* fecal samples obtained from a second study site, "False Bay," in which case the calculated DP = 25. Therefore, *A. aegyptiaca* inhabiting the first sample site have a higher DP for resting eggs of *Daphnia*. Similarly, 60 yellow-billed duck, *Anas undulata*, were sampled at site one, with 15 *Daphnia* eggs being recovered. A resulting DP of 75 was calculated, indicating a marginally higher DP for *Daphnia* eggs by *A. undulata* relative to both populations of *A. aegyptiaca*. Moreover, examination of DP for *Daphnia* resting eggs in relation to all waterbird species sampled at site one, while retaining our assumption of bird numbers, i.e., one fifth of all birds present were sampled for each species, allows the importance of the vectors to be ranked: *A. undulata* > *A. aegyptiaca* > white-faced duck, *Dendrocygna viduata* > spur winged goose, *Plectropterus gambensis* > red-billed teal, *A. erythrorhyncha* (DP scores: 75; 60; 20; 5; 0, respectively).

Similarly, Pellerin et al. (2016) documented the mean (± SE) combined recovery rate of the seeds from three plant species (*Plantago media*, *Prunella vulgaris,* and *Rubus fruticosus*) following gut passage through roe deer, *Capreolus capreolus*, red deer, *Cervus elaphus*, and wild boar, *Sus scrofa*, to be 5.6% (±4%), 13.1% (±4.9%), and 44.1% (±9.5%), respectively. In areas of co-occurrence, such as north-eastern France, *C. capreolus*, *C. elaphus,* and *S. scrofa*, attain calculated mean densities of 0.51 ind. $km^{-2}$ (range = 0.46–0.55: Richard et al., 2010), 0.96 ind. $km^{-2}$ (0.69–1.23: Garel et al., 2010; Baltzinger et al., 2016), and 6.46 ind. $km^{-2}$ (5–7.92: Lang et al., 2000; Baltzinger et al., 2016), respectively. Taking the mean seed recovery rates, and vector species density values, the DP of the dispersers can be ranked as: *S. scrofa* > *C. elaphus* > *C. capreolus* (DP scores: 284.89; 12.58; 2.86).

In an additional example, utilizing the data presented in Soons et al. (2016), we rank the DP of the European breeding populations of several dabbling duck species. Taking the highest estimate of the breeding population and the average number of seeds for all plant species recorded within the diet of each vector species, the DP of the waterbirds is ranked as follows: mallard, *A. platyrhynchos* > common teal, *A. crecca* > Eurasian wigeon, *A. penelope* > northern pintail, *A. acuta* > gadwall, *A. strepera* > northern shoveler, *A. clypeata* (DP scores: $3.27 \times 10^9$; $1.75 \times 10^9$; $2.24 \times 10^8$; $1.84 \times 10^8$; $6.14 \times 10^7$; $3.12 \times 10^7$).

## METRIC TWO: THE RELATIVE DISPERSAL POTENTIAL (RDP)

Although the DP can be used to quantify the importance of vector species, the comparative relationship between different vectors also requires consideration. Ranking of vector DP alone does not inform on the extent to which a species likely influences dispersal. However, the RDP is a ratio-based metric that can facilitate the assessment of DP for one vector relative to that of another. Simply, when the calculated value for RDP of Species A is < 1, then its comparative DP is less than that of Species B. However, RDP > 1 indicates a superior DP of Species A to that of Species B. Finally, when RDP = 1, both species have an equal DP. The RDP can be calculated as:

$$RDP = \left(\frac{DP\ Species\ A}{DP\ Species\ B}\right) \tag{2}$$

For example, taking the above calculated DP scores for ungulate species, the RDP of *S. scrofa* (Species A) to that of *C. capreolus*

(Species B) is 99.61, which suggests the DP of *S. scrofa* is almost 100 times greater than that of *C. capreolus*. Similarly, for the previously assessed European dabbling duck species, the RDP of *A. platyrhynchos* (Species A) in comparison to *A. crecca* (Species B) is 1.87, suggesting the DP of *A. platyrhynchos* is almost 1.9 times greater than that of *A. crecca*, at the level of the estimated breeding population. Interestingly, "species" could be replaced with different age cohorts, sample sites, or populations to allow for in-depth examination of comparative DP within and between species, across both community and ecosystem levels. In a more refined hypothetical example, concerning transport distances beyond 1,000 km, we will arbitrarily assume that 20 and 80% of the European breeding populations for *A. platyrhynchos* and *A. crecca* populations engage in such movements, respectively. Accordingly, the calculated RDP for *A. platyrhynchos* (Species A: 20% = $6.54 \times 10^8$) and *A. crecca* (Species B: 80% = $14 \times 10^8$) is 0.47. This would indicate that at distances >1,000 km, *A. crecca* have a predicted DP which is approximately double that of *A. platyrhynchos*.

Furthermore, an improved understanding of propagule retention/attachment times, their subsequent viability, and vector directional movements will facilitate further refinement of such calculations. For instance, with an average flight speed of 78 and 58 km $h^{-1}$ (Clausen et al., 2002), flight-times of 13 and 17 h would be required for *A. platyrhynchos* and *A. crecca*, respectively, to surpass a distance of 1,000 km. As the probability of seed germination decreases with longer gut retention times (Soons et al., 2008), we will hypothetically assume that seed mixtures transported by *A. platyrhynchos* and *A. crecca* for 1,000 km will have successful germination rates of 10 and 5%, respectively. Accordingly, a conceptually more refined RDP for *A. platyrhynchos* (Species A: 10% = $6.54 \times 10^7$) and *A. crecca* (Species B: 5% = $7 \times 10^7$) is 0.934, which would predict an almost equal DP for a distances >1,000 km.

Alternatively, assessment of the RDP can also be performed visually via biplot, as per **Figure 2**. Using the waterbird species data reported by Reynolds and Cumming (2015) for *Daphnia* sp. dispersal at first study site, "Barberspan," we present Np (here the *per capita* number of propagules each vector species) on the x-axis and Tv (the abundance of each vector species at the study site) on the y-axis. By scanning the biplot from bottom left to top right, we clearly see that while *D. viduata* has the highest Np, its low Tv means it has only the third highest dispersal potential. While *A. undulata* and *A. aegyptiaca*, each with much higher Tv, have the highest and second highest dispersal potentials, respectively. *P. gambensis*, having a moderate Tv, but a low Np, had the fourth highest dispersal potential. As *A. erythrorhyncha* was found to have carried zero *Daphnia* sp. resting eggs by Reynolds and Cumming, and hence having no dispersal potential, this species was omitted from **Figure 2**.

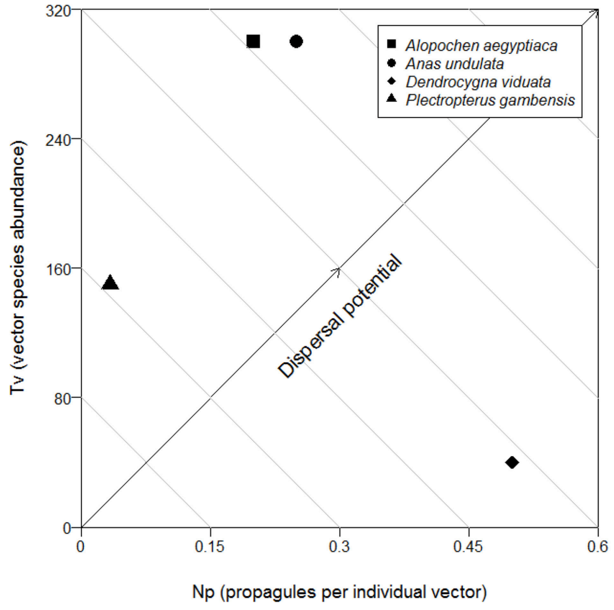

**FIGURE 2 |** Biplot showing the Relative Dispersal Potential potential of yellow-billed duck, *A. undulata;* Egyptian goose, *A. aegyptiaca;* white-faced duck, *D. viduata;* and spur-winged goose, *P. gambensis,* with *Daphnia* sp. propagules. The red-billed teal, *A. erythrorhyncha,* had zero *Daphnia* propagules and hence was omitted from the biplot. Dispersal potential increases from bottom left to top right.

## METRIC 3: THE COMBINED DISPERSAL POTENTIAL (CDP)

Multiple different vectors may contribute to the dispersal process, increasing the DP for a particular propagule type. Accordingly, we propose a third metric, the CDP, whereby the cumulative DP of several vectors may be considered:

$$CDP = \left(DP\ Species\ A + DP\ Species\ B + DP\ Species\ C \ldots\right) \quad (3)$$

As an example, we use the DP calculations derived above for *A. undulata*, *A. aegyptiaca,* and *D. viduata* in relation to *Daphnia* eggs (see above). The CDP of these three vector species is 155. Similarly, based on our derived DP values concerning the dispersal rate of seed mixtures for three plant species (*P. media*, *P. vulgaris,* and *R. fruticosus*) by multiple ungulate species, the CDP of *C. capreolus* and *C. elaphus* is calculated as 15.44. Further, as an additional example, we will consider the data presented by Banha et al. (2016) concerning the mean number of zebra mussel, *Dreissena polymorpha*, larval stages found adhering to an *A. platyrhynchos* carcass, an angler's waders and a fishing keep net, following 10 min exposure to infested water. In calculating the DP, due to lack to known abundance data, let us assume that the number of adult *A. platyrhynchos* will consistently outnumber anglers at an arbitrary ratio of 7.5 to 1 at this site. Accordingly, using an abundance of two anglers, the DP for each vector is calculated as follows: *A. platyrhynchos* = 120*;* angler's waders = 36; keep net = 44. When taken together these vectors have a CDP of 200. Secondary dispersal of propagules by additional vectors can be accounted for through addition of the DP for a primary disperser (Species A) and that of the secondary vector (Species B).

## METRIC FOUR: RELATIVE COMBINED DISPERSAL POTENTIAL (RCDP)

Similar to the concept of RDP, we suggest that a ratio-based metric to facilitate comparison of the CDP for multiple vectors to that of one or more others is worthwhile.

$$RCDP = \left( \frac{DP\ Species\ A\ +\ DP\ Species\ B}{DP\ Species\ C} \right) \quad (4)$$

Therefore, using our above calculated DP values for the South African waterbirds, a RCDP of 6.77 can be calculated for combined *A. undulata* and *A. aegyptiaca* in relation to the DP score of *D. viduata*. Similarly, an RCDP of 31 can be derived for all three of these species when compared to the DP of *P. gambensis*. Further, the RCDP of ungulate species *C. capreolus* and *C. elaphus*, when compared to *S. scrofa*, is calculated as 0.054. This indicates that *C. capreolus* and *C. elaphus* have a CDP which is equivalent to 5.4% of the DP shown by *S. scrofa*. Moreover, as a final example, the CDP of both angling equipment items relative to the DP of *A. platyrhynchos* (see above), results in a RCDP of 0.666. This suggests the CDP of the combined angling equipment is approximately two thirds of the DP predicted for *A. platyrhynchos* at the examined abundances. Additionally, RCDP could also be used to compare the DP of biodiverse systems, with numerous vector species present, to the DP of systems dominated by a single or relatively few vector species. Further, the impacts of range shifts, invasive species and defaunation, on vector assemblages and the subsequent dispersal of propagules, can also be explored in relation to the spread, introduction or loss of dispersers.

## DISCUSSION AND SYNTHESIS

Here, we have proposed a series of novel metrics to predict and classify likely dispersal and vector importance. The proposed metrics offer a basic straightforward assessment of dispersal, with potential for more in-depth network analysis concerning the relative importance of vector species. In addition to vector species abundances, the *per capita* effect of dispersers may also be assessed through use of other proxies, such as density or vector biomass (Dick et al., 2017c; Dickey et al., 2018). However, these calculations are context dependant on disperser and propagule interactions underpinned by both biotic and abiotic conditions. For instance, in certain scenarios "Vector Species A" may have a higher DP in some ecosystems than "Vector Species B," but in other systems this may be completely different. In addition, initial contact between disperser and propagule, particularly for more incidental vector species such as carnivorous secondary dispersers, may be a matter of coincidence and a rare event. Ultimately, the availability of propagules and their rate of uptake by vector species will vary. However, differential seasonal DP calculations reflective of both propagule and vector availability can be examined through the RDP, the CDP, and the RCDP.

So far, we have shown that the DP metrics can be used to quantitatively assess and predict likely dispersal, and classify vector importance. In the examples provided, differential dispersal of propagules of a single taxon or multiple taxa has been examined in relation to the calculated DP for various vector species, which allows for an improved understanding of disperser functional roles. However, the proposed analogous series of metrics can also be used to examine DP in the context of differential dispersal of multiple propagule combinations transported by one or more dispersers. For example, following the ingestion of plant seeds by adult *A. platyrhynchos*, Soons et al. (2008) observed mean germination rates of 32 and 8% for *Lycopus europaeus* and *Lythrum salicaria*, respectively. Although unknown, let us assume that smaller juvenile *A. platyrhynchos* will facilitate greater germination rates of these plants due to shorter gut retention times, in the arbitrary order of 64 and 16%, respectively. Accordingly, in the context of 10 adult and 10 juvenile *A. platyrhynchos*, which have each consumed exactly 100 seeds of each plant species, we would expect a germination based DP of: *L. europaeus* by juvenile mallard $>$ *L. europaeus* by adult mallard $>$ *L. salicaria* by juvenile mallard $>$ *L. salicaria* by adult mallard (DP scores $= 640 > 320 > 160 > 80$, respectively). As before, variation of the in-field *per capita* propagule load (Np) and vector abundances (Tv) will alter these outputs. Yet, as highlighted, assessment of DP in relation to different propagules carried by a single disperser, a vector species, or numerous combinations of both propagules, and dispersers can be performed. Thus, the metrics can facilitate an improved understanding of biological connectivity within meta-community dynamics.

Overall, the final fate of propagules is not a simple function of the *per capita* acquired propagule load and vector abundances. Propagule retention or adherence times, propagule release, subsequent viability, disperser distances traveled, failure to relocate, and suitability of new environments will also influence dispersal success (Vidal et al., 2013; Coughlan et al., 2017a,b). Therefore, the proposed DP metrics always have to be considered in the context of the data used in the calculation. Currently, a variety of studies have documented the abundance and richness of propagules carried by different vector species, and increasingly, quantitative data detailing propagule survival, and viability rates is being reported (e.g., Farmer et al., 2017; Bartel et al., 2018; Lovas-Kiss et al., 2018a,b,c). These are mostly recovered from coat or feather brushings, and regurgitated pellets, fecal or lower intestine gut samples. Although one vector species may carry more propagules than another different vector species, the relative abundance of vectors appears to be rarely considered. Our proposed metrics provide a blend of individual and population-level effects, which could be further refined beyond the concept of dispersal "potential" by reducing the knowledge gap between potential and realized dispersal, through uncertainty analysis techniques and further integration of factors such as post-dispersal viability, vector movements, and frequency of time spent by vector within habitat patches suitable for dispersed propagules. In particular, as briefly highlighted through an RDP example, these metrics could be used to assess the number of potential dispersal events across distance gradients, i.e., within different spatial gradients of seed (propagule) shadows. Equally, the propagule shadow created by multiple vector species across such spatial and temporal gradients can also be considered with the CDP.

Although vector species assemblages and their proportionality of interactions with propagules are frequently assessed in the context of ecological networks, the relative importance of individual species or functional groups at the community and population scale remain inadequately defined (Farwig and Berens, 2012; Vidal et al., 2013; Hämäläinen et al., 2017). For example, through a bipartite network analysis of recovered scat samples, Peredo et al. (2013) documented that red fox *Vulpes vulpes* facilitated a slightly higher proportion of *Rubus* sp. dispersal than wild boar *S. scrofa*, in relation to the total number of seeds recovered. However, the mean number (± SE) of *Rubus* seeds per scat sample was higher for *S. scrofa* (984.3 ± 299.2) than for *V. vulpes* (444.2 ± 90.7). Therefore, if the number of co-existing boars outnumbers those of territorial *V. vulpes* within a shared range, then RDP analysis would indicate that *S. scrofa* has a higher DP. Additionally, a single vector species may facilitate dispersal across multiple ecological networks, e.g., *S. scrofa* can disperse propagules originating from both terrestrial and aquatic systems (Vanschoenwinkel et al., 2008; Peredo et al., 2013). As generalists, high abundances of omnivorous *S. scrofa* may provide for greater levels of realized dispersal, in comparison to lower abundances of more specialist propagule consumers. Accordingly, the relative importance of *S. scrofa* within and across dispersal networks could be assessed with DP based metrics.

Even within relatively well-studied mutualistic frugivorous networks, interactions are generally sampled by recording the number of foraging visits by potential dispersers to source plants, with interaction frequency taken as a proxy for vector species abundances (Simmons et al., 2018). Often, subsequent calculations rely on the assumption that similar propagule uptake rates occur with each visit (Schupp et al., 2010). However, this assumption is likely less reliable for chance ectozoochorous dispersal, and also fails to account for opportunistic feeding, gorging, selectivity, and prey switching. Although dispersers can visit propagule source sites multiple times, with increased frequency of visitation likely resulting in greater occurrence of dispersal events, this detailed data is not always available for understudied systems. Accordingly, the proposed metrics are basic and require minimal data, as more complex metrics such as the Seed Disperser Effectiveness (SDE) as proposed by Schupp et al. (2010), cannot always be calculated from available data. The proposed series of DP metrics estimate likely disperser functional roles based on the result of a single source site visitation, when the *per capita* consumer uptake of propagules has occurred. However, multiplication of DP by the number of visits, or accumulative CDP and RCDP calculations, can be used to account for differential dispersal caused by visitation events. Nevertheless, we also suggest that the SDE metric could be considered in the context of the relative role played by multiple vector species. For example, calculations of: (1) Relative SDE (RSDE); (2) Combined SDE (CSDE); and (3), the Relative Combined SDE (RCSDE), could greatly enhance understanding of dispersal facilitated by different vector groups. Assessment of relative dispersal potential could also be examined for the propagule Ingestion and Excretion equations derived by (Kleyheeg et al., 2017).

Although further development and validation will be required, the proposed metrics represent a novel starting point for greater consideration of disperser functional roles across spatial and temporal gradients. As the relative capacity for assisted dispersal will differ between vectors, these metrics provide a means to quantify and rank the importance of different dispersers. This will facilitate an improved assessment of the prominence of different dispersers for the maintenance of connectivity, both within and between ecosystems. Furthermore, in studies of dispersal networks, the inclusion of these metrics will provide a more realistic measure of the functional role of different species than through examination of interaction intensities alone, since the metrics can account for vector abundance. Finally, incorporation of vector life history data, such as fecundity and lifespan (see Dickey et al., 2018), and spatial or temporal changes in interaction opportunities, such as shifts in vector diets in response to propagule availability (e.g., seasonal changes) or preferential interaction with invasive species by dispersers (Green, 2016; van Leeuwen, 2018), will present a more realistic representation of dispersal with increased prediction power.

## AUTHOR CONTRIBUTIONS

NC conceived and designed the study. JWD, RC, TK, MJ, and JTD assisted in developing the study. All authors contributed to the writing of the manuscript, which was led by NC.

## ACKNOWLEDGMENTS

NC and JTD are supported by the Irish Environmental Protection Agency research grant 2015-NC-MS-4. JWD acknowledges support from Queen's University Belfast and Inland Fisheries Ireland. RC is supported by the Department for the Economy, Northern Ireland. JTD also acknowledges funding received from Natural Environment Research Council. We particularly wish to thank Casper H.A. van Leeuwen and our two reviewers for helpful comments.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Ecology and Evolution

REVIEW
published: 20 March 2019
doi: 10.3389/fevo.2019.00074

# Zooplankton Diversity and Dispersal by Birds; Insights From Different Geographical Scales

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Given the major ecological and evolutionary role of dispersal abilities for organisms, as well as the current interest in species' potential for further migration and colonization as a result of climatic changes or human-mediated invasions, our knowledge about dispersal abilities on spatial and temporal scales in many taxa is surprisingly limited. Zooplankton inhabit lakes and ponds that functionally are "aquatic islands" in the landscape, and both community composition and richness depend on their ability to disperse, and their post-dispersal colonization abilities. We here assess the diversity and dispersal of freshwater microcrustaceans based on three types of data; (1) > 2000 lakes on mainland Norway spanning a wide range in longitude, latitude and altitude, (2) a more limited number of ponds at Svalbard that are differently affected by migrating birds, and (3) immigration and colonization of recently constructed wetlands and man-made ponds. At all scales we discuss whether observed patterns in diversity can be explicitly linked to birds as vectors, or if confounding factors such as climate, productivity, age of locality—or other means of immigration, precludes conclusive evidence. The spatial patterns of zooplankton distribution strongly suggest that local sorting is a major determinant of richness and community composition. This sorting may not necessarily lead to similar community composition (the "quorum effect") however. Despite the fact that rapid colonization occurs at local scales, and that birds undoubtedly can transmit animals or resting stages, their role in modulating community structure and richness is still an unsettled issue due to the many confounding parameters. The fact that birds often play a dual role in shaping diversity and community composition, first by direct dispersal, and secondly via affecting post-dispersal species sorting by changing water quality and productivity, is an important aspect of zoochory. Direct experimental evidence (colonization with and without bird exclusion), or genetic analysis of zooplankton species along migration routes, would however be the only ways to establish firm evidence for this case of zoochory.

**Keywords: birds, dispersal, lakes, ponds, sorting, microcrustacean zooplankton**

***Edited by:***
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***Specialty section:***
*This article was submitted to*
*Biogeography and Macroecology,*
*a section of the journal*
*Frontiers in Ecology and Evolution*

***Received:*** *25 September 2018*
***Accepted:*** *26 February 2019*
***Published:*** *20 March 2019*

***Citation:***
*Hessen DO, Jensen TC and Walseng B (2019) Zooplankton Diversity and Dispersal by Birds; Insights From Different Geographical Scales. Front. Ecol. Evol. 7:74. doi: 10.3389/fevo.2019.00074*

## INTRODUCTION

There are two principal drivers of biogeographical distribution patterns: the ability of species to disperse to new ecosystems, and the ability to establish permanent populations post dispersal. For the biota of lakes and ponds, living in confined "aquatic islands," the ability to spread, colonize and become established in new sites is a strong fitness component, provided post-dispersal establishment. The ability and mechanisms for dispersal of aquatic organisms may differ with geographical range, ecosystem connectivity, dispersal vectors, and lake specific properties. One would suspect that absence or presence in nearby lakes can be attributed to lake-specific properties. On the other hand, dispersal abilities may be hard to separate from "species sorting" due to water quality, lake or pond morphometry, flushing rate, climatic patterns, and landscape history.

The dispersal abilities of aquatic organisms is a matter of long-standing interests since Darwin's seminal studies demonstrated how birds may serve as dispersal vectors for organisms attached to plumage or feet (Darwin, 1859; Bilton et al., 2001; Bohonak and Jenkins, 2003; Simonis and Ellis, 2014). Fish and birds may also spread especially resting stages via gut survival (Banarescu, 1990; Green and Figuerola, 2005; Frisch et al., 2007; Green, 2016; Coughlan et al., 2017), which should give an advantage to species forming tough resting stages (like most cladocerans and some copepods). Resting eggs (ephippia) of cladocera may resist freezing and desiccation and hatch after extended periods (Weider et al., 1997). Ephippia may disperse by wind, water or by biological vectors (Gray and Arnott, 2011; Coughlan et al., 2017), and their facultative asexual mode of reproduction and fast growth rates (classical properties of "r-selected" organisms) should be an advantage to cladocera relative to most copepods. However, widespread endemism and provincialism and allopatric speciation within restricted areas has been reported among *Daphnia* populations, arguing for low gene-flow between populations (Hebert and Wilson, 1994), while also copepods may rapidly colonize recently established ponds (Cácares and Soluk, 2002; Louette and De Meester, 2005). Resting eggs in copepods may also promote dispersal, yet the comparison of the two calanoids *Eudiaptomus graciloides* (with diapausing eggs) *E. gracilis* (without) suggested that diapausing eggs was only beneficial for short distance dispersal (Zeller et al., 2006).

Given the wide, sometimes cosmopolitan, distribution of several zooplankton species (Flössner, 2000; Dussart and Defaye, 2006), the question is really why not "everything is everywhere," but rather what is the actual role of dispersal constraints and landscape history relative to colonization constraints for these tiny crustaceans? Also, the role of environmental factors relative to dispersal abilities may differ between regions and geographical scales even for the same group of organisms (de Mendoza et al., 2015).

Successful dispersal does not necessarily imply successful colonization however, and a suite of local factors including abiotic and biotic (productivity, food, competition, and predation) may shape community structure and diversity (Louette and De Meester, 2005). Thus, the real dispersal capacity can best be assessed in recently established sites with favorable conditions for most species. Over time, as these systems become "saturated," the likelihood of dispersal will remain unchanged, but the likelihood of permanent establishment will be reduced as interspecific competition increases with increased richness.

Separating between dispersal and colonization is one key issue in this context; others are the role of spatial structure and scale. Clearly higher colonization will occur in areas with high beta diversity, high density of localities and high degree of connectivity. For long distance dispersal, landscape constraints and barriers as well as climate gradients are important, and so are the migration routes of waterfowls or other vectors. Species sorting will likely also increase along spatial scales due to abiotic factors, i.e., increasing distance normally imply increasing differences in water quality properties, seasonality, productivity, and temperature. This holds especially for gradients toward lower productivity and harsher climate. Most taxa show decreased poleward diversity (Gaston, 2000), so also for freshwater zooplankton (Hessen et al., 2006). This is partly accredited to confounding factors such as temperature, productivity, and covariates of these, but also to ecosystem age and landscape history. Whether this to some extent also reflects e.g., post glaciation migration constraints, remains unsettled.

One important issue is whether local sorting also promotes community similarity which could be anticipated if local habitats shared common biotic or abiotic properties. Such a "quorum effect" (Jenkins and Buikema, 1998) would be expected to be more likely late in succession in nearby sites, while less so early in succession among distant sites (Jenkins, 2006). Jenkins (2006) did however not find support for a local sorting quorum effect in a number of experimental ponds, and both colonization events and community development appeared rather stochastic.

Despite strong evidence for high local dispersal capacities of zooplankton, notably cladocera (Louette and De Meester, 2005), the role of birds in this story remains somewhat speculative (Coughlan et al., 2017). Indeed, Louette and De Meester (2005) in their thorough study did not at all discuss the mechanisms for dispersal, and did not consider this as a potential explanation for the contrasting results between their own study and that of Jenkins and Underwood (1998), who reported slow dispersal rates. They do however point to the role of local connectivity, as does Cottenie and DeMeester (2004), who also found strong impacts of local, biotic sorting. Experiments provide evidence for efficient dispersal also when cages or small ponds are covered with nets to exclude birds (Cácares and Soluk, 2002; Cohen and Shurin, 2003). For long-distance dispersal (LDD), Green and Figuerola (2005) in their thorough review of bird-mediated dispersal of zooplankton states that "... studies of LDD in aquatic systems remain in their infancy". Recent studies addressing the role of waterfowl and shorebirds for seed dispersal confirm a strong potential of endozoochory over short to moderate ($<$ 20 km) distances (Bartel et al., 2018), but also long-distance dispersal across Europe (Lovas-Kiss et al., 2018). While it could be argued that plant seeds have a higher likelihood of being ingested and dispersed than aquatic animals, also resting stages of zooplankton may withstand gut passage. Moreover, this demonstrates that gut evacuation not necessarily prohibit long distance dispersal (cf. Clench and Mathias, 1992).

One could argue that what matters is the ability to disperse and colonize, irrespective of what kind of mechanism is responsible. Given the current changes in bird populations, it is however relevant to know if birds *per se*, and which species, that may can serve as important vectors. While there are striking declines in many common bird species, including shorebirds, associated with the cultural landscape (Donald et al., 2001; Inger et al., 2014), others are increasing. The strong increase in geese

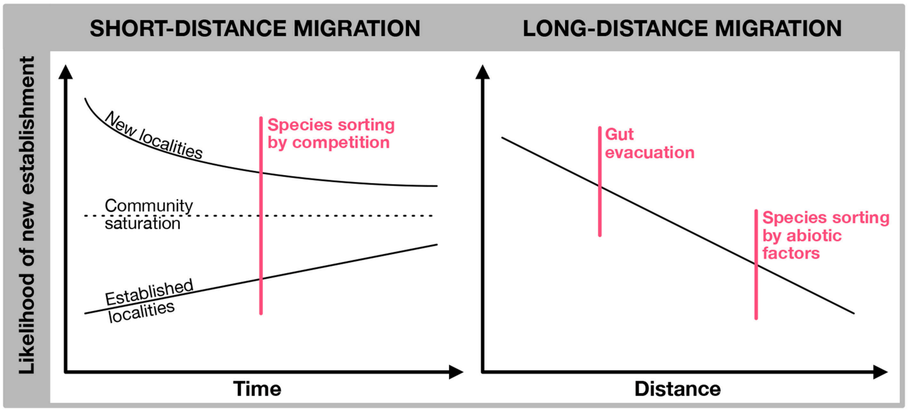

**FIGURE 1 |** Conceptual diagram for likelihood of dispersal, local sorting and establishment of zooplankton mediated by birds. Right panel displays the likelihood of local dispersal and establishment in recently formed and established (and species saturated) systems, respectively. In new localities with many available niches and low competition, initial likelihood of establishment is high, but will decrease over time as the system approach "saturation". A high turnover of species may still occur, and community composition may change, but the species richness does not increase. As the systems becomes established, the chance of new establishment will be small, but still the cumulative likelihood of establishment will increase over time. The right panel illustrate how likelihood of establishment decrease with migration distance, partly owing to increased likelihood of complete gut evacuation prior to arrival at the recipient site, partly due to increased likelihood of different abiotic conditions in donor sites and recipient sites. The vertical red lines represent constraints that in principle operate gradually over time and distance.

populations at high latitudes (Fox, 2010; Madsen et al., 2013) does indeed impact freshwaters over vast areas, i.e., provide LDD of freshwater taxa from overwintering sites in southern or central Europe, to stopover sites during migration, and finally to breeding sites in the high Arctic (Hessen et al., 2017). Thus, the development of bird populations may have strong impact also on aquatic biodiversity.

We here utilize three sources of data to assess if zooplankton diversity patterns and colonization can be evidently linked to birds as vectors: (1) The Norwegian mainland where zooplankton data exist from > 2000 lakes spanning a wide range in longitude, latitude and altitude. (2) The more isolated and local Svalbard case where there are links between species and clonal richness and impact by migrating bird populations, and (3) recently established ponds in the agricultural landscape, where colonization has been monitored over some years. We then discuss evidence—or lack of such—of bird-induced zoochory and propose how to proceed in this long-lasting debate, which indeed is important for understanding biodiversity patterns and also has conservation management implications.

A conceptual illustration of different mechanisms operating on different spatial and temporal scales is provided in **Figure 1**. This covers both the likelihood of dispersal *per se*, and physical or biological filtering or sorting mechanisms that may determine whether or not dispersed organism actually establish permanent populations. Throughout the text we will refer to these tentative mechanisms.

## The Norwegian Mainland

We here explored a database on pelagic zooplankton diversity and community composition data that has been sampled since the early 1980ies. This includes altogether 2,031 localities, covering the entire mainland of Norway (58°3′ to 71°4′N) and spanning a wide range in terms of altitude, area, and water quality (for details, see Walseng et al., 2006 and Hessen et al., 2007). If birds are major determinants of zooplankton dispersal, one could, based on this dataset covering such a wide geographical range, predict that this could be reflected in local diversity and community composition. More specifically, it could be expected that on top of gradients related to climate (and thus implicitly altitude, latitude and partly longitude), it should be possible to track local diversity hotspots related to migration and stopover sites, or at least temporal occurrence of species outside their natural range of distribution. For areas where there are no obvious constraints related to climate or productivity, like the costal lakes with favorable climate and often nutrient impacts from human activity, efficient zoochory by birds would even out regional differences. Confounding factors related to impact of lake size, local climate, fish predation etc. could clearly pose problems in the interpretation of bird impact, however. Also, species may be transferred but then fail to establish, or being established in particular lakes, but still unable to spread further. The likelihood of establishment will clearly be both species-specific and depend on the frequency and number of propagules.

**Figure 2** clearly demonstrates the wide range in species richness, with the highest diversity in the south-east and a general decline with latitude, altitude and longitude (for the central areas). Notably altitude, but also longitude and latitude, provide a pronounced upper bound of species richness, but there is a major scatter due to a full range of species diversity also at low altitudes, latitudes and longitudes. Diversity and species composition changed along these geographical clines, while there were no general differences in the relative abundance of cladocera vs. copepods. The mean ratio between cladocera and copepods, including both pelagic and littoral species, was 2.8, and with no systematic change over latitude, longitude or

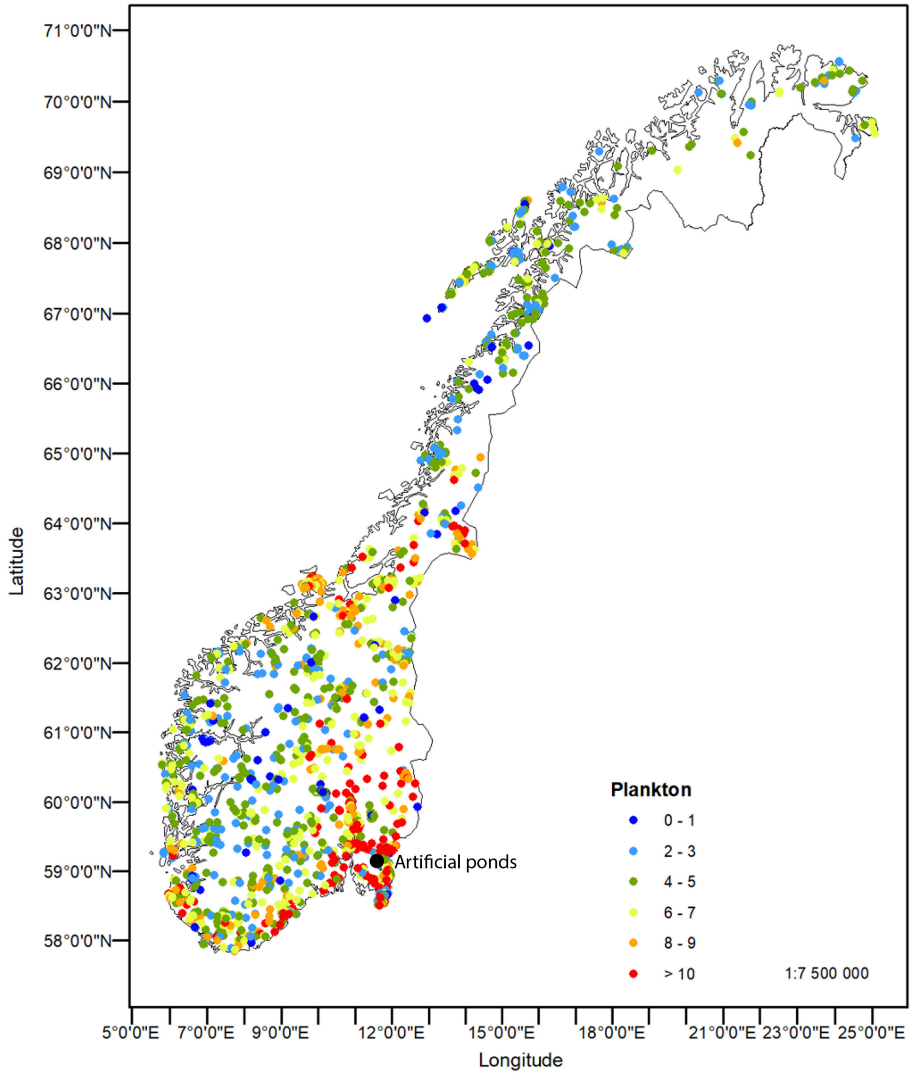

**FIGURE 2 |** Species richness of pelagic crustacean zooplankton across the Norwegian mainland. Color codes represents the number of species per locality. The site of the constructed, artificial ponds is indicated.

altitude. A multivariate analysis using latitude, longitude, altitude and lake area as determinants of total zooplankton (pelagic and littoral microcrustaceans) diversity in 2.937 localities, explained 22% of observed variability. The fact that 78% of observed variability in species diversity (represented by species numbers) remained unexplained, suggests that a major part of variability must be explained by local or site-specific properties like water quality, productivity, connectivity, species sorting, competition (and monopolization), or other unidentified drivers.

An extensive database of band and recapture sites for all Norwegian bird species, including shorebirds and waterfowl, is accessible (http://stavangermuseum.no/ringmerkingssentralen/ringmerkingsatlas). While most water-related birds are coastal and display north-south migration routes, there are also common species with an extensive distribution and more random migration pattern, such as mallard (*Anas platyrhynchos*) and common gull (*Larus canus*) (**Figure 3**). These and a number of other water-related species should in principle provide key vectors for rapid dispersal of zooplankton throughout Norway. Most shore-birds and waterfowl perform seasonal, long distance migration, and linking central Europe, Norway and the Svalbard archipelago, e.g., barnacle geese (*Branta leucopsis*) and purple sandpiper (*Calidris maritima*) (**Figure 3**).

Both inland and subalpine wetlands are nesting sites for a large number of waders and ducks, but still possess modest or low zooplankton richness. Of course, this does not imply that birds do not act as vectors, since species sorting due to low temperature, low productivity, competition, predation, or other site-specific properties, may constrain permanent establishment. Hotspots for crustaceans are documented from two well-recognized stop-over sites for waterfowl in central, southern Norway. With 80 recorded species the Lake Randsfjorden (including Dokka delta) is the

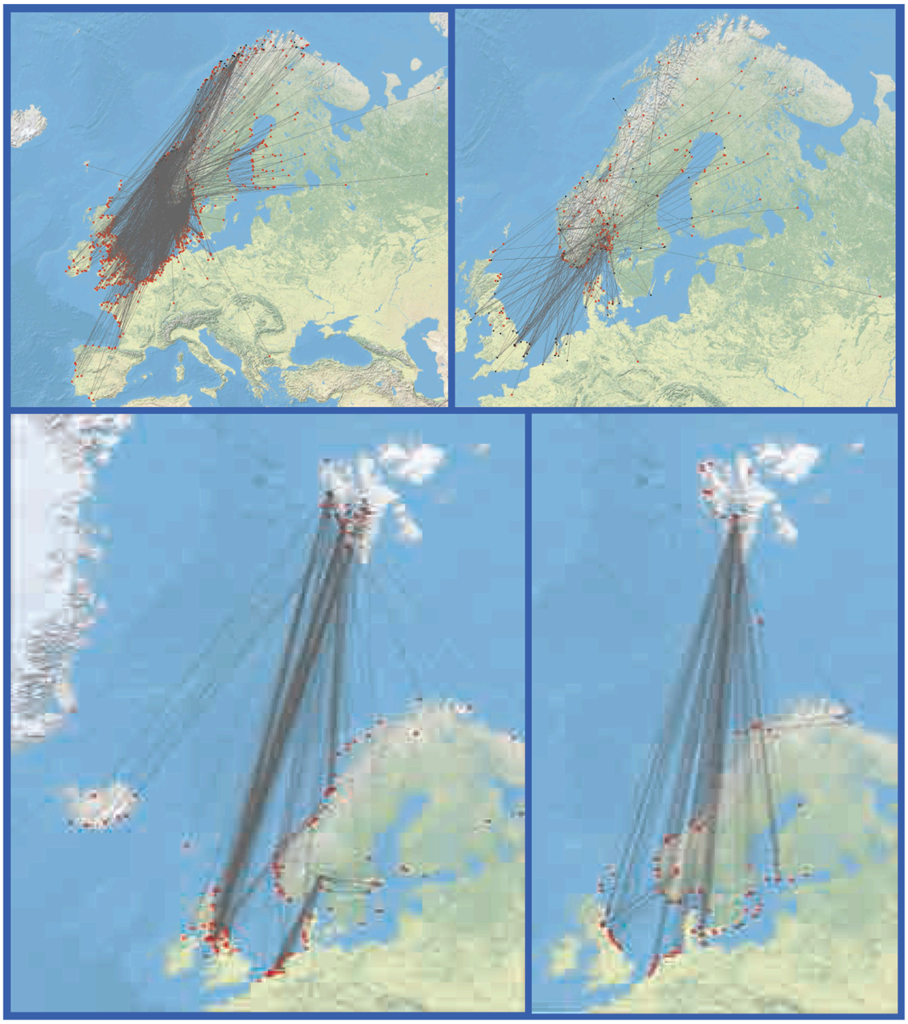

**FIGURE 3 |** Examples of migration routes for ringed waterfowl. Upper panel show very abundant species with a widespread migration "all over" (left: common gull—*Larus canus*; left: mallard *Anas platyrhynchos*). Lower panel are species with a coastal preference that typically link northern Europe, Norway and the Svalbard archipelago (left: purple sandpiper—*Calidris maritima*; right: barnacle geese—*Branta leucopsis*).

site with the highest number of recorded microcrustaceans in Norway, followed by the Lake Øyeren with its delta (62 species). This high diversity is partly a result of high spatial heterogeneity in habitats and water quality in the two large lakes. However, both these deltas are important stopover sites for migratory water birds, and bird-mediated dispersal could also partly explain the high microcrustacean diversity where local sorting is less pronounced owing to the favorable habitats with a high number of available niches. This is still at best circumstantial evidence since there is no conclusive evidence of bird-mediated zoochory in shaping community composition or richness at these scales, not the least due to the large number of confounding factors.

## The Svalbard Archipelago

The high Arctic lakes and ponds at Svalbard provide a more species poor and geographically isolated and constrained area for testing the potential role of bird-mediated dispersal. The fact that lakes, ponds and birds are all localized in constrained coastal areas, and there is an intensive monitoring of bird populations and activities, offers a good opportunity for linking freshwater fauna to birds at a local scale. This holds especially for the geese populations that have increased dramatically over the past 30 years (Pedersen et al., 2013), is well-monitored, and that is actively exploiting and affecting the aquatic habitat (Van Geest et al., 2007; Hessen et al., 2017). Hence, this offers a possibility to link both spatial and temporal changes in the zooplankton community to the prevalence of birds within a climatologically constrained area. The presence of geese often overlaps with activity of other water-related birds, and yet waders forage directly in the freshwaters sites, geese also frequently spend time near or at the ponds where they contribute to increased concentrations of nitrogen (N) and phosphorus (P) in the water bodies (Van Geest et al., 2007; Alfsnes et al., 2016).

**TABLE 1 |** Parameter estimates for linear regression models relating cladoceran species richness to the goose abundance (judged from droppings), total-N and total-P [all transformed, $\log_{10}(X + 1)$].

| Response variable | Coefficients | Estimate (± SE) | *t*-value | *p* |
|---|---|---|---|---|
| Cladoceran species richness | Intercept | −1.913 (0.804) | −2.379 | 0.026 |
| | Goose abundance | 0.711 (0.313) | 2.271 | 0.033 |
| | Total-N | 1.295 (0.366) | 3.533 | 0.002 |

*A backward selection procedure was applied for the multiple regression (p > 0.1). Only goose dropping abundance and total-N were included in the model.*

In this context we used a dataset (Walseng unpublished) from 25 Svalbard ponds along a gradient of goose impact (abundance of goose droppings) including also data on nutrient concentration and cladoceran and copepod species richness. We tested if goose impact affected species richness of cladocerans and copepods differently.

The relationship between cladoceran and copepod (cyclopoids calanoids and harpacticoids) species richness, respectively and goose abundance (using absolute dropping abundance), total nitrogen and total phosphorus was analyzed with univariate multiple linear regression. A backward selection procedure was used to exclude predictors in the multiple regression ($P > 0.1$). Number of goose droppings, total-N and total-P were transformed [log10(X + 1)] prior to analysis due to data skewness. The multiple regression of cladoceran species richness included goose dropping abundance and total-N [$F_{(2,\,22)} = 22.161$, $p < 0.001$, $R^2 = 0.668$]. Total-N received a stronger weight in the model than goose dropping abundance (**Table 1**) and accounts uniquely for 18.8 and 7.8%, respectively of the variance in cladoceran species richness. An additional regression including the interaction between goose dropping abundance and total N, showed that this term was not significant. Hence, cladoceran species richness increased with increasing goose abundance and increasing total N concentration, but total N seemed to be the most important of the two predictors. Neither goose abundance, total N or total P turned out to be significant contributors for copepod species richness. Alfsnes et al. (2016) recorded the highest species and clonal diversity of *Daphnia* in nutrient-rich and bird—impacted localities at Svalbard and concluded that an increased species richness and clonal changes since 1992 could likely be attributed to climate change and increased bird impacts.

A recent survey of the freshwater invertebrate fauna on Svalbard (Walseng et al., 2018), demonstrated that some microcrustaceans had expanded their distributions. Additionally, a number of new species was recorded on the archipelago (Dimante-Deimantovica et al., 2018). The increasing goose populations on Svalbard during the past few decades, likely in combination with the climate warming, may have contributed to the local spreading of the native species as well the establishment of new species. In fact, the goose population may play an important role in the colonization of biota of the many newly formed water bodies as the glaciers retract due to the rising temperatures, but again we lack firm evidence for this.

## Recent Pond Colonization

Six constructed ponds within a constrained watershed were studied over a 8-year period (1998–2005) (Ekeberg and Walseng, 2000; Hov and Walseng, 2003; Walseng unpubl.). These ponds are located in southern, central Norway (cf **Figure 2**), close to the Lake Øyeren with highest recorded diversity of freshwater taxa in Norway, and high abundance of water-fowl. There were no permanent water-bodies prior to the establishment of the ponds, but a tiny stream. Sampling of crustaceans and macroinvertebrates was performed in this stream the year before the ponds were established in 1999.The main purpose of the ponds was to increase the retention of sediments and nutrients from the stream, and they were all located within a landscape with moderate agricultural influence, pronounced topography, minimizing the dispersal by wind, or floods. The ponds differed somewhat in nutrient contents, yet this was not correlated with the number of taxa or species recorded. Rather the opposite. Following construction, the ponds were rapidly colonized, starting with oligochaets, and chironomids, followed by crustaceans, while the colonization of insects other that chironomids was slower. During the study period, 29 species of cladocerans and 26 species of copepods were identified.

Prior to the establishment of these ponds and wetlands, a close examination of four ponds draining to the local stream revealed a total of 19 cladocerans and 12 copepods (Ekeberg and Walseng, 2000). Hence, there was a restricted local inoculum of species. However, the very same year as the first sites were established in 1999, the diversity (13 cladocerans and 14 copepods) was already comparable with the very species-rich lake Lake Øyeren (situated ca 1.5 km from the ponds, but without direct contact). Six years later the accumulated number of species was 29 cladocerans and 25 copepods, respectively demonstrating a very fast colonization rate. We found no evidence of cladoceran dominance among the early settlers in any of the ponds. In fact, despite their lower species numbers in Norwegian freshwater (80 cladocera, 50 species of copepods, including littoral species), there was a slight copepod dominance in most of the samples (**Figure 4**). The cumulative number of recorded species indicated a very fast colonization up to species saturation and a likely local sorting due to competition. No specific survey of bird vectors of dispersal was performed, but the ponds were regularly visited by ducks (notably mallard), gulls and waders. The proximity to the large lake Øyeren implies a high likelihood of local dispersal by birds.

# DISCUSSION

By addressing the issue of bird-mediated zoochory of zooplankton at three widely different geographical scales, we conclude (in line with many previous studies) that there are indications of this at all scales, notably on small scales, but really no firm evidence. At the largest scale, despite strong regional and clinal patterns in diversity, it is impossible to separate bird-mediated dispersal from confounding variables related to

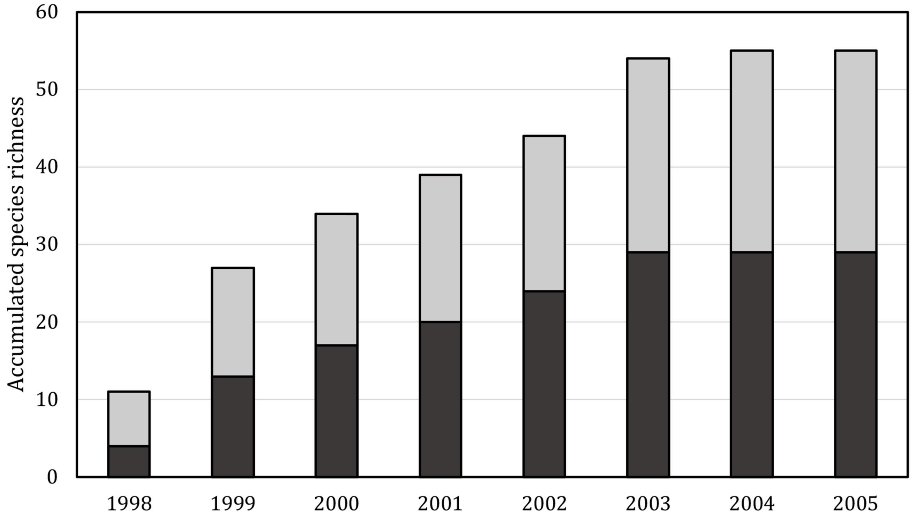

**FIGURE 4 |** Mean, cumulative number of observed species in the artificial pond. Black: copepods, gray: cladocera.

climate, productivity, site heterogeneity or other confounding variables. No doubt post-dispersal species sorting (e.g., failure of establishing permanent populations) add further constraints on the interpretation of zoochory. Local or regional founder effects may determine zooplankton communities for extended periods even under high dispersal rates (Shurin, 2000; De Meester et al., 2002; Ventura et al., 2014). Inland deltas that are important stop-over sites for birds possess very high diversity, but these are also productive and heterogenous habitats that anyway would offer suitable niches to many species. The main migration route of migrating water-fowls follows the coastline of Norway, but there is also an extensive migration by very abundant duck and gull species all over Norway, including inland lakes and east-west routes (cf. **Figure 3**). (The full range of migrations as well as zooplankton species distributions can be explored and visualized on http://stavangermuseum.no/ringmerkingssentralen/ringmerkingsatlas and https://www.biodiversity.no/Pages/231126, respectively). Despite this, the diversity on the west-coast is strikingly lower than further east, and also diversity generally decreases with latitude and altitude (Hessen et al., 2007). The extensive data on bird migration does not include quantitative data, however, and access to mark or recapture sites can only be achieved manually, hence a formal analysis of linking zooplankton distribution to migration is not possible, but nevertheless the migration data very well-illustrate the widespread migration across spatial scales.

Judged from such wide-spread migrations across geographical gradients, there should in principle have been sufficient time over the thousands of years since last glaciation to find "everything, everywhere," i.e., a general community homogenization—if birds act as efficient long-distance dispersers, and if not strong, local species sorting was operating. Whether local sorting also promotes community similarity and "quorum effects" is a matter of scale and time, but Jenkins (2006) found poor evidence for such effects in his experimental study. Stochastic events and monopolization effects seem to override quorum effects. While our data not really address community similarity at different scales, we still will argue that local sorting is important for species richness. E.g., it is less likely to become established in a "saturated" community than one with open niches. There clearly also is an abiotic sorting that reduces the likelihood of species to become established in alpine and northern sites compared to southern, productive, warmer and more productive sites, at least during early succession. This could be anticipated if local habitats shared common biotic or abiotic properties. This is simply reflected by the sheere number of species with a southern relative to a northern (or alpine) distribution (Hessen et al., 2007). While there also is a large potential for dispersal from the mainland to the Svalbard archipelago, the very low diversity here is presumably related to climatic factors and even with successful dispersal, there would be a strong constraint on the ability to establish permanent populations despite frequent migration by a number of species (e.g., examples provided in **Figure 3**).

It is hard to imagine alternatives to birds for long distance dispersal of aquatic crustaceans. We have to admit, however, that we are far from conclusive evidence as to the role of birds in shaping diversity and community patterns at such large, geographical scales. Since many birds, notable geese, mainly forage on terrestrial and coastal sites, and also have a rather fast gut evacuation rate, their ability to serve as long distance vectors may be quite restricted, yet not precluded (Clench and Mathias, 1992).

The more constrained study site at Svalbard, where data on bird influence on specific ponds, as well as data on recent increase in goose populations can be provided, the zooplankton diversity were found to be clearly related to bird activity. An extensive monitoring of geese has been performed over the last years, covering different parts of the season (pre- and post-breeding, breeding, foraging, molting), revealing an extensive migration locally and regionally with a strong likelihood of visiting adjacent ponds and lakes (see: http://goosemap.nina.no/Kartogdata/Kart.aspx). This is also confirmed by recent satellite tracking (unpublished data). Despite these strong indications of zooplankton transmission, we cannot, however, separate the role of birds as vectors due to the confounding impact from productivity of the sites promoted by fertilization from bird droppings and other activities. Also passive transmission by wind is likely over such short distances. Species diversity was primarily related to bird activities, however, and this also holds for clonal (haplotype) diversity of the dominant group *Daphnia* (Alfsnes et al., 2016). The generally obligate asexual *Daphnia* spp is the dominant zooplankton and constitutes a large number of clones or haplotypes distributed over a few species or subspecies (Alfsnes et al., 2016). The largest haplotype diversity was always found in bird-influenced localities (Alfsnes et al., 2016). Certain lineages, like European *D. pulicaria*, has been observed in ponds along the migratory route of birds in northern Norway and the north-western part of Russia (Weider et al., 1999), and both species and haplotype richness is related to bird prevalence (Alfsnes et al., 2016). A more thorough assessment of genetic markers (e.g., haplotypes) along migration routes would, along with experimental studies, serve as the best approach to settle the role of bird zoochory at different geographical scales. Since local species sorting is likely to occur, also this will be a minimum estimate of actual dispersal however. Studies on dispersal and clonal affinities of *Daphnia* population in Greenland ponds corroborate these findings, and also suggest a prominent role of birds for local dispersal (Haileselasie et al., 2016).

Studies on bird impacts on Arctic freshwaters are biased toward the impacts on aquatic productivity (Milakovic et al., 2001; Van Geest et al., 2007; Côté et al., 2010; MacDonald et al., 2015; Hessen et al., 2017). Some studies do address the impact of seabirds on the biodiversity of Arctic ponds (Keatley et al., 2009; Stewart et al., 2013; Gonzalez-Bergonzoni et al., 2017), but again primarily the indirect impacts by nutrient enrichment. Marine seabirds clearly do not serve as vectors of freshwater invertebrates, but may impact diversity negatively by reducing pH (Gonzalez-Bergonzoni et al., 2017). Also, within Svalbard a very strong local sorting is anticipated, reflected by the strikingly lower diversity in cold sites close to glaciers (Walseng et al., 2018).

One important aspect here is however that birds clearly may play a dual role in shaping diversity and community composition, first by direct dispersal, and secondly by affecting post-dispersal species sorting by increasing water quality and productivity (Mariash et al., 2018). Birds may indeed affect their habitat not only by nutrient release and thereby productivity and community composition among autotroph and thus also zooplankton. They may also affect turbidity and serve as vectors of competitors, predators or parasites. This is also an important aspect of zoochory, since the main concern is primarily how the overall direct and indirect activity of birds affects changes in biogeographical patterns and species richness.

The colonization of artificial ponds over the course of a brief time period should a priori offer the best opportunity to reach conclusions about birds as vectors of invertebrates (Louette and De Meester, 2005). In this case, it is important that the ponds have some attractive properties for birds (i.e., not simply concrete basins), and also that they offer possibilities for species to establish populations, not only to be transmitted. As pointed out above, the initial likelihood of colonization should *a priori* be larger in recently established systems with few species and more empty niches. The recently established ponds displayed not only an almost immediate colonization, but also a very high site-specific turnover. In total one third of all recorded Norwegian zooplankton species were recorded in these tiny ponds over the course of the study period, illustrating the strikingly high dispersal ability. The high temporal species turnover likewise suggests that internal competition and species sorting are superimposed on dispersal. Absence from samples in single years does not mean absence from the locality however, and the species could likely be present as resting eggs or dormant stages in the local "seedbank" (Hairston, 1996). Still the high turnover strongly suggests frequent recolonization at the metapopulation level. The studies of these ponds strongly argue for a successful dispersal by birds, with a rapid increase in diversity due to low initial competition and sorting. Firm evidence of bird dispersal is lacking, however, because bird visits or samples from visiting birds were not included—as they rarely are. And this is a key point here. Despite the numerous studies and discussions on richness, diversity and colonization of zooplankton or other freshwater fauna on different spatial and temporal scales, firm evidence is still rare owing to the fact that the numerous studies on diversity and community composition in freshwater habitats so rarely include proper assessments on bird impacts.

It could be argued that since aquatic organisms clearly do disperse, few are endemic and most are widespread, it does not really matter what kind of mechanism mediates the dispersal. Clearly it does. First of all, birds operate over long distance, and as climate change proceeds, they may also serve as vectors of "alien" species. Secondly, if bird dispersal is dominant at all scales, the roles of connectedness are less critical (Havel and Shurin, 2004). Third, the fact that abundance of birds is in strong change, with some groups or species in strong declines, others in strong increase, these changes may profoundly affect freshwater invertebrate communities as well. Thus, it is indeed important also to provide firm evidence for the mode of dispersal, but the hard way of examining birds and their gut content for viable propagules does not prove anything beyond the capacity of birds to serve as vectors, which is hardly disputable (Figuerola et al., 2005).

Even for zooplankton, despite several examples of widespread, sometimes cosmopolitan distributions, it is clearly not so that "everything is everywhere". At local or regional scales, diversity and community composition may primarily be governed by biotic and abiotic properties of the water bodies, as well as connectivity and lake density. Flooding events or dispersal by fish, amphibians or mammals also becomes more likely at local scales. At larger spatial scales, colonization is likely related to bird migration routes (Figuerola and Green, 2002; Green and Figuerola, 2005). Our data suggest spatial explanations dominates over local, and that along longitudinal clines, and especially in the case of geographical barriers such as mountain ridges, even 8000 years of post-glaciation history has been insufficient to complete western colonization for most species.

Given the potential fitness-promoting effect of dispersal, one would expect a selective pressure toward abilities to disperse and colonize, and that certain species or taxa have evolved properties promoting "bird-hiking". It could also be anticipated that different taxonomical groups (i.e., cladocera vs. copepods) have different colonization abilities due to considerable differences in features, such as propagules, generation time, and sexual vs. asexual reproduction, implying that Allee effects would benefit asexual cladocera relative to sexually reproducing copepods (Pinel-Alloul et al., 2013; Henriques-Silva et al., 2016). From the literature there is mixed evidence for higher dispersal rates in cladocera. Comparison across taxa done by Cohen and Shurin (2003) could not show any consistent differences between these groups though, instead species of both cladocerans and copepods ranged from highly effective to slow dispersers. Our large-scale data gave no support for this, neither did the very small-scale pond colonization studies, while colonization events at Svalbard hinted on a stronger dispersal ability among cladocera.

At the species level, there are several cases of fast dispersal. E.g., the large, carnivorous cladoceran *Bythotrephes longimanus* represent a well-documented history with a progressive dispersal over few decades in North America (Yan et al., 2011). In Scandinavia, the herbivorous cladoceran *Limnosida frontosa* is a large herbivorous species which has colonized a number of Norwegian lakes up to 61° degree north during the past 100 years (Jensen et al., 2001). There are certain species that typically are confined to coastal areas or north-south valleys and thus implicitly migration routes (e.g. *Diaphanosoma brachurum, Daphnia cristata, Bosmina longiriostris,* and *Simocephalus serrulatus*). This may however also reflect temperature preferences, i.e., that colder, and often oligotrophic sites at higher altitudes are avoided.

Dispersal could also work in the opposite direction. The cladoceran *Macrothrix hirsuticornis* has a holarctic distribution on the northern hemisphere. It is also one of the most common microcrustaceans on Svalbard (Walseng et al., 2018). However, on the Norwegian mainland the species is almost entirely limited to the area between 67,5 and 69,6 northern latitude, except for one record 150 km further south (66,2 N). The main distributional area for the species on mainland Norway is also the area where the Svalbard geese leave/enter the Norwegian coast before heading for Svalbard. Hence, it seems possible that *M. hirsuticornis* colonized mainland Norway from Svalbard. Correspondingly, the calanoid copeod *Acanthodiaptomus tibetanus* is recorded in Norway only in a few localities in the northernmost county, Finnmark, and in one alpine lake further south. It was possible to relate this distribution to the main migration route of waterfowl from Siberia (Walseng et al., 1996). Similarly, single recordings of *Heterocope borealis* way off from its main area of distribution may be attributed to birds (Koksvik et al., 2017).

Thus, while assessing dispersal at the species level in specific cases can be tentatively related to birds, it is more difficult to relate general patterns of distribution to birds or any other means of zoochory. By including genetic analysis, not only may the dispersal *per se* be substantiated, but also the founder or source populations. E.g., the genetic main lineages of *Daphnia laevis* across North America was found to largely follow migratory patterns of waterfowls (Taylor et al., 1998). Also, Figuerola et al. (2005) related dispersal in *Daphnia* species to birds by assessing mtDNA to known migration routes, and microsatellite data of bryozoans has also provided strong evidence for bird-mediated dispersal (Okamura and Freeland, 2002).

## CONCLUSION

To assess the role of zoochory is important for several reasons, not the least for understanding the likelihood of "new" species arriving in a changing climate, but also for a full understanding of how ecosystems are connected via migrating animals (Bartels, 2012; Hessen et al., 2017). With dynamic population sizes of waterfowl, their roles as vectors for transmission of aquatic invertebrates is highly relevant for assessment of biogeographical patterns of richness and community composition. Despite the long-lasting interest in the topic, we are still far from a proper understanding of bird-mediated zoochory. By addressing this at three different scales, we illustrate the disparity between circumstantial and firm evidence of bird-mediated zoochory of freshwater zooplankton. We suggest that long distance dispersal is less likely due to the fact that water-fowl commonly forage in terrestrial or coastal habitats and also have a fast gut turnover time. Moreover, local sorting due to different abiotic or biotic properties of recipient water bodies will constrain the permanent establishment of new species, especially when donor and source populations are in different, climatic regions. Short distance zoochory is presumably common, and in recently established habitats this may result in a fast rate of colonization, while in species saturated, established habitats, species sorting is more likely due to strong competitions. Thus, the net impact of zoochory needs to consider both these processes; dispersal and establishment, and these will differ both spatially and temporally. Finally, we conclude that the best line of evidence for dispersal might not be at the species level, but at the genotype level.

## AUTHOR CONTRIBUTIONS

DH conceived the idea and wrote the first draft. TJ and BW have both been involved in data sampling, analysis and writing of the MS.

## FUNDING

The samplings at Svalbard have been funded by The Research Council of Norway (grant number 227024/E10 and 246726/E10).

## ACKNOWLEDGMENTS

This work has benefitted from a number of colleagues that has participated in sampling. In particular we would like to acknowledge Kirsten Christoffersen and Asbjørn Magnar Hov for contribution to the samplings at Svalbard and the southern ponds, respectively, and Håvard Husebø for data and maps on bird migrations.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Ecology and Evolution

ORIGINAL RESEARCH
published: 27 March 2019
doi: 10.3389/fevo.2019.00082

# Overlooked Parrot Seed Dispersal in Australia and South America: Insights on the Evolution of Dispersal Syndromes and Seed Size in *Araucaria* Trees

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**Specialty section:**
*This article was submitted to*
*Biogeography and Macroecology,*
*a section of the journal*
*Frontiers in Ecology and Evolution*

**Received:** *03 October 2018*
**Accepted:** *04 March 2019*
**Published:** *27 March 2019*

**Citation:**
*Tella JL, Blanco G, Dénes FV and*
*Hiraldo F (2019) Overlooked Parrot*
*Seed Dispersal in Australia and South*
*America: Insights on the Evolution of*
*Dispersal Syndromes and Seed Size*
*in Araucaria Trees.*
*Front. Ecol. Evol. 7:82.*
*doi: 10.3389/fevo.2019.00082*

While Psittaciformes (parrots and allies) are well-recognized as highly-mobile seed predators, their role as seed dispersers has been overlooked until very recently. It remains to be determined whether this role is anecdotic or is a key mutualism for some plant species. We recently found that the large nut-like seeds of the two South American *Araucaria* tree species (*Araucaria araucana* in Andean forests and *Araucaria angustifolia* in Atlantic forests, weighing c. 3.5 and 7 g, respectively) are frequently dispersed, and to long distances, by parrots. Moreover, both observational and experimental work demonstrated that dispersed seeds can germinate faster after partial predation by parrots. Here, we hypothesized that a third, even larger-seeded (17.5 g) congeneric Australian species (*A. bidwillii*) is also dispersed by parrots. We surveyed 52 *A. bidwillii* and 42 *A. cunninghamii* (a sympatric species with small winged seeds, c. 0.2 g) during the seeding period. We found that sulfur-crested cockatoos (*Cacatua galerita*) consumed large amounts of seeds from all of the *A. bidwillii* trees surveyed. Cockatoos dispersed ca. 30% of the seeds they removed from the mother tree, carrying the seeds to distant perches for handling or dropped them while flying. Dispersal distances ranged between 10 and 153 m (mean = 61 m). Most seeds handled for consumption (93%) were fully eaten but others were dropped intact (3%) or only partially eaten (4%), and germination was confirmed for both intact and partially-eaten dispersed seeds. Moreover, seeds dropped by cockatoos facilitated secondary seed dispersal by conspecifics and another three bird species. We found no evidence of other primary dispersal species for *A. bidwillii,* while the small, winged seeds of *Araucaria cunninghamii* were only dispersed through barochory and anemochory. The seed weight of the three *Araucaria* species dispersed by zoochory is strongly related to the body mass of their main seed-disperser parrot species. These results support a role for parrots as key dispersers of the three large-seeded *Araucaria* species around the world, and suggest that large seeds may have evolved–at least partially–as an adaptation that allows trees to attract parrots, satiate them, and benefit from their long-distance seed dispersal services.

**Keywords: cockatoo, conifers, dispersal syndromes, mutualism, Psittaciformes, seed dispersal, seed predation, seed size**

## INTRODUCTION

Plant investment in reproductive tissues is shaped by biotic and abiotic factors over evolutionary time. In particular, the variability in seed size among extant plants, which exceeds ten orders of magnitude (Moles et al., 2005), may result from multiple life-history trade-offs combined in complex ways with past and current ecological factors, both being influential (Leishman et al., 2000; Díaz et al., 2016). Conifers, and other non-flowering plants (gymnosperms), provide good models with which to study the evolution of seed size. These plants have several specific features that make them highly suitable for such studies: they bear seed-producing organs as separate, compact structures (seed cones), evolved 225 Myr before flowering plants (angiosperms), and have shown much larger seeds than angiosperms from the time of their origin until the present day (Leslie et al., 2017). Comparative phylogenetic studies combining fossil records and extant plants have identified several drivers in seed size evolution. In conifers, there was an increase in the amount of cone tissue devoted to seed protection (robust, tightly packed scales) without a concomitant increase in seed size, which has been interpreted as an evolutionary response to the diversification of vertebrate and insect seed predators (Leslie, 2011). These antagonistic plant-animal interactions were moreover combined with climatic factors and mutualistic plant-animal interactions, as seeds are generally larger in animal-dispersed than in wind-dispersed conifers (Leslie et al., 2017). However, seed size is expected to be constrained by the gape size of those vertebrates (birds and mammals) that could ingest and disperse them, thus maintaining relatively small propagule sizes (Leslie et al., 2017).

The patterns described above differ in several ways from that found in the family Araucariaceae, an ancient conifer clade with 37 extant species that currently occurs in southern South America, Australia and some Pacific islands (Farjon, 2017; Gleiser et al., 2019). This is the only conifer family in which evolution in seed size was apparently not influenced by climate (Leslie et al., 2017). Moreover, although most species show unspecialized, wind-dispersed, seeds of relatively small size (Leslie et al., 2017), a small clade section composed of *Araucaria* species (*Araucaria araucaria, Araucaria angustifolia, Araucaria bidwillii,* and *Araucaria hunsteinii*) evolved the largest seed cones (Gleiser et al., 2019) and seeds of any living conifer (Farjon, 2017). These large seeds are expected to be part of an animal-mediated dispersal syndrome (Leslie et al., 2017; Gleiser et al., 2019), and thus more detailed information on their seed-dispersing species is needed in order to understand their evolutionary trajectory (Gleiser et al., 2019).

Araucariaceae was the first conifer family to develop large seed cones, where they appear as early as the Jurassic, coinciding with the diversification of large sauropod dinosaurs that could act as their seed predators and dispersers (Leslie, 2011). To our knowledge, however, there is no evidence that these large seeds were dispersed by extinct megafauna and no extant vertebrate can ingest and defecate them intact, although some mammals and birds currently act as seed predators and external seed dispersers (Vieira and Iob, 2009; Shepherd and Ditgen, 2013; Dénes et al., 2018). Recent studies, however, have added new, thus far overlooked actors to this scenario, demonstrating that parrots (Psittaciformes) may not act only as plant antagonists (as pervasive seed predators) but also as legitimate seed dispersers for a variety of plant species (see review in Blanco et al., 2018), as it has been also shown for some Neotropical primates (Barnett et al., 2012). In a recent study, we showed that the only parrot species living in the monkey puzzle (*Araucaria araucana*) forests of the Andes, the Austral parakeet (*Enicognathus ferrugineus*), feeds mostly on the large seeds (mean 3.5 g) of this tree during the seeding period, and disperses them at higher rates and for longer distances than do rodents (Tella et al., 2016a). Also in South America, we found that two other, larger parrot species (red-spectacled amazon, *Amazona pretrei,* and vinaceous amazon, *Amazona vinacea*) base their diets on the large seeds (7 g) of the Paraná pine (*Araucaria angustifolia*) in Atlantic forests, dispersing them at high frequencies and over long distances and more efficiently than the previously recognized bird dispersers (Tella et al., 2016b). Moreover, both observational and experimental work on these two *Araucaria* species has shown that seeds partially eaten by parrots germinate well and even faster than undamaged seeds (Tella et al., 2016b; Speziale et al., 2018). This robust evidence allowed us to hypothesize that large *Araucaria* seeds could have evolved to attract parrots, satiate them, and benefit from their long-distance and legitimate seed dispersal services (Tella et al., 2016b).

Given the above hypothesis, we expected that the Bunya pine (*Araucaria bidwillii*), which is distributed throughout eastern Australia and has the largest seeds of any extant congener (averaging 17.5 g), would also be dispersed by a large parrot species. Here, we present the results of a field work expedition designed to test this prediction. The little information available suggested that *A. bidwillii* seeds mainly disperse by gravity (barochory), with poorly-known secondary dispersal by water and small mammals (Smith and Butler, 2002; Smith et al., 2005, 2007; Picone, 2014). We investigated seed predation and seed dispersal in *A. bidwillii* and, as a control, in the coexisting hoop pine (*Araucaria cunninghamii*), whose small (0.2 g) winged seeds are considered to be wind-dispersed (anemochory) (Leslie et al., 2017). Following this rationale, we predicted a positive relationship between *Araucaria* species seed weights and the body mass of their main seed-disperser parrot species, supporting a role for animal dispersal as a driver in the evolution of large seeds and seed cones in this Araucariaceae clade (Gleiser et al., 2019).

# MATERIALS AND METHODS

## Field Work Procedures

Field work was conducted between 12 and 25 May 2017, coinciding with the end of the seeding period of *Araucaria bidwillii* that year. This sampling timing facilitated the detection of seed predators and dispersers (Tella et al., 2016a,b). We traveled throughout the distribution range of these species in Queensland, Australia (Thomas, 2011a,b), but concentrated our surveys in three areas: (1) the region between Lamington National Park and Canungra, southern Queensland, inhabited by *A. cunninghamii*, (2) Bunya Mountains, occupied by the latter species but also host to one of the largest populations of *A. bidwillii*, and (3) the surroundings of Cannabullen Falls, about 1,000 km north of the Bunya Mountains, where a relict population of <100 mature *A. bidwillii* persists (Thomas, 2011b).

Surveys replicated our previous work on seed dispersal of *Araucaria* species in South America see (Tella et al., 2016a,b). Briefly, we drove a car at low speed through unpaved and secondary roads to increase the chances of finding highly mobile flocks of foraging parrots. When we located parrots feeding on *Araucaria* trees, we stopped and observed them with telescopes from a distance to avoid disturbance. When good visibility allowed, we recorded dispersal rates by counting the number of seeds consumed in the mother tree and the number of seeds transported in flight to distant perches. In many other instances, we could only see parrots flying with seeds in their beaks, without evaluating the actual dispersal rates. Dispersal distances were measured with a laser rangefinder (Leica Geovid 10x42x, range: 10–1,300 m) as the distance from the mother tree to the perching site (exact distance) or up to where the seed-carrying flying parrot went out of sight (minimum distance). Once perching sites were identified, we looked underneath them for additional dispersed seeds and measured the distance to the nearest seeding tree (i.e., we conservatively recorded a minimum dispersal distance). These perching sites were repeatedly used by parrots (cockatoos), so we can satisfactorily assume that seeds found there were moved by them but not by secondary disperser species. We also recorded whether the dispersed seeds were fully consumed, dropped intact, or partially eaten. Intact and partially-eaten seeds were apparently viable (i.e., they were not fungal-infested or rotten).

The proportion of seeding trees on whose seeds parrots fed was also obtained following our previous work (Tella et al., 2016a,b; Dénes et al., 2018). Briefly, we selected well-spaced seeding trees (separated > 40 m from the closest seeding tree) and well spatially distributed across the study areas to cover potential effects of spatial heterogeneity in parrots' distributions and movements. We then looked below their canopies for signals of seed predation, the forms of which allowed unambiguous distinction between the different bird and mammal seed predators (Tella et al., 2016a,b; Dénes et al., 2018). In this study, fieldwork was facilitated by the low number of predator species found (see Results) and by the relatively low number of seeds produced by *A. bidwillii*. Therefore, we were able to count all the seeds found under *A. bidwillii* canopies and recorded whether they were preyed upon, dropped intact, or partially eaten by cockatoos (after observing the characteristic way cockatoos opened the seeds, see Results). Dropped seeds further consumed by non-native wild boars (*Sus scrofa*) and native rodents were easily identified, as described in our previous studies (Tella et al., 2016a,b). Only a few seeds of *A. bidwillii* were consumed by unidentified mammals. The number of seeds of *A. cunninghamii* was much greater, so we restricted our sampling to 50–100 seeds per tree.

## Data Analyses

Contingency tables and Chi-square tests were used for testing differences in proportions. Dispersal distance distributions were right-censored as they included a number of minimum distances, so we employed an adaptation of Kaplan-Meier estimators for survival functions (Klein and Moeschberger, 2003) to estimate dispersal functions, $D(d)$ see (Tella et al., 2016b) for the same approach. Mean and median dispersal distances were obtained from the estimated function, as the integral of the dispersal curve, conservatively restricting the mean to the larger distance recorded, and as the intersection of the curve with a horizontal line drawn at 0.5, respectively (Therneau, 2015). We used the package *survival* (Therneau, 2015) in R (R Core Team, 2015) to estimate the dispersal function.

## Data Compilation

For comparative purposes, information on seed dispersal rates and dispersal distances of *A. araucana* and *A. angustifolia* was obtained from Tella et al. (2016a) and Tella et al. (2016b), respectively. Body masses of the parrot species recorded in these previous studies, and in the current one, independent of whether they acted as seed dispersers, were obtained from Forshaw (2006). Seed weights of each *Araucaria* species were also obtained from the literature (Ntima, 1968; Henderson, 1979; Muñoz, 1984; Mantovani et al., 2004; Burrows et al., 2017). In all cases, the midpoint was used when a range of weights was provided for a given species.

# RESULTS

## Seed Predation and Dispersal of *Araucaria bidwillii*

During field work in the Bunya Mountains, we only observed sulfur-crested cockatoos (*Cacatua galerita*) in a highly mobile flock of ca. 70 individuals, preying upon *A. bidwillii* seeds (**Figure 1A**). Cockatoos perched on the large female cones to extract the seeds (**Figure 1B**), and opened the pericarp in a characteristic way to gain access to the seed content and consume it (**Figure 1C**). This allowed us to clearly identify the seeds preyed upon by this species. Individuals from another four

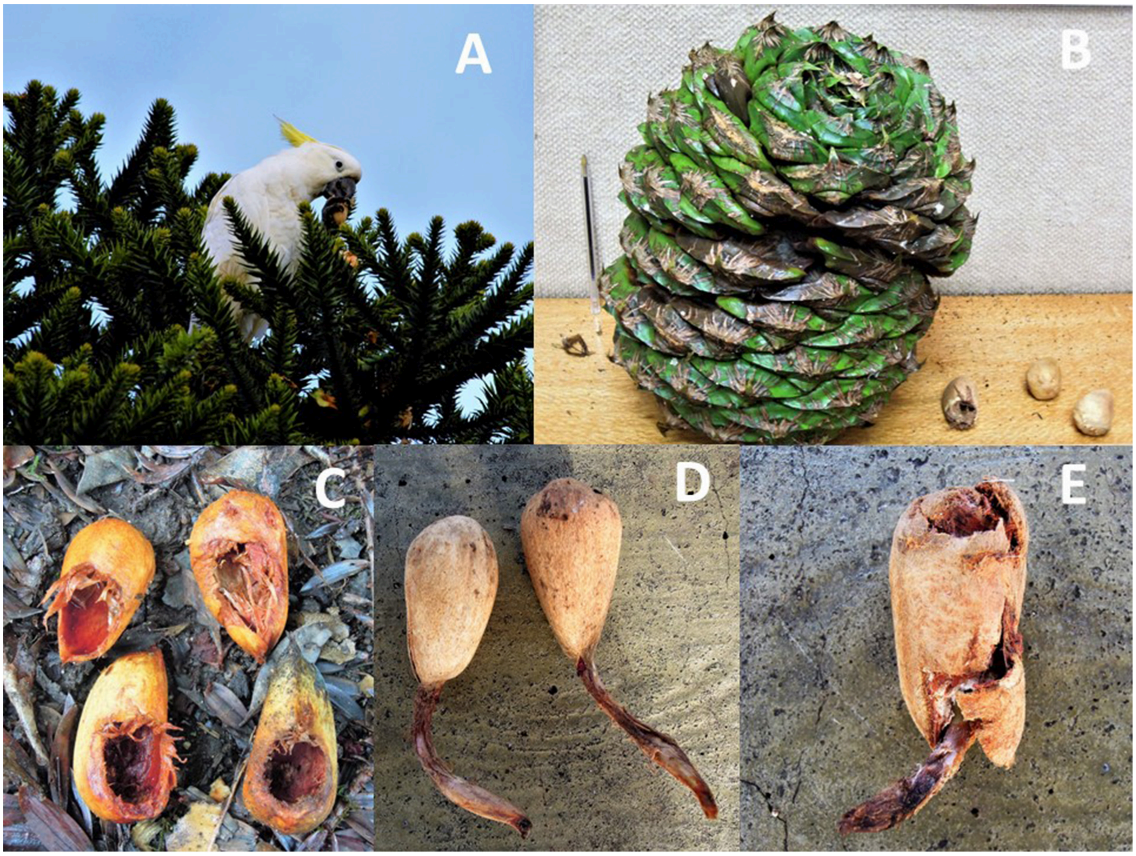

**FIGURE 1 |** A sulfur-crested cockatoo handling a Bunya pine (*A. bidwillii*) seed **(A)** after extracting it from the large and strong female cone **(B)**. Note the size of the cone related to the size of the seeds, in the bottom right corner of the picture. Cockatoos open the pericarp to consume the endosperm in a characteristic way **(C)**. Entire **(D)** and partially-eaten **(E)** seeds germinating after dispersal by cockatoos (Pictures: GB and JT).

parrots species (12 galahs *Eolophus roseicapilla*, 10 Australian king parrots *Alisterus scapularis*, 8 crimson rosellas *Platycercus elegans*, and 3 red-winged parrots *Aprosmictus erythropterus*) were also observed, but none of them were seen preying upon *A. bidwillii* seeds. We observed Torresian crows (*Corvus orru*) obtaining the remainders of seeds discarded by sulfur-crested cockatoos in the same tree canopy, and Australian ravens *Corvus coronoides* and Australian brush-turkeys (*Alectura lathami*) taking seeds dropped by cockatoos under the trees. The latter two species repeatedly pecked the seeds but seemed unable to open the pericarp for obtaining seed fragments to ingest them, so they acted as secondary seed dispersers when moving the seeds to distant sites.

The detailed observations of cockatoos foraging on female cones, totaling 2.15 h, gave a seed dispersal rate of 29.63% ($n = 27$); 19 seeds were picked and consumed in the mother tree while 8 seeds were transported with the beak, flying to distant perches to handle and consume them. Only five cockatoos were observed looking for seeds dropped by others, despite the fact that this species often forages on the ground. Four of these cockatoos consumed the seeds on the ground, while the other (20%) dispersed one seed by flying to a distant perch. Dispersal distances, including exact measurements ($n = 115$) and minimum distances (i.e., from birds lost in flight or from the perching site to the closest seeding female tree, $n = 126$) ranged from 10 to 153 m ($n = 241$) (**Figure 2A**). Kaplan-Meyer analysis for right-censored data allowed us to estimate a mean dispersal distance of 60.86 m (SE = 4.57) and a median dispersal distance of 40 m (95% CI = 40–72 m) (**Figure 2B**).

In three instances (1.2%) cockatoos dropped seeds in flight, while the rest were handled for consumption at distant perches. Perching sites ($n = 48$) included branches of non-seeding *A. bidwillii* trees (89.6%), *Eucalyptus* spp. trees (2.1%), other species of trees (6.2%) and electricity poles (2.1%). Most dispersed seeds (92.9%, $n = 241$) were fully eaten but others were dropped intact (3.3%) or only partially eaten (3.7%). Two undamaged seeds and one partially-eaten seed were **already** germinating under perches after dispersed by cockatoos (**Figures 1D,E**).

About half (52 out of 109) of the well-spaced *A. bidwillii* trees inspected produced seeds during the study season. All of these seeding trees were previously visited by cockatoos, as indicated by the characteristically predated seeds (**Figure 1C**) we found below their canopies. We only found the remainders of a single case of barochory, i.e., when the entire mature female cone falls to the ground and the contained seeds disaggregate due to strong

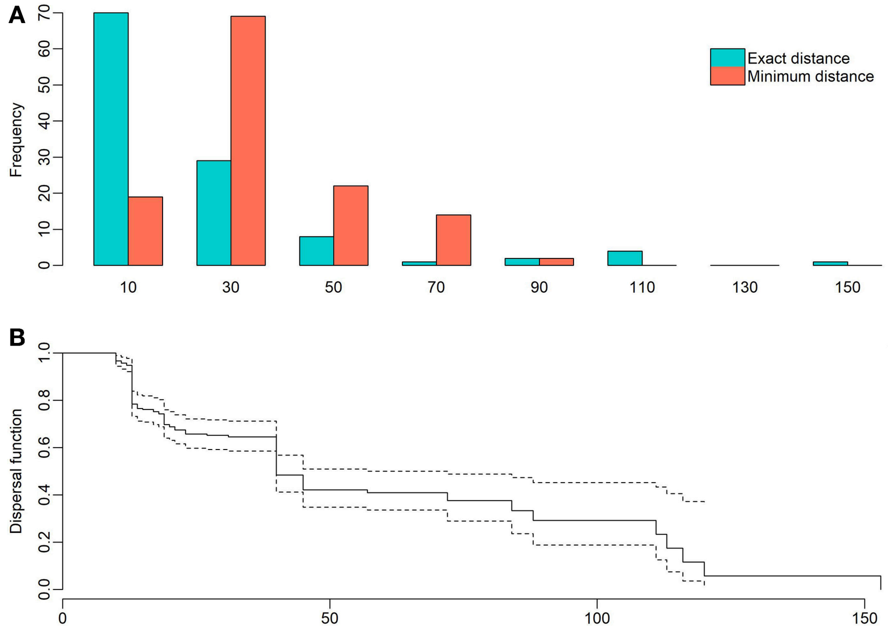

**FIGURE 2 |** Dispersal distances (in m) of *A. bidwillii* seeds transported by sulfur-crested cockatoos. **(A)** Distribution of the exact and minimum dispersal distances recorded ($n = 241$). **(B)** Kaplan-Meyer estimate of the dispersal function. Dashed lines show 95% confidence bounds.

impact. Therefore, almost all the seeds produced by the 52 trees were preyed upon, dropped (intact or partially eaten) *in situ* or dispersed by cockatoos before naturally falling to the ground. The proportion of seeds preyed upon (97.8%), undamaged (1.9%), or partially eaten by cockatoos (0.3%) found under the canopies ($n = 1{,}283$ seeds) differed from those regarding dispersed seeds (see above, $\chi^2 = 30.4$, df $= 2$, $p < 0.001$), due to a lower proportion of undamaged and partially-eaten seeds. This was probably due to the predation of the seeds dropped by cockatoos by terrestrial mammals attracted by the residual material they left. In fact, we found clear evidence of seed predation by wild boars (*Sus scrofa*), rodents and unidentified mammals under 15.5% (5.8, 1.9, and 7.7%, respectively) of inspected trees.

Apart from the *A. bidwillii* population in the Bunya Mountains, we could only inspect 14 adult trees in the northern relict population, and none of them produced seeds successfully.

## Seed Predation and Dispersal of *Araucaria cunninghamii*

We did not observe predation of *A. cunninghamii* seeds by any of the species of parrots recorded in the Bunya Mountains (see above), or in the Lamington-Canungra area where we also recorded a number of foraging granivorous parrots (140 sulfur-crested cockatoos, 113 galahs, 24 Australian king parrots, 4 crimson rosellas, 3 pale-headed rosellas *Platycercus adscitus*, and 2 yellow-tailed black cockatoos *Calyptorhynchus funereus*). We observed sulfur-crested cockatoos feeding on fresh and dry branches and on gum of several *A. cunninghamii* trees, but not on their abundant seeds.

We inspected a sample of 2,550 seeds from 42 seeding trees (13 in Lamington–Canungra area and 29 in the Bunya Mountains). No seeds showed signs of predation by parrots, and only 0.8% of them had been preyed upon by rodents, with the rest being undamaged. These trees were isolated or separated >40 m from the closest seeding tree, thus avoiding the possibility that seeds found under a particular tree came from another tree. Most seeds were dispersed by barochory (i.e., they were found just below the mother canopy tree), while in a few cases the wind seemed responsible of dispersing seeds in a radius of up to 30 m (anemochory).

## Comparison of Parrot-Mediated Seed Dispersal in *Araucaria* Species

**Table 1** summarizes the available information on seed dispersal of *Araucaria* species by parrots. The Austral parakeet (*E. ferrugineus*) was the only bird species recorded dispersing seeds of *A. araucana*, which constitutes the main food resource for this species during the seeding period (Tella et al., 2016a). Primary dispersal rate performed by this species was extremely low, while secondary dispersal (i.e., after mature seeds fall to the ground) showed the highest dispersal rate for the three *Araucaria* species. Maximum and mean dispersal distances are the lowest, although they were clearly underestimated since only minimum distances (i.e., right-censored) could be recorded. In the case of *A. angustifolia*, two amazon species (*A. pretrei* and *A. vinacea*) are strongly linked to this species, as its seeds constitute the bulk of their winter diet (Tella et al., 2016b). Other parrot species play a minor role as dispersers and/or their distributions barely overlap with the distribution of *A. angustifolia* forests (Tella et al., 2016b). Amazons exclusively act as primary seed dispersers, with moderate dispersal rates and the largest dispersal distances recorded for *Araucaria* species. Finally, based on the

**TABLE 1 |** Comparison of seed dispersal traits by parrots of the three *Araucaria* species dispersed by zoochory.

| | Dispersal | Dispersal rate (%) | Dispersal distances (m) | | | Source |
|---|---|---|---|---|---|---|
| | | | Min. | Max. | Mean | |
| *A. araucana* | Primary | 0.1 | | | | Tella et al., 2016a |
| *A. araucana* | Secondary | 57 | 5 | 50* | 15** | Tella et al., 2016a |
| *A. angustifolia* | Primary | 22.5 | 5 | 500* | 247 | Tella et al., 2016b |
| *A. bidwillii* | Primary | 29.6 | 10 | 153 | 61 | This study |
| *A. bidwillii* | Secondary | 20 | | 84 | | This study |

**Underestimated as they were minimum dispersal distances (right censored), **Underestimated as it was obtained from right-censored distances.*

present study, *C. galerita* seems to be the only primary seed-disperser parrot of *A. bidwillii*, showing moderate dispersal rates and dispersal distances compared to the rest of parrot species. This cockatoo also acts, although at a much lower frequency, as a secondary seed disperser.

**Figure 3** shows the variability in seed weight between the three zoochoraceous species of *Araucaria* (*A. araucana, A. anfustifolia,* and *A. bidwillii*) compared to those exclusively dispersed by barochory and anemochory (*A. cunninghamii*). As expected from a potential role of parrots in the evolution of seed size, the relationship between the seed weights of the three zoochorous *Araucaria* species and of their main parrot-disperser species (Austral parakeet, amazons and sulfur-crested cockatoo) closely matches the diagonal line between plant and disperser traits (i.e., the line depicting a theoretical full correlation, $r = 1$; **Figure 3**). The body mass of most parrot species that contribute little or nothing to *Araucaria* seed dispersal largely departs from the diagonal line (**Figure 3**).

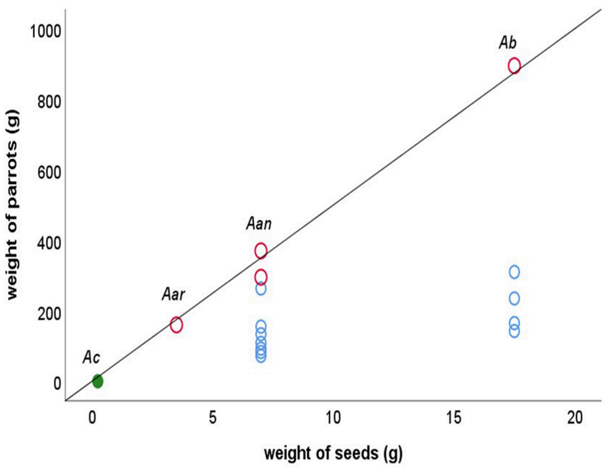

**FIGURE 3 |** Relationship between the seed weight of three *Araucaria* species (*Aar, A. araucaria; Aan, A. angustifolia; Ab, A. bidwillii*) and the body mass of parrot species that disperse them (red circles). Blue circles represent parrot species that have a small (for *Aan*) or null seed dispersal role (for *Ab*). The diagonal line represents the expected perfect correlation between seed size and parrot size. A. *cunninghamii* (*Ac*) is depicted (green dot) as a reference, since no parrots disperse its seeds (they are only dispersed by barochory and anemochory).

# DISCUSSION

## Seed Dispersal in *Araucaria bidwillii*

Little information is available on seed dispersal strategies of *A. bidwillii*. Although some authors have argued that dinosaurs and large mammals may have dispersed its seeds in the past (Smith et al., 2007), the prevailing view is that the species is now mostly gravity-dispersed (Smith and Butler, 2002; Pye, 2005; but see Farjon, 2017). The large seeds are retained in similarly large female cones (which can weigh in excess of 10 kg) until they mature and fall intact to the ground; therefore, excluding instances of rolling down slopes or falling into watercourses, the majority of seeds are expected to initiate an intra-cone competition to germinate beneath the parent tree (Pye, 2005). It has been suggested that this limited dispersal capacity explains the poor ability of the species to recolonize areas following its past range contraction (Smith and Butler, 2002). However, observations of rodents caching seeds suggested that additional mechanisms might be available for dispersal (Pye, 2005). Smith et al. (2005) tagged seeds and placed automatic cameras for monitoring seed predation and secondary dispersal by mammals, showing that a small proportion of seeds were handled and eaten by rodents and that some seeds were dispersed at least 16 m. Additional work by the same authors (Smith et al., 2007) showed that some seeds were carried up to 8 m outside the parent tree canopy, and that the short-eared possum (*Trichosurus caninus*) is able to disperse the seeds. Moreover, Picone (2014) observed cockatoos, though not indicating the species, feeding on seeds and suggested that they could also act as dispersal vectors.

Here, we demonstrate that sulfur-crested cockatoos are both pervasive seed predators and legitimate seed dispersers of *A. bidwillii*. These cockatoos are able to open the strongly compacted female cones with their strong beaks to extract the seeds, something that other large bird species such as crows and ravens, or smaller parrot species, cannot do. Notably, cockatoos preyed upon seeds on all the well-spaced trees surveyed well before dispersal by gravity, as we only found a single fallen mature female cone at the end of the seeding period, while the rest of the seeds were predated, dropped, or dispersed by cockatoos when cones were still in the canopy. Therefore, at least in the study year, gravity played a marginal role compared to the primary seed dispersal performed by cockatoos, challenging the prevailing idea of barochory being the main dispersal syndrome for this species (Smith and Butler, 2002; Pye, 2005; Picone, 2014). Also notably, cockatoos dispersed seeds at a high rate (30%) and at distances that exceed by one order of magnitude those reported for secondary dispersal by small mammals (Smith et al., 2005, 2007). A relatively high proportion of the dispersed seeds (7%) were dropped intact or partially eaten in flight or under perches, thus having the potential to germinate, as germination was corroborated for both kinds of seeds despite the short-term nature of our survey. Moreover, cockatoos also dropped intact and partially-eaten seeds when extracting them from the cones, making them available under parent trees to other bird species as well as cockatoos (this study) and small mammals (Smith et al., 2005, 2007) that can act as secondary seed dispersers. Overall, our results add to recent findings showing that parrots are not merely seed predators but can be involved in plant-parrot mutualism-antagonism continuums (Montesinos-Navarro et al., 2017) where they can play a key role as seed dispersers (Boehning-Gaese et al., 1999; Blanco et al., 2015, 2016, 2018; Tella et al., 2015, 2016a,b; Baños-Villalba et al., 2017).

## Dispersal of Large-Seeded *Araucaria* Trees by Parrots

The identification of the main seed dispersal syndromes and vectors has been controversial not only for *A. bidwillii*, but also for the whole Araucariaceae family. In a recent comparative phylogenetic study, Contreras et al. (2017) identified most species of the family as being dispersed by anemochory, with a few species relying on barochory for their seed dispersal. Simultaneously, other analyses suggested that the ancestral seed dispersal strategy for the large-seeded clade of *Araucaria* (*A. araucana, A. angustifolia, A. hunsteini, A. bidwillii*) was zoochory, with a reversal to anemochory in *A. hunsteini* (Leslie et al., 2017), and that animals are still the main dispersal vectors for these species (Gleiser et al., 2019). In fact, there was accumulated evidence supporting current secondary seed dispersal of *A. araucana, A. angustifolia,* and *A. bidwillii* by vertebrates (Smith et al., 2005, 2007; Vieira and Iob, 2009; Shepherd and Ditgen, 2013). Our results show that a typical small-winged *Araucaria* species (*A. cunninghamii*) is mainly dispersed by barochory and anemochory, as expected given its diaspore morphology (Leslie et al., 2017; Gleiser et al., 2019). On the other hand, parrots seem to play a greater role as seed dispersers than the previously identified animal vectors for the three large-seeded species (Tella et al., 2016a,b; this study). The Austral parakeet is the only bird species dispersing seeds of *A. araucana*, and over much larger distances (Tella et al., 2016a) than the only rodent species that effectively disperse its seeds (Shepherd and Ditgen, 2013). The same is true for *A. bidwillii* (see above). A larger number of bird and mammal species were identified as seed dispersers of *A. angustifolia* (Vieira and Iob, 2009; Dénes et al., 2018). However, large amazon parrots were shown to be more efficient at dispersing seeds than jays, which were previously thought to be the main animal vectors for this species (Tella et al., 2016b). Remarkably, the three large-seeded species are dispersed at high rates by parrots and, perhaps more importantly, to long, underestimated, distances. Several reviews have highlighted the difficulties of measuring long-distance dispersal, as well as its pivotal importance: just a very small proportion of seeds effectively dispersed at long distances is key to maintaining gene flow and facilitating forest regeneration (Cain et al., 2000; Nathan and Muller-Landau, 2000; Howe and Miriti, 2004; Schurr et al., 2009; Jordano, 2017).

Large *Araucaria* seeds partially eaten by parrots germinate well and even faster than intact seeds, probably because the partial removal of the seed coat eliminates the main barrier to moisture, while favoring subsequent water intake and seedling emergence (Tella et al., 2016a; Speziale et al., 2018). This allowed us to hypothesize that their large seeds evolved to attract parrots—and perhaps also some unknown extinct vertebrate-, satiate them and benefit from their long-distance dispersal services (Tella et al., 2016b). These highly nutritive seeds (Brand et al., 1985; Conforti and Lupano, 2011) are covered by a relatively thin coat, but retained within strongly compacted cones until dispersal by barochory. Since seed size correlates with cone size (Gleiser et al., 2019), larger species of parrots, bearing stronger beaks, would be necessary to open the larger cones, access the seeds and eventually disperse them. Despite the unavoidably small sample size, the strong covariation found between the weight of *Araucaria* seeds and the body weight of their main seed-disperser parrot species supports our hypothesis of a role for parrots as drivers in the evolution of seed size in *Araucaria* species. Fossil records suggest that the family Araucariaceae may have originated in the Triassic, achieving its maximum diversity during the Jurassic and Cretaceous periods (Kershaw and Wagstaff, 2001), while molecular studies point to the origin and radiation of parrots in the late Cretaceous-Paleogene periods (Wright et al., 2008). Leslie et al. (2017) showed that dispersal syndromes are good predictors of seed size and cone morphology in conifers, including Araucariaceae. In line with these results, an increase in genome size, which correlates with seed size, may have moved the large-seeded *Araucaria* clade from the ancestral anemochory/barochory dispersal strategies to zoochory, by making their larger seeds attractive to seed predators (Gleiser et al., 2019). Therefore, there were opportunities during a long geological period for parrots to contribute to the evolution of seed size in *Araucaria* through seed predation and dispersal. One could also argue that current parrot communities may largely differ from the oldest ones, and thus that the relationship shown in **Figure 3** may not result from a long-term but from a contemporary process. This is also a possibility, as seed and cone morphology (e.g., Benkman et al., 2003; Dylewski et al., 2017), and even more complex reproductive strategies such as serotiny (an ecological adaptation exhibited by some plants, in which seed release occurs in response to an environmental trigger such as fire; Talluto and Benkman, 2014), are known to be shaped by seed predators/dispersers at short temporal and small spatial scales in conifers.

## Future Research Avenues

Clearly, more research is required to confirm the potential role of parrots in the evolution of seed size and seed dispersal strategies in Araucariaceae. A puzzling question is related to the dispersal syndrome of *A. hunsteinii*, whose range is restricted to New Guinea. This is the only species within the large-seeded Araucaria clade that shows a relatively small and winged seed, suggesting a reversal from zoochory to anemochory dispersal (Leslie et al., 2017). However, its nut-like seed is also highly nutritive and weighs ca. 2 grams (Henderson, 1979), thus being potentially attractive to the large community of parrots, including two cockatoo species inhabiting New Guinea (Forshaw, 2006). In fact, Ntima (1968) vaguely mentioned that cockatoos damage *A. hunsteinii* seeds. Field work would be needed to assess the relative contribution of wind dispersal and presumably parrot seed dispersal in this species, for a better understanding of the evolution of seed dispersal strategies, seed and cone size in Araucariaceae (Gleiser et al., 2019).

Our results show that parrots currently are frequent, long-distance and legitimate dispersers of the three large-seeded *Araucaria* species. Future work should assess to what extent parrots may be shaping the spatial recruitment of trees, as has been recently shown for a palm tree species mostly dispersed by large parrots (Baños-Villalba et al., 2017). Moreover, this role for parrots could be translated to the genetic population structure of *Araucaria* populations. Population genetic studies were carried out on the four species studied here (Bekessy et al., 2002; Pye and Gadek, 2004; Stefenon et al., 2007; Pye et al., 2009; Souza et al., 2009), and results were interpreted under the assumption that only pollen dispersal is responsible for long-range gene flow in these species. This assumption comes from a thorough paternity study conducted on seeds, seedlings and juveniles of *A. angustifolia* (Bittencourt and Sebbenn, 2007). It contrasts with the fact that pollen grains in Araucariaceae are among the largest non-saccate (i.e., without inflated air bladders) grains of any conifer (Leslie, 2010), raising questions about the effectiveness of wind dispersal for these large, non-floating pollen grains (Sousa and Hattemer, 2003), and its role in long-range gene flow (Pye, 2005). Results indicating that pollen dispersal is more important than seed dispersal for long-distance gene flow were obtained from two small, highly fragmented and isolated patches of *A. angustifolia* (Bittencourt and Sebbenn, 2007), where seed-disperser parrots could be scarce or even absent. In fact, the two parrot species that act as the main seed dispersers of this species have experienced substantial population declines and range contractions and, as for other parrot-plant (Luna et al., 2018) and plant-animal mutualisms (Valiente-Banuet et al., 2015), the local extinction of these species may have disrupted key seed dispersal processes (Tella et al., 2016b). The fragmented distribution of *A. angustifolia* thus offers a unique natural experiment for assessing the actual role of parrots as long-distance dispersers of this critically endangered species (Thomas, 2013), by replicating the work of Bittencourt and Sebbenn (2007) in several areas with and without the presence of parrots. Moreover, as *Araucaria* trees are extremely long-lived (e.g., >1,000 year reported for *A. araucana*, Aguilera-Betti et al., 2017), the comparison within areas of the genetic arrangement of seedlings and juveniles with that of centenary adults would further deepen the disruption of dispersal processes at long-term temporal scales.

A deeper understanding of the consequences of seed dispersal by parrots could lead to improved design of conservation strategies for these tree species, since *A. Araucana* and *A. angustifolia* are listed as Endangered and Critically Endangered species, respectively, by the IUCN (Premoli et al., 2013; Thomas, 2013), while *A. bidwillii* is listed as Least Concern but has a fragmented distribution and even an isolated, genetically distinct population (Pye and Gadek, 2004; Thomas, 2011b).

## ETHICS STATEMENT

This study relies on observational data obtained in areas unrestricted to people and thus did not require special permits.

## AUTHOR CONTRIBUTIONS

JT, FH, and GB designed the expedition. GB and JT conducted field work. FD analyzed the data. JT wrote a first draft of the manuscript and all authors contributed to improve it.

## ACKNOWLEDGMENTS

We thank R. Heinsohn for his initial advice on preparing the field work expedition, and G. Gleiser and two reviewers for their valuable comments to the manuscript. Loro Parque Fundacion funded the publication of this paper.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Ecology and Evolution

ORIGINAL RESEARCH
published: 18 April 2019
doi: 10.3389/fevo.2019.00133

# Beyond Scatter-Hoarding and Frugivory: European Corvids as Overlooked Vectors for a Broad Range of Plants

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It is well-known that some members of the crow family (Corvidae) are important for seed dispersal either via frugivory (e.g., when feeding on berries) or by scatter hoarding (e.g., of nuts). Dispersal via gut passage of seeds within a fleshy fruit can be considered "classical endozoochory." However, corvids are rarely recognized as vectors of plants lacking a fleshy fruit, or a large nut (such as plants with a dry achene, capsule or caryopsis). Dispersal of such seeds via gut passage can be considered "non-classical endozoochory." A century ago, Heintze (1917a,b); Heintze (1918) reported on extensive field studies of seed dispersal by 11 species of European Corvidae. His work is overlooked in contemporary reviews of corvid biology. We resurrect his work, which suggests that contemporary views about seed dispersal by corvids are too narrow. Heintze identified 157 plant taxa from 42 families which were dispersed by corvids by endozoochory, as well as another nine taxa only dispersed by synzoochory (which includes scatter-hoarding). Most (54%) of the plant species dispersed by endozoochory lack a fleshy fruit and have previously been assigned to other dispersal syndromes, mainly associated with wind (10%), self-dispersal (22%) or epizoochory (18%). Plants lacking a fleshy fruit were particularly well-represented from the Caryophyllaceae (12 species), Poaceae (14 species), and Polygonaceae (8 species). Of 27 taxa germinated by Heintze from seeds extracted from corvid pellets or feces (71% of those tested), 20 lack a fleshy fruit. Similarly, of 32 taxa he recorded as seedlings having germinated from pellets in the field, 11 lacked a fleshy fruit. However, Heintze's quantitative data show that classical endozoochory is dominant in Magpies *Pica pica* and Hooded Crows *Corvus cornix*, for which 97% of seeds dispersed were fleshy-fruited. Corvids overlap with waterfowl as vectors of terrestrial plants dispersed by non-classical endozoochory, and 56 species are dispersed by both corvids and dabbling ducks according to the lists of Heintze and Soons et al. (2016). Finally, Heintze's data show that corvids were already dispersing alien plants in Europe a century ago, such as the North American Dwarf Serviceberry *Amelanchier spicata*.

**Keywords: Corvidae, gut-passage, Hooded Crow, Magpie, non-classical endozoochory, non-standard dispersal, pellets, seed dispersal**

***Edited by:***
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*Wageningen University & Research,*
*Netherlands*

***Reviewed by:***
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***Specialty section:***
*This article was submitted to*
*Biogeography and Macroecology,*
*a section of the journal*
*Frontiers in Ecology and Evolution*

***Received:*** *03 January 2019*
***Accepted:*** *03 April 2019*
***Published:*** *18 April 2019*

***Citation:***
*Green AJ, Elmberg J and Lovas-Kiss Á (2019) Beyond Scatter-Hoarding and Frugivory: European Corvids as Overlooked Vectors for a Broad Range of Plants. Front. Ecol. Evol. 7:133. doi: 10.3389/fevo.2019.00133*

## INTRODUCTION

Plants disperse their diaspores ("seeds" from here on) by many means (Ridley, 1930), including via animal vectors ("zoochory"). By virtue of their flight, birds are excellent vectors, and their ability to disperse seeds via gut passage ("endozoochory") is particularly well-known, and was already recognized by the ancient Greeks (Theophrast who died in 287 BC described how birds disperse mistletoe, Holmboe, 1900). Seed dispersal is considered one of the most important of avian ecological functions (Sekercioglu, 2006). Most contemporary research on avian endozoochory is focussed on frugivores, and fruit has been reported to make up 10% or more of the diet of 83 species in the crow family (Corvidae) (Table 5.1 in Wenny et al., 2016). Dispersal of seeds embedded in the fleshy pulp of an edible berry or fruit has been called "classic endozoochory" (McPartland and Naraine, 2018). This corresponds to the long-standing practice of assigning "dispersal syndromes" to plant species on the basis of diaspore morphology, in which only fleshy-fruited diaspores are assigned to a "endozoochory syndrome" (van der Pijl, 1982; Perez-Harguindeguy et al., 2013). On the other hand, the Corvidae are the bird family most associated with plant dispersal by seed-caching or scatter-hoarding. This dispersal mode (a form of "synzoochory") occurs when jays, nutcrackers or other corvids carry large diaspores, such as nuts, acorns or pine seeds in their bill and then bury them in caches (Pesendorfer et al., 2016; Tomback, 2016).

Corvids are particularly familiar birds to people, and have influenced human culture (Marzluff and Angell, 2007). They provide many ecosystem services, for example through their value in forest regeneration from scatter-hoarding (Tomback, 2016). On the other hand, corvids are partly granivorous and have long had a reputation for causing damage to cereal crops (Barrows, 1888; Hadjisterkotis, 2003), hence the term "scarecrows." In this paper, we highlight the importance of corvid endozoochory for a broader spectrum of plants, many of which are dispersed by granivory rather than by frugivory. We refer to dispersal of plants that do not have a fleshy fruit as "non-classical endozoochory." Granivory by corvids can lead to endozoochory of plants assigned to other dispersal syndromes, such as barochory, and which are therefore assumed to disperse by other mechanisms with less capacity to disperse seeds over long distances (Czarnecka and Kitowski, 2013). In order to understand the broader importance of corvid endozoochory, we resurrect the work of Sven August Heintze, a Swedish pioneer in the study of avian seed dispersal a century ago (**Figure 1**). Heintze's contributions have long been overlooked and are not cited in major reviews of corvid biology or their role in seed dispersal (e.g., Coombs, 1978; Cramp and Perrins, 1994; Birkhead, 2010; Tomback, 2016).

**FIGURE 1 |** Sven August Heintze (1881–1941) demonstrated the importance of corvids for seed dispersal in a wide range of plant species. Heintze was trained at Lund University, Sweden, where he defended his doctorate in botany in 1913. He published extensively about seed dispersal by birds and mammals in the years 1910-1918 (in Swedish), and later reached out for a wider audience in a two-volume synthesis about plant dispersal (in German; Heintze, 1932, 1935). Three of his papers provide data used for analyses in the present paper, resurrecting Heintze's legacy as a pioneer in this field.

Although the term "endozoochory" did not yet exist at the time, this dispersal process was considered by Holmberger (1785), a student of Linnaeus who reviewed dispersal strategies in plants, who mentioned seed consumption by Magpies *Pica pica*, and discussed seed survival after gut passage by birds in general. More than a century later, Holmboe (1900) applied the term "endozoochory" (which he attributed to A.P. de Candolle) to corvids. Holmboe recovered many seeds from the gizzards of Northern Nutcracker (*Nucifraga caryocatactes caryocatactes*) from Norway (see **Table S1**), but did not demonstrate that they survived gut passage. Soon after, Heintze (1917a,b); Heintze (1918) went much further than Holmboe in the study of endozoochory by corvids, and reported on his own field data for the following seven species: Magpie, Hooded Crow (*Corvus cornix*), Jackdaw (*C. monedula*), Rook (*C. frugilegus*), Common Raven (*C. corax*), European Jay (*Garrulus glandarius*), and Siberian Jay (*Perisoreus infaustus*). Heintze's dataset was most extensive for Magpies and Hooded Crows, allowing us to conduct detailed quantitative analysis of seeds dispersed by these two species. In addition to extensive field studies of plants and bird behavior during all seasons, Heintze carried out germination experiments with seeds extracted from pellets and feces of Magpies and Hooded Crows (Heintze, 1917a,b, 1918).

On top of his own thorough field and experimental work, he reviewed data previously provided by others on seed dispersal by additional corvid species [the seven listed above plus Carrion Crow (*Corvus corone*), Northern Nutcracker, Red-billed Chough (*Pyrrhocorax pyrrhocorax*), and Alpine Chough (*P. graculus*)] (Heintze, 1918). The latter paper ends in a synthetic discussion in which Heintze argues at length that European corvids have a wide role as dispersers of a long list of plant species in a wide range of habitats. Not only is his own research remarkable, the literature he reviewed 100 years ago shows that avian endozoochory was already a well-established research field at that time. Regrettably, these insights published in Swedish were largely neglected in his later seminal books written in German on plant dispersal *sensu latu*. Heintze (1932, 1935) mentioned the role of corvids only in passing, which is another reason his insights on this topic have been overlooked.

In this paper, we resurrect Heintze's work to show how important European corvids are for both classical and non-classical endozoochory. We identify the plant species he considered to be dispersed by endozoochory by 11 different European corvid species, quantifying the numbers of taxa recorded with different fruit types, morphological dispersal syndromes, habitat requirements and seed size. In the case of his more detailed datasets for pellets and feces of Magpies and Hooded Crows (Heintze, 1917a,b, 1918), we compare the relative abundance of intact seeds from each of the above categories, and compare seeds in pellets with those in feces.

# METHODS

## Methods Used by Heintze

Sven August Heintze was born in Skurup in southern Sweden in 1881 and earned a doctorate in botany at Lund University in 1913. His thesis was on zonation in alpine plants in Swedish Lapland, but he soon changed focus to study seed dispersal by birds. In 1912, Heintze already had the ambition to study the general role of corvids as dispersers of seeds in "Holarctic and Neoboreal" regions. His main contributions to this field were made in 1910–1917, when he collected feces, pellets and gut samples from European corvids (Heintze, 1917a,b, 1918). He also carried out extensive literature compilations, using sources in at least eight languages.

A description of his objectives and methods is found (in Swedish) on page 210 in Heintze (1917a). He traveled extensively in Sweden to collect corvid feces and pellets and examined the gastrointestinal tract of shot corvids. In his exhaustive review of terms for different plant dispersal processes, Heintze (1932) even proposed separate terms for seeds dispersed by egestion in pellets ("hemiendozoochory") and feces ("euendozoochory"). Heintze was well aware of the role of corvids as scatter-hoarders, which was already widely acknowledged in the literature in the early 1900's, and this is why he focused on endozoochory instead. He emphasized the importance of carrying out these studies "in all seasons," as he recorded the occurrence of germinated plants in nature (in excreta, at corvid nests, roosts, and at feeding sites) which had been dispersed by corvids. In addition, Heintze made ambitious germination experiments to study the implications of endozoochory; "*fresh regurgitates and excrements were subjected to a rinsing/washing process* [in the lab] *similar to that accomplished by rain- and snowmelt water in nature*" (Heintze, 1917a, p. 210). He carried out germination experiments of the contents of regurgitated pellets and excrements from Magpie and Hooded Crow. Most germination trials were carried out in early spring, of seeds that had been collected the preceding autumn or winter. Although many seeds germinated within a few weeks, trials were run throughout summer and autumn if necessary to detect later germinations (e.g., in 1916 the germination experiments were run until October 16th, Heintze, 1917a, p. 240). Further details of these experiments (e.g., substrate, indoor/outdoor, water supply, etc.) are unknown.

## Methods Used in Our Review of Heintze's Work

We extracted empirical data from the Swedish texts of Heintze explicitly focussing on seed dispersal by corvids (Heintze, 1917a,b, 1918). These sources list observations about regurgitated pellets, sampling of feces (mainly at roost and nest sites), and on contents of the upper (gizzard) and lower (intestines) gut. These data were originally reported in a rather unsystematic way, with variation in the level of detail and the extent to which different sample types were described. Interfoliated among Heintze's own data are references to research by colleagues, mainly in Central Europe, which are included in his summaries of the general role of corvids as seed dispersers. Many of his original sources are obscure and difficult to trace.

From Heintze's texts, we extracted details of those plant species with strong evidence of endozoochory, in particular cases where details of the bird species were provided, together with good evidence for endozoochory either because seeds were found in an intact state in pellets, feces or the alimentary canal, or they were germinated after removal from pellets or feces. Heintze stated that his list of species he considered to be dispersed by endozoochory "*contains plant species that with full certainty or with high degree of certainty are spread endozooically by European corvids*" (Heintze, 1918, p. 35). He also specified which plants were dispersed by synzoochory (i.e., scatter hoarding or for direct consumption at roosts without caching), especially by European Jays and Northern Nutcrackers, and we summarize these data for comparison.

We present detailed results for two different sets of Heintze's data. Firstly, a complete list of the plant taxa dispersed by endozoochory in Northern and Central Europe by any of the 11 species of corvids which he reviewed (Heintze, 1918). Secondly, his own detailed empirical dataset on pellets and fecal samples of Magpies and Hooded Crows in Sweden, for which he provided the number of intact seeds extracted for each plant species. The first dataset allows us to analyse what kinds of plant taxa were dispersed by endozoochory. The second dataset allows us to do a quantitative analysis of the relative abundance of seeds from each plant category (i.e., of seed size, fruit morphology, dispersal syndrome or habitat requirements) in pellets and feces for both species. The vast majority of this second dataset was collected from a restricted area on the west coast of southern Sweden.

The categories of fruit type and dispersal syndrome were assigned for each plant species from the Baseflor database (Julve, 1998). Seed length data were from the LEDA traitbase (Kleyer et al., 2008). When multiple measurements were available, we used their mean. Ellenberg moisture values were obtained from Hill et al. (1999).

For Heintze's detailed dataset for Magpies and Hooded Crows, we compared the frequency of occurrence of intact seeds of different plant species between pellets and feces using Fisher exact tests using online software (http://vassarstats.net/tab2x2.html).

We then compared the frequency of occurrence of different plants in pellets of the two corvid species. We did not repeat this for feces because of small sample sizes. We also compared the paired frequencies of occurrence of each plant species between pellets (fp) and feces (fe) for each corvid species using a matched paired *t*-test (i.e., testing fp-fe), to see if there was a consistent difference between sample types (e.g., higher frequency of seeds in pellets). We then tested if the difference between the frequencies in pellets and feces (i.e., fp-fe) for individual plant taxa was related to seed size, using a non-parametric correlation (between fp-fe and seed length). *T*-tests and correlations were carried out in R (R Core Team, 2017).

Unfortunately, it was not possible for us to realize all the statistical tests of interest, as many of the details of which seeds were present in each individual sample were lacking, even for Magpies and Hooded Crows. Heintze did not always report which plant species were found together, or the combined total number of seeds, in each sample.

# RESULTS

## Diversity of Plants Dispersed by Endozoochory and Their Relation With Dispersal Syndromes

When combining Heintze's reviews of the literature with his own data, a total of 157 plant species from 42 angiosperm families were recorded as dispersed by endozoochory by at least one corvid species (**Table S1**). In contrast, Heintze cited nine taxa as dispersed exclusively by synzoochory (including scatter-hoarding): *Aesculus hippocastanum*, *Carpinus betulus*, *Castanea sativa*, *Corylus avellana*, *Fagus silvatica*, *Juglans regia*, *Pinus cembra*, *Quercus robur*, and *Q. sessiliflora*.

Of those species dispersed by endozoochory, 91 (56%) do not have a fleshy fruit and hence do not belong to the "endozoochory syndrome," and were dispersed by non-classical endozoochory (**Table S1**; **Figure 2**). Many taxa with epizoochory, barochory and anemochory syndromes were recorded (**Figure 2**). Hence, many plants generally assumed to disperse via other mechanisms, such as wind, attached to fur, or self-dispersal, were dispersed by non-classical endozoochory. Those plant species dispersed by the most corvid species were fleshy-fruited, with up to nine different vectors per plant (**Table S1**). Nevertheless, some species with dry achenes and associated with barochory or epizoochory syndromes were dispersed by as many as six different corvid species (e.g., *Polygonum aviculare*, *Rumex acetosella*, *Urtica urens*, **Table S1**). Amongst the corvid species, the Magpie (114 plant species) and Hooded Crow (105) were recorded as vectors for the most plant species, reflecting Heintze's extensive work with those corvid species. They were followed by the Raven (71), Carrion Crow (33) and Jackdaw (29), whereas the Red-billed Chough (4) dispersed the fewest plant species (**Table S1**). The relative importance of classical and non-classical endozoochory varied between species. For example, of 29 species dispersed by Jackdaws, 18 (62%) were via non-classical endozoochory, compared to 36 (51%) of 71 species dispersed by Ravens.

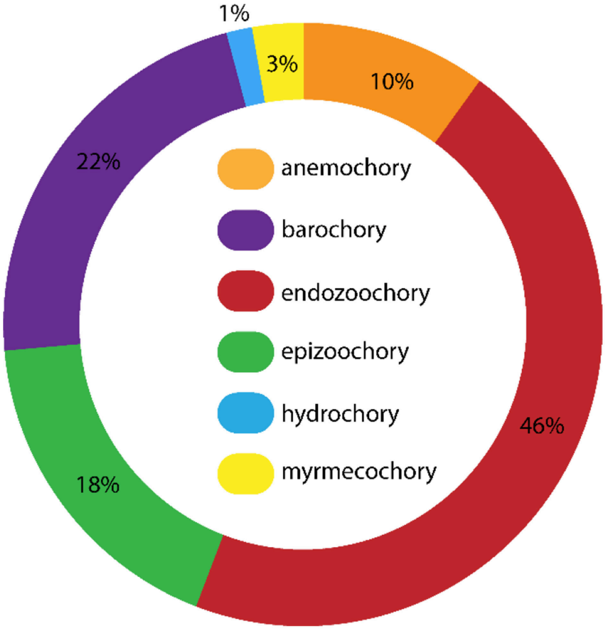

**FIGURE 2 |** Relative frequencies of different dispersal syndromes (Baseflor, Julve, 1998) assigned to all the plant taxa dispersed by corvids through endozoochory, according to Heintze's complete list ($n = 148$ taxa, another nine species were missing because their dispersal syndrome was unknown, see **Table S1**). Anemochory is wind dispersal, barochory is self-dispersal, hydrochory is water dispersal, epizoochory is dispersal stuck on the outside of animals, myrmecochory is dispersal by ants (see Julve, 1998; Perez-Harguindeguy et al., 2013).

Five species alien to the European continent were included amongst the plants dispersed by corvid endozoochory (**Table S1**). Aliens to Europe included the Dwarf Serviceberry *Amelanchier spicata* which is native to North America, the other four being cultivated species, such as the Cucumber *Cucumis sativus*, White Mulberry *Morus alba*, and Buckwheat *Fagopyrum esculentum*. Amongst the species recorded in Heintze's samples from Sweden, there were also an additional 10 species alien to Sweden but native to other parts of Europe, such as the grass *Bromus arvensis* (**Table S1**).

For the quantitative dataset on pellets and feces of Magpie and Hooded Crow produced from intensive study by Heintze, intact seeds from a total of 65 plant species (plus 5 plants identified to genus and one to family) were recorded, of which 37 (56%) do not have a fleshy fruit and so do not belong to an "endozoochory syndrome" (**Table 1**). Many taxa assigned to epizoochory (24% of taxa), barochory (20%) and anemochory (10%) syndromes were dispersed by non-classical endozoochory (**Table 1**). Nevertheless, when considering the total abundance of seeds from each syndrome, and not merely the number of taxa, the pattern changed markedly, and 97% of seeds were from an endozoochory syndrome (**Table 1**).

Germination tests confirmed the viability of seeds from 25 plant species (from a total of 35 species tested, i.e., 71%) plus two taxa identified to genus whose seeds were recovered from pellets and feces of Magpies and Hooded Crows (**Table 1**). Viability

**TABLE 1** | Abundance and frequencies of different plant taxa recovered by Heintze from the pellets and feces of Magpies and Hooded Crows, showing: Dispersal syndrome (DS, an, anemochory; ba, barochory; en, endozoochory; ep, epizoochory), Fruit type (FT, ac, achene; be, berry; ca, capsule; car, caryopsis; co, cone; dr, drupe; po, pod; si, silique), Fruit length (FL), Number of intact seeds in pellets (SP), Number of pellets containing seeds (P), Number of seeds germinated from pellets (SPG), Number of intact seeds found in feces (SF), Number of fecal samples containing seeds (F), Number of seeds germinated from fecal samples (SFG).

| | | | | | **Magpie** | | | | | | **Hooded Crow** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Plant family** | **Plant species** | **DS** | **FT** | **FL** | **SP** | **P** | **SPG** | **SF** | **F** | **SFG** | **SP** | **P** | **SPG** | **SF** | **F** | **SFG** |
| Adoxaceae | **Sambucus racemosa** | en | dr | 2.85 | 219 | 2 | – | | | | | | | | | |
| | **Viburnum opulus** | en | be | 7 | 2 | 1 | 2 | | | | | | | | | |
| Amaranthaceae | *Chenopodium album* | ba | ac | 1.24 | 4 | 3 | 3 | | | | 15 | 6 | 8 | | | |
| Apiaceae | *Carum carvi* | ba | ac | 4.44 | 1 | 1 | 0 | | | | | | | | | |
| Asparagaceae | **Polygonatum odoratum**[2] | en | be | 3.98 | | | | | | | 51 | 1 | – | | | |
| | **Polygonatum verticillatum** | en | be | 3.32 | 6 | 2 | – | | | | | | | | | |
| | **Maianthemum bifolium** | en | be | 3.15 | 7 | 1 | – | | | | | | | | | |
| Asteraceae | *Cyanus segetum* | an | ac | 3.59 | 1 | 1 | 0 | | | | | | | | | |
| | *Centaurea jacea* | ep | ac | 4.55 | 3 | 1 | 2 | | | | | | | | | |
| | *Leucanthemum vulgare* | ba | ac | 2.45 | 1 | 1 | 0 | | | | | | | | | |
| | *Tripleurospermum inodorum* | ba | ac | ND | | | | | | | 1 | 1 | 0 | | | |
| Berberidaceae | **Berberis vulgaris** | en | be | 5 | 19 | 6 | 12 | | | | | | | | | |
| Betulaceae | *Alnus glutinosa* | an | ac | 2.77 | 1 | 1 | – | | | | | | | | | |
| Brassicaceae | *Capsella bursa-pastoris* | an | si | 0.94 | 6 | 1 | 3 | | | | | | | | | |
| Caryophyllaceae | *Cerastium fontanum* subsp. *vulgare*[1] | an | ca | 0.65 | 3 | 1 | 3 | | | | | | | | | |
| | *Scleranthus annuus* | ep | ca | 3.82 | 3 | 2 | 0 | | | | | | | | | |
| | *Stellaria media*[1] | ba | ca | 0.99 | 4 | 1 | 0 | | | | | | | | | |
| Cupressaceae | **Juniperus communis**[1] | en | be | 4.5 | 44 | 10 | – | | | | 9 | 3 | – | | | |
| Cyperaceae | *Carex* sp. | – | – | – | 1 | 1 | 0 | | | | | | | | | |
| Ericaceae | **Arctostaphylos uva-ursi** | en | be | 3.5 | | | | | | | 1 | 1 | – | | | |
| | **Empetrum nigrum**[2] | en | be | 1.65 | 25 | 3 | – | | | | 1,027 | 9 | – | 20 | 3 | |
| | **Vaccinium myrtillus** | en | be | 1.28 | 400 | 10 | 19 (of 58) | | | | 1,500 | 15 | – | 127 | 4 | 32 (of 41) |
| | **Vaccinium sp.** | – | – | – | 25 | 4 | 12 (of 15) | | | | | | | 4 | 1 | 2 (of 4) |
| | **Vaccinium uliginosum** | en | be | 1.25 | Many | 1 | | | | | | | | | | |
| Fabaceae | *Astragalus alpinus* | ep | po | 2.25 | 3 | 2 | 2 | | | | | | | | | |
| | *Trifolium pratense* | ep | po | 1.73 | 1 | 1 | 0 | | | | | | | | | |
| | *Vicia sativa* | ba | po | 3.9 | 1 | 1 | 0 | | | | | | | | | |
| Lamiaceae | *Galeopsis tetrahit*[1,2] | ba | ac | 2.86 | 14 | 7 | 3 | | | | | | | | | |
| Melanthiaceae | **Paris quadrifolia** | en | be | 2.38 | 121 | 20 | – | | | | | | | | | |
| Pinaceae | *Pinus sylvestris* | an | co | 4.01 | 2 | 2 | 1 (of 1) | | | | | | | | | |
| Plantaginaceae | *Plantago maritima* | ba | ac | 2.33 | | | | | | | 3 | 1 | 2 | | | |
| Poaceae | *Avena sativa* | ep | car | 8 | 6 | 6 | 0 | | | | 7 | 1 | 2 (of 7) | | | |
| | *Bromus arvensis* | ep | car | 5.75 | 8 | 2 | 0 | | | | | | | | | |

*(Continued)*

**TABLE 1 |** Continued

| | | | | | Magpie | | | | | | Hooded Crow | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Plant family** | **Plant species** | **DS** | **FT** | **FL** | **SP** | **P** | **SPG** | **SF** | **F** | **SFG** | **SP** | **P** | **SPG** | **SF** | **F** | **SFG** |
| | *Poaceae* | – | – | – | 1 | 1 | 0 | | | | 2 | 2 | 0 | | | |
| | *Hordeum vulgare* | ep | car | 7.69 | 11 | 5 | 1 | | | | 2 | 1 | 0 | | | |
| | *Phleum pratense* | ep | car | 1.53 | 5 | 1 | 2 | | | | 7 | 1 | 4 | | | |
| | *Poa* sp. | – | – | – | | | | | | | 6 | 2 | 2 | | | |
| | *Secale cereale*[2] | ep | car | 8.95 | 3 | 1 | 1 | | | | 23 | 3 | – | 5 | 3 | 1 (of 5) |
| | *Triticum aestivum* | ep | car | 6.75 | | | | | | | 4 | 2 | | 10 | 7 | 3 |
| Polygonaceae | *Polygonum aviculare*[1] | ba | ac | 2.94 | 5 | 4 | 1 | | | | 8 | 5 | 1 | | | |
| | *Fallopia convolvulus* | an | ac | 3.7 | | | | | | | 1[d] | 1 | | | | |
| | *Persicaria maculosa* | ba | ac | 2.98 | 2 | 1 | 1 | | | | 4 | 3 | 0 | | | |
| | *Rumex acetosella*[1,2] | ba | ac | 1.2 | 9 | 2 | 7 | | | | 8 | 5 | 4 | 4 | 2 | 1 (of 2) |
| | *Rumex* sp. | – | – | – | 1 | 1 | 0 | | | | | | | | | |
| Primulaceae | *Lysimachia maritima* | ba | ca | 1.5 | | | | | | | 6 | 1 | 0 | | | |
| Ranunculaceae | *Ranunculus acris* | ep | ac | 2.79 | | | | | | | 2 | 2 | – | | | |
| | *Ranunculus repens* | ep | ac | 3.84 | 9 | 8 | 2 | | | | 6 | 4 | 1 | 2 | 1 | 0 |
| Rhamnaceae | ***Frangula alnus*[1]** | en | be | 4.77 | 5 | 2 | – | | | | | | | | | |
| Rosaceae | ***Cotoneaster integerrimus*[2]** | en | dr | 3.83 | 5 | 4 | – | | | | | | | | | |
| | ***Crataegus laevigata*** | en | dr | 5.88 | | | | | | | 5 | 3 | – | | | |
| | ***Fragaria vesca*[1]** | en | ac | 1.15 | 51 | 7 | – | 10 | 1 | | | | | | | |
| | ***Prunus avium*[1]** | en | dr | 9 | 38 | 10 | – | | | – | 186 | 19 | – | | | |
| | ***Prunus domestica*** | en | dr | 11.5 | 1 | 1 | – | | | | | | | | | |
| | ***Prunus padus*[1]** | en | dr | 6.25 | 122 | 23 | – | | | | | | | | | |
| | ***Prunus spinosa*[1]** | en | dr | 7.5 | 21 | 12 | – | | | | 12 | 2 | – | | | |
| | ***Rosa canina*[1]** | en | ac | 1.4 | 27 | 11 | – | | | – | 27 | 6 | – | | | |
| | ***Rosa cf. mollis*[2]** | en | ac | 4.95 | 4 | 1 | – | 1 | 1 | – | | | | | | |
| | ***Rubus fruticosus*[1]** | en | dr | 3.08 | 1 | 1 | – | | | – | | | | | | |
| | ***Rubus idaeus*[1,2]** | en | dr | 2.58 | 1,352 | 45 | – | 44 | 6 | – | 1,050 | 26 | – | 289 | 17 | 6 (of 28) |
| | ***Rubus saxatilis*** | en | dr | 3.5 | 9 | 3 | – | | | | | | | | | |
| | ***Sorbus aucuparia*[1,2]** | en | dr | 3.5 | 253 | 46 | 2 (of 10) | | | – | 32 | 10 | – | 5 | 1 | |
| Rubiaceae | *Galium aparine* | ep | ca | 3.2 | 1 | 1 | 1 | | | | | | | | | |
| Solanaceae | ***Solanum dulcamara*** | en | be | 2.26 | 497 | 13 | 76 (of 85) | 5 | 2 | 5 | 406 | 1 | 19 (of 20) | | | |
| Urticaceae | *Urtica dioica* | ep | ac | 1.205 | 7 | 1 | 3 | | | | | | | | | |
| Violaceae | *Viola* sp. | – | – | – | | | | | | – | 1 | 1 | | 1 | 1 | 0 |
| Total | | | | | 3,370 | 339 | | 60 | 9 | | 4,411 | 270 | | 467 | 165 | |

*When not all seeds recovered were tested for germination, sample sizes are given in brackets. Total sample sizes including those without seeds were: Magpie pellets 339, Magpie feces 9, Hooded Crow pellets 270, Hooded Crow feces 165. Most samples were from the West coast of southern Sweden. Species shown in bold are fleshy fruited. See* ***Table S1*** *for sources for DS, FT, FL.* [d]*Broken seed. Superscript numbers on plant species names indicate cases where Heintze also reported seedlings growing in the field, surrounded by remains of the pellet the seeds were dispersed inside:* [1]*Magpie pellet,* [2]*HC pellet. See* ***Table S2*** *for details.*

was confirmed for taxa from four dispersal syndromes, including all of 6 species with an endozoochory syndrome, 6 of 12 with barochory, 3 of 4 with anemochory and 10 of 13 with epizoochory (note, the number of seeds tested per species varied, **Table 1**). Overall, two taxa were only germinated from feces, 16 were only germinated from pellets, and 5 species (20% of the total) were germinated from both pellets and feces (**Table 1**). In addition, Heintze germinated *Polygonum aviculare* from the rectum of a European Jay, and *Vaccinium* cf *vitis-idea* from the feces of a Raven.

In addition, Heintze (1917a, 1918) recorded seedlings of at least 32 plant species in the process of becoming established in nature after germinating within the remnants of excreta (27 plant species in Magpie pellets and at least 10 in Hooded Crow pellets). These species included at least 11 that lack fleshy fruits and have been assigned to epizoochory, anemochory and barochory syndromes (**Table 1**; **Table S2**), confirming the importance of non-classical endozoochory.

## Fruit Types

Plant taxa dispersed by corvid endozoochory were from a broad range of fruit types, of which berries, drupes and achenes were the most frequent (**Figure 3**). Only seven of 45 taxa with achenes had a fleshy achene, corresponding to the "endozoochory syndrome." When considering fruit types for plant taxa recovered from pellets and feces of Magpies and Hooded Crows (**Figure 4**), berries and drupes were more dominant, together with smaller numbers of achenes (only 11% of achenes recovered were fleshy). This is consistent with the dominance of seeds with an endozoochory syndrome in these samples (**Table 1**). For Hooded Crows, drupes appeared more likely to be recorded in feces and berries more likely to be recorded in pellets (**Figure 4**), although we were unable to test the statistical significance of this difference.

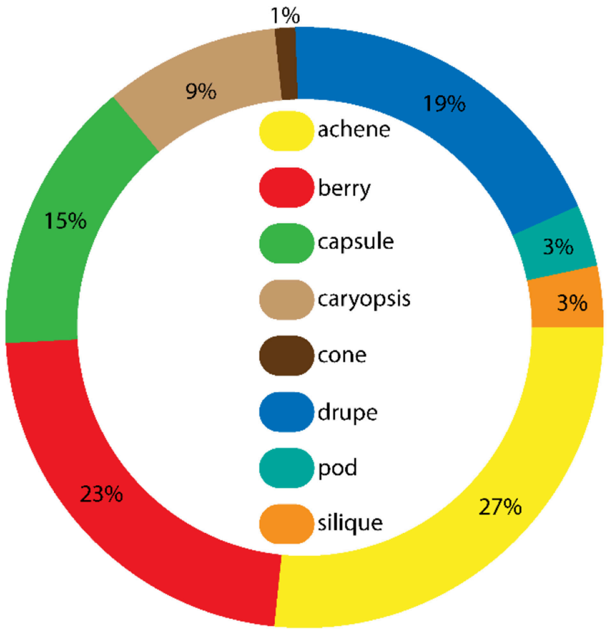

**FIGURE 3 |** Relative frequency of different fruit types (Baseflor, Julve, 1998) for all the plant taxa dispersed by European corvids through endozoochory, according to Heintze's complete list ($n = 150$ taxa, excluding seven species with unknown fruit types, see **Table S1** for details). Fleshy fruits are drupes, berries, and seven of the 45 species with achenes (such as the fig *Ficus carica*).

## Seed Size

European corvids dispersed seeds with a broad size range by endozoochory, from a minimum seed length of 0.75 mm (*Cerastium fontanum*) to a maximum length of 11.5 mm (*Prunus domestica*), although most taxa had a length of <4 mm (**Figure 5**). In the detailed dataset for Magpies and Hooded Crows, the size distribution was more skewed toward smaller seeds (**Figure 6**). Comparing the size distribution for all taxa dispersed by corvids (**Figure 5**) with the data on relative abundance (**Figure 6**) suggests that taxa with relatively small seeds (<4 mm in length) were dispersed more often.

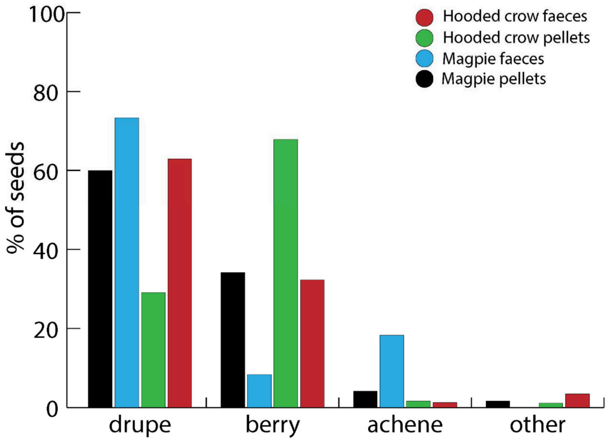

**FIGURE 4 |** Relative abundance of different fruit types (Baseflor, Julve, 1998) among intact seeds recovered from pellet and fecal samples of Magpies and Hooded Crows by Heintze. Fleshy fruits are drupes, berries and three species with fleshy achenes (such as dog rose, *Rosa canina*). "Other" includes small numbers each of siliques, capsules, pods, cones, and caryopsis. Sample sizes: Magpie pellet: 3,370 seeds; Magpie feces: 60 seeds; Hooded Crow pellet: 4,411 seeds; Hooded crow feces: 467 seeds.

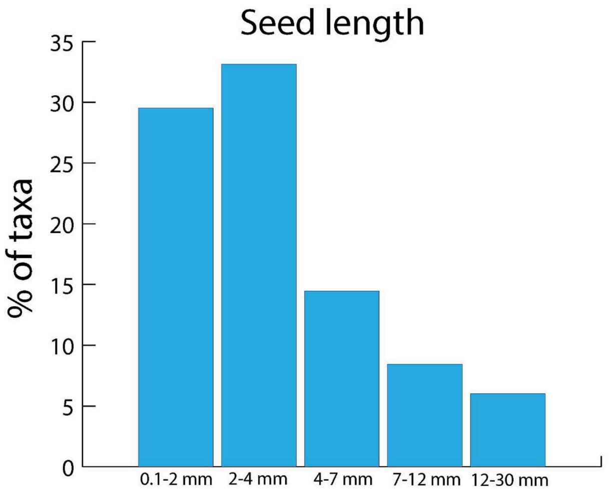

**FIGURE 5 |** Seed length distribution for all the plant taxa dispersed by European corvids through endozoochory, according to Heintze's complete list ($n = 143$ taxa, 14 species were excluded because their seed length was unknown, see **Table S1**).

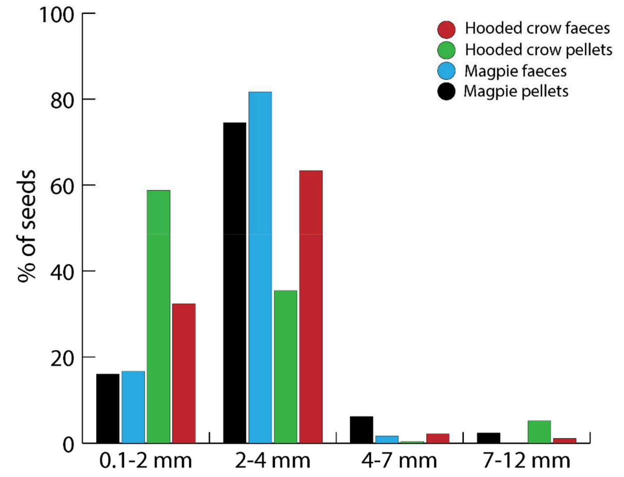

**FIGURE 6 |** Size distribution of all intact seeds recovered from pellets and fecal samples of Magpies and Hooded Crows by Heintze. See **Figure 4** for sample sizes.

Seeds dispersed by Magpies tended to be larger than those dispersed by Hooded Crows, with a higher proportion of seeds of 2–4 mm for Magpies. Surprisingly, seeds tended to be larger overall in the fecal samples of Hooded Crows than in their pellets, although the largest seeds of >4 mm were more frequent in pellets (**Figure 6**).

## Plant Habitat Requirements

Plant taxa dispersed by corvid endozoochory were from a broad range of terrestrial habitats, as indicated by Ellenberg moisture values (**Figure 7**). When considering the numbers of seeds from pellets and feces of Magpies and Hooded Crows assigned to each Ellenberg value, there was a tendency for higher soil moisture, and values 6 and 8 were better represented (**Figure 8**). No aquatic plants were recorded.

## Comparing the Detailed Composition of Pellets and Feces of Magpies and Hooded Crows

Overall, on average there were more intact seeds in Magpie pellets (9.9 seeds per sample) than their feces (6.6 seeds). Similarly, there were more seeds in Hooded Crow pellets (16.3 seeds per sample) than in their feces (2.8 seeds). However, since Heintze did not provide the combined total number of seeds in individual samples, we could not test these differences statistically.

For Magpies there were relatively few fecal samples, but *Rubus idaeus* was significantly more prevalent in feces than in pellets (Fisher exact, $P = 0.0004$). For Hooded Crows, *Triticum aestivum* was more prevalent in feces ($P = 0.001$), whereas *Prunus avium* was more prevalent in pellets ($P = 0.0003$). Eight plant species were significantly more frequent in Magpie pellets than in Hooded Crow pellets, and two species were more frequent in Hooded Crow pellets (**Table 2**).

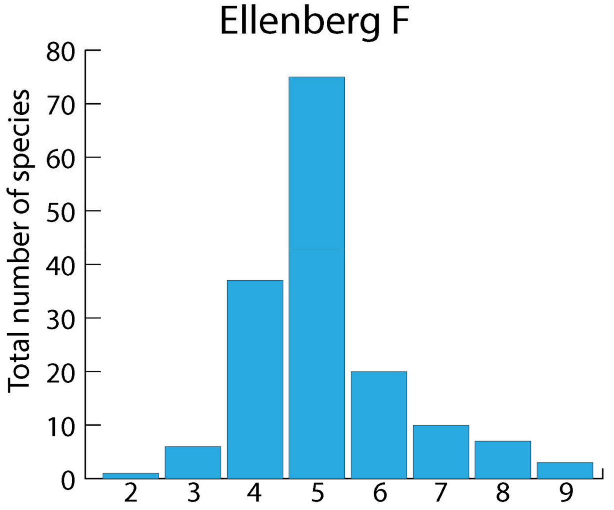

**FIGURE 7 |** Relative frequencies of Ellenberg moisture indicator values (F) for plant taxa dispersed by corvids through endozoochory, according to Heintze's complete list. $N = 150$ taxa (excluding seven species with an unknown $F$ value, see **Table S1**). This scale goes from 1 (dry soils) to 12 (submerged macrophytes). Plants with $F \geq 10$ can be considered aquatic.

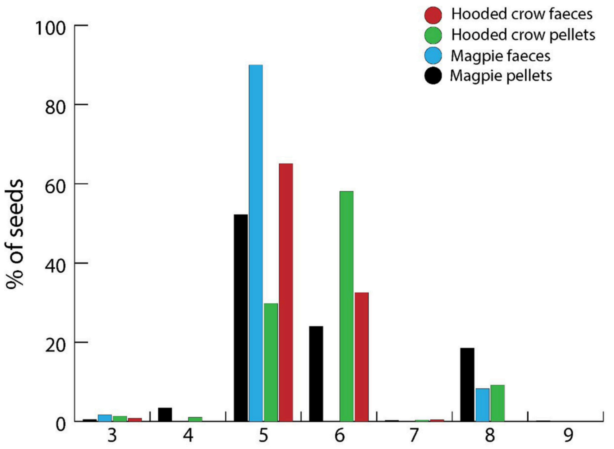

**FIGURE 8 |** Relative abundance of intact seeds recovered from pellet and fecal samples of Magpies and Hooded Crows by Heintze assigned to different Ellenberg moisture indicator values ($F$). This scale goes from 1 (dry soils) to 12 (submerged macrophytes). For Ellenberg 9 there was only 1 seed (0.02% of the total) of alder *Alnus glutinosa* from a Magpie pellet. See **Figure 7** for sample sizes.

In Hooded Crows, there was a higher overall frequency of seeds in pellets than in feces (comparing the frequencies of occurrence for each plant taxon with a matched paired $t$-test: $t = 2.73$, $df = 64$, $p = 0.0082$). In Magpies, there were few fecal samples and no significant difference in frequencies with pellets ($t = 0.373$, $df = 63$, $p = 0.71$). There was no relationship between the seed size of a particular plant species and the difference in its frequency of occurrence between pellets and feces, whether for Magpies ($\rho = 0.106$, $p = 0.48$) or Hooded Crows ($\rho = 0.074$, $p = 0.72$).

**TABLE 2 |** Plant species with a significant difference in the frequency of occurrence (given as %) of intact seeds between the pellets of Magpies and Hooded Crows.

| Plant species | Magpie | Hooded Crow | *P*-value |
|---|---|---|---|
| *Berberis vulgaris* | 1.77 | 0 | 0.0365 |
| *Empetrum nigrum* | 0.88 | 3.33 | 0.0395 |
| *Galeopsis tetrahit* | 2.06 | 0 | 0.0193 |
| *Paris quadrifolia* | 5.9 | 0 | 0.00002 |
| *Fragaria vesca* | 2.06 | 0 | 0.0193 |
| *Prunus avium* | 2.95 | 7.04 | 0.0215 |
| *Prunus padus* | 6.78 | 0 | 0.000003 |
| *Prunus spinosa* | 3.54 | 0.74 | 0.0274 |
| *Sorbus aucuparia* | 13.57 | 3.7 | 0.00002 |
| *Solanum dulcamara* | 3.83 | 0.37 | 0.0047 |

*P-values were calculated with Fisher Exact tests. All species listed are fleshy-fruited, with the exception of Galeopsis tetrahit which has a dry achene.*

# DISCUSSION

Heintze's work shows that corvids disperse a broad taxonomic and ecological range of plants through gut passage, and slightly more than half of the taxa are dispersed by non-classical endozoochory (i.e., through granivory). The importance of classical endozoochory by corvids has adequately been demonstrated by much contemporary research (Nogales et al., 1999; Wenny et al., 2016; Bai et al., 2017). Heintze (1918, p. 29) also wrote: "*Magpies, Hooded Crows, Carrion Crows, Rooks, Jackdaws, Ravens, Choughs, Yellow-billed Choughs, and Siberian Jays disperse seeds of pretty much all European trees, bushes, shrubs, and herbs with berries* [fleshy fruits]." The importance of synzoochory by corvids has also been demonstrated by much contemporary research (Pesendorfer et al., 2016; Tomback, 2016). Heintze's dataset identifies few plant species dispersed by synzoochory, but this was probably a consequence of his deliberate focus on endozoochory.

Non-classical endozoochory can also be considered a form of "non-standard dispersal" because the means of dispersal does not match the dispersal syndrome (Higgins et al., 2003; Nathan et al., 2008). However, syndromes are commonly assigned based solely on a botanist's interpretation of fruit morphology, and in the absence of empirical data on dispersal mechanisms. Hence, syndromes may not identify what mechanisms are "standard" in a sense that has any true ecological or evolutionary meaning, and they have proved to be poor predictors of mechanisms for long-distance dispersal (Higgins et al., 2003; Nogales et al., 2012; Lovas-Kiss et al., 2018a, 2019). Syndromes recognize the importance of frugivory (through the "endozoochory syndrome"), but ignore the role of granivory as a dispersal mechanism.

Our results are supported by a careful reading of other corvid literature showing that corvids can be important vectors by non-classical endozoochory. In his seminal review of plant dispersal, Ridley (1930) recognized the importance of different corvid species around the world for frugivory and scatter-hoarding, but he also provided a list of over 50 plant taxa with dry fruits and grains that may be dispersed within pellets. Ridley cited Heintze (misspelled as "Heinitz") and listed some of the plant species he recorded in the diet of Hooded Crow and Magpie. Herb seeds were the most abundant food item in gut contents of adult Magpies from the former Soviet Union (Cramp and Perrins, 1994). Unique modern research shows the importance of non-classical endozoochory in Rooks (Czarnecka and Kitowski, 2010, 2013; Czarnecka et al., 2013). Other passerines with similar ecology to corvids can also be important vectors of non-classical endozoochory, as illustrated by the Australian Magpie *Gymnorhina tibicen* (Artamidae, Twigg et al., 2009).

## Classical vs. Non-classical Endozoochory

Based on presence/absence of plant taxa dispersed by endozoochory, classical and non-classical endozoochory by corvids appear to be equally important. However, the quantitative dataset for Magpies and Hooded Crows suggest that classical endozoochory events are considerably more frequent. Heintze found a similar diversity of plant taxa with and without a fleshy-fruit to be dispersed by these two species, but seeds from the latter were much less abundant. Heintze's extensive dataset suggests important differences among different corvid species as plant vectors by endozoochory, although this dataset is influenced by differences in sampling effort and relative abundance of each corvid in the habitats where he and his colleagues worked. His data do not allow us to fully assess the relative importance of classical vs. non-classical endozoochory in the other nine species, but more recent research demonstrates that classical endozoochory is not always dominant. Czarnecka and Kitowski (2013) found for Rooks in Poland that seeds were present in 18% of pellets, and half of all seeds belonged to dry-fruited species.

Differences among corvid species in their roles as plant vectors are inevitable, given their differences in habitat use. Heintze argued that in landscapes transformed by agriculture in southern Sweden, the role of Magpie and Hooded Crow as plant vectors by endozoochory was equivalent to that played by the Raven and European Jay in forest landscapes. He also observed that some corvids disperse plants by secondary endozoochory, a form of diplochory (Vander Wall and Longland, 2004). Magpies, Hooded Crows, Rooks, and Jackdaws frequently extracted seeds from the dung of horses, cows, sheep, goats, and wild ungulates, and Ravens consumed seeds from dung of reindeer, cows, and horses (Heintze, 1917a,b, 1918). Similarly, seeds in the feces of herbivores, such as ungulates or elephants are often secondarily dispersed by rodents that extract them from the dung (Jaganathan et al., 2016).

Heintze recognized that differences between corvid species in the amounts of grit in the gizzard are likely to influence seed survival (as shown for Anatidae, Figuerola et al., 2002), and suggested that Magpie, Raven, Siberian Jay, and Northern Nutcracker all have little or no grit. In contrast, he found Eurasian Jays to contain the most grit, and combined with their habit of rarely producing pellets he concluded "*the Jay surely has a much lesser importance as an endozoochoric species than do other corvids*" (Heintze, 1917a, pp. 209, 225; Heintze, 1918, pp.7, 11, 15), although this species is a major plant vector by synzoochory (Pesendorfer et al., 2016).

## Corvids Are Effective Dispersers Over Long Distances

For dispersal to be effective, seeds must be dispersed into a suitable habitat allowing germination and subsequent growth. Heintze frequently reported field observations on established plants growing near Magpie nests, which he concluded had been dispersed there by the birds (Heintze, 1917a). His extensive observations of seedlings growing among the remnants of Magpie and Hooded Crow pellets are particularly important (**Table 1**; **Table S2**), confirming that not only are viable seeds dispersed by classical and non-classical endozoochory, but that they are moved into suitable microhabitats. Heintze also considered Raven pellets to be important in the establishment of plants in nature, and cited Holmboe as recording many germinated plants under Raven nests, e.g., *Actea spicata* in Norway and *Ficus caria* and *Olea europea* at nests in Cyprus (Heintze, 1918, p. 7–8).

Central to the role of corvids as endozoochoric seed dispersers are their movements between ingestion and egestion of a viable seed. Heintze knew that the Magpies and Hooded Crows he studied mainly undertook daily, local movements rather than long-distance migrations. He wrote that Magpies "*regularly transport seeds ca 2 km, rarely more than 5 km*" (Heintze, 1917a, p. 229–230). He found seeds from seashore plants in Magpie pellets retrieved 5 km from the closest seashore (Heintze, 1917a, pp. 217 and 230). For Hooded Crow, he stated "*they frequently fly 10–20 km, or even longer, to reach their regular night roost*" (Heintze, 1918, p. 2). He also provided known dispersal distances for Raven; "*often 20–30 km*" (Heintze, 1918, p. 9) and Rook [50–60 km, citing Altum (Heintze, 1918, p. 6)]. Moreover, Heintze was interested in endozoochory over longer distances during seasonal migrations by the Hooded Crow. He suggested that this species has the capacity for overseas seed dispersal between land masses, particularly since his dissections showed that seeds may remain in the gizzard for up to 24 h (Heintze, 1918, pp. 34–35). Some northern populations of Hooded Crow still undertake seasonal migrations.

The likely dispersal distances identified by Heintze a century ago are remarkably similar to known movement distances in present-day corvids in northern Europe (see also Cramp and Perrins, 1994; e.g., Wernham et al., 2002; Fransson and Hall-Karlsson, 2008; Czarnecka and Kitowski, 2010; Pesendorfer et al., 2016; Marchand et al., 2018). This confirms that corvids are important vectors for long-distance dispersal of plants, and offer a maximum dispersal distance far greater than is generally recorded for other mechanisms including epizoochory, anemochory, and obviously barochory (Bullock et al., 2017).

Corvids can be important vectors for alien species (Nogales et al., 1999), especially in urban environments (Czarnecka et al., 2013). Interestingly, Heintze showed they were already dispersing numerous alien plants a century ago. Heintze also stated that Magpies were responsible for spreading "*our cultivated garden trees, bushes and herbs with berries into the wild*" (1917a, p. 230). Furthermore, many of the native species dispersed by corvids in Europe by non-classical endozoochory are alien species in other continents, where they may be spread by corvids in a manner not predicted by diaspore morphology. For example, the Hemp-nettle *Galeopsis tetrahit* has a dry achene that was recorded from six corvid species by Heintze (**Table S1**), was particularly frequent in Magpie pellets (**Table 2**), and is a widespread and problematic alien in North America (USDA and NRCS, 2019).

## Seed Size

Plants with the largest seeds can be expected to be dispersed less often by corvid endozoochory since they are more likely to be destroyed in the foregut, and also because of the general negative relationship between seed size of a given species and the numbers of seeds produced by individual plants (Bruun and Poschlod, 2006; Green et al., 2016). Comparing the seed size distribution for all plants dispersed by corvid endozoochory (**Figure 5**) with the relative abundance of seeds dispersed by Magpies and Hooded Crows (**Figure 6**) does provide indirect support for a greater dispersal frequency of smaller seeds. Heintze (1917a, 1918) suggested that larger seeds were more likely to be egested and dispersed via pellets and smaller ones in the feces. Heintze stated that "*all larger, most middle-sized, and more than half of the small-sized seeds are spread hemiendozoically* [i.e., in pellets], *whilst the rest comes out in the excrements*" (1918, p. 30). This would be consistent with expectations based on gut morphology, since the sphincter passage between the gizzard and intestines should make it harder for larger seeds to pass intact. It is also consistent with literature for other birds, such as gulls and shorebirds, in which larger seeds were egested in pellets (Lovas-Kiss et al., 2018b, 2019). However, such a clear pattern is not supported from our analyses of Heintze's dataset for Magpies and Hooded Crows (**Figure 6**).

Heintze suggested (at least for Magpie and Hooded Crow) that seed dispersal via regurgitated pellets was more important than seed dispersal via feces (Heintze, 1917a, p. 228). We found evidence that seeds are more frequent in pellets than in feces, especially for Hooded Crows. However, rates of endozoochory events depend on the rate of egestion, and it is likely that each pellet sample contains a higher proportion of the daily regurgitated output than each feces sample contains of daily fecal output. Some birds produce <1 pellet a day (e.g., White Storks, Rosin and Kwiecinski, 2011). Based on dissections, Heintze concluded that seeds may remain in the gizzard of a Hooded Crow for up to 24 h (Heintze, 1918, pp. 34–35), suggesting they produce at least one pellet a day.

## Comparison Between Corvids and Waterfowl

Owing to the focus on classical endozoochory in the literature, little information is available about the importance of non-classical endozoochory by other granivorous birds so as to enable a comparison with corvids. The best known vectors of non-classical endozoochory are the waterfowl Anatidae (Green et al., 2016). In particular, the dabbling ducks *Anas* spp. are likely to disperse over 500 plant species in Europe, and only a small fraction of seeds they disperse have an endozoochory syndrome

(Soons et al., 2016; Lovas-Kiss et al., 2018a). Interestingly, there is a considerable overlap in plant species dispersed by corvids and waterfowl, mainly being cases of non-classical endozoochory (see **Table S1** for full details). Of the 157 plant species listed by Heintze as dispersed by corvid endozoochory, 56 are also thought to be dispersed by dabbling ducks (Soons et al., 2016), and 20 were listed as dispersed by Greylag Geese *Anser anser* in the Stockholm archipelago in Sweden (Jerling et al., 2001). We found corvids to disperse more seeds from large size categories (most of which are of fleshy-fruited plants) than dabbling ducks (see **Figure 2** in Soons et al., 2016). Plant species common to corvid and waterfowl endozoochory are terrestrial species. Not surprisingly, the distributions of Ellenberg moisture values differ among the plant taxa and seeds dispersed by corvids and dabbling ducks, because ducks disperse many moist soil and aquatic taxa with an Ellenberg value of 9–12 (see **Figure 3** in Soons et al., 2016).

## Conclusions

Based on the work of others and his own extensive research Heintze concluded (1917a, p. 210) that "*It appears that within these zoogeographic areas* [the Holarctic and the Neoboreal] *corvids are the most important seed dispersers among all landbirds*." This article lends support to this statement, given the demonstrated importance of corvids as vectors for an extensive diversity of plants dispersed by both classical and non-classical endozoochory. Much has changed in European habitats over the past century, and it would be interesting to conduct similar research again in the same parts of Sweden where Heintze collected his own data.

It is unfortunate that the work of Heintze and co-workers a century ago or before has been overlooked for so long. The scientific community has effectively lost their understanding about the role of corvids as seed dispersers for a very wide range of terrestrial plants, and we hope our paper helps to restore this knowledge. The effort and knowledge required by Heintze to retrieve and study thousands of pellets and fecal samples is awe-inspiring, and should humble us all to pay more attention to the work of such pioneers. Increasing access to digitalized older literature is making this easier. His work also illustrates the immense value that natural history plays in ecology, and the serious consequences of its current decline (Tewksbury et al., 2014).

Many corvid species are likely to be vectors for a broad range of plants through both frugivory and granivory because they are opportunistic, generalist foragers able to exploit a range of habitats in both natural and human-created landscapes. In urban environments, Magpies, Jackdaws and other species can be amongst the most abundant birds. Corvids worldwide may be one of the most important bird groups in providing a vital seed dispersal service that maintains and restores a wide range of plants with a variety of growth forms and other traits, and in forested and open landscapes, as well as urban habitats. Corvids are heavily hunted in many countries, and this may negatively impact their important role as native plant vectors. On the other hand, corvids also have a role in unwanted spread of alien plants.

As shown previously for waterfowl, shorebirds and gulls (Jerling et al., 2001; Lovas-Kiss et al., 2018a,b, 2019), non-classical endozoochory by corvids is a major dispersal process that requires more attention if we are to fully understand plant dispersal processes and plant-animal interactions. We hope this paper will inspire others to look beyond frugivory and synzoochory and pay more attention to the importance of non-classical endozoochory by corvids, as well by other little-studied granivorous birds, such as galliformes and small passerines (Swank, 1944; Orlowski et al., 2016).

## AUTHOR CONTRIBUTIONS

AG, JE, and ÁL-K conceived the study. JE and ÁL-K collected the data. AG and ÁL-K analyzed the data. AG, JE, and ÁL-K wrote the manuscript.

## ACKNOWLEDGMENTS

AG was supported by Spanish National Plan project CGL2016-76067-P (AEI/FEDER, EU). Peter Bengtsson at Kristianstad University library kindly provided bibliographic assistance. ÁL-K was supported by NKFIH FK-127939 project. We acknowledge support of the publication fee by the CSIC Open Access Publication Support Initiative through its Unit of Information Resources for Research (URICI).

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2019.00133/full#supplementary-material

**Table S1 |** Complete list of plant taxa whose seeds are dispersed by endozoochory according to data in Heintze (1917a,b); Heintze (1918).

**Table S2 |** Complete list of plant taxa recorded as seedlings in the field within remnants of pellets of Magpies and Hooded Crows, and considered by Heintze to have been dispersed within those pellets.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Ecology and Evolution

ORIGINAL RESEARCH
published: 24 May 2019
doi: 10.3389/fevo.2019.00161

# Non-native Mammals Are the Main Seed Dispersers of the Ancient Mediterranean Palm *Chamaerops humilis* L. in the Balearic Islands: Rescuers of a Lost Seed Dispersal Service?

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OPEN ACCESS

**Edited by:**
*Casper H. A. Van Leeuwen,*
*Netherlands Institute of Ecology*
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**Reviewed by:**
*Carl R. Gosper,*
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*Alejandro Valenzuela,*
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**Specialty section:**
*This article was submitted to*
*Biogeography and Macroecology,*
*a section of the journal*
*Frontiers in Ecology and Evolution*

**Received:** *31 October 2018*
**Accepted:** *23 April 2019*
**Published:** *24 May 2019*

**Citation:**
*Muñoz-Gallego R, Fedriani JM and Traveset A (2019) Non-native Mammals Are the Main Seed Dispersers of the Ancient Mediterranean Palm Chamaerops humilis L. in the Balearic Islands: Rescuers of a Lost Seed Dispersal Service? Front. Ecol. Evol. 7:161. doi: 10.3389/fevo.2019.00161*

Megafauna extinctions often lead to the disruption of plant-animal interactions, such as the seed-disperser mutualisms, which might entail severe consequences for plant populations and entire communities. Interestingly, the contemporary persistence of anachronistic plant species might be possible thanks to surrogate dispersers or seed dispersal "rescuers". We know very little on how these relevant functional replacements are contributing to the performance of present-day plant-frugivore networks. The dwarf palm *Chamaerops humilis* L. is a Mediterranean endemism with fleshy fruits and typically dispersed by mammals. Despite its ecological importance and wide distribution in some of the Mediterranean islands, no information exists about its seed dispersal on these depauperated-fauna systems. In this study, we aim at identifying and quantifying the relative importance of introduced frugivores on the island of Mallorca (Balearic Islands), where no native terrestrial mammals exist. Specifically, we assess for the first time the seed dispersal effectiveness (SDE) for *C. humilis* on islands; we evaluate the quantitative component by fecal and regurgitation sampling surveys, and the qualitative component by means of seed germination experiments and seedling growth measures. Introduced goats (*Capra hircus* L.) and pine martens (*Martes martes* L.) were the local mammal fruit consumers of *C. humilis* identified in our study sites. Results suggest that goats are much more important quantitatively than pine martens, due to the high number of fruits handled in each foraging bout and their extremely high abundance on the island. However, pine marten-ingested seeds showed the highest final seedling emergence success and seedling growth, thus its qualitative contribution on *C. humilis* seed dispersal is higher than that of goats. Overall, SDE was almost 9-fold higher for goats than for pine martens. We conclude that these two non-native mammal species are effective seed dispersers of *C. humilis* in this and probably other Mediterranean islands, where humans led to the extinction of its native seed dispersers, as it was probably the case of the goat-like *Myotragus balearicus* in the Balearic Islands.

**Keywords: anachronism, goat, megafauna extinction, *Myotragus balearicus*, pine marten, seed dispersal effectiveness, seedling emergence, seedling growth**

## INTRODUCTION

Numerous extinctions of large terrestrial vertebrates driven by human activity have taken place during the Late Pleistocene and Early Holocene (Ceballos et al., 2015; Faurby and Svenning, 2015). This has occurred in most continents, and well-studied cases are the extinctions of megafauna species in the American continent, such as saber-toothed cats (*Smilodon* spp.), mammoths (*Mammuthus* spp.), and giant ground sloths (*Megalonyx jeffersonii*) (Janzen and Martin, 1982; Sodhi et al., 2012). On islands worldwide, the rate of extinction of terrestrial mammals after the arrival of humans was c. 30%, being fateful for the orders Proboscidea, Xenarthra and Bibymalagasia and quite strong for Artiodactyla, Carnivora, and the rodent infraorder Hystricognathi (Alcover et al., 1998). These events not only led to the vanishing of many populations and species, but also to the disruption and reshaping of ecological interactions which likely altered the structure and function of entire communities and ecosystems. In fact, severe impacts of large vertebrate extinctions on the ecosystem physical and trophic structure, plant community composition, and diversity, ecosystem biogeochemistry, and regional and global climate have been described (Dirzo et al., 2014; Bello et al., 2015; Ripple et al., 2015; Malhi et al., 2016; Smith et al., 2016). In addition, the loss of megafauna can affect ecological interactions in three ways: leading to co-extinctions, adaptative shifting, and evolutionary anachronisms (Galetti et al., 2018). This last concept, described for the first time several decades ago by Janzen and Martin (1982), is defined in a context of frugivory as “extant interactions between animal frugivores and plants involving traits that show striking unfit patterns to an extant fauna” (Guimarães et al., 2008, p. 2). Typical examples are fruits with huge seeds which are dispersed inefficiently by the contemporary frugivore assemblages, like avocado *(Persea americana)* (Cook, 1982), osage orange (*Maclura pomifera*) (Janzen and Martin, 1982), or Florida torreya (*Torreya taxifolia)* (Barlow, 2001). Anachronisms can also show up through antagonisms, such as the presence of spines or other plant defenses against large herbivores that no longer exist (e.g., Bond et al., 2004). These unfit patterns could be explained if we consider all the extinct megafauna which would have acted as legitimate dispersers or herbivores in the past, exerting selective pressures on plant traits (Barlow, 2000; Guimarães et al., 2008).

Seed dispersal is a key ecological process in the life cycle of plants because it allows propagules to move away from the maternal environment, providing a higher propagule survival probability, maintaining the genetic flow, and enabling the colonization of vacant habitats (Levey et al., 2002; Medel et al., 2009; Schupp et al., 2010). Therefore, the disruption of this relevant ecological service due to the extinction of effective seed dispersers can result in serious consequences for plant populations: modification of range and spatial distribution (e.g., Meehan et al., 2002; Rotllàn-Puig and Traveset, 2016), decrease in the total number of seed successfully dispersed, germinated and established (e.g., Galetti et al., 2006; Wotton and Kelly, 2011; Traveset et al., 2012), alteration of the seed shadow (e.g., Beaune et al., 2013; Bueno et al., 2013), restriction of the gene flow and genetic structure increase (e.g., Collevatti et al., 2003; Caughlin et al., 2015), interruption of long-distance dispersal (e.g., Pérez-Méndez et al., 2016; Pires et al., 2018), and shift of phenotypic selection on seed size (e.g., Galetti et al., 2013). This often ends in a collapse in the life cycle and species decline (Valiente-Banuet et al., 2015; Rumeu et al., 2017; Galetti et al., 2018). On islands, where communities are depauperate and plants may be very specialized in their dispersers (González-Castro et al., 2012), the negative outcomes from such disruptions are even greater (Traveset and Richardson, 2014). The disappearance of frugivorous forest birds (e.g., moas, piopio *Turnagra capensis*, huia *Heterolocha acutirostris*) as important dispersers of many woody plants on New Zealand (Clout and Hay, 1989; Wood et al., 2008), or the reduction of flying foxes on tropical Pacific islands (McConkey and Drake, 2006) are some examples of disrupted dispersal service on islands, although the list is long and increases (e.g., Rogers et al., 2017; Rumeu et al., 2017). In the Mediterranean basin, few studies have been carried out on the disruption of plant-disperser mutualisms (e.g., Traveset, 2002; Traveset et al., 2012). Interestingly, the persistence of large-fleshy-fruited species has often been possible thanks to extant small-sized frugivores mammals such as rodents (Jansen et al., 2012), and also to introduced carnivores or livestock (Janzen and Martin, 1982), which may act as “surrogate Pleistocene dispersal agents” (*sensu* Janzen, 1982). Other examples have been documented from other systems. For instance, in the Balearic Islands, introduced pine martens (*Martes martes*) replace, at least partially, the native lizards which presumably used to be the only legitimate dispersers of the native shrub *Cneorum tricoccon* until recently (Traveset, 1995; Celedón-Neghme et al., 2013). Therefore, contemporary frugivore assemblages, even though in a suboptimal way (Guimarães et al., 2008), can act as “rescuers” of the seed dispersal service of anachronistic species, mitigating the effects of previous animal extinctions, and avoiding the vanishing of ecological interactions (Zamora, 2000; Valiente-Banuet et al., 2015; Rumeu et al., 2017; Cares et al., 2018).

On Mallorca island (Balearic archipelago), the native vertebrate fauna of the Upper Pleistocene and the Holocene was composed only of three endemic species: the cave goat *Myotragus balearicus* (Artiodactyla: Bovidae; 25.19–33.34 kg, Palombo et al., 2008), the giant dormouse *Hypnomys morpheus* (Rodentia: Gliridae; 0.23 kg, Moncunill-Solé et al., 2014) and the Balearic shrew *Nesiotites hidalgo* (Soricomorpha: Soricidae; 0.023–0.031 kg, Bover et al., 2008; Moncunill-Solé et al., 2016). The cave goat was thus the only medium-sized herbivorous mammal present on the islands (specifically, on Mallorca, Menorca and Cabrera). Its extinction took place during the Holocene, around 3000–2000 BC, and has been associated to the first arrival of humans to these islands (Bover and Alcover, 2008; Bover et al., 2016). Nowadays, the only non-flying mammals present in the Balearics have all been introduced by man, including goats, sheep, cows, pigs, dogs, as well as rodents (Bover and Alcover, 2008; Valenzuela and Alcover, 2013a). The wild carnivore the weasel (*Mustela nivalis*) appears to have been imported by Talaiotic people (prior to 123 BC) (Valenzuela and Alcover, 2013b). Subsequently, during the Roman period (123 BC-534 AD), the domestic cat (*Felis catus*) and the pine marten (*M. martes*) were introduced (Valenzuela and Alcover, 2015), and the common genet (*Genetta genetta*) was also probably introduced previously to the Muslims arrival (656–773 AD; Delibes et al., 2017b). The raccoon (*Procyon lotor*) and the coati (*Nasua nasua*) have been established very recently (in 2006 and 2004, respectively) on the island (Mayol et al., 2009; Pinya et al., 2009). However, very little is known about the ecological role of these introduced vertebrate species on the dynamics and composition of island communities.

In the present study, we aim at identifying and quantifying the relative importance of introduced seed dispersers for the dwarf palm *Chamaerops humilis* L. on Mallorca. This endemic palm is known to be mammal-dispersed elsewhere (Fedriani and Delibes, 2011) and we thus expect that non-native mammals play the role of dispersers in this island. In the few areas of continental Europe where its seed dispersal has been studied (**Table 1**), carnivorous mammals such as the European badger (*Meles meles*) and the red fox (*Vulpes vulpes*) have been described as its legitimate dispersers, and rabbits (*Oryctolagus cuniculus*) and some ungulates, like the wild boar (*Sus scrofa*), deer (Cervidae) and the domestic goat (*C. hircus*), act as occasional and suboptimal dispersal agents (Fedriani and Delibes, 2008, 2011; Delibes et al., 2017a). On islands, for which information is less available, only a few species have been described as consumers of the palm fruits (see **Table 1**). In Mallorca, its seeds have only been documented from *G. genetta* feces (Alcover, 1984; Clevenger, 1995), and no study has been carried out on the seed dispersal of this plant despite its high ecological, cultural and economic value (Guzmán et al., 2017).

The specific questions addressed in this study are the following: (1) Who are currently the main vertebrate fruit consumers of *C. humilis* on Mallorca? (2) How quantitatively and qualitatively effective are such fruit consumers as seed dispersers? To estimate the quantitative component of the seed dispersal effectiveness (SDE) of potential seed dispersers, we searched for mammal droppings and regurgitations, throughout linear transects in areas where this plant is abundant. On the other hand, the qualitative component of SDE for each seed disperser was assessed by means of a seed germination experiment using *C. humilis* seeds extracted from mammal samples as well as control (i.e., non-ingested) seeds. From our results, we discuss on the functional role of non-native mammal species as seed dispersers of this ancient Mediterranean palm on islands.

## MATERIALS AND METHODS

### Study Area

The study was carried out in the Northeast of the Mallorca island, specifically in the North of Serra de Tramuntana (municipality of Pollença) and in the North of Serra de Llevant (municipality of Artá). Four study sites, called “Cala Bóquer”, “Cap de Formentor”, “Ermita de Betlem” and “Es Caló” (**Figure 1**), were chosen. The predominant forest species are Aleppo pine (*Pinus halepensis*) and oak (*Quercus ilex*). Several shrub species, such as *C. humilis, Olea europaea var. sylvestris, Pistacia lentiscus, Erica multiflora, Cistus monspeliensis, Ampelodesmus mauritanicus,* and *Calicotome spinosa* are abundant over this territory. The climate of the Balearic archipelago is typically Mediterranean, characterized by two rainy seasons, a hot dry summer and a soft winter. Monthly rainfall on Mallorca varied between 0 and 107.2 mm during 2017, with the most rain falling in January and September, and extreme drought in May. Annual average temperature was 18.2°C (data from *Red de Estaciones Meteorológicas de Baleares*[1]).

### Study Species

The endemic Mediterranean dwarf palm *Chamaerops humilis* L. (Arecaceae) is a representative species of the Pre-Pliocene paleotropical ancestral lineages (Thompson, 2005). It is very likely that this ancient palm was in the Balearic Islands long before the arrival of the first settlers since its presence in the eastern coast of the Iberian Peninsula (Arroyo et al., 2004; Pérez-Obiol et al., 2010) and in other Mediterranean islands (Sardinia; Biondi and Filigheddu, 1990) is very old, previous to the Mediterranean Sea desiccation. Nevertheless, this remains uncertain so far due to the lack of any pre-anthropic evidence in both pollen and fossil records (Carrión, 2012), which is not surprising because of the poor preservation of this species and the little amount of pollen that scatters (F. Burjachs, *pers. comm.*) due to, probably, its insect-pollinated syndrome (Carrión, 2002). In Mallorca, this dioecious palm is relatively abundant currently, especially in Serra de Tramuntana and in the North of Serra de Llevant (Garcia and Morey, 1989; Bioatles—Govern de les Illes Balears[2]). It flowers during spring (March–May) and the fruits ripen in autumn (September–November). Its fleshy fruits are poly-drupes (Herrera, 1989). Unripe fruits are bright green, turning to dull yellow to brown when ripe. The seed (usually 0.6– 0.8 g, 16.3 × 11.0 mm average dimensions, Delibes et al., 2017a) comprises a small cylindrical embryo (2 mg), which is surrounded by several layers, from inner to outer: (1) a nutritious endosperm, (2) a

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[1] http://balearsmeteo.com/

[2] http://bioatles.caib.es/serproesfront/VisorServlet

**TABLE 1 |** Mammal species that consume *C. humilis* fruits described in the Mediterranean basin so far, indicating the system (island or mainland), the region, whether they are native or not, the frequency of occurrence of dwarf palm fruits in their diet, and the reference of the study.

| System | Mammal consumer | Native or non-native | Region | Frequency of occurrence | References |
|---|---|---|---|---|---|
| Island | Common genet (*Genetta genetta*) | Non-native | Balearic Islands (Spain) | $8.0^{a}$ | Clevenger, 1995 |
| | Sardinian fox (*Vulpes vulpes* subsp. *ichnusae*) | Non-native | Sardinia (Italy) | $<4.0^{a}$ | Farris et al., 2017 |
| | Sardinian wild boar (*Sus scrofa meridionalis*) | Non-native | Sardinia (Italy) | $3.0^{b}$ | Pinna et al., 2007 |
| Mainland | European badger (*Meles meles*) | Native | Almería (Spain) | $\sim30.0^{a}$ | Requena-Mullor et al., 2016 |
| | | | Huelva (Spain) | $<20.0^{c}$ | Revilla and Palomares, 2002 |
| | | | | Presence | Fedriani and Delibes, 2008 |
| | | | | Presence | Fedriani and Delibes, 2011 |
| | | | | Presence | Perea et al., 2013 |
| | | | | Presence | García-Cervigón et al., 2018 |
| | Red fox (*Vulpes vulpes*) | Native | Almería and Murcia (Spain) | $2.77 \pm 1.69^{a}$ | Cancio et al., 2017 |
| | | | Barcelona (Spain) | $35.05^{a}$ | Martín, 2008 |
| | | | Huelva (Spain) | $8.0^{d}$ | Fedriani, 1996 |
| | | | | Presence | Fedriani and Delibes, 2008 |
| | | | | $5.0^{e}$ | Perea et al., 2013 |
| | | | | Presence | García-Cervigón et al., 2018 |
| | | | Murcia (Spain) | Presence | V. M. Zapata, *pers. comm.* |
| | | | Sevilla (Spain) | Presence | J.M. Fedriani, *pers. obs.* |
| | Wild boar (*Sus scrofa*) | Native | Huelva (Spain) | Presence | Fedriani and Delibes, 2008 |
| | | | | $2.0^{e}$ | Perea et al., 2013 |
| | | | | Presence | García-Cervigón et al., 2018 |
| | Barbary macaque (*Macaca sylvanus*) | Native | Cascades d'Ouzoud (Morocco) | Presence | El Alami and Chait, 2017 |
| | | Non-native | Gibraltar (United Kingdom) | Presence | Schurr et al., 2012 |
| | African golden wolf (*Canis anthus*) | Non-native | Tlemcen (Algeria) | $34.96^{a}$ | Eddine et al., 2017 |
| | Egyptian mongoose (*Herpestes ichneumon*) | Non-native | Huelva (Spain) | $1.0^{d}$ | Palomares and Delibes, 1991 |
| | Domestic goat (*Capra hircus*) | Native | Sevilla (Spain) | Presence* | Delibes et al., 2017a |
| | Red deer (*Cervus elaphus*) | Native | Albacete (Spain) | Presence* | Castañeda et al., 2018 |

**Experimental study.*
*[a] Percent in feces.*
*[b] Percent in stomach.*
*[c] Percent estimated biomass ingested.*
*[d] Minimum number of seeds in feces.*
*[e] Number of seeds per feces (log scale).*

wide woody layer or endocarp, (3) a fleshy and fibrous mesocarp (the pulp, that smells strongly of rancid butter when ripe), and (4) the thin outer layer or exocarp (González-Benito et al., 2006; Hasnaoui et al., 2009). The medium-sized seed is usually large enough to preclude their consumption by birds, so this plant species belongs to the known "mammal-dispersal syndrome". Occasionally, though, birds may remove some fruits and thus transport seeds to different distances; specifically, one songbird (*Turdus merula*) was once observed picking one fruit and leaving with it in its bill (R. Muñoz-Gallego, *pers. obs.*). The dwarf palm seeds are commonly predated by beetles. At least, two non-native scolytine species have been described to bore into *C. humilis* seeds in the continent, *Coccotrypes dactyliperda* and *Dactylotrypes longicollis*, taking place as both pre- and post-dispersal events. Thus, these beetles bore the pulp and endocarp of fruits still attached to the mother plant, dropped from inflorescences, and also of dispersed (and depulped) seeds (Rodríguez et al., 2014; this study). Fruits are attached to infructescences (or ramets) of up to 30 cm long (20–40 fruits per ramet, Fedriani and Delibes, 2011) and located at 10–30 cm from the ground level. Germination is hypogeal and remote, with most seedlings emerging during spring time. Moreover, it has a well-known ability to thrive on poor nutrient soils (Herrera, 1989).

## Seed Dispersal Effectiveness: Quantitative and Qualitative Components

Seed dispersal effectiveness (SDE) is defined as the "number of new adult plants produced by the dispersal activities of a disperser" (Schupp, 1993, p. 16). SDE can be quantified as the number of seeds dispersed by a dispersal agent (quantitative

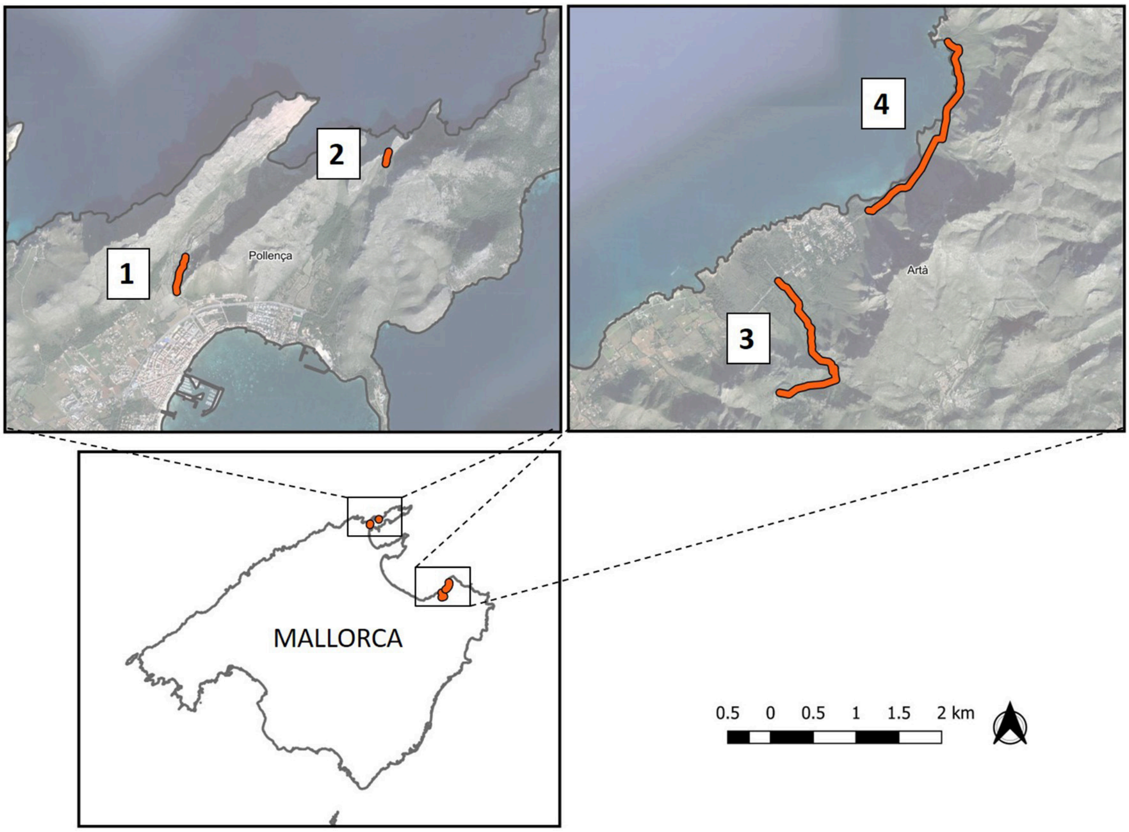

**FIGURE 1 |** Study area located in the Northeast of the Mallorca island. Orange lines are the four linear transects used for the fecal and regurgitation sampling surveys: (1) Cala Bóquer, (2) Cap de Formentor, (3) Ermita de Betlem, (4) Es Caló.

effectiveness) multiplied by the probability that a dispersed seed produces a new adult (qualitative effectiveness): SDE = Quantity · Quality. In our study, the quantitative component of SDE was obtained by recording the number of mammal samples that contained *C. humilis* seeds multiplied by the mean number of seeds per sample (**Figure 2**). This metric was then corrected by the distance (km) of each transect (e.g., Suárez-Esteban et al., 2013). On the other hand, the qualitative component (i.e., quality of treatment in the mouth and gut) was assessed by recording seedling emergence and seedling growth. The latter was determined by measuring both seedling length and dry weight (**Figure 2**). Finally, the SDE landscape (sensu Schupp et al., 2010), a visual representation of effectiveness and the relative contribution of each disperser, was estimated by multiplying the quantity component (number of dispersed seeds per distance sampled) by the quality component (emergence success). The landscape was plotted with the code provided by Jordano (2014) with Rstudio v.1.1.383 (R Core Team, 2017).

## Fecal and Regurgitation Sampling Surveys

In November of 2017, we searched for fresh mammal droppings and regurgitations belonging to the second half of the 2017 fruiting season (determined by their non-dry appeareance), along forest tracks and adjacent forest and garrigues. The four transects surveyed are shown in **Figure 1**: "Cala Bóquer" with 0.44 km in length (start point: 39° 54′ 53.55″ N, 3° 4′ 55.60″ E), "Cap de Formentor" with 0.15 km (start point: 39° 55′ 46.54″ N, 3° 6′ 39.93″ E), "Ermita de Betlem" with 2.25 km (start point: 39° 44′ 56.80″ N, 3° 18′ 41.23″ E), and "Es Caló" with 2.6 km (start point: 39° 45′ 23.60″ N, 3° 19′ 25.80″ E). The first two are located in Serra de Tramuntana and the last two in Serra de Llevant. Mammal feces were identified by their color, shape, smell and associated footprints (Bang and Dahlstrom, 2001). Fecal and regurgitated samples were air dried at room temperature and stored individually in paper bags. Each fecal sample was later washed using a sieve under running water, and seeds were immediately and carefully removed and dried (Fedriani and Delibes, 2009).

## Seed Germination Experiment

We sowed *C. humilis* mammal-ingested seeds as well as control seeds (i.e., non-ingested). To obtain control seeds, ripe fruits were collected during the sampling surveys from individuals distributed along the transects. Fruit ingestion and digestion have two effects on seeds that usually enhance their germination: (1) neutralization of the pulp inhibitory effect due to the removal of the pulp, and (2) seed coat scarification effect (Samuels and Levey, 2005). To separate both effects, we used two types of

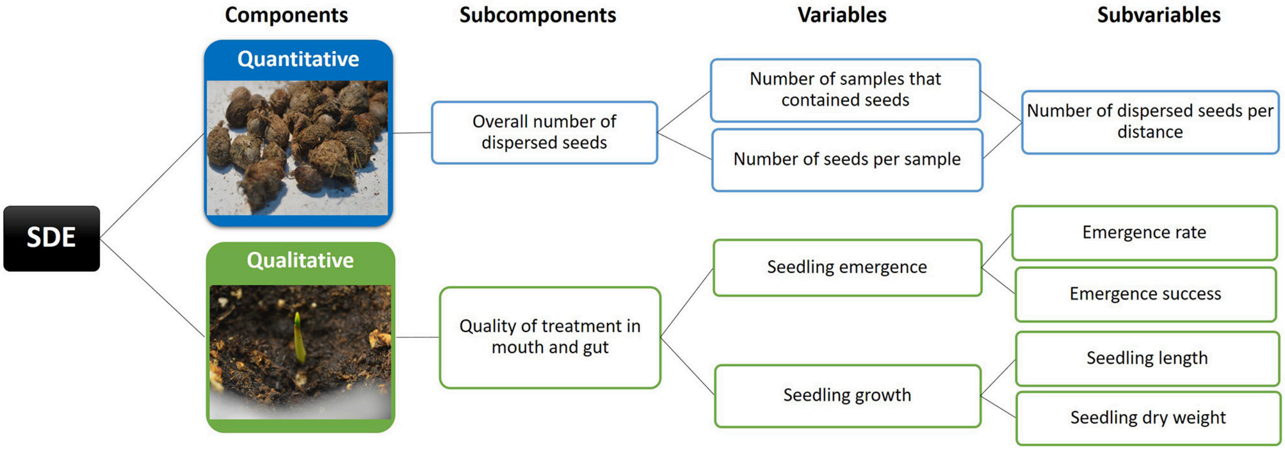

**FIGURE 2 |** Summary of methods used to assess each of the SDE (Seed Dispersal Effectiveness) components: quantitative and qualitative (*sensu* Schupp et al., 2010).

control seeds: intact fruits and fruits with the pulp manually removed. Therefore, we established five seed treatments for the germination experiment: (1) control 1 or "control seeds with pulp" (*n* = 43), (2) control 2 or "manually-depulped control seeds" (*n* = 44), (3) seeds regurgitated by goats (*n* = 99), (4) seeds spit out by goats (*n* = 80), and (5) pine marten-ingested seeds (*n* = 24). Distinction between seeds spit out and seeds regurgitated was required as the former experience a mechanical and chemical treatment only in the mouth while the latter spend also a given amount of time within the stomach in contact with the gastric juices. So, we categorized spit and regurgitated seeds based on our previous field observations and our work on other similar systems (Delibes et al., 2017a; Castañeda et al., 2018). Regurgitated seeds showed little persisting pulp, a wear out aspect, and often an irregular surface. Spit seeds have generally attached most of the pulp and a much less wear out aspect. Firstly, collected seeds were examined visually with 20–40 x magnification glasses, and traits which could affect germination, such as seed predation by insects, were recorded. Seed predation was estimated from the presence and number of holes on the endocarp (Rodríguez et al., 2014). In January 2018, both control (*n* = 87) and mammal-ingested seeds (*n* = 203) were sown individually in pots within 18 pot-trays (18 × 8 × 8 cm) with commercial substrate. Sown seeds (*n* = 290) were incubated in a chamber under controlled environment (25 ± 5°C, 50 ± 5% relative humidity, 12-h dark/12-h light photoperiod, uninterrupted ventilation, watering periodically; Salvador and Lloret, 1995; González-Benito et al., 2006; Hasnaoui et al., 2009). We monitored seedling emergence weekly for 9 months (from January to September), recording the date that any seedling part first emerged from the substrate surface. At the end of the experiment (33 weeks after sowing), we measured the length of emerged seedlings and extracted them and separated the aboveground part (i.e., leaves), the underground part (i.e., root), and the seed. Both aboveground and underground parts were dried for 72 h at 50°C and weighed to determine dry biomass.

## Statistical Analyses

Data on seedling emergence and seedling growth were analyzed through linear mixed models using the "lme4" package in Rstudio v. 1.1.383 (R Core Team, 2017). To analyze the final proportion of seedling emergence (or emergence success), we constructed generalized linear mixed models (GLMMs) with binomial error distribution (and associated logit link). To the response variable, the value "0" was assigned to those seeds that had no emerged and "1" when it did emerge. "Seed treatment" was the only predictor variable. To analyze seedling growth (using as response variables "Seedling length" and "Seedling dry weight"), we constructed linear mixed models (LMMs) with a gaussian error distribution, using as predictor variables "Seed treatment" and "Emergence time" (i.e., number of weeks between sowing and emergence). Due to the fact that seedlings developed either one or two leaves, three linear models were constructed for the response variable "Seedling length": model 1 with the length of the first (i.e., the highest) leaf as response variable, model 2 with the additive length (i.e., the addition of the length of the two leaves), and model 3 with the absence or presence of the second leaf as a binomial distribution. Regarding the variable response "Seedling dry weight", we constructed three linear models as well: model 1 with the aboveground dry weight, model 2 with the underground dry weight, and model 3 with the total weight. The variable "Study site" was included as random effect in all models. We fitted the GLMMs using Laplace approximation to maximum likelihood and the LMMs using restricted maximum likelihood (REML). Adjusted means and standard errors were calculated using the package "lsmeans", as well as the contrasts among the different levels of significant main factors through a Tukey *post-hoc* test.

On the other hand, to assess the potential effects of the different treatments on the emergence rate (speed), we used failure-time analyses by fitting a Cox proportional hazard regression mixed model (Therneau and Grambsch, 2000) to data consisting of the number of weeks between sowing and seedling emergence. To separate the effects on emergence rate from those on emergence success, we only considered seeds that had emerged by the end of our germination experiment (e.g., Fedriani et al., 2012). "Study site" was included as frailty factor. The significance of the target factor was evaluated by backwards stepwise elimination from the full model. In comparing successive models, we calculated the double absolute difference of their respective expectation maximization (EM) likelihood algorithms and compared that value against a chi-square with $k-1$ degrees of freedom, $k$ being the number of levels of the factor being tested. For the frailty factor we also assumed a chi-square distribution with one degree of freedom (Therneau and Grambsch, 2000). For this purpose, we used the package "survival" in Rstudio v. 1.1.383 (R Core Team, 2017).

## RESULTS

### Quantitative Effectiveness: Seeds per Sample and per Distance

Overall, we collected 56 mammal samples, of which 17 belonged to goat (*Capra hircus* L.) samples (both spits and regurgitations, **Figures 3A,B**) and 39 were pine marten (*Martes martes* L.) feces (**Figure 3C**). A total of 203 seeds of *C. humilis* were recovered from the samples: 179 seeds from goat samples, specifically 99 regurgitated seeds and 80 spit out seeds, and 24 seeds from pine marten feces. Goat samples were present in three of four study sites, with a frequency of seed occurrence of 100%. Pine marten feces were found in all study sites, although the frequency of *C. humilis* seed occurrence was 50% in Cala Bóquer, 25% in Ermita de Betlem and 0% in the rest of the study sites (**Table S1**). Therefore, the mean number of samples that contained seeds per transect was 4.25 ± 1.65 for goat and 2.25 ± 1.65 for pine marten (**Figure 4A**). Regarding the number of seeds per sample, it was much lower for pine marten, 2.2 ± 1.8, than for goat, 8.1 ± 3.6 (**Figure 4B**). The difference increases when considering the total number of seeds dispersed by each species (i.e., multiplying the number of samples with seeds by the number of seeds per sample), being 44.7 ± 19.7 for goat and 6.0 ± 3.7 for pine marten (**Figure 4C**). Finally, the total number of seeds dispersed corrected by the length (km) of each transect shows again that goats are much more important quantitatively (165.6 ± 138.5) than pine martens (9.5 ± 8.2) (**Figure 4D**). Specifically, 94.6 ± 79.8 seeds per km were regurgitated and 71.0 ± 58.8 seeds per km were spit out by goats.

### Qualitative Effectiveness: Seedling Emergence

Overall, 43.45% of the sown seeds ($n = 290$) germinated. Treatment-specific final emergence percentages were, on average, 56.3 ± 13.6% ($n = 43$), 81.1 ± 8.4% ($n = 44$), 27.9 ± 7.9% ($n = 99$), 49.4 ± 4.4% ($n = 80$), and 63.3 ± 3.3% ($n = 24$) for control seeds with pulp, manually-depulped control seeds, seeds regurgitated by goats, seeds spit out by goats, and pine marten-ingested seeds, respectively. Nonetheless, if we consider seeds regurgitated and spit out by goats together, the final emergence percentage of seeds dispersed by goats is 32.7 ± 5.7% ($n = 179$). Seedling emergence started 6 weeks after sowing (March 2018) for all treatments except for control seeds with pulp (C1), which started 3 weeks later (April 2018; **Figure 5A**). No seedling emerged in the last 8 weeks of monitoring. The Cox regression analyses indicated that, once corrected for the effect of study site (random or frailty factor), there were strong and significant differences among treatments in emergence rate ($\chi^2 = 29.43$, df = 4, $P < 0.0001$). Thus, on average, manually-depulped control seeds and pine marten-ingested seeds emerged 2.4 weeks earlier than control seeds with pulp. Seeds regurgitated and seeds spit out by goats emerged 5.0 and 3.3 weeks earlier than control seeds with pulp, respectively (**Figure 5B**). The hazard ratio of control seeds with pulp was <1 (0.4), which suggests that the presence of fruit pulp, and thus germination inhibitors, delayed seedling emergence.

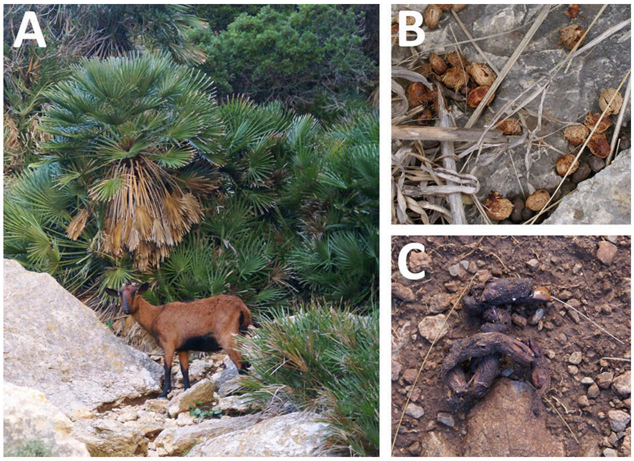

**FIGURE 3 |** Local mammal fruit consumers of *Chamaerops humilis* identified in the study. **(A)** A goat (*Capra hircus*) in front of several individuals of dwarf palm. **(B)** *C. humilis* seeds expeled by goat. **(C)** Pine marten (*Martes martes*) feces with *C. humilis* seeds.

Our mixed model revealed that seed treatment had a significant effect ($\chi^2 = 41.37$, df = 4, $P < 0.0001$) on final emergence success (**Table 2**), being highest for manually-depulped control seeds and lowest for seeds regurgitated by goats (**Figure 6A**). In pairwise comparisons (**Table S2**), differences were significant between control seeds with pulp and seeds regurgitated by goats, manually-depulped control seeds and seeds regurgitated by goats, manually-depulped control seeds and seeds spit out by goats, seeds regurgitated and seeds spit out by goats, and seeds regurgitated by goats and pine marten-ingested seeds. About 24.6% of mammal dispersed seeds collected in the field ($n = 203$) were predated by insects (i.e., they had at least one beetle exit hole). Specifically, 18.7% ($n = 80$) of seeds spit out by goats and 35.3% ($n = 99$) of seeds regurgitated by goats had between 1 and 4 holes made by scolitine beetles, probably introduced (Rodríguez et al., 2014). No predated seeds were found for other treatments. To better understand the

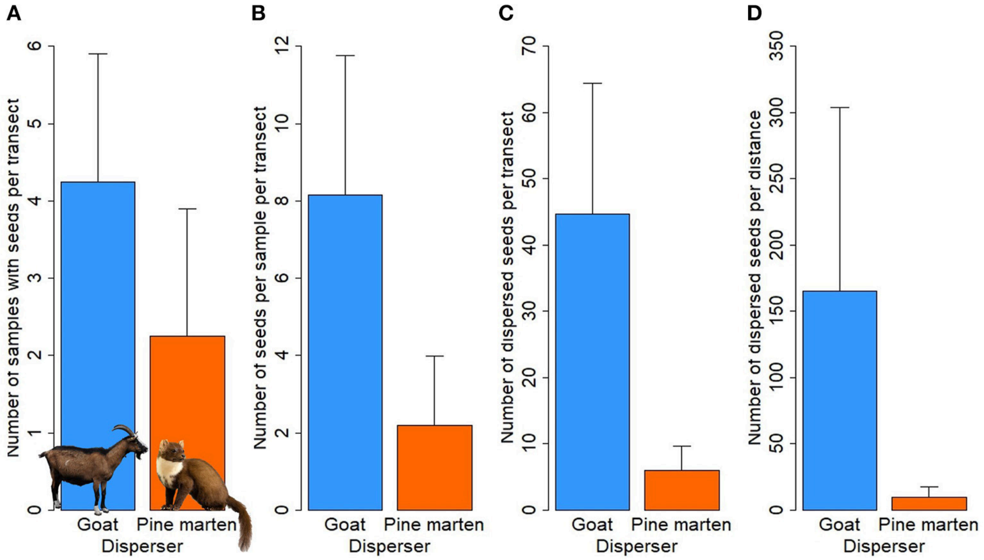

**FIGURE 4 |** Means (± SE) among the four transects of different quantitative variables for the two dispersers, goat (blue bars) and pine marten (orange bars). **(A)** number of samples with seeds per transect; **(B)** number of seeds per sample per transect; **(C)** number of dispersed seeds per transect; **(D)** number of dispersed seeds per transect corrected by distance (km).

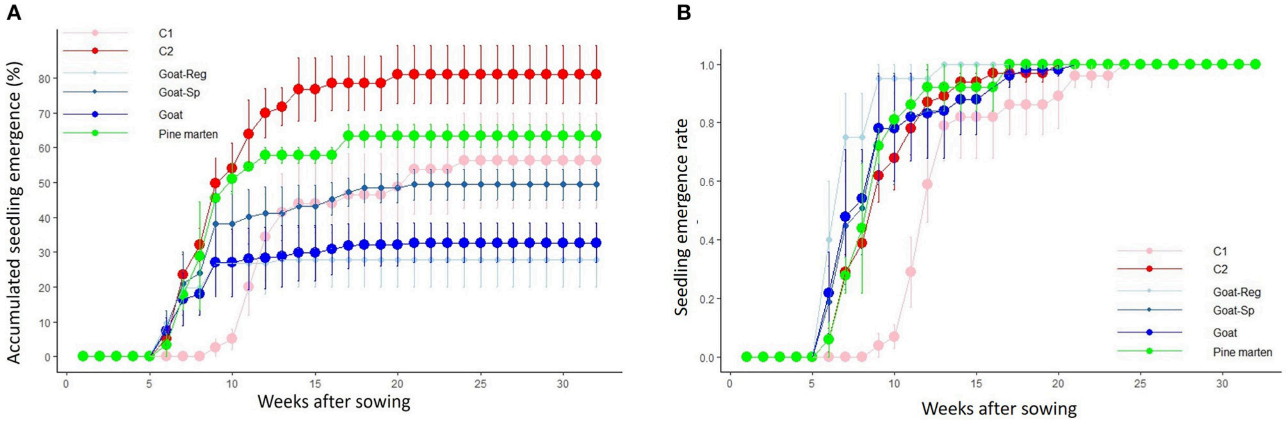

**FIGURE 5 | (A)** Cumulative percentages (mean ± SE) of seedling emergence (i.e., emergence success) for the five treatments, adding the net percentages for the goat, over the weeks after sowing. **(B)** Emergence rate, i.e., emerged seedlings each week divided by the total emerged seedlings, for the five treatments over the weeks after sowing. Pink line, C1 or "control seeds with pulp"; red line, C2 or "manually-depulped control seeds"; light blue line, seeds regurgitated by goats; dark blue line, seeds spit out by goats; blue line, seeds dispersed by goats; and green line, pine marten-ingested seeds.

relative qualitative effectiveness of seed dispersers in scenarios of absence of scolitine beetles, we ran the linear mixed model again but without considering scolitine-predated seeds. Results were very similar to the previous model, again with a significant effect of seed treatment ($\chi^2 = 28.14$, df = 4, $P < 0.0001$) on emergence success (**Table 2**), but differences were significant only in pairwise comparisons between each treatment and seeds regurgitated by goats (**Table S2**). Adjusted means of seeds regurgitated and spit out by goats were slightly higher in this case, but without substantial changes with respect to the other treatments (**Figure 6B**).

## Qualitative Effectiveness: Seedling Growth

Once the study site effect was corrected for, our linear mixed models revealed that seed treatment only had a marginal effect on seedling length for the first (and largest) leaf ($\chi^2 = 8.74$, df = 4, $P = 0.07$; **Table 3**). However, emergence time had a significant effect on seedling length in the three models ($\chi^2 = 16.43$, df = 1, $P < 0.0001$), always reaching higher values those that emerged earlier. In order to evaluate whether the relationship between emergence time and seedling length was consistent among treatments, we fitted simple linear regressions using the length of the first leaf as variable. The correlation

**TABLE 2 |** Main results of the generalized linear mixed models testing the effects of emergence success, with and without predated seeds by insects (response variable), for each treatment (C1 or "control seeds with pulp", C2 or "manually-depulped control seeds", Goat-Reg or seeds regurgitated by goat, Goat-Sp or seeds spit out by goat, and Pine marten or pine marten-ingested seeds) (predictor variable).

| | Seedling emergence | | | | Seedling emergence without predated seeds by insects | | | |
|---|---|---|---|---|---|---|---|---|
| Predictor variable | $\chi^2$ | df | | ***P*-value** | $\chi^2$ | df | | ***P*-value** |
| Seed treatment | 41.37 | 4 | | **<0.0001** | 28.14 | 4 | | **<0.0001** |
| | **Estimate** | **Standard error (SE)** | ***Z*-value** | ***P*-value** | **Estimate** | **Standard error (SE)** | **Z value** | ***P*-value** |
| Intercept | 0.17 | 0.34 | 0.48 | 0.63 | 0.18 | 0.39 | 0.48 | 0.63 |
| Treatment C2 | 1.25 | 0.49 | 2.54 | **0.01** | 1.29 | 0.50 | 2.58 | **0.01** |
| Treatment Goat-Reg | −2.00 | 0.47 | −4.25 | **<0.0001** | −1.42 | 0.47 | −3.03 | **0.002** |
| Treatment Goat-Sp | −0.37 | 0.43 | −0.84 | 0.40 | −0.03 | 0.44 | −0.07 | 0.95 |
| Treatment pine marten | 0.38 | 0.54 | 0.70 | 0.48 | 0.49 | 0.55 | 0.88 | 0.38 |

*Above, the results of Chi-square tests ($\chi^2$, DF, P-value). Below, the output of the model summaries [estimate, standard error (SE), Z value, P-value]. Treatment C1 acts as the intercept in these models. Significant differences ($P < 0.05$) are marked in bold.*

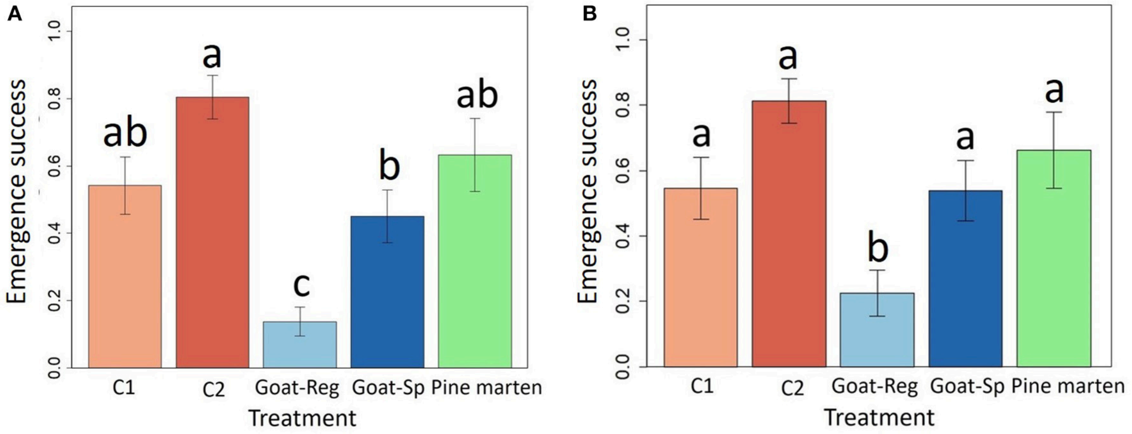

**FIGURE 6 |** Model-adjusted means (± SE) of the final emergence success, with **(A)** and without predated seeds by insects **(B)**, for the five treatments (C1 or "control seeds with pulp", C2 or "manually-depulped control seeds", Goat-Reg or seeds regurgitated by goat, Goat-Sp or seeds spit out by goat, and Pine marten or pine marten-ingested seeds). Different letters denote significant differences between paired levels in Tukey test.

was significantly negative for the whole dataset including all treatments and controls ($P < 0.0001$, **Figure S1A**) and for both control treatments "C1" and "C2" ($P < 0.0001$, **Figure S1B** and $P < 0.05$, **Figure S1C**, respectively). Nevertheless, it was non-significant for seeds regurgitated by goats, seeds spit out by goats, and pine marten-ingested seeds (**Figures S1D–F**), suggesting that seed processing by dispersers altered the negative relationship between emergence time and seedling length.

Seed treatment had a significant effect on seedling underground dry weight and total dry weight ($\chi^2 = 12.94$, df = 4, $P = 0.01$ and $\chi^2 = 10.29$, df = 4, $P < 0.05$, respectively), being highest for pine marten-ingested seeds and lowest for seeds spit out by goats (**Figures 7A,B**), but showed no effect on seedling aboveground dry weight ($\chi^2 = 4.89$, df = 4, $P = 0.3$; **Figure 7C**). In addition, emergence time had a significant effect on seedling aboveground, underground and total dry weight ($P < 0.01$; **Table 4**); seedlings emerging earlier showed higher dry weights. Pairwise comparisons through Tukey test (**Table S3**) indicated that differences were significant between manually-depulped control seeds—seeds spit out by goats, and seeds spit out by goats—pine marten-ingested seeds for underground dry weight. However, for aboveground dry weight differences among seed treatments were non-significant. Finally, for total dry weight, differences were only significant between manually-depulped control seeds and seeds spit out by goats.

## Total Seed Dispersal Effectiveness (SDE)

The SDE landscape shows that goats are, overall, about nine times more effective seed dispersers (SDE = 54.14) than pine martens (SDE = 6.03) (**Figure 8**). Goats are high-quantity and medium-quality dispersers, whereas pine martens are high-quality but low-quantity dispersers. Moreover, if goat-treatments are separated, seeds spit out are better qualitatively (SDE = 35.07) than seeds regurgitated (SDE = 26.4). The higher overall dispersal effectiveness of goats occurred even though some goat-processed seeds were predated by scolitines.

**TABLE 3 |** Main results of the linear mixed model testing the effects of seed treatment (C1 or "control seeds with pulp", C2 or "manually-depulped control seeds", Goat-Reg or seeds regurgitated by goat, Goat-Sp or seeds spit out by goat, and Pine marten or pine marten-ingested seeds) and emergence time (predictor variables) on seedling length for the first leaf (response variable).

| Predictor variables | $\chi^2$ | df | *P*-value |
|---|---|---|---|
| Seed treatment | 8.74 | 4 | 0.07 |
| Emergence time | 16.43 | 1 | **<0.0001** |

| | Estimate | Standard error (SE) | *t*-value | *P*-value |
|---|---|---|---|---|
| Intercept | 35.56 | 2.48 | 14.35 | **<0.0001** |
| Treatment C2 | 0.10 | 1.48 | 0.07 | 0.94 |
| Treatment Goat-Reg | −3.40 | 1.87 | −1.82 | 0.07 |
| Treatment Goat-Sp | −2.63 | 1.47 | −1.78 | 0.08 |
| Treatment Pine marten | 0.23 | 1.85 | 0.12 | 0.9 |
| Emergence time | −0.59 | 0.14 | −4.05 | **<0.0001** |

*Above, the results of Chi-square test ($\chi^2$, DF, P-value). Below, the output of the summary [estimate, standard error (SE), t value, P-value]. Treatment C1 acts as the intercept in this model. Significant differences ($P < 0.05$) are marked in bold.*

# DISCUSSION

Island systems are well-known to be fauna-depauperated and, consequently, to have highly specialized plant-animal mutualisms, such as plant-frugivore interactions (e.g., González-Castro et al., 2012). Therefore, past fauna extinctions may lead to important ecological consequences for plant communities on islands (Hansen and Galetti, 2009; Traveset and Richardson, 2014), like the functional replacement of the extinct seed dispersers by contemporary frugivore assemblages (e.g., Traveset, 1995; Celedón-Neghme et al., 2013). This study assesses for the first time the SDE for *C. humilis* on islands, and describes understudied dispersal systems, that is, goats (*C. hircus*) and pine martens (*M. martes*) as seed dispersers of this palm in the largest Balearic island. Moreover, this study contributes with a valuable example of how non-native species can be exerting an important ecosystem function that was likely lost thousands of years ago, when humans led to the extinction of the unique native mammals on many Mediterranean islands.

## Quantitative Effectiveness

Our results indicate that the quantitative component of SDE was high for goats and low for pine martens. Several variables could be explaining these large differences between both species. First, the local abundance of the disperser is a relevant variable for SDE (Schupp et al., 2010). In this case, an extremely high abundance of goats has been reported in Mallorca (it is estimated that several tens of thousands live in the island—exact amount not known; Vives and Baraza, 2010). They are heterogeneously distributed throughout Serra de Tramuntana and Serra de Llevant (Bioatles—Govern de les Illes Balears[2]). Second, the number of fruits handled per visit seems to be much higher for goats than for pine martens, and the handling behavior could also influence on the final number of seeds dispersed per feeding bout. Given that humans are largely determining the number of goats on Mallorca (Mayol et al., 2017), they are probably influencing the "relative seed dispersal effectiveness", not only for *C. humilis*, but also for many other systems where livestock "rescues" the seed dispersal service of anachronistic plant species (Janzen and Martin, 1982; Cares et al., 2018).

Goats have been described to expel the ingested seeds while defecating (e.g., Mancilla-Leytón et al., 2011) or ruminating (e.g., Delibes et al., 2017a); however, we found no goat feces with *C. humilis* seeds in our study sites. On the other hand, introduced common genets (*G. genetta*) were scarce in the study area and no feces were found during the surveys; moreover, this species usually defecates in rather unsuitable sites for seed germination and seedling establishment (rocks and cliffs; Traveset, 2002). Likewise, we detected no signs of rabbit or rodents fruit consumption in our transects. We must note, however, that the sampling surveys were focused on forest tracks, which could be overestimating the quantitative contribution of some frugivore species—like the pine marten, which is known to use tracks to fecal marking (Barja, 2005) -, at the same time that we could have underestimated the contribution of others, like the common genet. On the other hand, pulp feeders (rabbits and rodents) could not have been identified through the fecal sampling surveys since they usually consume the pulp, leaving the seeds under the mother plant (Fedriani et al., 2012).

## Qualitative Effectiveness

Seed dispersers can affect germination via two effects: (1) neutralization of the pulp inhibitory effect due to the removal of the pulp, and (2) seed coat scarification effect (Samuels and Levey, 2005). In our study, the earliest emergence of both manually-depulped control seeds and ingested seeds indicates that disperser species influence emergence time by removing the pulp inhibitory effect (Fedriani and Delibes, 2009). In relation to seed coat scarification, seed treatment in goat-stomach during rumination (i.e., digestive contractions and fermentation) could be accelerating seed germination as seed regurgitated by goats showed the highest emergence speed. However, this treatment is sometimes too harsh for seed viability, leading to the damage of seed embryos (i.e., seed predation) (e.g., Mancilla-Leytón et al., 2015; Jara-Guerrero et al., 2018) and, consequently, decreasing the final emergence success. Pine marten-ingested seeds showed the highest emergence success (after the manually-depulped control seeds); however, this disperser does not seem to be exerting any effect on emergence speed through seed scarification. Regarding seedling growth, seeds spit out by goats showed the lowest values of seedling dry weight, whereas pine marten-ingested seeds the highest ones. This could suggest that treatment in the mouth of goats could be more aggressive than treatment in the gut of pine martens, since chewing process is very intensive in ruminant species (Mancilla-Leytón et al., 2015). Nonetheless, the seedling dry weight of seeds regurgitated by goats was rather similar to that of pine marten-ingested seeds (**Figures 7A,B**), thus further research is required to go in depth in these results. In addition, it is necessary to consider that the method used to classify seeds regurgitated vs. seeds spit out by goats might be not entirely objective. As it is already known, seed treatment in mouth and gut differs between frugivore species

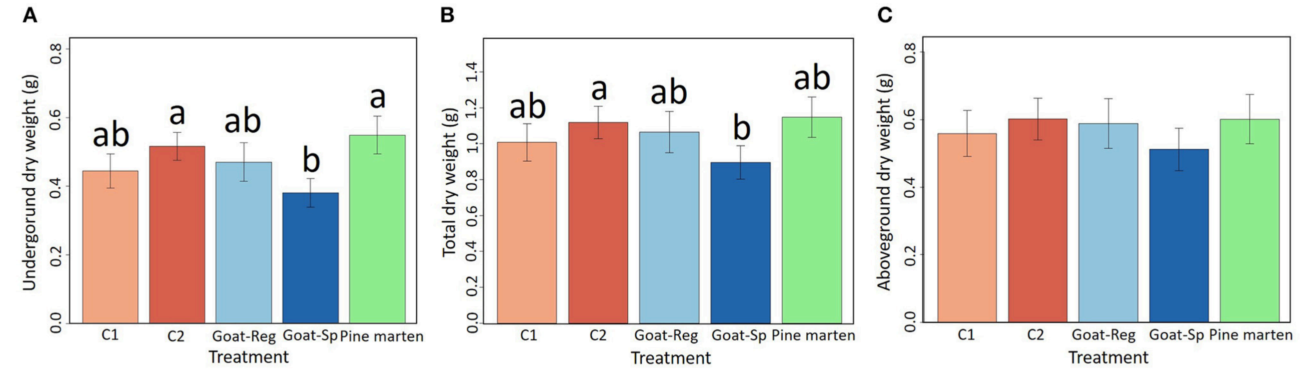

**FIGURE 7 |** Model-adjusted means (± SE) of seedling underground dry weight **(A)**, seedling total dry weight **(B)**, and seedling aboveground dry weight **(C)** for the five treatments (C1 or "control seeds with pulp", C2 or "manually-depulped control seeds", Goat-Reg or seeds regurgitated by goat, Goat-Sp or seeds spit out by goat, and Pine marten or pine marten-ingested seeds). Different letters denote significant differences between paired levels in Tukey test.

**TABLE 4 |** Main results of the linear mixed models testing the effects of seed treatment (C1 or "control seeds with pulp", C2 or "manually-depulped control seeds", Goat-Reg or seeds regurgitated by goat, Goat-Sp or seeds spit out by goat, and Pine marten or pine marten-ingested seeds) and emergence time (predictor variables) on seedling growth: aboveground, underground, and total seedling dry weight (response variable).

| | **Aboveground dry weight** | | | **Underground dry weight** | | | **Total dry weight** | | |
|---|---|---|---|---|---|---|---|---|---|
| Predictor variables | $\chi^2$ | **df** | ***P*-value** | $\chi^2$ | **df** | ***P*-value** | $\chi^2$ | **df** | ***P*-value** |
| Seed treatment | 4.89 | 4 | 0.3 | 12.94 | 4 | **0.01** | 10.29 | 4 | **0.04** |
| Emergence time | 7.14 | 1 | **0.007** | 13.36 | 1 | **0.0002** | 13.28 | 1 | **0.0003** |

| | **Estimate** | **SE** | ***t*-value** | ***P*-value** | **Estimate** | **SE** | ***t*-value** | ***P*-value** | **Estimate** | **SE** | ***t*-value** | ***P*-value** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Intercept | 0.70 | 0.10 | 7.22 | **<0.0001** | 0.63 | 0.08 | 7.61 | **<0.0001** | 1.34 | 0.16 | 8.47 | **<0.0001** |
| Treatment C2 | 0.04 | 0.05 | 0.80 | 0.42 | 0.07 | 0.05 | 1.40 | 0.16 | 0.11 | 0.09 | 1.20 | 0.23 |
| Treatment Goat-Reg | 0.03 | 0.07 | 0.44 | 0.66 | 0.03 | 0.07 | 0.40 | 0.69 | 0.06 | 0.12 | 0.50 | 0.62 |
| Treatment Goat-Sp | −0.05 | 0.05 | −0.90 | 0.37 | −0.06 | 0.05 | −1.23 | 0.22 | −0.11 | 0.09 | −1.22 | 0.22 |
| Treatment Pine marten | 0.04 | 0.07 | 0.63 | 0.53 | 0.10 | 0.06 | 1.62 | 0.11 | 0.14 | 0.11 | 1.22 | 0.22 |
| Emergence time | −0.01 | 0.005 | −2.67 | **0.0003** | −0.02 | 0.005 | −3.65 | **0.0004** | −0.03 | 0.01 | −3.64 | **0.0004** |

*Above, the results of Chi-square test ($\chi^2$, DF, P-value). Below, the output of the summary [estimate, standard error (SE), t value, P-value]. Treatment C1 acts as the intercept in these models. Significant differences ($P < 0.05$) are marked in bold.*

–and might differ as well among conspecific individuals- as a consequence of differences in morphological and physiological traits, as well as in retention times (Traveset et al., 2007). In conclusion, the monitoring of seedling emergence and seedling growth suggests a higher qualitative seed dispersal effectiveness for pine martens than for goats.

Frugivore-plant interactions can turn out into trade-offs, i.e., benefits and costs, for plant fitness (e.g., Fedriani and Delibes, 2011). Regarding the quality of seed deposition, all the seeds predated by scolitine beetles found in the field had been dispersed by goats, specifically 18.7% ($n = 80$) of the seeds spit out and 35.3% ($n = 99$) of the seeds regurgitated. This might be related with seed covering, since pine-marten dispersed seeds are embedded in the dung (Traveset et al., 2007), but might also be due to persisting pulp surrounding the seed, which is probably higher for seeds spit out by goats and pine marten-ingested seeds than for those regurgitated by goats. Thus, pulp removal by frugivorous vertebrates appears to enhance germination, but at the same time it represents a risk due to the cost of insect predation increase (Rodríguez et al., 2014). Moreover, seeds dispersed by goats are deposited in aggregates of ∼8 seeds on average, and it is very likely that high seed density has a negative effect on seedling establishment by intraspecific competition (Murray, 1998). Finally, it is well-known that the microsite of seed arrival is a key qualitative aspect of seed dispersal effectiveness (García-Cervigón et al., 2018), so it must be considered in future studies where seedling emergence and survival are monitored under field conditions.

## Total Seed Dispersal Effectiveness (SDE)

Overall, the relative contribution of goats to the seed dispersal effectiveness of *C. humilis* is more important than that of pine martens, mainly due to the quantitative component. Goat long-distance mobility, the great amount of fruits per time that they can consume, and a long seed retention time in the gut support

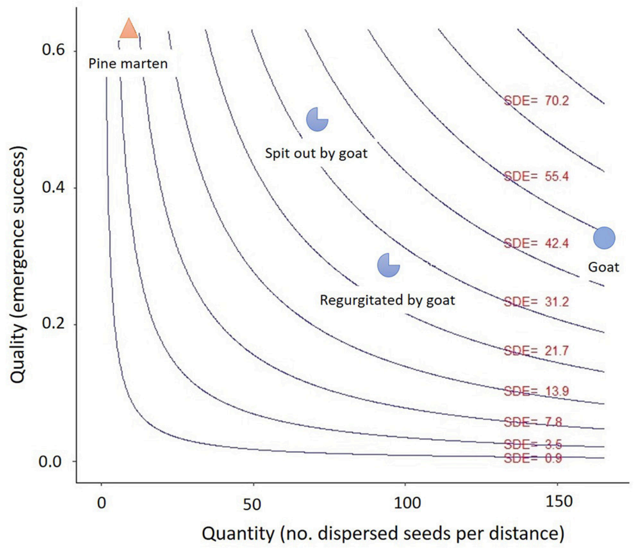

**FIGURE 8 |** Seed dispersal effectiveness (SDE) landscape. The blue circles indicate the quantitative and qualitative components for goat-treatments, both separated (regurgitated and spit out seeds) and together, and the orange triangle the quantitative and qualitative components for pine marten. The isoclines (blue lines) and the SDE values (red) show the seed dispersal effectiveness (quantity x quality) throughout the SDE landscape.

their functional relevance in many dispersal systems (Mancilla-Leytón et al., 2011; Grande et al., 2013). In Spain, goats have been shown to disperse effectively the seeds of at least 12 species, including *C. humilis* (Robles et al., 2005; Mancilla-Leytón et al., 2011, 2015; Delibes et al., 2017a). Pine martens also transport seeds over long distances (González-Varo et al., 2013; up to ~1,200 m) and have long retention times in the gut (Schaumann and Heinken, 2002). Moreover, they are known to consume many fleshy-fruited species in Northern Spain (Clevenger, 1993; Bermejo and Guitián, 1996; Rosellini et al., 2007), as well as *C. humilis* among other species in the Balearic Islands (Clevenger, 1995); however, the number of fruits per feeding bout is usually very low, and their population densities are not as high as those of goats.

We must consider, however, that goats not only act as effective seed dispersers, but also as herbivores, browsing frequently on *C. humilis* (Rivera Sánchez, 2014) and devastating leaves and inflorescences of many other plants (Mayol et al., 2017); and also as pre-dispersal seed predators (Mancilla-Leytón et al., 2011) like other ruminant species (e.g., Giordani, 2008). These ungulates have been reported to cause much damage on a number of plant species (Rivera-Sánchez et al., 2015), many of them threatened (Mayol et al., 2017). On the other hand, endemic plant species in Mallorca seem to have evolved under the selective pressure of herbivore ungulates (Vives and Baraza, 2010; Bover et al., 2016), and moderate browsing by goats indeed helps to maintain plant communities (Johnson, 2009; Rosa García et al., 2012). All these potential goat negative effects on other life plant stages of *C. humilis* as well as goat impact on other plant populations prevent considering them as necessarily positive from a conservation point of view.

## Goat and Pine Marten: Surrogate Dispersers of *Myotragus balearicus*?

The extinct Balearic goat-like *Myotragus balearicus* Bate, the only medium-sized mammal present on the island in the Pleistocene, was a generalist species with a broad dietary niche, encompassing leaves, stems and inflorescences of diverse plant species (Bartolomé et al., 2011; Winkler et al., 2013a; Rivera et al., 2014; Welker et al., 2014). Several authors have suggested the pivotal role of this species as shaper of vegetation dynamics, acting as a keystone species in these insular ecosystems during the Pleistocene and Holocene (Palmer et al., 1999; Winkler et al., 2013b; Welker et al., 2014). Although no seeds have been found so far in any coprolites (Rivera et al., 2014; Welker et al., 2014), it is not discarded that *M. balearicus* could also feed on fruits (as many goats do today, e.g., Mancilla-Leytón et al., 2015; Delibes et al., 2017a) and discard seeds intact, thus acting also as a legitimate disperser. On the other hand, even though the origin of *C. humilis* in the Balearic Islands remains uncertain (Carrión, 2012), its old presence in the eastern coast of the Iberian Peninsula (Arroyo et al., 2004; Pérez-Obiol et al., 2010) would make more plausible the hypothesis of a Balearic existence before the recent human arrival. García-Castaño et al. (2014) even suggest that Balearic dwarf palm populations might appeared because of microplate migrations from the Oligocene or during the Messinian salinity crisis. In addition, an active human transport of *C. humilis* should have taken place along the Mediterranean basis in the Quaternary (García-Castaño et al., 2014; Guzmán et al., 2017).

Our study demonstrates that both goats and pine martens are effective seed dispersers of *C. humilis* in Mallorca, and we thus hypothesize that these introduced frugivores, especially the goat, could be replacing the function probably carried out by the extinct *M. balearicus* in the past, acquiring the role of seed dispersal "rescuers". This role has already been suggested for the goat in other systems (e.g., Mancilla-Leytón et al., 2015; Cares et al., 2018). So, despite the fitness costs imposed by both introduced mammals, they could be providing an indispensable service to this ancient palm by dispersing it on island systems, where native dispersers are extinct and the alternatives are very limited.

## CONCLUSIONS

In spite of the myriad negative impacts widely described for non-native species on islands (Reaser et al., 2007; Mayol et al., 2017; Rogers et al., 2017; Weller et al., 2018), their role as rescuers of ecological functions, lost in the past with fauna extinctions, acquires great relevance under the current scenario of increasing defaunation (Dirzo et al., 2014; Malhi et al., 2016). Our results here support the Janzen and Martin (1982) anachronism hypothesis and suggest that non-native species can disperse effectively anachronistic fruits, exerting a fundamental role in insular ecosystems. Thus, more studies are required to assess the role of non-native species in depauperated communities and to establish suitable management programs to preserve plant species and their associated ecological functions in Mediterranean ecosystems.

## AUTHOR CONTRIBUTIONS

JMF and AT: experimental design and field work. RM-G and JMF: laboratory work. RM-G: statistical analyses and original paper draft. RM-G, JMF, and AT: paper review and editing.

## ACKNOWLEDGMENTS

We are grateful to J. A. Alcover, E. Baraza, and J. Mayol for information about the current situation of *C. hircus* on Mallorca island, to F. Burjachs and J. Rita for solving some doubts about the *C. humilis* pollen dating in the Balearics, and to J. P. González-Varo for contributing to the last paper review. Thanks also to S. Santamaría for helping with the field work. This study is framed within project CGL2017-88122-P financed by the Spanish Government. JMF was funded by Portuguese Foundation for Science and Technology (grant IF/00728/2013).

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2019.00161/full#supplementary-material

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Ecology and Evolution

REVIEW
published: 17 July 2019
doi: 10.3389/fevo.2019.00265

# Spitting Seeds From the Cud: A Review of an Endozoochory Exclusive to Ruminants

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Given their strong masticatory system and the powerful microbial digestion inside their complex guts, mammalian ruminants have been frequently considered seed predators rather than seed dispersers. A number of studies, however, have observed that ruminants are able to transport many viable seeds long distances, either attached to the hair or hooves (i.e., epizoochory) or inside their body after ingesting them (i.e., endozoochory). However, very few studies have investigated a modality of endozoochory exclusive to ruminants: the spitting of usually large-sized seeds while chewing the cud. A systematic review of the published information about this type of endozoochory shows a marked scarcity of studies. Nonetheless, at least 48 plant species belonging to 21 families are dispersed by ruminants in this manner. Most of these plants are shrubs and trees, have fleshy or dry fruits with large-sized seeds, and are seldom dispersed via defecation. Many cases have been observed in tropical areas, where more frugivorous ruminant species occur, but other records are from temperate and dry areas, covering thus all continents except Antarctica. Twenty-one species of ruminants from 18 genera have been reported as endozoochore spitters. They involve domestic and wild species belonging to the families Tragulidae, Cervidae, and Bovidae. This suggests that almost any ruminant species could potentially eat fruits and regurgitate large hard seeds during rumination. Likely, this seed dispersal mechanism has been neglected due to the difficulty of observing rumination behavior and locating spat seeds. Further research on the potential of wild and domestic ruminant species as long-distance seed dispersers through spitting seeds from the cud appears particularly important given their increasing pervasiveness and abundance worldwide.

**Keywords: mutualism, plant-animal interactions, rumination, seed regurgitation, seed dispersal**

**Edited by:**
*Casper H. A. Van Leeuwen,*
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*Christophe Baltzinger,*
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**Specialty section:**
*This article was submitted to*
*Biogeography and Macroecology,*
*a section of the journal*
*Frontiers in Ecology and Evolution*

***Received:*** *12 November 2018*
***Accepted:*** *24 June 2019*
***Published:*** *17 July 2019*

**Citation:**
*Delibes M, Castañeda I and Fedriani JM (2019) Spitting Seeds From the Cud: A Review of an Endozoochory Exclusive to Ruminants. Front. Ecol. Evol. 7:265. doi: 10.3389/fevo.2019.00265*

## INTRODUCTION

Reproductive plants frequently benefit from moving their seeds away from their immediate vicinity. Such benefits include the increase of seed and seedling survival, enhanced germination, reduced sibling competition, increased gene flow, and the colonization of vacant habitats. Not surprisingly, plants use myriad mechanisms for seed dispersal, such as the transport outside and inside vertebrates' bodies (Herrera, 2002). To attract vertebrate seed dispersers, many plants have evolved edible fruits covering seeds, so that these nutritious fruits are ingested by animals that later eject seeds in suitable conditions to germinate (Herrera, 2002). However, fruits are attractive also for some other frugivores that kill seeds during ingestion and/or digestion, acting thus as seed predators. Given their strong masticatory system and the powerful microbial digestion inside their complex guts, mammalian ungulates, and particularly those that are ruminants, have been frequently considered predators of the large-sized seeds characteristic of many woody plant species. Indeed, many authors consider that ungulates maximize nutritional intake from fruits by digesting the entire resource, including seeds (e.g., Bodmer, 1991).

The antagonistic role of ungulates as plant consumers and seed predators has been frequently highlighted. As a result, the environmental risk of overgrazing derived from the recent increase in numbers of some wild ungulate species is receiving increasing research attention (e.g., Côté et al., 2004; Perea et al., 2014a; Lecomte et al., 2016). In the same way, global livestock production is an important cause of environmental concern, either for climate change, reactive nitrogen mobilization, or appropriation of plant biomass at planetary scales (Pelletier and Tyedmers, 2010). Nevertheless, the mutualistic role of ungulates as effective seed dispersers (*sensu* Schupp et al., 2010) has been also investigated, especially for grasses and some other plants whose small seeds often escape mastication and pass intact through the gut (i.e., endozoochory; e.g., Janzen, 1984; Myers et al., 2004; Mouissie et al., 2005). Also, ruminants can move many viable seeds at long distances attached to the fur and the hooves (i.e., epizoochory; e.g., Manzano and Malo, 2006). Recent reviews analyzing the main seed traits that facilitate dispersal by ungulates concluded that seeds having hooks or an elongated shape would be likely dispersed by epizoochory, while rounded and small seeds would be dispersed via endozoochory (Albert et al., 2015a,b).

Interestingly, a particular group of ungulates, the ruminants, can disperse large-sized seeds via a "less well-reported form of endozoochory specific to ruminants: large viable seeds are spat out after some time in the rumen" (Feer, 1995). In this case, seeds are released without completing the whole digestion process (i.e., by defecation), but ejected from the regurgitated bolus (the cud) while ruminating. Ruminants using this modality of seed dispersal were named by Forget et al. (2007) "endozoochore spitters" (a type of dispersal similar to the regurgitation of many bird species; Levey, 1987). Here we estimate for the first time the global importance of such a peculiar kind of endozoochory exclusive to ruminants.

Ruminants, i.e., the members of the mammalian Order Artiodactyla that include a rumen, reticulum, omasum (or some part homologous to the omasum) and abomasum in their digestive system, are abundant and frequently large body sized mammals that are able to severely influence ecosystem structure and functioning (e.g., Danell et al., 2006). According to Hackmann and Spain (2010), there are about 200 wild species of ruminants distributed in six families (Tragulidae, Moschidae, Bovidae, Giraffidae, Cervidae, and Antilocapridae), although most of them are Bovidae (140 species) and Cervidae (41). Besides, there are nine species of domestic ruminants (eight Bovidae and one Cervidae). Their estimated population numbers are impressive: about 75.3 million wild ruminants and 3.6 billion domestic ones (Hackmann and Spain, 2010). Importantly, only 10% of these domestic ruminants are raised as industrial livestock (i.e., detached from the land base of feed supply and waste disposal), with the remaining 90% being raised in mixed and grazing land-based systems (http://www.fao.org/ag/againfo/themes/en/meat/backgr_productions.html).

As ruminants are abundant and rather large body sized animals (up to 900 kg; median of extant wild species is 45 kg; Hackmann and Spain 2010), their effects on the diversity and dynamics of plant communities are usually very relevant (e.g., McNaughton et al., 1988), either directly affecting plant demography through grazing, browsing and seed dispersal, or indirectly by modifying the plant environment, such as soil, nutrient flows, and water cycle (Hobbs, 1996). Thus, attaining a comprehensive understanding of the patterns, mechanisms, and consequences of plant-ruminant interactions is an important challenge for ecologists, conservationists and managers (Danell et al., 2006; Foster et al., 2014; Bernes et al., 2018).

As is known, the rumen (or first chamber of the ruminant stomach) delays plant food at the gut for enough time to allow symbiotic microbes to ferment it. The delaying mechanism relies on the orifice between the rumen and the omasum, which limits the size of the food particles that can pass on to successive stomach chambers and intestines (Wenninger and Shipley, 2000). Ruminants ingest many whole fruits with scarce or no mastication. In their forestomach, sized particles are stratified into small and large, and the latter are regurgitated and then remasticated to smaller, easier-to-digest particles (Schwarm et al., 2008). In this way, hard small seeds can pass directly all along the gut and be defecated, relatively soft seeds are crushed or digested, and well-protected large ones must be regurgitated at the cud (one or several times), to be expelled from the mouth or swallowed again and destroyed by rumen microbes (Bodmer, 1991; Sridhara et al., 2016). In addition to the typically large size of regurgitated seeds, this modality of seed dispersal is likely characterized by other parameters, such as retention time, dispersal distance, condition of seed deposition (e.g., level of aggregation), seed survival, and germination success, which must differ from those of seeds dispersed through defecation (Sridhara et al., 2016).

Given the high and increasing pervasiveness and abundance of domestic and wild ruminants worldwide, their capacity to ingest daily a large number of fruits, and the large extent of their movements, their role as potential long-distance seed dispersers deserves particular attention. In this sense, if regurgitating viable seeds while chewing the cud is commonplace, ruminants would be much more important dispersers than previously considered. So far, however, research on this particular kind of endozoochory is largely lacking.

Here we present the results of a review including all available information about, exclusively, dispersal of seeds while chewing the cud (i.e., we do not consider the short-distance dispersal of seeds ejected during fruit mastication) from both wild and domestic ruminant species.

## METHODS

A literature review was carried out to know the state of knowledge about regurgitation and spitting of seeds during rumination, without considering *a priori* if the articles refer specifically to seed dispersal. We consulted reference databases such as Science Citation Index, Science Direct, Scopus, and Google Scholar, among others. Search terms included several combinations of "seeds," "seed dispersal," "endozoochory," "rumination," "spitting," "regurgitation," "ungulates," "ruminants," "livestock," "cattle," "goat," "sheep," "deer," and "antelope." We also searched for articles citing Feer (1995) and Prasad et al. (2006) and examined all references contained in each of the previously selected articles mentioning spitting of seeds during rumination. More than 1000 papers were considered, but only 40 (plus two communications *in litt.)* were useful for this review (**Table 1**). Some of them simply quoted other authors to say that seeds are regurgitated during rumination (e.g., Krefting and Roe, 1949; Corlett, 1998), others alluded to indirect observations of "apparent" seed regurgitation by some species of ruminant (e.g., Jordano, 1987), and finally some others assumed seed spitting, for instance because they found undamaged seeds at the rumen of dead animals but never at the dung (e.g., Slater and du Toit, 2002). Only 25 papers included direct assertions on original observations or experimental evidence of spitting seeds from the cud.

To unify scientific names, we used as reference for plants (**Table S1**) the Plant List (www.theplantlist.org) created by the Royal Botanic Gardens of Kew and the Missouri Botanical Garden, and the list of Mammal Species of the World of (Wilson and Reeder, 2005), third edition (www.departments.bucknell.edu/biology/resources/msw3/) for mammals. Seed mass was obtained from the original papers on seed dispersal, when available, or from the Seed Information Database of the Royal Botanic Gardens, Kew (http://data.kew.org/sid/). For ruminants' body weight and other characteristics we used Bodmer (1990) and the Handbook of the Mammals of the World, vol. 2 (Wilson and Mittermeier, 2011; **Table 2**).

## RESULTS AND DISCUSSION

### A Brief History of Seed Spitting During Rumination

As a matter of fact, the places where domestic ruminants lay down for rumination do appear frequently covered by spat seeds mixed with dung. Then, likely human herders at least since the Neolithic knew that ruminants were able to spit undamaged seeds while ruminating. In the XVIII century, Antonio Sánchez-Valverde, a priest and lawyer in the Hispaniola Island (current Haiti and Dominican Republic), wrote (in Spanish) about *corozo* palm (*Acrocomia aculeata*) fruits: "Bovine livestock, that swallow these globes very barely chewed, digests the flesh and throw up the remains, i.e., the hard nut" (Sánchez-Valverde, 1785, p. 56). According to our knowledge, the first scientific report on this kind of endozoochory corresponds to Troup (1921) in a treatise on silviculture of Indian trees. He described that piles of stones (of *Spondias mangifera,* today *Spondias pinnata*) "are continually met with in place where deer have lain ruminating and bringing them up" (p. 247, vol. 1), and also that the seed of *Acacia arabica* (at present, *Acacia nilotica*) "seldom passes completely through sheep and goats, but is ejected by them from the mouth during rumination" (p. 427, vol.2). Besides, he added that "the fermentation and moistening which the seeds undergo before their ejection undoubtedly assists germination". In his seminal review on seed dispersal, Ridley (1930, p. 372) quoted Troup mentioning briefly that seeds of some fruits consumed by deer in Asia were not defecated, but "disgorged during rumination." The findings of Troup were also mentioned by Krefting and Roe (1949), but later they would be practically forgotten. Wilson and Clarke (1962) reported this type of endozoochory in captive duikers (*Sylvicapra grimmia*) in Africa and Möhring (1963) in a captive roe deer (*Capreolus capreolus*) in Europe. Later, Janzen (1982, 1985) experimentally investigated the passing of seeds of guanacaste (*Enterolobium cyclocarpus*) through cattle guts and of nuts of *Spondias mombin* through white-tailed deer (*Odocoileus virginianus*) guts. Most seeds of guanacaste were defecated by cattle and very few spat from the cud; contrarily, white-tailed deer "regurgitate the nuts (of *Spondias*) while chewing their cud" and these "nuts never pass into the lower digestive tract." Since the middle of the 1980s, a few authors described this overlooked behavior in African ruminants (e.g., Gautier-Hion et al., 1985; Feer, 1995), and at the beginning of the XXI century in Australian sheep and goats (e.g., Tiver et al., 2001) and wild Asian ruminants (e.g., Chen et al., 2001; Prasad et al., 2006; Brodie et al., 2009a). More recently, endozoochorous spitters have received attention in Europe and North Africa (e.g., Grünewald et al., 2010; Delibes et al., 2017; Castañeda et al., 2018).

Some recent reviews on frugivory and seed dispersal in tropical areas have considered this type of endozoochory (e.g., Forget et al., 2007), especially in Asia (Corlett, 2011, 2017; Sridhara et al., 2016). However, other reviews refer only to seed regurgitation by birds, primates and fish (e.g., Parolin et al., 2013), mention ungulates mainly as seed predators (e.g., Stoner et al., 2007) or refer exclusively to plant species identified in dung, overlooking spat seeds (e.g., Miceli-Méndez et al., 2008, which introduced the term "bovinochory" to refer to seed dispersal by cattle in the Neotropics).

### Plant Species Dispersed During Rumination

At least 48 plant species belonging to 21 families are likely dispersed from the cud by domestic and wild ruminants (**Table 1** and **Table S1**). These include some plants spontaneously consumed and some others fed to domestic and captive animals in more or less controlled conditions. Curiously, only two of these species (*Crataegus laevigata* and *Prunus avium*), both European, are considered in the meta-analysis carried out by Albert et al. (2015a,b) to compare, at community level, the traits of seeds dispersed and not dispersed by ungulates, and both are cataloged in their study as "never dispersed in endozoochory, fur-epizoochory and hoof-epizoochory studies taken into account in the analysis." At the same line, Albert et al. (2015a,b) reported the dispersal by ungulates of 278 plant species belonging to 42 families, but only two of these (Fabaceae or *Leguminosae*, and *Rosaceae*) are represented among the 21 families we reported as dispersed through endozoochore spitting. This supports the

**TABLE 1 |** Families of plants whose seeds are spat by different genus of ruminants while chewing their cud.

| Plant family | Plant lifestyle | Fruit type | Ruminant genus | References |
|---|---|---|---|---|
| *Anacardiaceae* | Tree/small tree | Drupe | *Hyemoschus, Muntiacus, Axis, Rusa, Odocoileus, Mazama, Philantomba, Cephalophus* | 3, 4, 6, 7, 8, 13, 14, 15, 16, 21, 24, 39 |
| *Annonaceae* | Tree | Berry | *Muntiacus, Rusa* | 25 |
| *Arecaceae* | Palm/dwarf palm | Drupe | *Cervus, Bos, Capra, Cephalophus* | 5, 12, 13, 19, 34, 35, 41 |
| *Burseraceae* | Tree | Drupe | *Tragulus, Muntiacus, Rusa, Philantomba, Cephalophus* | 7, 13, 15, 23, 42 |
| *Calophyllaceae* | Tree | Drupe | *Cephalophus* | 13 |
| *Combretaceae* | Tree | Drupe | *Tragulus, Axis* | 30, 42 |
| *Elaeocarpaceae* | Tree/small tree | Drupe | *Muntiacus* | 7 |
| *Euphorbiaceae* | Tree/small tree | Drupe/schizocarp | *Moschiola, Muntiacus, Axis, Sylvicapra, Cephalophus* | 7, 9, 13, 30, 31, 32, 40 |
| *Irvingiaceae* | Tree | Drupe | *Cephalophus* | 13, 16 |
| *Lamiaceae* | Shrub/small tree | Drupe | *Cephalophus* | 13 |
| *Leguminosae* | Tree/small tree | Pod | *Cervus, Bos, Capra, Ovis, Sylvicapra, Cephalophus,* | 1, 5, 12, 13, 20, 26, 38, 39 |
| *Linaceae* | Shrub | Drupe | *Philantomba, Cephalophus,* | 13; 16 |
| *Meliaceae* | Tree | Drupe | *Muntiacus* | 7 |
| *Oleaceae* | Tree/small tree | Drupe | *Capra* | 12, 22 |
| *Putranjivaceae* | – | – | *Cephalophus* | 13 |
| *Rhamnaceae* | Shrub/small tree | Drupe | *Axis, Taurotragus, Aepycerus* | 30, 31, 36 |
| *Rosaceae* | Tree/small tree /shrub | Pome/drupe | *Cervus, Capreolus, Capra,* | 5, 12, 18, 27 |
| *Rubiaceae* | – | – | *Philantomba, Cephalophus,* | 13 |
| *Sapotaceae* | Tree | Berry | *Philantomba, Cephalophus, Cervus, Gazella, Capra,* | 11, 12, 13, 16 |
| *Ulmaceae* | Tree | Drupe | *Capra,* | 12, 22 |
| *Vitaceae* | Shrub | Berry | *Capreolus* | 27 |

*The plant lifestyle and the type of fruit are also indicated. The specific names of plants and ruminants can be found in **Table 2** and **Table S1**. Numbers of references correspond to: 1. (Biosecurity Queensland, 2016), 2. (Bodmer, 1991), 3. (Brodie et al., 2009a), 4. (Brodie et al., 2009b), 5. (Castañeda et al., 2018), 6. (Chanthorn and Brockelman, 2008), 7. (Chen et al., 2001), 8. (Corlett, 1998), 9. (Corlett, 2011), 10. (Corlett, 2017), 11. Cuzin in litt., 12. (Delibes et al., 2017), 13. (Feer, 1995), 14. (Feer et al., 2001), 15. (Forget et al., 2007), 16. (Gautier-Hion et al., 1985), 17. (Gill and Beardall, 2001), 18. (Grünewald et al., 2010), 19. Hiraldo, in litt., 20. (Janzen, 1982), 21. (Janzen, 1985), 22. (Jordano, 1987), 23. (Kitamura et al., 2006), 24. (Mandujano et al., 1994), 25. (McConkey et al., 2018), 26. (Miller, 1995), 27. (Möhring, 1963), 28. (Myers et al., 2004), 29. (Pile et al., 2015), 30. (Prasad et al., 2004). 31. (Prasad et al., 2006), 32. (Prasad et al., 2010), 33. (Ridley, 1930), 34. (Sánchez-Valverde, 1785), 35. (Scariot, 1998), 36. (Slater and du Toit, 2002), 37. (Sridhara et al., 2016), 38. (Tiver et al., 2001), 39. (Troup, 1921), 40. (Wilson and Clarke, 1962), 41. (Yamashita, 1997), 42. (Yasuda et al., 2005).*

idea that plants dispersed by ruminants while chewing their cud are a different set of species than those usually dispersed by conventional endozoochory (i.e., defecated seeds). The contrast is evident when comparing traits of plants whose seeds are dispersed in ungulate dung and those of plants dispersed while ruminating. For instance, through the review of 52 studies, Albert et al. (2015a) concluded that plants dispersed by ungulates are mainly grasses typical of open habitats (93%), while most (but not all) species dispersed from the cud are forest trees (**Figure 1A** and **Table S1**). Also, most (85.2%) of the fruit consumed by wild ruminants in Asia, according to the review carried out by Sridhara et al. (2016), corresponded to trees.

## Fruit and Seed Characteristics of Plants Dispersed While Ruminating

Most plants dispersed by ungulates inside the dung respond to the "foliage is the fruit" hypothesis enunciated by Janzen (1984), i.e., they are grasses with small seeds which are ingested inadvertently while eating the foliage. In the case of plants dispersed by ruminants via regurgitation, the reward is the fruit pulp, as for other vertebrate seed dispersers (e.g., birds, carnivores). So, fruits must be attractive to ruminants and usually they include nutritious pulp or pod (**Table 1** and **Figure 1B**).

According to Prasad et al. (2006), fruit traits of species dispersed while ruminating "appear to converge toward being green or brown, drupaceous, with fibrous pulp and strong seed protection." Sridhara et al. (2016) found more variation in the fruits consumed by wild large ruminants in Asia, 36.7% being yellow and most of them drupes (50.3%) and berries (27.2%). In contrast, seeds dispersed through defecation usually correspond to fruit without pulp (**Figure 1B**).

Besides, dispersal units (i.e., individual seeds or cocci including a small number of them) must be large enough to avoid being passed out of the ruminant forestomach. The contrast between the sizes of the propagules dispersed by ruminants through defecation and those spat from the cud is very evident (**Figure 1C**). In spite of these differences, some plant species can be dispersed through both types of endozoochory by the same or different ruminant species. For example, cattle defecated most seeds of *Enterolobium cyclocarpum*, but spat from the cud a small portion of them (Janzen, 1982). Contrarily, deer spat most *Chamaerops humilis* seeds, but defecated a small percentage (Castañeda et al., 2018). In general, large ruminants defecate

**TABLE 2 |** Species of ruminants reported as spitters of seeds while ruminating, and some taxonomic, biological, and ecological information about them (from Bodmer, 1990, complemented with Wilson and Mittermeier, 2011).

| Ruminant species | Family | Weight Kg | Habitat | Feeding strategy | Zoogeographical range | References to spitting |
|---|---|---|---|---|---|---|
| *Moschiola indica* | Tragulidae | 3 | Forest | Frugivore | Indo-Malayan | 32 |
| *Tragulus javanicus* | Tragulidae | 1–2 | Forest | Frugivore | Indo-Malayan | 23, 42 |
| *Hyemoschus aquaticus* | Tragulidae | 8–13 | Forest | Frugivore | Afrotropical | 16 |
| *Muntiacus muntjak* | Cervidae | 15 | Forest | Frugivore | Indo-Malayan | 3, 4, 6, 7, 10, 15, 23, 25, 30 |
| *Axis axis* | Cervidae | 45–85 | Forest/grassland | Browser/Grazer | Indo-Malayan | 30, 31, 39 |
| *Rusa unicolor* | Cervidae | 130–260 | Forest/grassland | Browser/Grazer | Indo-Malayan | 3, 4, 6, 15, 23, 25 |
| *Cervus elaphus* | Cervidae | 160–260 | Forest/grassland | Browser/Grazer | Palaearctic | 5, 22 |
| *Capreolus capreolus* | Cervidae | 17–23 | Forest | Browser | Palaearctic | 18, 27 |
| *Odocoileus virginianus* | Cervidae | 30 | Forest/grassland | Browser | Nearctic/Neotropical | 21, 24, 28, 29 |
| *Mazama* spp. | Cervidae | 15–35 | Forest | Frugivore | Neotropical | 2, 14, 15 |
| *Bos taurus* | Bovidae | 900 | Grassland | Grazer | (domestic) | 19, 20, 34, 35, 41 |
| *Taurotragus oryx* | Bovidae | 300–900 | Savanna | Browser | Afrotropical | 36 |
| *Aepycerus melampus* | Bovidae | 45–80 | Savanna | Browser/Grazer | Afrotropical | 36 |
| *Gazella cuvieri* | Bovidae | 30–82 | Savanna | Browser/Grazer | Palaearctic | 11 |
| *Capra hircus* | Bovidae | 40–100 | Grassland/altiplan | Browser | (domestic) | 1, 12, 22, 39 |
| *Ovis aries* | Bovidae | 40–140 | Grassland/altiplan | Browser/Grazer | (domestic) | 1, 26, 38, 39 |
| *Sylvicapra grimmia* | Bovidae | 10–18 | Savanna | Browser/Frugivore | Afrotropical | 26, 40 |
| *Philantomba monticola* | Bovidae | 5 | Forest | Frugivore | Afrotropical | 13 |
| *Cephalophus dorsalis* | Bovidae | 20 | Forest | Frugivore | Afrotropical | 13 |
| *Cephalophus silvicultor* | Bovidae | 60 | Forest | Frugivore | Afrotropical | 13 |
| *Cephalophus callipygus* | Bovidae | 20 | Forest | Frugivore | Afrotropical | 13 |
| *Cephalophus* spp. | Bovidae | 4–60 | Forest | Frugivore | Afrotropical | 13, 16 |

*Numbers of references are the same as in* ***Table 1****.*

some seeds that are usually spat by small ruminants (e.g., Slater and du Toit, 2002; see below).

Frequently large seeds are held in large fruits, hence the plants dispersed from the cud often have rather large fruits (e.g., 50 mm diameter in *Acrocomia aculeata*). However, as expected, the size of regurgitated seeds tends to increase with the size of the ruminant consumer (Gautier-Hion et al., 1985). That is evident also in our sample (**Figure 2**) and contrasts markedly with the conclusion of the global analysis of Chen and Moles (2015) wherein ungulates showed a negative relationship between body mass and ingested seed size. Indeed, the fact that Chen and Moles (2015) did not include any information on seeds dispersed through regurgitation biased their results.

To some extent the relative sizes of seeds and ruminants will determine whether a hard seed is handled without swallowing or swallowed, and in the second case if it is later spat during rumination, defecated, or completely digested. For example, while cattle (900 kg) pass through the gut most *Acacia nilotica* seeds, the smaller sheep (around 50 kg) reject many of them during pod ingestion and mastication, spit some others during rumination, and scarcely 1% are delivered in the dung (Tiver et al., 2001).

This suggests strong context-dependency of these fruit-frugivore interactions, as seed fate (dispersal mechanism/predation) would vary with partner identities (Perea et al., 2014b). Nevertheless, relatively small seed size does not guarantee that seeds will be defecated. For instance, captive red deer (*Cervus elaphus*) spat from the cud 19% and defecated 25% of the ingested seeds of *Ceratonia siliqua* (seed weight = 0.18 g; Castañeda et al., 2018). Future studies should add information about the relationship between ruminant body size and spat seed size and the extent to which this relationship shapes seed fate.

Some other fruit characteristics of plants dispersed while ruminating seem to be shared with those of plants dispersed via endozoochory by mammalian carnivores (e.g., Herrera, 1989), such as having scented fruits that fall to ground when ripe, "possibly to attract terrestrial dispersers" (Brodie et al., 2009a). In addition, some of the fruits eaten by ruminants are also edible by people and have economic value, such as *Choerospondias axillaris* (Chen et al., 2001) or *Phyllanthus emblica* (Prasad et al., 2004).

Interestingly, some traits of fruits dispersed from the cud by ruminants match those of the so-called megafaunal fruits, i.e., those dispersed during the Pleistocene by now-extinct megafauna (Janzen and Martin, 1982). In particular, "ruminant fruits" resemble the type 1 megafaunal fruits described by Guimarães et al. (2008): usually brown or green large-sized fleshy fruits with either a single or few large and hard seeds. Janzen and Martin (1982) mention 14 plant families of Costa Rica, which include species "probably dispersed by extinct megafauna;" seven of these families (e.g., Anacardiaceae, Arecaeae, Sapotaceae) are quoted in our list of plants dispersed through spitting by ruminants (**Table 1**), and only one (*Leguminosae*) does appear in the list of families dispersed by ungulates in the dung according to

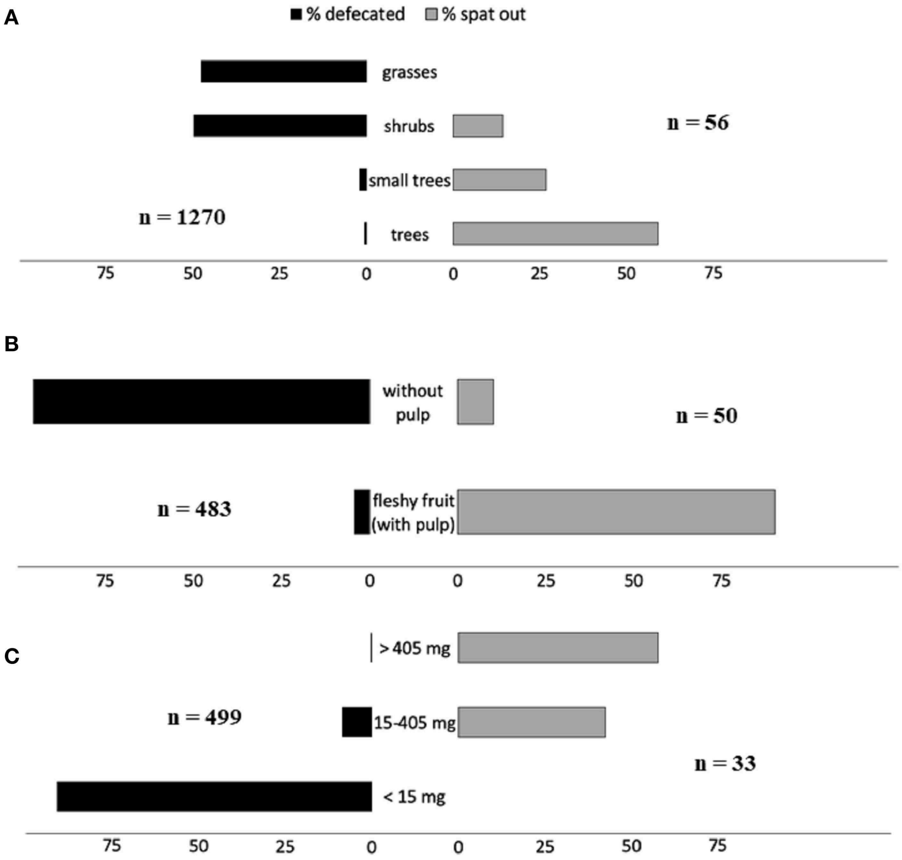

**FIGURE 1 |** Distribution of frequencies (percentages) of **(A)** type of plants, **(B)** type of fruits, and **(C)** propagule weights, whose seeds are dispersed by ruminant defecation (black bars; data in Albert et al. 2015b, Appendix 5) and spitting from the cud (gray bars; data in this review). Plants under 0.4 m in Albert et al. (2015b) were associated with grasses, from 0.4 to 2 m to shrubs, from 2 to 5 m to small trees and above 5 m to trees. Some species were included in several categories (e.g., small trees and trees).

Albert et al. (2015a,b). Frequently, studies of current Neotropical communities consider only the tapir (*Tapirus terrestris*) and exotic livestock (including feral pigs) to be capable of dispersing megafaunal fruits by endozoochory (e.g., Donatti et al., 2007), but the potential role of wild ruminants such as *Mazama* spp. and *Odocoileus virginianus* should not be ignored anymore (Janzen, 1985; Mandujano et al., 1994).

## Disperser Ruminants

At least 21 species included in 18 genera of ruminants have been reported as endozoochorous spitters (**Table 2**). They include domestic ($n = 3$) and wild ($n = 18$) species, belonging to the families Tragulidae (three species, but the taxonomy of *Tragulus* is not clear; Wilson and Reeder, 2005), Cervidae ($n = 7$) and Bovidae ($n = 11$). They range in body size from 1 to 2 kg for the small mousedeer (*Tragulus* spp.) of Southeast Asia to near 900 kg for some domestic cows (*Bos taurus*). According to Bodmer (1990), ungulates could be distributed along a linear continuum ranging from fruit feeders to browsers and then grazers, with the ruminants included mainly in the browsers and grazers categories. Assuming the classification proposed by Bodmer (1990), 5–7 of the genera we identified as spitters of seeds (**Table 2**) would belong to the frugivores (*Mazama*, *Tragulus*, probably including *Moschiola*, *Muntiacus*, *Hyemoschus,* and *Cephalophus*, including *Philantomba*), one would be browser/frugivore (*Sylvicapra*), four browsers (*Odocoileus*, *Taurotragus*, *Capreolus* and *Capra*), 4–6 browser/grazers (*Cervus*, probably including *Axis* and *Rusa*, *Aepyceros*, *Gazella,* and *Ovis*) and 1 grazer (*Bos*). It seems that almost any ruminant species could consume fruits and eventually regurgitate their seeds during rumination. Thus, future studies should identify ruminant and fruit traits that play a major role in this mutualistic interaction.

It must be emphasized that seed dispersal while chewing the cud does appear in the Tragulidae, the most primitive family of extant ruminants, whose members lack a true omasum and have been considered "living fossils" (Hackmann and Spain, 2010). That means that this particular type of endozoochory is a rather ancestral behavior in the group.

## Geographical Area and Habitat

Spitting seeds from the cud seems to be a universal seed dispersal mechanism among ruminants, as we recorded cases in all continents except Antarctica (**Table S1**). In particular,

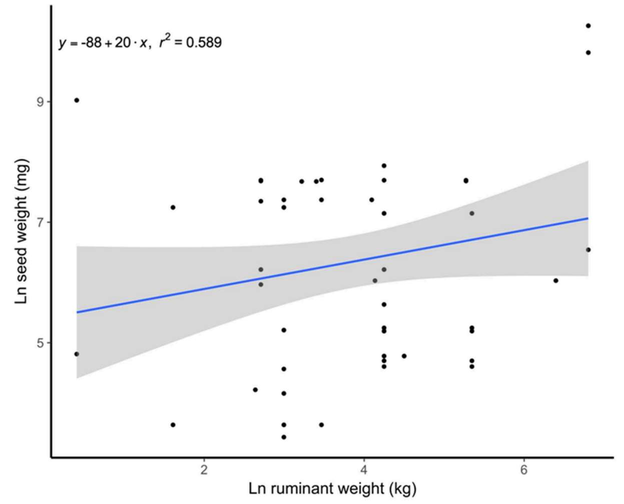

**FIGURE 2 |** Positive relationship between the weight of spat seeds and the size of the spitter ruminants. Data from **Table S1**.

we found 10 plant species dispersed in Europe, 12 in Asia, 21 in Africa, nine in America and one in Oceania (some species were dispersed in more than one continent). Many plant species are from tropical forests, where a higher diversity of frugivorous ungulates exist (Bodmer, 1990), suggesting their feeding habits make them prone to regurgitate seeds (**Table 2**). In all, we found 40 dispersed plant species from tropical areas (forests and wooded savannas) and 13 species from non-tropical areas (Mediterranean scrubland, temperate forests, and subdeserts).

Apart from the number of involved plant species, it is very difficult to speculate about the relevance of this modality of endozoochory in different continents or ecosystems. A priori, tropical forests should be favored because of the high number of frugivores living there, but it can be suspected, for instance, that African ungulates could disperse during rumination many seeds of dry and fleshy fruits (e.g., *Leguminosae*, *Arecaceae*). Additionally, it must be considered that a given plant species is often dispersed by contrasting types of seed dispersers. For instance, some seeds regurgitated by ruminants can be dispersed also through defecation by other mammals, such as mammalian carnivores (Order Carnivora). So, in Europe *Chamaerops humilis* can be regurgitated by goats and deer (**Table S1**), but also defecated by foxes (*Vulpes vulpes*) and badgers (*Meles meles*) (Fedriani and Delibes, 2011); also, roe deer does spit out while ruminating viable seeds of *Prunus avium*, while foxes and badgers defecate them (Grünewald et al., 2010). In the same way, frugivores with different dispersal abilities (including several ruminants) can compete for the same fruit. The relative importance of seed dispersal of any plant species by ruminants and other mammals (or large birds) should be analyzed with more attention, emphasizing the context-dependence of fruit-animal interactions affecting seed fate.

### Quantity of Seed Dispersal

Seed dispersal effectiveness has a quantitative (number of seeds dispersed) and a qualitative (probability that a dispersed seed produces a new adult) component (Schupp et al., 2010). For endozoochorous plants, the number of dispersed seeds largely depends of the amount of ingested fruit. Because ruminants cannot take most fruit directly from the trees, they often rely on either primates (or other arboreal vertebrates) or natural falling of the fruit onto the ground to consume them (see Prasad and Sukumar, 2010, for a discussion of the topic). Despite these limitations, they can ingest large quantities of fruit when available (e.g., Johnsingh, 1981; Bodmer, 1991; Brodie et al., 2009a). For instance, two species of ruminants accounted for over 95% of the total *Phyllanthus emblica* fruits removed by frugivores in India (Prasad et al., 2010).

However, in the case of ruminants, ingestion does not guarantee that seeds will be dispersed, because a particular seed can be ejected while foraging, spat from the cud, defecated or digested, as previously said (Castañeda et al., 2018). The fate relies on many factors, not only the relative size of seeds and consumers (Forget et al., 2007), but also on consumer satiation, foraging speed, and availability of alternative foods, among others. In addition, direct observation of seed regurgitation by ruminants is very difficult, even in captivity (Prasad et al., 2006; McConkey et al., 2018). Because of this, data about the individual rate of seed spitting often come from captive or semi-captive individuals (e.g., Möhring, 1963). Consequently, quantitative information about seed dispersal while ruminating is scarce.

The percentages of ingested seeds of different plants that were spat from the cud by several domestic and captive wild ruminants are summarized in **Table 3**. They are quite variable (ranging from 2 to 100%). Even if the per capita percentages of spat seeds are generally low, the high abundance of wild and domestic ruminants and the potential high number of consumed fruits per individual suggest that the quantity of dispersed seeds by this type of endozoochory is important. Thus, when overlooking spitting by ruminants, a relevant fraction (quantitative and qualitative) of seed dispersal by these animals is likely missed by researchers. Besides, several authors (e.g., Möhring, 1963; Janzen, 1985; Prasad et al., 2006) emphasized that the seeds of some species spat from the cud were never detected at the dung (see **Figure 1**).

## Quality of Seed Dispersal

### Rumen Retention Time and Dispersal Distance

Usually, ruminants ingest fruits with limited or no mastication and eject clean seeds some time later while bedding and ruminating. Observations on the distance between the foraging and the ruminating points are logistically very difficult. Then, available direct information on dispersal distance is reduced, but several authors reported that seeds were spat far from the mother plant. Chen et al. (2001) stated that “the whole fruit (of *Choerospondias axillaris*) is eaten (by *Muntiacus muntjak*); then, the stone is regurgitated at a different location.” In a small plot of central Thailand, *Rusa unicolor* and *Muntiacus muntjak* dispersed while ruminating 83–98% (2 years) of the seeds of *Choerospondias axillaris* to distances up to 70 m from the nearest fruiting tree (Chanthorn and Brockelman, 2008), but the authors

**TABLE 3 |** Percentages of the ingested seeds of several plant species that were spat from the cud by domestic and wild captive ruminant species.

| Ruminant species | Plant species | % Spat seeds | References |
|---|---|---|---|
| *Axis axis* | *Phyllanthus emblica* | 78 | Prasad et al., 2006 |
| *Rusa unicolor* | *Platymitra macrocarpa* | >10 | McConkey et al., 2018 |
| *Cervus elaphus* | *Chamaerops humilis* | 7 | Castañeda et al., 2018 |
| *Cervus elaphus* | *Crataegus monogyna* | 2 | Castañeda et al., 2018 |
| *Cervus elaphus* | *Celtis australis* | 5 | Castañeda et al., 2018 |
| *Cervus elaphus* | *Ceratonia siliqua* | 19 | Castañeda et al., 2018 |
| *Capreolus capreolus* | *Prunus avium* | 70–89 | Grünewald et al., 2010 |
| *Odocoileus virginianus* | *Spondias mombin* | up to 100% | Janzen, 1985 |
| *Bos taurus* | *Enterolobium cyclocarpum* | 3 | Janzen, 1982 |
| *Capra hircus* | *Chamaerops humilis* | 30–45 | Delibes et al., 2017 |
| *Capra hircus* | *Olea europea var. domestica* | 30–45 | Delibes et al., 2017 |
| *Capra hircus* | *Celtis australis* | <10 | Delibes et al., 2017 |
| *Capra hircus* | *Crataegus monogyna* | <10 | Delibes et al., 2017 |
| *Capra hircus* | *Ceratonia siliqua* | <10 | Delibes et al., 2017 |
| *Capra hircus* | *Olea europea var. sylvestris* | <10 | Delibes et al., 2017 |
| *Ovis aries* | *Acacia nilotica* | 14 | Tiver et al., 2001 |

considered that "deer are capable of longer distance dispersal than was recorded in this study." According to Feer (1995), dispersal by duikers (Cephalophinae) in Gabon is characterized by "scattered deposition sites and long distance." Gautier-Hion et al. (1985) wrote that seed spitting during rumination "always occurs away from the fruit source." In peninsular Malaysia, hard and large seeds of *Canarium littorale* and *Terminalia citrina* "are likely to be regurgitated (by *Tragulus javanicus*) from the mouth in rumination and dispersed at a distance of the mother tree" (Yasuda et al., 2005). In southern Spain, we have found many clean seeds of *Chamaerops humilis* at sheep and goat pens, very far (i.e., kilometers) from the places where the fruits were likely consumed, suggesting long retention times, and dispersal distances.

Alternatively, seed retention time at the rumen can provide a surrogate of dispersal distance, assuming a positive relationship between elapsed time and distance traveled. Feeding and ruminating rhythm of ungulates (hence, also retention time) were influenced by body size and feeding type, typically grazers having longer lapses than browsers and concentrate selectors (i.e., those with a mixed diet, tending to avoid fiber; Hofmann, 1989). Additionally, retention time can be affected by particle size, or in this case seed size (e.g., Clauss et al., 2009). However, most of these studies refer to retention time before defecation (e.g., Picard et al., 2015), while references to retention time at the rumen are scarce. Estimations about the time elapsed from fruit ingestion to seed regurgitation from the cud go from scarcely 35 min for a young roe deer eating cherries to ten days for a red deer eating hawthorn pomes, but typically range between 3 h and 2 days (**Table 4**). This rather long seed retention time has a strong potential to facilitate long-distance seed dispersal. As an indication, with average retention times of several hours, Couvreur et al. (2005) estimated that at least half of the epizoochorous seeds attached to the fur of some horses and cattle were released from 47 to 3,080 m from the source site. For defecated seeds and passage times of 48 h, maximal dispersal distances of 3.5 km by red deer and 2.0 km by roe deer were estimated by Pellerin et al. (2016). Obviously, higher distances should be expected for domestic ruminants driven hundreds of kilometers (transhumance; e.g., Manzano and Malo, 2006) and for migratory wild ruminants (e.g., Berger, 2004).

**TABLE 4 |** Elapsed time between fruit ingestion and seed release from the cud (i.e., seed retention time at the rumen) according different authors.

| Ruminant species | Plant species | Rumen retention time | References |
|---|---|---|---|
| Tragulidae | Undetermined | Many hours | Corlett, 2017 |
| *Muntiacus muntjak* | *Choerospondias axillaris* | Several h | Chen et al., 2001 |
| *Axis axis* | *Phyllanthus emblica* | 7–27 h | Prasad et al., 2006 |
| *Rusa unicolor* | *Platymitra macrocarpa* | 6–11 h | McConkey et al., 2018 |
| *Cervus elaphus* | Undetermined | 1–4 days | Castañeda et al., 2018 |
| *Cervus elaphus* | *Chamaerops humilis* | up to 8 days | Castañeda et al., 2018 |
| *Cervus elaphus* | *Ceratonia siliqua* | up to 9 days | Castañeda et al., 2018 |
| *Cervus elaphus* | *Crataegus monogyna* | up to 10 days | Castañeda et al., 2018 |
| *Capreolus capreolus* | *Prunus avium* | 0.6–1.8 h | Möhring, 1963 |
| *Capreolus capreolus* | *Prunus avium* | 1–3 h | Grünewald et al., 2010 |
| *Capra hircus* | Undetermined | hours to days | Delibes et al., 2017 |

*Most ruminants used in the trials were tame or captive individuals.*

## Deposition Patterns and Seed Fate

There is very scarce information about the destination microhabitat of spat seeds. Domestic species that forage at day often ruminate at night in pens or farmyards (e.g., Troup, 1921; Yamashita, 1997), where there is little or no probability of seed germination. Brodie et al. (2009a) conclude that sambar deer regurgitated higher numbers of seeds of *Choerospondias axillaris*, but muntjak was the only disperser moving them to open microhabitats, where germination was enhanced. Feer (1995) suggested that nocturnal duikers (Cephalophinae) choose to ruminate (and to deposit regurgitated seeds) places favorable for plant species needing improvements in light conditions for their establishment.

Typically, regurgitated seeds are clumped. Different authors refer to "small piles" or "loose clusters" of clean seeds (e.g., Corlett, 2011), but others mention dense clusters and "very shiny piles" (Brodie et al., 2009a). Piles of five seeds of *Platymitra macrocarpa* regurgitated by sambar deer were found by McConkey et al. (2018) in Thailand. The same deer species, also in Thailand, deposited seeds of *Choerospondias axillaris* in piles containing "between 14 and 140 seeds, with the exception of one pile which was found to contain 750 seeds;" in the same area muntjak "tended to deposit seeds in smaller piles (of usually <100)" (Chanthorn and Brockelman, 2008). At chital deer bedding sites, clusters of 4–193 (median = 15, $n$ = 23) cocci of *Phyllanthus emblica* can be found, along with cocci of other plant species (Prasad et al., 2004). Seeds of *Acrocomia aculeata* fruit consumed by cattle during the day are regurgitated at night in piles of up to 85 seeds (Scariot, 1998). In Morocco, we found at field piles of 15–30 clean seeds of *Chamaerops humilis* where goats were ruminating. White-tailed deer make piles of 15–62 seeds of *Spondias purpurea*, but as a consequence of deer sociality, large numbers of seeds can be concentrated in a few square meters (Mandujano et al., 1994). Similarly, Janzen (1985) reported the following: "A portion of a 2–4-nut-deep layer of *Spondias mombin* nuts on the forest floor beneath a parent tree; this accumulation was produced by fruit drop and regurgitation by white-tailed deer 8 months previously." These observations introduce a new and often neglected factor to consider in a qualitative assessment of seed dispersal: social species of ruminants (e.g., sheep) and species or individuals using recurrent rumination sites will accumulate dispersed seeds, reducing the quality of dispersal.

As expected, groups of clean seeds can attract different seed-eaters, so post-dispersal predation (and likely secondary dispersal, e.g., by rodents; Vander Wall et al., 2005; Jansen et al., 2012) is usually high among endozoochorous spat plants. Brodie et al. (2009a) found that 30-40% of seeds of *Choerospondias axillaris* were removed from their primary local deposition, but seed pile size did not influence germination or first year seedling survivorship. More than 80% of seeds of *Chrysophillum beguei* regurgitated by duikers in Gabon were eaten or removed by rodents in the next 60 days (Feer, 1995). Most of the dispersed seeds of *Spondias mombin* in Costa Rica were killed by bruchid beetles (Janzen, 1985). In Thailand, bruchids attack the dispersed seeds of *Platymitra macrocarpa* in 6–22 days (McConkey et al., 2018). In Brazil, >50% of seeds of the palm *Acrocomia aculeata* chewed by cattle were infested by bruchids, and this proportion increased to 99% after 27 days; regurgitated seeds had a significant higher rate of insect predation than seeds of non-chewed fruit (Scariot, 1998). Additionally, piles of seeds of the palms *Acrocomia aculeata*, *Attalea phalerata,* and *Syagrus coronata* regurgitated by cattle were regularly visited by macaws (*Anodorhynchus leari* and *A. hyacinthinus*) to feed on them, cracking the nuts (Yamashita, 1997). This last author hypothesizes that macaws could track the movements of the extinct Pleistocene megaherbivores in order to collect the large seeds they dispersed.

### Germinability of Seeds Spat From the Cud

Different studies have stated that seeds were intact and in a great proportion alive after being regurgitated from the rumen (e.g., Delibes et al., 2017). Some others indicated that spat seeds germinated under field conditions (e.g., Feer, 1995; Castañeda et al., 2018; McConkey et al., 2018). In his pioneer description of seed dispersal while chewing the cud, Troup (1921) indicated that retention at the rumen improved germination of *Acacia nilotica* from 7 to 35%, concluding: "The superiority of seed collected from goat and sheep pens is generally recognized and seed so collected is extensively used for artificial sowing." Also, it was said that treatment by goats was necessary for the germination of *Argania spinosa* seeds (Morton and Voss, 1987). Seeds of *Spondias purpurea* spat by deer germinated better than those defecated by the iguana *Ctenosaura pectinata* (Mandujano et al., 1994). In other cases, treatment seemed to be unimportant, as it occurs with the seeds of *Choerospondias axillaris* that "germinate equally well whether they are defecated by gibbons, regurgitated by deer, or the fruits are uneaten" (Brodie et al., 2009a). Finally, other authors found that rumen retention influences negatively the germination; unconsumed seeds of *Phyllanthus emblica* germinated more (72%) than pulp-removed seeds (58%) and deer-regurgitated seeds (22%); latency period, however, was shorter for deer-regurgitated and depulped seeds than for those of unconsumed fruit (Prasad et al., 2006).

We can speculate that retention time at the rumen will affect the germination of spat seeds, either improving (by scarification) or decreasing (by damaging the embryos) it. Thus, future studies should quantify these effects.

## CONCLUSIONS

Many species of ruminants are potential dispersers of numerous species and families of plants by spitting their seeds while chewing the cud (**Tables 1**, **2** and **Table S1**). Until now, this behavior had been described mainly in tropical habitats, but it has also been found in other ecosystems in all continents except Antarctica. Given the abundance of wild and free-living domestic ruminants and their high rate of food consumption, it can be suspected they are able to mobilize great numbers of seeds during rumination. Recent reviews on frugivory and seed dispersal by vertebrates in tropical Asia have recognized the importance of this type of endozoochory (Sridhara et al., 2016; e.g., Corlett, 2017), but in general it has been overlooked in many other reviews, even devoted to tropical areas (e.g., Parolin et al., 2013) or specifically to ungulates (e.g., Miceli-Méndez et al., 2008; Albert et al., 2015a). The difficulty in monitoring rumination, a process that usually occurs in sheltered and quiet places, may explain why this modality of endozoochory has been overlooked so far. In fact, a significant part of the data that we reviewed came from captive animals (**Table S1**). Besides, methods to study seed rain or deposition patterns are biased toward avian or arboreal dispersers (e.g., seed traps) or rely on fecal surveys, being unable to capture seeds spat from the cud by ruminants.

By neglecting the seeds spat from the cud, the quantity and quality of seed dispersal by ruminants could have been severely underestimated until the present. The dispersal of some plant species can persist undetected because they are exclusively spat while ruminating. In other cases, underestimation may result in considering only seeds contained in feces, ignoring that some others of the same species are being spat from the cud. For instance, Miller (1996) investigated the dispersal of seeds of *Acacia tortilis* and *A. nilotica* in the dung of South African ungulates: at least five species of ruminants consumed *Acacia* pods in her study parcel, but we can speculate that probably some of them will spit seeds during rumination, as sheep and goats in Australia do (Tiver et al., 2001). In the same way, by overlooking endozoochorous spitting, the dispersal by ruminants of large-sized seeds of fleshy-fruited plants, frequently shrub and trees, will be missed. This is the case of many comprehensive reviews and meta-analysis of seed dispersal by ungulates that consider exclusively seeds released inside the dung (e.g., Mouissie et al., 2005; Albert et al., 2015a,b) and conclude that tiny seeds of grasses are favored. As reported decades ago by Janzen (1985), some large seeds are solely ejected during rumination, never passing "into the lower digestive tract," and are thus absent in the feces. As previously said, by ignoring the seeds dispersed from the cud, some studies about the size of seeds dispersed by ungulates arrive at wrong conclusions (e.g., Chen and Moles, 2015).

Another potential bias resulting from ignoring the spitting of seeds from the cud appear when ruminants in experimental cages are fed with different fruits, and the manure collected to investigate the proportion of released seeds and their potential germination (e.g., Grande et al., 2013). Already, Troup (1921) signaled that "the seeds are, it is true, found among their droppings (from sheep and goats), but this is because of the fact that rumination ordinarily takes place where the animals are herded." This means that the manure of captive ruminants usually includes spat and defecated seeds, which should be considered in a different way in any analysis.

Indeed, the ability of ruminants to disperse large-sized seeds of fleshy and dry fruits must be kept in mind in multi-species mutualistic studies (e.g., Bascompte and Jordano, 2007), instead of treating them exclusively as plant antagonists. However, our review shows that the interaction of ruminants with seeds is very complex. On the one hand, seeds of the same species can be dispersed at short distance while eating, dispersed at long distance while ruminating, dispersed at long distance via defecation, or totally digested (i.e., predated). The proportions of seeds in each of these categories will depend on the relative sizes of both seeds and consumers (Gautier-Hion et al., 1985), but also on other plant- and animal-related factors, such as plant chemical and physical defenses, rate of ingestion, satiation and physical condition. On the other hand, the access of particular ruminants to fruits will be severely influenced by the spatiotemporal context (e.g., plant aggregation, crop size, alternative food availability, numbers of competitors, and predators; Prasad and Sukumar, 2010; Perea et al., 2014b).

Finally, high levels of post-dispersal predation on clumped spat seeds could reverse the sign of the plant-ruminant interaction from mutualistic to antagonistic. Thus, future studies should address the complexities of this fascinating type of plant-animal interaction and quantify the variable fate of seeds ingested by ruminants in different ecosystems.

## AUTHOR CONTRIBUTIONS

MD conceived the original idea and wrote the first draft. MD and IC did the literature review. MD, IC, and JF reviewed and edited the manuscript several times.

## FUNDING

JF was funded by the Portuguese FCT (grant IF/00728/2013).

## ACKNOWLEDGMENTS

Many herders in Morocco and Spain helped us to obtain information from their animals. F. Cuzin and F. Hiraldo offered us unpublished data on gazelles and cattle spitting seeds from the cud. G. Calvo and M. Jácome helped us in fieldwork and S. Conradi in the laboratory work. S. Sridhara and C. Baltzinger significantly improved a previous version of the manuscript.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2019.00265/full#supplementary-material

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Ecology and Evolution

ORIGINAL RESEARCH
published: 29 August 2019
doi: 10.3389/fevo.2019.00328

# Multiple External Seed Dispersers Challenge the Megafaunal Syndrome Anachronism and the Surrogate Ecological Function of Livestock

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The dispersal of many large-seeded plants is thought to have been handicapped by the extinction of megafauna in the late Pleistocene, and due to the ongoing defaunation of the largest of the extant dispersers. Oversized fruits defined as "megafaunal" provide variable amounts of flesh even though many of them cannot be ingested entirely, nor their seeds defecated, by any extant vertebrate. This apparent mismatch lead to the hypothesis of anachronisms involving extinct megafauna as dispersal-mediated selective agents on fruit traits shaped through endozoochory. It has been suggested that free-ranging livestock partially supply the dispersal functions previously provided by those globally or regionally extinct species. However, there is little knowledge on the role of livestock as a surrogate for megafauna dispersal agents relative to living wild dispersers. Here, we focus on seed dispersal of six palm species (*Attalea eichleri*, *Attalea barreirensis*, *Attalea speciosa*, *Attalea princeps*, *Mauritia flexuosa*, *Acrocomia totai*) with large fruits that conform to the so-called "megafaunal syndrome". Data on seed dispersal were obtained by observations and camera trapping in the Cerrado, Pantanal and Amazonia biomes in Bolivia and Brazil. Rich communities of wild seed dispersers differing among palm species and study areas were recorded, including rodents, monkeys, canids, and a wide variety of birds, especially parrots. Long-distance primary dispersal was mainly conducted by parrots, while multiple species acted as short- and medium-distance secondary dispersers. Among livestock, dispersal was limited to seeds of *A. totai* and *A. princeps* moved by several species through stomatochory and endozoochory (mainly regurgitation). These results show that the large seeds can be efficiently dispersed externally by a wide array of present-day vertebrates of variable size but much smaller than extinct megafauna and livestock. A knowledge gap of the natural history of these and other plants with oversized fruits assumed to be maladapted for contemporary dispersal may have been partially favored by neglecting some key disperser guilds (e.g., parrots) and dispersal mechanisms (e.g., ectozoochory). The evaluation of historic and ongoing defaunation of key external dispersers is advocated to understand the influence of actual (rather than putative) dispersers on contemporary frugivore-plant mutualistic interactions.

**Keywords: disperser assemblages, ectozoochory, extinct megafauna, livestock, oversized fruits, parrots, palms, tapir**

OPEN ACCESS

***Edited by:***
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***Reviewed by:***
*Jo Carpenter,*
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***Specialty section:***
*This article was submitted to*
*Biogeography and Macroecology,*
*a section of the journal*
*Frontiers in Ecology and Evolution*

***Received:*** *04 January 2019*
***Accepted:*** *16 August 2019*
***Published:*** *29 August 2019*

***Citation:***
*Blanco G, Tella JL, Díaz-Luque JA and Hiraldo F (2019) Multiple External Seed Dispersers Challenge the Megafaunal Syndrome Anachronism and the Surrogate Ecological Function of Livestock. Front. Ecol. Evol. 7:328. doi: 10.3389/fevo.2019.00328*

# INTRODUCTION

Trade-offs between fruit size and seed dispersal ability have been repeatedly highlighted as governing plant-frugivore mutualistic interactions (Wheelwright, 1985; Lord, 2004, Bruun and Poschlod, 2006; Burns, 2013). A number of extant plants show fruit size, phenological patterns and other traits that have been argued to better reflect adaptations to past than present-day ecological conditions (Barlow, 2000). These "anachronisms" are generally suggested to explain traits that are unexpected or not understood based on their interactions with extant assemblages of fruit-eating vertebrates (Janzen and Martin, 1982; Barlow, 2000). In particular, the megafaunal seed dispersal hypothesis states that some extant plants show "overbuilt" fruits apparently adapted for seed dispersal by very large mammals such as elephant-like gomphotheres and giant ground sloths that went extinct during the Pleistocene in the Neotropics (Janzen and Martin, 1982). These "anachronistic" fruits were assumed to be ecologically ineffective today because of the lack of present-day seed dispersal mechanisms (Janzen and Martin, 1982).This argument implies that the extinction of megafrugivores resulted in marked shifts in the patterns of seed dispersal observed today in extant plants with oversized fruits (Janzen and Martin, 1982; Howe, 1985; Barlow, 2000), with important implications in the ecology, evolution, and conservation of biodiversity. However, this hypothesis has remained controversial given its vagueness, the discrepancies in its assumptions and the limited or contrary evidence for many of its predictions (Howe, 1985; Hunter, 1989).

A comparative study of Neotropical large-fruited plants revisited and refined the megafaunal seed dispersal hypothesis, introducing an operational definition and classification of megafaunal fruits, like oversized fruits with fleshy pulp packaging extremely large individual seeds or large numbers of moderately-sized seeds (Guimarães et al., 2008). In using these criteria, subsequent studies have adopted the conjecture that extinct megafauna acted as legitimate past dispersers of many oversized fruits (Onstein et al., 2018; van Zonneveld et al., 2018), while others critically tested its assumptions given knowledge on the effectiveness of smaller contemporary dispersal agents using variable dispersal mechanisms (Jansen et al., 2012; Boone et al., 2015; Baños-Villalba et al., 2017; Rebein et al., 2017; Carpenter et al., 2018; McConkey et al., 2018). Multiple variably-sized extant dispersers and abiotic factors have been recognized in present-day dispersal interactions with "megafaunal plants," while assuming the existence of body-size trade-offs constraining dispersal ability via fruit ingestion and seed defecation (Donatti et al., 2007; Guimarães et al., 2008; Pires et al., 2018). Thus, the main challenge facing the megafaunal hypothesis remains the establishment of the disperser size that represents a functionally pervasive trait for each plant species (Howe, 1985, 2016; Jansen et al., 2012; Carpenter et al., 2018; McConkey et al., 2018), without succumbing to the circular rationale that assumes that large-sized vertebrates are major seed dispersers of large-fruited plants (Burns, 2013; Chen and Moles, 2015; Sebastián-González, 2017). Therefore, since Howe's (1985) critical review, the testing of the megafaunal seed dispersal hypothesis continues to be challenged by a general lack of knowledge on dispersers and dispersal strategies of many large-fruited plants (Fleming and Kress, 2013; Jordano, 2014).

Different fruit-exploiting and seed-dispersal mechanisms have important implications for seed viability, dispersal distance and recruitment (Howe and Smallwood, 1982). However, the definition of megafaunal fruit restrictively uses criteria matching African elephant-dispersed fruits, implying endozoochory as a dispersal mechanism (Guimarães et al., 2008; Bunney et al., 2017). Owing to their relatively large size, it has been suggested that domestic livestock serve as contemporary substitutes of extinct megafauna (Janzen, 1982; Janzen and Martin, 1982; Guimarães et al., 2008). The intensive contemporary harvesting of oversized fruits by large mammals, such as tapir and livestock acting as endozoochorous dispersers, has been highlighted to support this rationale (Janzen and Martin, 1982; Guimarães et al., 2008). This conflicts with evidence indicating that seeds of many of these fruits may not require gut passage to be efficiently dispersed by multiple legitimate external dispersers (Dominy and Duncan, 2005; Jansen et al., 2012; Blanco et al., 2018). Indeed, external dispersal may be the major mechanism exploited by large-fruited plants when a variable quantity of flesh is provided to dispersers (Dominy and Duncan, 2005; Baños-Villalba et al., 2017), and even when seed predators are the main dispersers (Jansen et al., 2012; Tella et al., 2016a,b, 2019). In this context, assessing the role of livestock as seed dispersers of oversized fruits was proposed as a feasible approach to test the megafaunal hypothesis (Janzen, 1982; Janzen and Martin, 1982; Howe, 1985). To date, however, there is still little information on seed dispersal by particular livestock species like cows, horses, sheep, goats, and pigs, on their external or internal seed dispersal mechanisms, as well as on their impact on seed germination and recruitment of particular large-fruited plants (Janzen, 1982; Boone et al., 2015; Baños-Villalba et al., 2017; Delibes et al., 2019). The ways in which different livestock species exploit large fruits and disperse their seeds can be especially informative for understanding their current role in plant population dynamics, and to assess the past role of extinct megafauna in the evolutionary ecology of their presumed food plants. This includes the determination of whether seeds are defecated (endozoochory), cleaned by stripping the pulp from the fruit in the mouth and then ejected (stomatochory), regurgitated after a variable period in the upper digestive system (endozoochory) or dispersed by a combination of these methods. This remains generally unknown for most livestock-large fruit interactions (Delibes et al., 2019).

Among large-fruited plants providing abundant food for multiple organisms, including human populations, palms (Arecaceae) have been highlighted as a keystone group of generally poor conservation status (Henderson, 2002; Smith, 2015). Information on the seed dispersal of large-fruited palms is limited (Zona and Henderson, 1989; Eiserhardt et al., 2011; Muñoz et al., 2019). In fact, previous studies attributed to extinct megafauna the dispersal of palm species because of the lack of essential natural history observations on the identity of current seed dispersers. Moreover, one of the main conclusions of a synthesis of palm-frugivore interactions published very recently is that "massive knowledge gaps of interaction diversity remain in the tropics" (Muñoz et al., 2019), which may be partially due to the recent regional and global declines and extinctions of many of their main primary dispersers (de Andrade et al., 2015; Lanes et al., 2016; Baños-Villalba et al., 2017).

Here, we focus on seed dispersal of six palm species (*Attalea barreirensis*, *Attalea eichleri, Attalea speciosa*, *Attalea princeps*, *Mauritia flexuosa*, *Acrocomia totai*) with large fruits that conform to the so-called "megafaunal syndrome" (Guimarães et al., 2008), and thus putatively anachronistic. We assessed whether contemporary wild vertebrates exert the seed dispersal function attributed to extinct megafauna in the past, and whether the assumption of endozoochory of large seeds by extinct megaherbivores can be supported by their presumed domestic substitutes, as a model to investigate past and ongoing seed dispersal strategies of these large-fruited plants. We hypothesized that seeds of large-fruited palms are currently dispersed by rich communities of wild vertebrates rather than by livestock acting as megafauna substitutes. This predicts (a) that multiple variable-sized contemporary fruit and seed consumers smaller than extinct megafauna and livestock act as reliable primary and secondary dispersers at variable distances using different dispersal mechanisms, not only endozoochory. In addition, (b) the largest among the extant potential dispersers, especially tapir, should act as reliable dispersal agents of these plants. Finally, if we assume that livestock act as disperser surrogates of extinct megafauna, we predict (c) that large seeds of the study palms are reliably dispersed by livestock using similar mechanisms (endoozochory implying defecation) as those presumably used by extinct megaherbivores.

## MATERIALS AND METHODS

### Study Areas and Palm Species

Fieldwork was conducted in six different study areas within the Cerrado tropical dry forest, Pantanal and Amazonia biomes, including transitional habitats with Beni savannas and Chiquitano dry forest, according to the microhabitat requirements of the studied palm species (Lorenzi et al., 1996). **Table 1** shows the details of the habitat and study periods in each locality in the departments of Beni and Santa Cruz in Bolivia, and the state of Piaui in Brazil (**Figure 1**). Sampling dates corresponded to the fruiting periods of the study palms. Given the long fructification periods of these palm species, which also greatly varied between years and regions, we attempted to cover several sampling periods in different areas to attain a more general view of the dispersers' communities.

The study areas within the Cerrado biome and their transitional habitats with Amazonian forest and Chiquitano dry forest are characterized by a heterogeneous savanna landscape including grasslands, shrublands, gallery forests, and dry and humid forests, with palm patches of variable size in locations with suitable soil microclimate (Oliveira and Marquis, 2002; de Carvalho and Mustin, 2017). The habitat in these areas shows a variable degree of fragmentation and defaunation (especially hunting pressure for bush meat and parrot poaching for the pet trade) depending on its inclusion within protected areas, human population density, nearness to inhabited nuclei, and their influences on the frequency of fires and livestock density (Silva et al., 2006; de Carvalho and Mustin, 2017; Mistry et al., 2019; see **Table S1** for details). The study area within the Beni savannas are characterized by wide areas of seasonal flooded grasslands dotted with forest islands dominated by palms and semi-deciduous groves used historically by indigenous human communities (Langstroth and Riding, 2011; Hordijk et al., 2019). The study area in San Matías, Ángel Sandoval (Bolivia) correspond to a transition between the Bolivian Pantanal, with roughly 80% of its floodplains submerged during the rainy season, and the Chiquitano dry forest characterized by shrublands, gallery forests, and dry forests (Power et al., 2016; de Carvalho and Mustin, 2017). Extensive ranching in large properties (generally >1,000 hectares) is the main livestock operation in these areas. Free-range cattle and equids move through the territory with temporal periods of grouping in corrals, while much less abundant herds of sheep and goats are locked in corrals every night to avoid predation. Pigs are restricted to the surroundings of small communities and isolated houses. The sampling areas where observations and camera trapping were conducted in each study site covered between 130 hectares (Yotaú, Guarayos, Bolivia) and about 15,000 hectares (San Matías, Ángel Sandoval, Bolivia; **Table S1**).

**Table 2** shows the characteristics of the studied palm species, their fruits and seeds, which were obtained from the literature (Lorenzi et al., 1996) and our own measurements. The fruits of these palm species are categorized as "megafaunal fruits" Type I, defined as fleshy fruits 4–10 cm in diameter with up to 5 large seeds (generally 2.0 cm diameter; Guimarães et al., 2008) Although the mean size of fruit and seed of *A. totai* are smaller, the ranges can reach the values considered to be "megafaunal fruits" (**Table 2**).

### Direct Observations on Wild Dispersers and Dispersal Mechanisms

In each study area, we actively searched for frugivorous and omnivorous wild vertebrates feeding on palm fruits. **Table 1** shows the sampling effort, in days of observation, conducted by a team of 2–4 persons for each palm species in each study area. To increase the likelihood of encountering frugivorous dispersers and observing dispersal events, surveys were conducted between sunrise and midday and late in the afternoon by slowly driving along unpaved and secondary roads crossing or parallel to palm forests, or by walking in and around palm forests. Once frugivorous vertebrates were located foraging on the palms, observations were conducted with a telescope and binoculars to record foraging behavior while avoiding disturbance. Specifically, we focused on determining whether they moved the seed away from the parent or dropped it beneath the parent canopy, or secondarily after the seeds had been disseminated by primary dispersers or by abiotic factors coupled with physical fruit features (e.g., sphericity favoring rolling). We recorded whether the dispersal event occurred internally by swallowing seeds (endozoochory) or externally (ectozoochory) by transporting them in the beak or mouth (stomatochory or synzoochory), or by hand by monkeys, even when fruits were dropped beneath the palms thus promoting secondary dispersal by

**TABLE 1 |** Location, habitat, study periods, and sampling effort (number of days of observation, number of camera-trap days, and number of camera locations) for each palm species in each study area.

| Palm species | Study site | Habitat | Study period (*n* observation days) | camera-trap days (*n* locations) |
|---|---|---|---|---|
| *A. eichleri* | Sao Gonçalo da Gurguéia, Piaui, Brazil | Cerrado | May 2015 (4), October 2016 (14), January 2017 (20) | 83 (17) |
| *A. barreirensis* | Sao Gonçalo da Gurguéia, Piaui, Brazil | Cerrado | May 2015 (4), October 2016 (14), January 2017 (20) | 73 (23) |
| *A. speciosa* | San Ramón, Mamoré, Bolivia | Cerrado-Amazonian forest transition | October 2017 (6) | 25 (7) |
| | Yotaú, Guarayos, Bolivia | Amazonian forest-Chiquitano dry forest transition | May–June 2018 (7) | 354 (32) |
| *A. princeps* | Loreto, Marbán, Bolivia | Beni savanna | June–October 2013 (70)[a], August–October 2016, 2017 (90) | 107 (36)[a] |
| | San Ramón, Mamoré, Bolivia | Beni savanna | August–October 2016, 2017 (25) | – |
| *M. flexuosa* | Sao Gonçalo da Gurguéia, Piaui, Brazil | Cerrado | May 2015 (4), October 2016 (14), January 2017 (20) | 126 (26) |
| | Exaltación de la Santa Cruz, Yacuma, Bolivia | Cerrado | October 2017 (10) | 65 (16) |
| *A. totai* | San Ramón, Mamoré, Bolivia | Cerrado-Amazonian forest transition | August–October 2016, 2017 (31) | 38 (9) |
| | Exaltación de la Santa Cruz, Yacuma, Bolivia | Cerrado | October 2017 (10) | 70 (17) |
| | San Matías, Ángel Sandoval, Bolivia | Cerrado, Pantanal-Chiquitano dry forest transition | November 2017 (4), August-October 2018 (19) | – |
| | Loreto, Marbán, Bolivia | Beni savanna | August–October 2016-2017 (90) | – |

[a] *Data partially included in* Baños-Villalba et al. (2017).

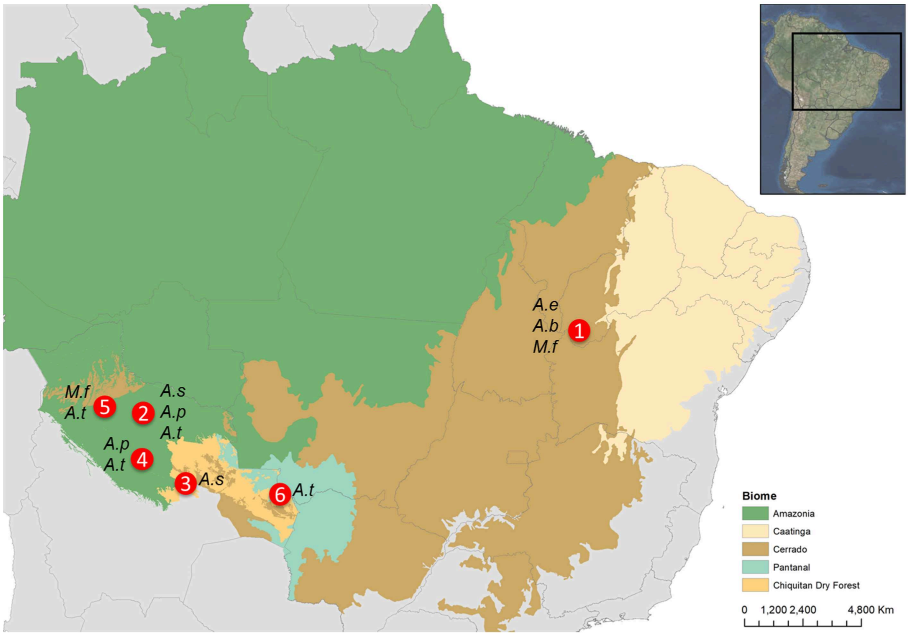

**FIGURE 1 |** Map indicating the location of the study areas (1: Sao Gonçalo da Gurguéia, Piaui, Brazil; 2: San Ramón, Mamoré, Bolivia; 3: Yotaú, Guarayos, Bolivia; 4: Loreto, Marbán, Bolivia; 5: Exaltación de la Santa Cruz, Yacuma, Bolivia; 6: San Matías, Ángel Sandoval, Bolivia) for each palm species: *A.e*, *Attalea eichleri*; *A.b*, *Attalea barreirensis*; *A.s*, *Attalea speciosa*; *A.p*, *Attalea princeps*; *M.f*, *Mauritia flexuosa*; *A.t*, *Acrocomia totai*.

**TABLE 2 |** Details of the characteristics of the studied palm species, their fruits, and seeds.

| | ***Attalea eichleri*** | ***Attalea barreirensis*** | ***Attalea speciosa*** | ***Attalea princeps*** | ***Mauritia flexuosa*** | ***Acrocomia totai*** |
|---|---|---|---|---|---|---|
| Palm height (m) | 0.5–2 | 0.5–2 | 6.5–30 | 9–15 | 3–25 | 3–15 |
| Biome | Cerrado | Cerrado | Mixed | Mixed | Mixed | Mixed |
| Fruit type | Drupe | Drupe | Drupe | Drupe | Drupe | Drupe |
| Fruit size (mm) | 60.5 × 40.5 | 59.1 × 40.7 | 88.8 × 69.9 | 63.3 × 35.2 | 49.1 × 40.0 | 27.8 × 27.8 |
| Range of fruit size (cm) | 5–7 × 4–5 | 5–7 × 4–5 | 7–12 × 4–10 | 6–9 × 3–5 | 4–7 × 3–6 | 2–4.5 × 2–4 |
| Range of fruit weight (gr) | 10–80 | 10–80 | 90–280 | 35–85 | 30–95 | 10–24 |
| Epicarp | Medium, fibrous | Medium, fibrous | Medium, fibrous | Medium, fibrous | Scaly | Fine, fibrous |
| Mesocarp (mm) | Fleshy (<2) | Fleshy (<2) | Fleshy (<4) | Fleshy (2–4) | Fleshy (<4) | Fleshy (9–11) |
| Endocarp (cm) | Very hard and thick (≥1) | Very hard and thick (≥1) | Very hard and thick (≥1) | Very hard and thick (0.3) | Soft and fine (≤0.4) | Hard and fine (≤0.15) |
| Number of seeds | 1–3 | 1–4 | 3–8 | 1–4 | 1 | 1 |
| Seed size (mm) | 37.5 × 11.5 | 32.0 × 11.5 | 44.1 × 15.2 | 10.9 × 9.9 | 32.0 × 25.0 | 16.1 × 16.1 |
| Fruit color | Brown | Brown | Green-yellow/Brown | Green-yellow/Brown | Red-brown | Green-brown |
| Pulp color | Orange | Orange | White | Orange | Orange | Orange |

*Data obtained from Lorenzi et al. (1996) and our own measurements.*

other organisms. When the foraging bout ended, remains of dispersed fruits and entire fruits dropped under the perching trees were inspected to assess which fruit parts were consumed and the form in which the consumer species consumed the fruits, including whether the pulp and seed were ingested and whether the discarded seeds were damaged by mastication or pecking. Random night surveys were conducted sporadically right after dawn along unpaved roads using flashlights to attempt to localize ground- and arboreal-dwelling frugivores feeding on fruits over or below fruiting palms and record seed dispersal events.

## Camera Trapping

We used camera trapping to detect elusive, scarce or nocturnal potential dispersers like ground-dwelling mammals mainly acting as secondary dispersers of seeds from fruits passively falling from the palms or dispersed primarily by other frugivores. The cameras (model Bushnell 6 MP Trophy Cam Essential) were placed hidden at ground level close (3–5 m) to fleshy-fruited palms with presence of a variable number of fallen mature palm fruits (10–30) to increase the likelihood of detecting secondary dispersers (Baños-Villalba et al., 2017). The cameras were directed toward the fruits, and ran automatically for 5 consecutive days. Cameras were programmed to detect fruit consumers and dispersers with multiple instantaneous digital captures and short video recordings (video length = 10 s, interval between videos = 60 min) aimed to determine the manner in which fruits were used and moved; this time interval between videos or "trigger delay" was programed to prevent the card from filling up with too many redundant images of a single animal or group, also thus preventing the batteries from running out. **Table 1** shows the sampling effort, as the number of camera-trap days (during daylight and night) and the number of camera locations, for each palm species in each study area.

## Disperser Identification by Beak Marks and Feces

As part of the surveys, dispersed seeds found under perches (trees, cliffs, and fence poles) and foraging sites were attributed to disperser species based on distinctive beak marks found on fruits and seeds (see Baños-Villalba et al., 2017; Montesinos-Navarro et al., 2017 for parrots). Direct observations of fruit consumption allowed us to ascertain the types of marks produced by each species by examining fruit and seed remains and entire pecked and wasted fruits collected below fruiting palms after the foraging bouts. We searched for seeds dispersed by rodents and carnivorous mammals within and around palm patches. The identification of these disperser species were based on tooth marks on the pulp of partially consumed fruit and on seeds (see examples in **Figure S1**), and on the presence of other distinctive signs like traces, footprints, droppings and warrens (Eisenberg and Redford, 2000). This was enhanced in many cases by confirming presence, fruit consumption, and seed dispersal with camera traps, allowing us to subsequently search for dispersed fruits and seeds with typical tooth and beak marks of each species in the camera surroundings.

During the random surveys conducted in palm forests and their surroundings, we searched for feces of large-bodied vertebrates that could act as potential dispersers of palm seeds, especially carnivorous mammals, peccaries and Greater rhea (*Rhea americana*). These feces were examined *in situ* by breaking them up by hand to determine if they contained palm seeds. We paid particular attention to the qualitative role of South American tapir (*Tapirus terrestris*) as a reliable disperser of palm seeds. Given its elusive habits and scarcity in the study areas, we conducted specific and intensive searching with the help of local people in Yacuma Province, Beni Department, Bolivia to locate the latrines where this species generally defecate (Fragoso and Huffman, 2000; O'Farrill et al., 2013). This area was specifically selected to assess disperser assemblages of *M. flexuosa* and *A. totai*

(**Table 1**), although small and very localized patches of *A. princeps* also occur there. Once latrines were located, we proceeded to determine the presence of seeds by opening each scat individually and searching for the presence of whole and partially eaten seeds, as well as other remains from palm fruits, specifically the presence of epicarp fragments (scales) of *M. flexuosa*. We recorded the presence of germinating palm seeds or saplings within and around latrines. The presence of seeds of other large fruited plants not systematically identified or quantified was also recorded in the latrines.

## Dispersal Distances

Seed dispersal events that were directly observed allowed us to measure exact distances, from the location where the fruit was collected to where the fruit or its seeds were released, by using binoculars with a laser rangefinder (Leica Geovid 10 × 42) working in a range of 10–1,300 m. When the dispersers were lost from sight in the vegetation, we considered a minimum dispersal distance from the location where the fruit was collected to the location where the animal was no longer visible. For flying dispersers carrying fruits in the beak when they were first observed, we recorded the distance at which they were first detected by using reference points, and then followed them with binoculars. In these cases, we estimated minimum dispersal distances, as measured from the location of first sighting to where the disperser perched for handling the fruit, where they released the fruit in flight, or where they were lost from sight in flight or within the forest. When we observed frugivorous species feeding on palm fruits without knowledge of the location of the mother palm, we conservatively estimated the minimum dispersal distance as the distance to the closest fruiting palm. In addition, for dispersed seeds found under perches and foraging sites, and for those found in feces and warrens attributed to each disperser species, the minimum dispersal distance to the closest fruiting palm was considered. To avoid pseudoreplication, minimum dispersal distances were not estimated for seeds found in tapir latrines because they were not found at random but rather by focused intensive searching.

## Seed Dispersal by Livestock

Direct observations were conducted on livestock foraging on palm fruits to determine the form of fruit exploitation and seed dispersal of each palm species in each study area. This was complemented with video-recordings during daylight and at night by camera traps placed at ground level under fleshy-fruited trees with mature palm fruits fallen passively or discarded by primary dispersers (**Table 1**). A random sample of feces of free-ranging livestock found in the palm forests and their surroundings was shredded *in situ* to determine the presence of seeds. The presence of seeds was also recorded at outdoor resting sites used during daylight (cows) or in corrals used at night (sheep, goat) depending on livestock operations in each study area. In addition to feces, we also recorded the presence of seeds in regurgitations, especially cud-chewing regurgitations produced during rumination (Delibes et al., 2019). Dispersal distances were determined as detailed above for wild animals when seeds were found at random, but not when they were searched for specifically at resting sites.

To further assess the patterns of palm seed dispersal by livestock, we conducted an intensive sampling in two monotypic stands of *A. totai* (3 and 9 hectares of extension) regularly used by extensive cows and horses in Yacuma Province, Beni Department, Bolivia. This area includes well-conserved Cerrado vegetation where this palm grows patchily due to a localized humid soil microclimate. Walking transects were conducted at random from the palm patch border to about 700 m in the Cerrado vegetation to record the location of livestock feces and regurgitations with palm seeds using a GPS device. A random sampling of the numbers of seeds regurgitated by cows was conducted in 1 $m^2$ plots ($n = 9$) within *A. totai* patches. In addition, we recorded the location of excreta at greater distances (3–8 km) from the palm patch to confirm the presence of livestock and potential seed dispersal at longer distances. For each excreta, we recorded the livestock species (cattle or horse), and the presence and number of palm seeds. The minimum distance from each excreta to the border of the palm grove was calculated with ArcGIS 10.5 (ESRI; Redlands, California, USA).

## Data Analysis

Factors affecting dispersal distance by wild animals were analyzed by Generalized Linear Models (GLM) using a negative binomial distribution and a log-link function. Palm species, disperser species nested within dispersal mode (primary or secondary) and the precision of the distance measurement (minimum or exact) were considered as predictor variables (fixed factors).

The patterns of *A. totai* seed dispersal by livestock were assessed with a GLM (binomial error, logit function) where the presence or lack of seeds in excreta was the response variable. The type of excreta (fecal or regurgitated), livestock species (cow or horse), and the identity (a or b) of each palm grove sampled were considered as explanatory factors, while the distance from each excreta to the border of the palm patch was included as a covariate.

Statistical analyses and checking of model assumptions were performed using SPSS software v. 25 (IBM SPSS Statistics).

# RESULTS

## Disperser Assemblages and Dispersal Mechanisms

A total of 54 vertebrate species were confirmed as seed dispersers of the study palms (**Table S2**). Wild dispersers ranged from very small species like the bat *Artibeus* sp. (about 65 g) and a small parakeet (*Brotogeris chriri*, 72 g) to larger species like the tapir and livestock of variable sizes, but generally larger than wild dispersers (**Table S2**). The number of dispersers varied among palm species; parrots (Psittaciformes) and monkeys (Primates) were recorded as seed dispersers for all of the studied palm species, while other Orders varied in their impact as dispersers of each palm species (**Figure 2**, **Table S2**). Parrots (13 species) dominated among the primary dispersers for all palm species, ranging from one species (*A. hyacinthinus* for *A. eichleri* and *A. barreirensis*) to 12 species for *A. totai* (**Figures 2**, **3**, **Table S2**).

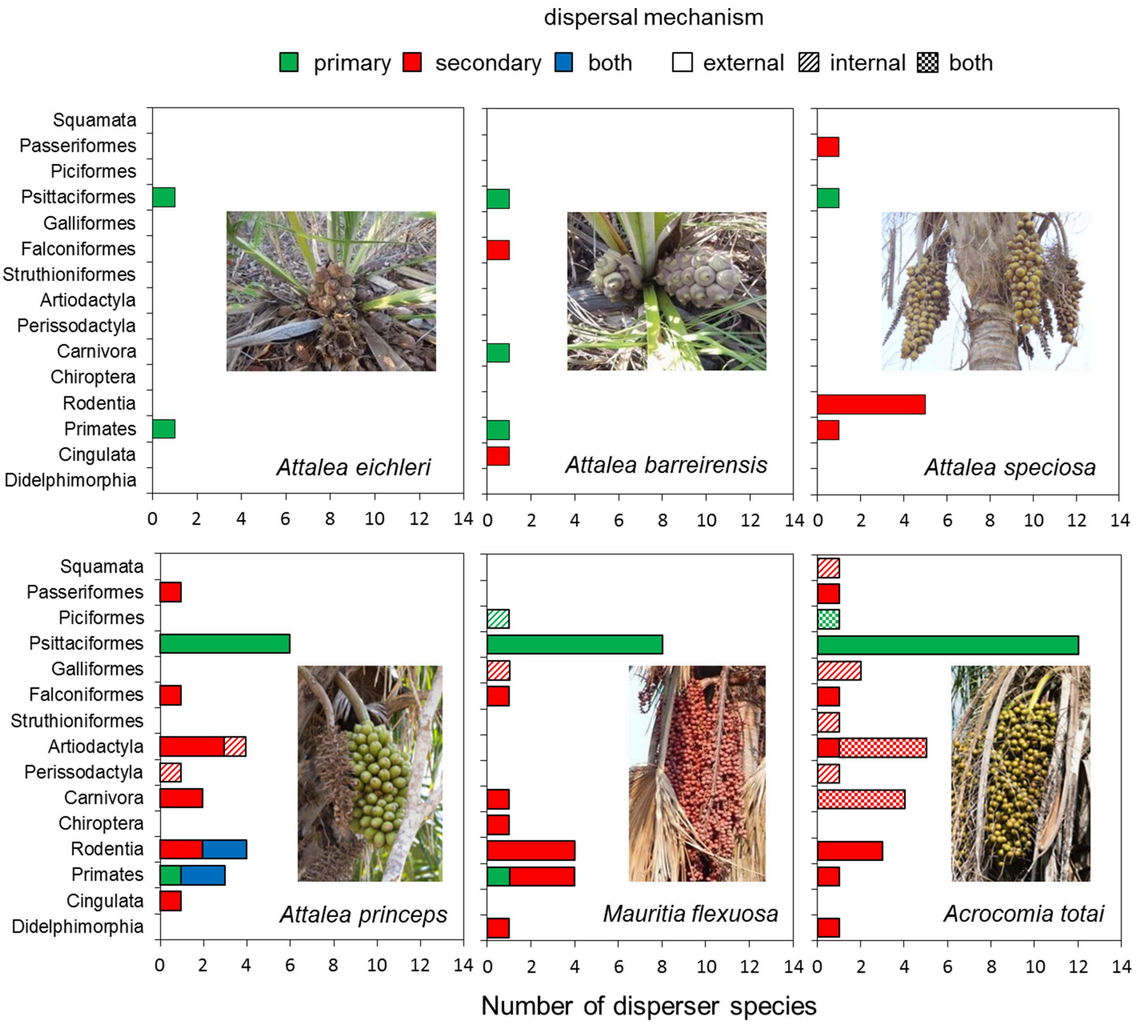

**FIGURE 2 |** Number of seed disperser species of each vertebrate Order for each palm species according to dispersal mechanism.

Several tree- and ground-dwelling primates and rodents acted as primary and secondary dispersers of *A. princeps*.

Most disperser species disseminated palm seeds externally by transporting them in the beak and mouth (stomatochory). Only external dispersers were recorded for *A. eichleri*, *A. barreirensis,* and *A. speciosa* (**Figures 2**, **4**, **Table S2**). Among wild dispersers, only tapirs acted as internal dispersers of *A. princeps*, while two bird species (*Mitu tuberosum* and *Ramphastos toco*) were observed swallowing entire fruits of *M. flexuosa*. The seeds of the smallest-fruited palm (*A. totai*) were dispersed externally (20 disperser species), internally (5 dispersers), and by both methods (9 dispersers, including wild and domestic species). Most dispersers (40 of 54 species, 74%) acted exclusively as external dispersers of 1–4 palm species each, while 5 of 54 (9%) were exclusively internal dispersers of 1–2 palm species each; the remaining species (9 of 54, 17%) acted both as primary and secondary dispersers of *A. totai* (**Figure 2**, **Table S2**). Overall, 49 of 54 species (91%) acted as external dispersers of 1–4 palm species, while 14 of 54 species (26%) acted as internal dispersers of 1–2 palm species.

A proportion of dispersers moved seeds of multiple palm species (21 of 54, 38.9%). Therefore, we pooled all palm species to summarize the role of primary and secondary dispersal, or both, in relation to the mechanism (endozoochory, ectozoochory, or both) used by each disperser species (**Figure 5**). All primary dispersers moved palm seeds externally (100%, $n = 16$ species), most being parrots (13 of 16 species, 81%). Other dispersers acting exclusively as primary dispersers included the black howler (*Alouatta caraya*), an unidentified *Artibeus* bat and the toco toucan (*R. toco*) for *M. flexuosa* and *A. totai* (**Table S2**). The toucan was considered both an external and internal primary disperser of *A. totai* (**Figure 5**, **Table S2**), because this species can fly with fruit in the beak regardless of whether the fruit is later swallowed or lost. Most secondary dispersers move seeds only externally (especially rodents), or both externally and internally (especially canids and livestock), while exclusively

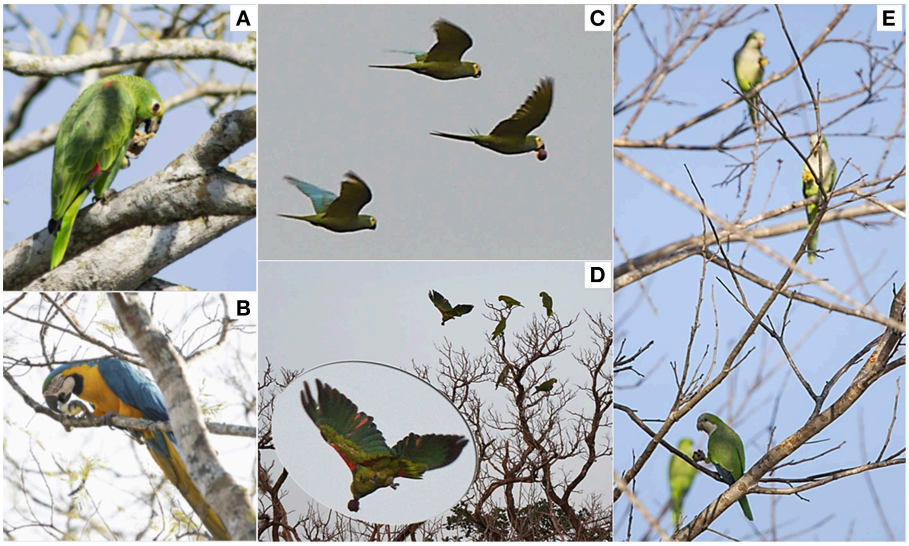

**FIGURE 3 |** Photographs illustrating examples of primary external dispersal of palm seeds by parrots: **(A)** *Amazona ochrocephala* and **(B)** *Ara ararauna* feeding on the pulp of *Attalea princeps* fruits moved from mother palms to perching trees; **(C)** *Orthopsittaca manilatus* flying with a *Mauritia flexuosa* fruit; **(D)** *Amazona aestiva* departing from a perching tree with a *Mauritia flexuosa* fruit (a two-phase dispersal); **(E)** three *Myiopsitta monachus* individuals feeding on the pulp of *Acrocomia totai* fruits moved from mother palms to a perching tree. Photographs taken by J. Salguero **(A,B,E)** and M. de la Riva **(C,D)**.

endozoochorous species included the tapir, a variety of large birds and the single reptile recorded. Finally, versatile ground- and tree-dwelling primary and secondary dispersers such as squirrels and primates moved palm seeds only externally (**Figure 5**).

## Dispersal Distances

Overall, we recorded the distances of seed dispersal events ($n$ = 2,504, **Table S3**) conducted by 21 wild disperser species for the six study palms (*A. barreirensis*, $n$ = 385, *A. eichleri, n* = 253 *A. speciosa*, $n$ = 719, *A. princeps*, $n$ = 363, *M. flexuosa*, $n$ = 400, *A. totai* $n$ = 384). Seed dispersal distance was influenced by palm species (binomial negative GLM, $\chi^2$ = 434.4, df = 5, $P$ < 0.0001, **Figure 6A**), and dispersal mode being higher when seeds were moved primarily ($n$ = 1,609) than secondarily ($n$ = 895) while controlling for disperser species ($\chi^2$ = 1579.1, df = 20, $P$ <0.0001, **Figures 6A,B**). The precision of the distance measurement (minimum, $n$ = 1,591 or exact, $n$ = 913) was not significant ($\chi^2$ = 0.08, df = 1, $P$ = 0.77). The dispersal mechanism was not included as a predictor because internal dispersal distance was only recorded for *A. totai* seeds found in a single scat of an unidentified carnivorous mammal. Dispersal distances ranged from a few meters when seeds were moved by a variety of rodents acting as secondary dispersers, to several kilometers when primarily dispersed by macaws (**Figure 6B**).

## Dispersal by Tapir

Half of the tapir latrines ($n$ = 20) contained seeds of *A. totai*, while about 26% of feces ($n$ = 744) showed between 1 and 5 *A. totai* seeds. The number of seeds was not recorded in all feces but at least 493 *A. totai* seeds were counted, pooling all latrines. Sixty-five seeds of *A. princeps* were found in two old latrines where the number of feces could not be determined (**Table 3**). Multiple epicarpus scales of *M. flexuosa* were found in a large proportion of latrines, but no intact seeds were found after examining all individual feces and the compacted unquantified fecal material found in the bottom of latrines (**Table 3**).

One dead seedling and two germinating seeds of *A. princeps* were found in a single latrine. A sample of the remaining seeds found in the same latrine ($n$ = 18) were opened to confirm their apparent viability (7 seeds, 38.9%) or unviability due to predation by invertebrate larvae (11 seeds, 61.1%). No *A. totai* seeds showed signs of germination, nor were any seedlings or saplings of old palm seeds found within the latrines or their surroundings (**Table 3**).

A variable number of seeds from other large-fruited plants were found in the latrines but not systematically identified or quantified. Among them, all seeds examined by opening and checking the endosperm were unviable (*Cariocar brasiliensis*, $n$ = 10; *Mauritella armata*, $n$ = 3; *Artocarpus* sp., $n$ = 2) or were found germinating at high densities (*Euterpe precatoria*, estimated $n$ > 1,500) or low densities (*Pouteria* sp., $n$ = 2) in the latrines. We found no older saplings of these species in the latrines and their close surroundings.

## Palm Exploitation by Livestock

Palm exploitation by each livestock species is shown in **Table 4**. Ruminants consumed saplings of all palm species, the leaves of mature bush layer palms and, when accessible, immature palms of the taller species. Cows also exploited flowers of the bush layer species. Most livestock species present in each study area consumed the fleshy pulp of the palm species with smaller fruits (*A. princeps*, *M. flexuosa*, *A. totai*), but none consumed the scarce pulp of the larger-fruited palms (**Table 4**). Pigs also consumed, or at least destructively masticated, the seeds of *A. princeps* and

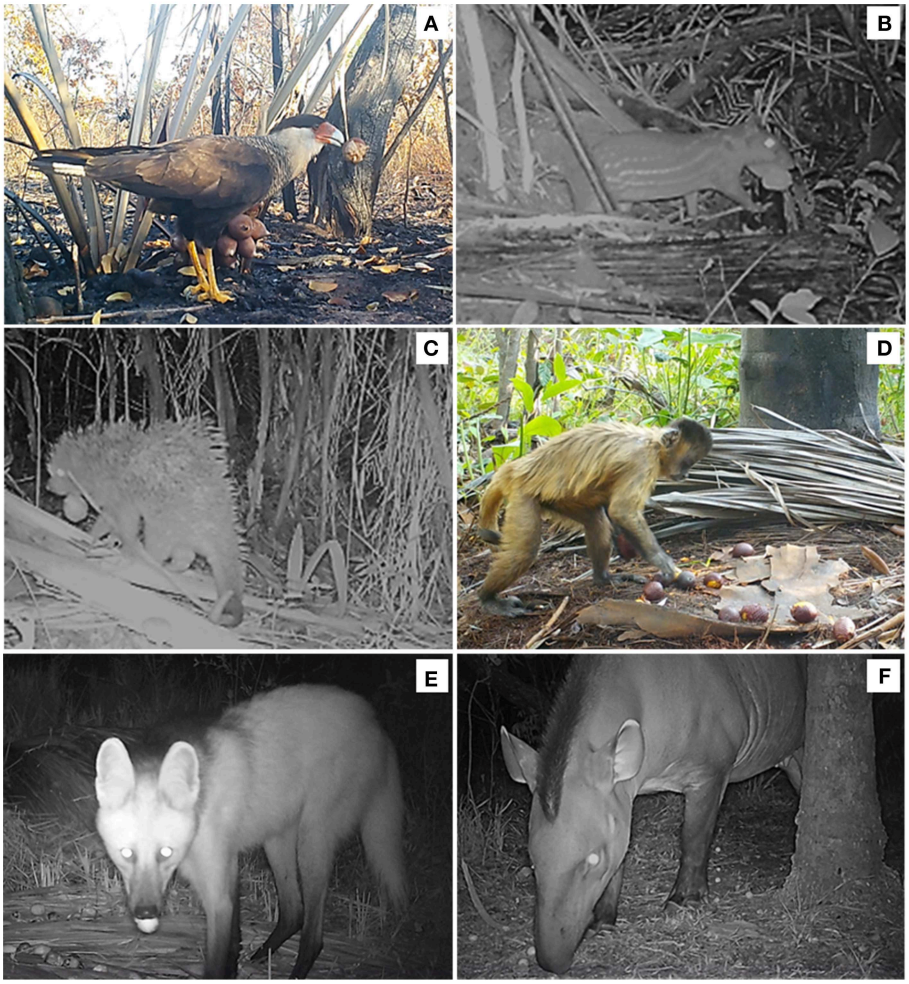

**FIGURE 4 |** Photographs illustrating examples of secondary external dispersal of palm seeds: **(A)** *Caracara plancus* dispersing *Attalea barreirensis*, **(B)** *Cuniculus paca* and **(C)** *Coendou prehensilis* dispersing *Attalea speciosa*; **(D)** *Sapajus libidinosus* and **(E)** *Chrysocyon brachyurus* dispersing *Mauritia flexuosa*; **(F)** *Tapirus terrestris* consuming and potentially dispersing *Acrocomia totai*. Photographs taken by camera traps.

*A. totai*, and likely those of *M. flexuosa* although this was not confirmed in this study.

## Seed Dispersal by Livestock

Livestock dispersal was restricted to the seeds of *A. totai* and *A. princeps* moved by endozoochory and ectozoochory. Specifically, cows, sheep and goats regurgitated large quantities of *A. totai* seeds after a period of rumination but never (sheep, goat) or rarely (cow) defecated seeds (**Table 4**). In addition, these livestock also exploited fleshy pulp without ingesting seeds, but transported and ejected them after a generally short period in the mouth. Pigs also chewed and sucked the pulp and spit out the seeds or ingested and defecated them. In other cases, livestock exploited the pulp and discarded the seeds of *A. totai* (equids) and *M. flexuosa* (cow, equids) without dispersing them. This was specifically confirmed by recording large numbers of *M. flexuosa* seeds stripped from the pulp ($n = 1{,}507$) or partially stripped ($n = 187$) after consumed by cows, compared with whole fruits ($n = 211$) below the canopy of particular fruiting palms ($n = 16$), but not in their surroundings.

## Seed Dispersal Frequency by Livestock

Estimating the frequency of each of these dispersal mechanisms was challenging because of the logistic difficulty in determining each seed's fate (i.e., whether or not seeds were ingested, and subsequent spitting, regurgitation, or defecation). This was further complicated by a likely combination of these dispersal mechanisms occurring for seeds consumed in a single feeding bout. The presence of palm seeds in feces was limited to a low proportion of *A. totai* in cow and pig feces (**Table 4**). Estimating the frequency of seed dispersal in regurgitations was hindered because seed regurgitation after rumination was apparently promoted by the ingestion of large quantities of palm fruits. In fact, all cow regurgitations recorded contained seeds of *A. totai* or *A. princeps* (**Table 4**). The mean number of *A. princeps* seeds per cow regurgitation was 3.3 (SD: 1.8, range: 1–6, $n = 6$).

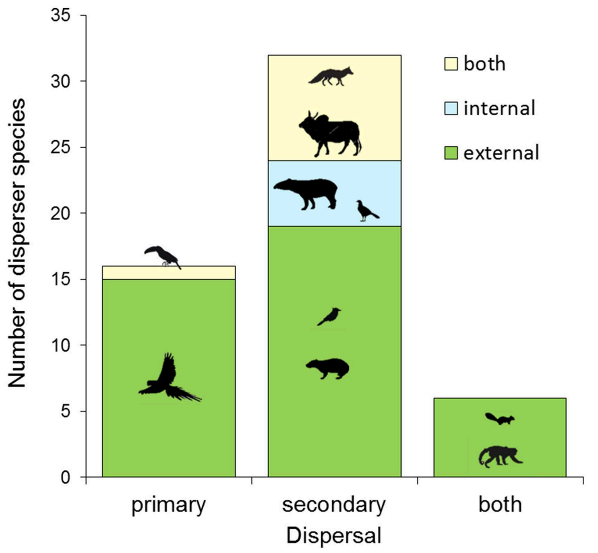

**FIGURE 5 |** Summary of the number of seed disperser species (pooling all palm species) according to dispersal mode (primary, secondary, or both) and mechanisms (external, internal, or both).

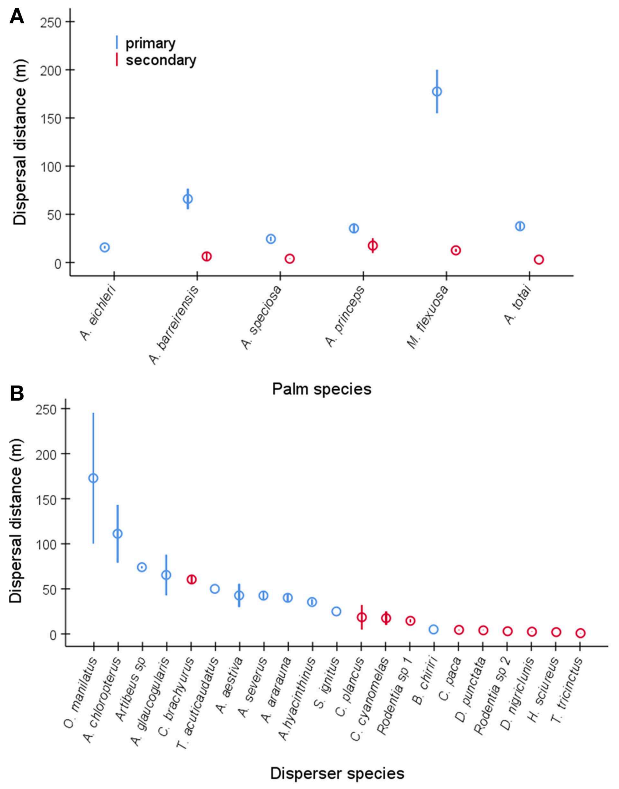

**FIGURE 6 |** Mean ± SE dispersal distances (in m) for **(A)** primary and secondary dispersions of seeds of each palm species, pooling disperser species, and for **(B)** dispersions conducted by each primary and secondary disperser species, pooling palm species. The complete scientific name of each disperser species is shown in **Table S2**.

## Seed Dispersal Sites and Distance by Livestock

Dispersal of *A. totai* seeds by cattle regurgitation mostly occurred below the source plant, especially along paths and at resting sites within the limits of the palm patches. The number of seeds counted in 1 $m^2$ plots within *A. totai* patches showed a high density of seeds regurgitated by cows (mean ± SD = 170 ± 82 seeds per $m^2$, range = 108–385, $n = 9$). Minimum dispersal distances were necessarily short in these cases because of the small size of the palm patches, but these distances were not measured to avoid introducing statistical bias related to the large number of seeds excreted as a consequence of artificially high cow densities. Pigs dispersed seeds of *A. totai* by spitting them out (all estimated minimum distances, mean ± SD distances = 27.8 ± 44.5 m, range = 4–185, $n = 215$) and defecation (17.7 ± 11.3 m, range = 6–35, $n = 9$). In several cases, we recorded sheep collecting *A. totai* fruits and spitting out seeds without ingestion at exact distances (45.0 ± 20.7 m, range = 20–60, $n = 8$). In addition, we observed thousands of *A. totai* seeds regurgitated during rumination in different sheep and goat corrals at minimum distances of 110 and 106 m, respectively, from the nearest palm patch.

## Intensive Sampling of *A. totai* Seed Dispersal by Livestock

The intensive sampling of livestock excreta conducted around two *A. totai* forest patches showed that seed dispersal depends mainly on the type of cow excreta (binomial GLM, $\chi^2 = 227.37$, df = 1, $P < 0.0001$). In fact, all regurgitations recorded contained seeds (100%, $n = 75$), while only one of the sampled feces contained seeds (0.9%, $n = 113$). The mean number of seeds per regurgitation was 24.1 (SD: 15.9, range: 4–68, $n = 30$), while the single cow scat with seeds contained five seeds. The presence or absence of seeds in cow excreta did not differ between palm patches ($\chi^2 = 0.96$, df = 1, $P = 0.33$) nor did it depend on the distance from each excreta to the palm patch ($\chi^2 = 1.83$, df = 1, $P = 0.18$). However, when the type of excreta (regurgitation or scat) was the response variable, a clear effect of the distance from the palm patch border was found (binomial GLM $\chi^2 = 15.18$, df = 1, $P < 0.0001$; palm patch effect: $\chi^2 = 1.07$, df = 1, $P = 0.30$). The frequency of regurgitations (all with seeds) decreased as the distance from the palms increased (**Figure 7**; the mean ± SD minimum seed dispersal distance in regurgitations was 107 ± 114, range = 7–358, $n = 75$), despite the fact that cows were present at larger distances as confirmed by the presence of their feces (**Figure 7**). No seeds of *A. totai* were found in horse feces sampled within and around the two palm patches ($n = 58$), and no regurgitations of horses were recorded.

# DISCUSSION

## External Dispersal as a Key Mechanism for Large-Fruited Plants

The large-fruited palms studied depend on a rich variety of variably sized vertebrates to efficiently disseminate their seeds

**TABLE 3 |** Frequency of latrines and individual feces of South American tapir with palm seeds and epicarp scales of *M. flexuosa*.

| Palm species | % of latrines *n* = 20 | % of feces[a] *n* = 744 | % of germinating seeds (*n*) | Presence of palm saplings |
|---|---|---|---|---|
| **Seeds** | | | | |
| *A. princeps* | 10.0 | Not quantified[b] | 3.1 (65) | No |
| *A. totai* | 50.0 | 25.9 | 0.0 (493) | No |
| *M. flexuosa* | 0.0 | 0.0 | – | – |
| **Fruit scales** | | | | |
| *M. flexuosa* | 70.0 | 57.1 | | |

*The frequency of germinating seeds and the presence of palm saplings within latrines and their close surroundings are also shown.*

[a] *Individual feces correspond to the same latrines analyzed.*

[b] *Sixty-five seeds were found in two old latrines where the number of feces could not be estimated.*

externally at variable distances, both primarily and secondarily. External dispersal has been generally under-appreciated as a major mechanism in the evolution of plants with large fleshy fruits (Fleming and Kress, 2013). Oversized fruits defined as "megafaunal" provide variable amounts of flesh even though many of them cannot be ingested entirely, nor their seeds defecated, by any extant vertebrate. This apparent mismatch lead to the hypothesis of anachronisms involving extinct megafauna as dispersal-mediated selective agents on fruit traits shaped through endozoochory (Janzen and Martin, 1982; Guimarães et al., 2008). Our results clearly challenge this contention by showing that large seeds of palms were dispersed externally by a wide array of vertebrates of variable size but much smaller than extinct megafauna and livestock.

The primary dispersers were generally birds and monkeys extracting fruits directly from the palm bunches and flying or moving over the canopy with them in the beak or mouth (stomatochory). In particular, rich communities of variably sized parrots (weighing between 70 and 1,500 g) were recorded dispersing seeds of all the study palms, with most species dispersing several palm species. This supports the key role of parrots as efficient dispersal agents of many large-fruited plants (reviewed in Blanco et al., 2018). Owing to their general wasteful feeding behavior, parrots disperse seeds at distances ranging from below the fruiting palm where secondary dispersers act, to long distances (up to several kilometers) that are generally underestimated due to logistic challenges of measurement (Tella et al., 2016a, 2019; Baños-Villalba et al., 2017). Among secondary dispersers, rich assemblages of frugivorous and omnivorous vertebrates mostly disseminated seeds externally, although some also ingested and defecated or regurgitated the seeds of the smallest-fruited palm species. As for other large-fruited palms, rodent species were especially frequent among the short-distance secondary dispersers (e.g., Jansen et al., 2012), while ground- and tree-dwelling squirrels and primates moved palm seeds externally, both primarily and secondarily (e.g., Chapman and Onderdonk, 1998; Wright and Duber, 2001).

Overall, external dispersal emerges as the major mechanism exploited by these large-fruited plants to disseminate their seeds at variable distances by an array of dispersers. Information on disperser assemblages of the study palms in other geographical areas (Zona and Henderson, 1989; Eiserhardt et al., 2011; Virapongse et al., 2017), as well as on other large-fruited plants (Jansen et al., 2012; Tella et al., 2015, 2019; Blanco et al., 2016; Rebein et al., 2017) support these mixed redundant and complementary dispersal systems by ectozoochory. Indeed, studies revisiting and adopting the megafaunal seed dispersal hypothesis recognized guilds of external dispersers with a role in population dynamics of these plants, although overlooked their potential impacts as selective agents shaping large size and other fruit traits (Guimarães et al., 2008; McConkey et al., 2018). More generally, it has often been emphasized that fleshy pulp evolved as a reward to gulpers ingesting entire fruits and to fruit mashers swallowing tiny seeds, both constrained by gape and gut size (Fleming and Kress, 2013). This assumes endozoochory as the main process driving trade-offs between body size and dispersal ability of contemporary dispersers (Fleming and Kress, 2013; Jordano, 2014) and extinct megafauna (Guimarães et al., 2008; Pires et al., 2018). These assumptions underestimated potential selective pressures imposed by frugivores moving large fleshy fruits to consume the pulp without ingesting or predating the seeds (Stevenson et al., 2005; Baños-Villalba et al., 2017; Delibes et al., 2019), or to predate and disperse seeds while ingesting or discarding the pulp (Jansen et al., 2012). This is striking because stomatochory allows the movement of fruits and seeds that are much larger than those dispersed by endozoochory (Lambert, 1999; Stevenson et al., 2005; Blanco et al., 2016; Castañeda et al., 2018; Fuzessy et al., 2018), thus promoting selective forces other than gape or gut size in the evolution of large-sized fleshy fruit and their seeds. Each particular species can be variably efficient in seed dispersal and recruitment, which deserves further investigation. However, whole rich assemblages of dispersers overlapping among palm species and study areas would guarantee the future dispersal-dependent survival of their populations. Therefore, the results of this study suggest that fruits of these palms cannot be further supported as anachronistic. These findings add to growing evidence indicating that assumptions related to extinct megafauna because of their large size are not necessary to understand all past and current mutualistic frugivore-large fruit interactions (Howe, 1985; Jansen et al., 2012; Baños-Villalba et al., 2017; Rebein et al., 2017; Carpenter et al., 2018).

In summary, our results agree with Howe's (1985) critical view that the megafaunal syndrome hypothesis suffers from a general lack of knowledge on the natural history of seed dispersal of many large-fruited plants. A knowledge gap in the number of interactions between Neotropical palms and vertebrates has been recently highlighted (Muñoz et al., 2019). For instance, Donatti et al. (2007) expressed their surprise about the survival of some plant species, exemplified by the apparent lack of contemporary dispersers and dispersal mechanisms in *A. speciosa*, and only three interactions with frugivorous species have been recorded for this palm in a recent review (Muñoz et al., 2019). However, we found that this palm is extensively dispersed primarily by a large macaw (*Ara ararauna*) and at least by seven secondary ground- and tree-dwelling dispersers, including rodents (see

**TABLE 4 |** Palm parts consumed, frequency of seeds dispersed in feces and regurgitations, and dispersal mechanism by each livestock species.

| Palm species | Livestock species | Palm use | Dispersal frequency % with seeds (*n*) | | Dispersal mechanism[g] |
|---|---|---|---|---|---|
| | | | Feces | Regurgitations[d] | |
| *A. eichleri* | Cow | Flowers, leaves, saplings | 0.0 (2,042)[b] | Not found | No |
| | Equids | Flowers, leaves, saplings | 0.0 (94)[b] | Not found | No |
| *A. barreirensis* | Cow | Flowers, leaves, saplings | 0.0 (2,042)[b] | Not found | No |
| | Equids | Flowers, leaves, saplings | 0.0 (94)[b] | Not found | No |
| *A. speciosa* | Cow | Leaves[a], saplings | 0.0 (71)[b] | 0.0 (13)[e] | No |
| | Equids | Leaves[a], saplings | 0.0 (3)[b] | Not found | No |
| *A. princeps* | Cow | Pulp, leaves[a], saplings | 0.0 (82) | 100 (6)[f] | E (spits), I (regurgitations) |
| | Equids | Leaves[a], saplings | – (Not quantified)[b] | Not found | No |
| | Pig | Pulp, seeds | 1.1 (90) | Not quantified | E (spits), I (feces) |
| *M. flexuosa* | Cow | Pulp, leaves[a], saplings | 0.0 (3,484) | 0.0 (91) | No |
| | Equids | Pulp, leaves[a], saplings | 0.0 (178) | Not found | No |
| | Pig | Pulp | 0.0 (9) | 0.0 (31) | No |
| *A. totai* | Cow | Pulp, leaves[a], saplings | 0.4 (1,588) | 100 (188) | E (spits), I (regurgitations, feces) |
| | Equids | Pulp, leaves[a], saplings | 0.0 (87) | Not found | No |
| | Pig | Pulp, seeds | 35.6 (90) | Not found | E (spits), I (feces) |
| | Goat | Pulp, leaves[a], saplings | 0.0[c] | Not quantified | E (spits), I (regurgitations) |
| | Sheep | Pulp, leaves[a], saplings | 0.0[c] | Not quantified | E (spits), I (regurgitations) |

[a] *Browsing leaves of immature palms.*

[b] *Despite no fruit consumption, feces evaluated for the presence of seeds of other palm species confirmed the lack of seed dispersal.*

[c] *Small size of feces makes it impossible to contain palm seeds; several hundred feces examined but not precisely quantified.*

[d] *Included regurgitations after rumination and spitting of seeds transported in the mouth.*

[e] *Corresponds to regurgitations with A. totai seeds in areas with presence of A. speciosa.*

[f] *Regurgitations with A. princeps seeds showed no seeds of A. totai, and vice versa.*

[g] *E: external dispersal (ectozoochory), I: internal dispersal (endozoochory). Regurgitations after rumination were distinguished from seeds spat out without swallowing.*

**Figures 4B,C**), a primate, a squirrel and a corvid species. This highlight how particular animal groups, traditionally neglected as reliable seed dispersers, can promote a crucial mutualistic role for palms and other large-fruited plants (Jansen et al., 2012; Blanco et al., 2015, 2018; Montesinos-Navarro et al., 2017; Albert-Daviaud et al., 2018).

## Role of Tapir as Dispersers of "Megafaunal Fruits"

Tapirs have been highlighted as the largest extant seed dispersers of large-fruited plants in the Neotropics, and thus their role as endozoochorous dispersers could resemble that of extinct non-ruminant megafauna (Fragoso and Huffman, 2000; Guimarães et al., 2008). We found that tapirs consume large amounts of palm fruits and defecate a few seeds of *A. princeps* and many seeds of the smallest-fruited palm species (*A. totai*). However, most seeds were unviable or lacked germination, and no saplings were found in abandoned or used latrines of variable size or their surroundings. In addition, although tapirs readily consumed fruits of *M. flexuosa*, as confirmed by abundant epicarp scales in a high proportion of excrement and latrines, the seeds were masticated and never survived gut passage. Thus, tapirs can be particularly destructive to seeds when feeding on these fruits (see also Bodmer, 1990; Fragoso and Huffman, 2000). Several studies have highlighted that different tapir species can be effective dispersers of small-medium seeds by passive browsing and defecation (Salas and Fuller, 1996; Fragoso and Huffman, 2000; Talamoni and Cançado, 2009; Campos-Arceiz et al., 2012; Barcelos et al., 2013; O'Farrill et al., 2013). However, seed recruitment has been generally evaluated under laboratory or greenhouse conditions rather than by monitoring the fate of seeds where they are excreted in the wild. Our results suggest that tapirs can be considered frequent consumers, effective predators, and poor dispersers of large-fruited palms, at least when their seeds are defecated in latrines where high densities of fecal nitrogen, pathogens and predators, as well as seed overcrowding and competition, may preclude or abort germination and seedling growth (Salas and Fuller, 1996; Campos-Arceiz et al., 2012). This was supported by the high number of old dead and unviable seeds of other plants with "megafaunal fruits" (Guimarães et al., 2008) found in the latrines. In conclusion, contrary to previous suggestions, the dispersal role of tapirs, as representative large-bodied non-ruminant frugivores, seems weak regarding large-seeded plants. Further research is required to confirm our results by considering these and other large-fruited plants dispersed outside latrines.

## Livestock as Substitutes of Extinct Megafauna for Dispersal of Large Seeds

Our results suggest that ruminants are poor seed dispersers of large-fruited palm species by endozoochory implying seed defecation. After examining hundreds of cattle, equids, goat

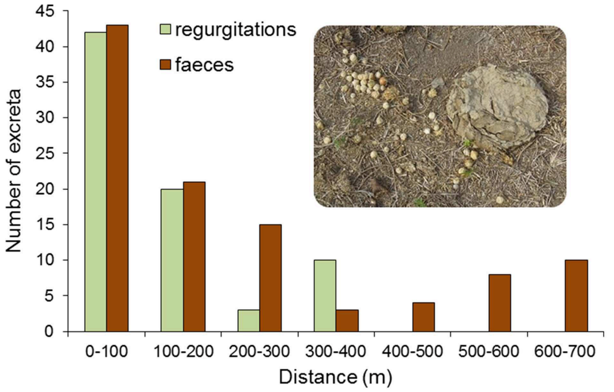

**FIGURE 7 |** Frequency of regurgitations containing seeds of *A. totai* regurgitated by cows during rumination, and frequency of cow droppings according to the distance from the border of the palm patch (two *A. totai* stands pooled). Both regurgitations and droppings were recorded in intensive walking surveys in the Cerrado vegetation around palm patches. The picture shows a cow regurgitation containing multiple *A. totai* seeds (left) close to a cow dropping without palm seeds.

and sheep scats, only a few seeds of the palm species with the smallest fruits and seeds were found at very low frequency in cow feces. Domestic pigs show a higher frequency of seeds of the same palm in feces, although they intensively masticate their food and can be particularly destructive to a much larger proportion of seeds of these and other species, similar to peccaries (Beck, 2006). Rather, livestock can regurgitate large quantities of seeds of *A. princeps* and *A. totai* after an unknown period of rumination, or spit out the seeds without swallowing them. Under normal browsing conditions on pastures, leaves, and twigs, rumen vomiting in livestock is a rare pathologic process associated with the accidental ingestion of unusually large items such as stones and artifacts causing blockages and injuries to the digestive system (Van Soest, 1994). This agrees with our results showing that palm seed regurgitation from the cud while ruminating was apparently provoked by the ingestion of large numbers of palm fruits, for which seed defecation appears anatomically impossible for smaller livestock (sheep, goat) or unnecessary due to nutritional and physiological costs for larger livestock (Van Soest, 1994; Feer, 1995; Clauss et al., 2009; Delibes et al., 2019).

Livestock ruminants chew their cud and ferment their food for variable periods of time (Van Soest, 1994), thus variably limiting seed survival through the upper gut passage (Janzen, 1982; Bodmer and Ward, 2006; Schwarm et al., 2009). In addition, pulp is often chewed and the cleaned seeds driven out without ingestion just below (without dispersal) or at a short distance from the fruiting palms. Although the viability of palm seeds dispersed after rumination or spitting without swallowing remains unknown, these different mechanisms for exploiting fruit can have important implications on seed dispersal distances (Castañeda et al., 2018; Delibes et al., 2019). Dispersal of *A. totai* seeds by cows mostly occurred below the source plant, as demonstrated the high density of seeds regurgitated in resting places within palm patches where plant recruitment is hindered by trampling, grazing and soil compaction (Montúfar et al., 2011; Smith, 2015; Baños-Villalba et al., 2017; Hordijk et al., 2019), or in locations unsuitable for germination outside palm patches within the dry Cerrado vegetation matrix. The frequency of regurgitations (all of *A. totai* seeds) decreased as the distance from the palms increased, with maximum dispersal distances of up to about 400 m in unsuitable sites, even though cow movement capability allows much longer daily travel distances. Cattle readily clean and spit out fruits of *M. flexuosa* below fruiting palms, without dispersing seeds, while they and other livestock can use this mechanism to disperse other palm seeds short distances. In any case, stomatochory leads to seed dispersal of large seeds at shorter distances than those dispersed by regurgitation after rumination, meriting focused research to adequately evaluate the dispersal role of livestock (Delibes et al., 2019). Seeds regurgitated by livestock can be potentially dispersed by other organisms. For instance, large macaws (*Anodorhynchus hyacinthinus* and *A. leari*) can act in two-phased (or tertiary) dispersal by moving palm seeds regurgitated by cattle and goats (authors' unpubl. data).

Our results did not agree with the prediction that seeds of large-fruited plants should be reliably dispersed by livestock exploiting similar mechanisms to those presumably used by extinct megaherbivores (Janzen and Martin, 1982; Guimarães et al., 2008). The logic of livestock as surrogate dispersers of seeds presumably disseminated by extinct megafauna implies gut passage through the digestive system and defecation of viable seeds (Janzen and Martin, 1982; Guimarães et al., 2008, Pires et al., 2018). Following this rationale, our results indicate that the complex digestive process of ruminants (Van Soest, 1994) hinders complete gut passage of large seeds (Feer, 1995; Clauss et al., 2009; Delibes et al., 2019). Therefore, like wild herbivorous ungulates (Feer, 1995; Bodmer and Ward, 2006; Castañeda et al., 2018), livestock are not effective long-distance endozoochorous dispersers of large seeds by defecation. Similar anatomical and physiological constraints and trade-offs governing fruit swallowing and seed defecation may also have operated in potential past interactions between large fruits and extinct megafauna (e.g., Carpenter et al., 2018). However, stomatochory by seed spitting without ingestion and endozoochory through regurgitation after a variable retention period in the upper digestive tract have been cited, but not comprehensively evaluated, as mechanisms used by extinct megafauna to exploit large fruits (Guimarães et al., 2008; Pires et al., 2018). In sum, livestock cannot be considered as ecological surrogates of extinct megafauna by exclusively assuming seed defecation as a dispersal mechanism.

## Rethinking the Megafaunal Seed Dispersal Hypothesis

This study negates the idea that overbuilt, fleshy fruits of the study palms are today maladapted for dispersal by contemporary fauna. Since the work of Janzen and Martin (1982), evidence has been building in support of seed dispersal of putative anachronistic plants by contemporary fauna (e.g., Jansen et al., 2012; Baños-Villalba et al., 2017; Rebein et al., 2017; McConkey et al., 2018), whose members existed in the same or much earlier geological periods as the extinct Pleistocene megafauna (Koch and Barnosky, 2006; Wright et al., 2008; Eriksson, 2016). Therefore, efforts are encouraged to rethink whether fruits assumed to have adjusted to a "megafaunal syndrome" actually required the past participation of extinct megafauna and to consider the contemporary function of domestic surrogates for seed dispersal.

Contrary to the assertion that seeds of many oversized fruits go undispersed due to the extinction of their legitimate megafaunal endozoochorous dispersers (Janzen and Martin, 1982; Guimarães et al., 2008), evidence suggests that mixed strategies combining dispersal mechanisms with asymmetric outcomes can be modulated depending on a complementary and/or redundant and rich variety of variably sized vertebrate groups (Wheelwright and Orians, 1982; Howe, 1985; Chapman and Chapman, 2002; McConkey et al., 2018). Therefore, oversized fruits can be better understood within a continuum ranging from species depending on few co-evolved dispersal partners to those depending on an array of unspecialized dispersers that can also exert selective impacts on plant fitness (for dry-fruited plants see Tella et al., 2016a,b, 2019; Gómez et al., 2018).

In conclusion, many oversized fruits assumed to be maladapted for contemporary dispersal are, in fact, misunderstood. This knowledge gap may have been partially favored by neglecting some key disperser guilds (e.g., parrots) and dispersal mechanisms (e.g., ectozoochory). By exclusively focusing in endozoochory via seed defecation as the mechanism shaping oversized fruits, the megafaunal dispersal hypothesis can fail to be adequately tested. This appealing hypothesis could attract renewed interest by considering ectozoochorous fruit mashers, thus reversing the view of past frugivore-plant mutualistic interactions markedly biased toward fruit gulpers. Alternative hypotheses are encouraged that incorporate regurgitation and spitting, and especially ectozoochory so commonly used by many vertebrates to get rid of large seeds (Fleming and Kress, 2013; Delibes et al., 2019). Thus, rather than (or before) relying on "ghosts of the past" in an attempt to explain traits that are unexpected or misunderstood, these refinements could provide a path to new fruitful research on frugivore-plant mutualistic interactions governing past and contemporary evolution of fruit traits.

### Conservation Implications

A proportion of species with "unfit" overbuilt fruits show restricted distributions and low genetic variability linked to reduced gene flow, which have been associated with the extinction of megafaunal dispersers (Guimarães et al., 2008; Johnson, 2009; Doughty et al., 2016). However, by relying on extinct megafauna for seed dispersal, the anachronism hypothesis overlooks the possibility that distribution patterns and genetic variability of many plants may be affected by historic defaunation of many large-sized vertebrates (other than extinct Pleistocene mammals) exploited by humans since their arrival to the Americas (Peres, 2000; Koch and Barnosky, 2006; Muller-Landau, 2007). Deforestation and defaunation have increased since then, further reducing distribution ranges and genetic variability of many plant species to the point that these patterns could be directly associated with the recent and ongoing extinction and decline of many contemporaneous dispersers (Peres and Roosmalen, 2002; Kurten, 2013). Among primary dispersers, several macaws and primates have become globally or regionally extinct recently, while most surviving species have suffered strong declines from much of their original distribution (Wich and Marshall, 2016; Berkunsky et al., 2017; Forshaw and Knight, 2017). The ecological functions of these organisms have been undervalued or neglected in population dynamics and genetic variability of their food plants (Chapman and Onderdonk, 1998; Blanco et al., 2015, 2018; Baños-Villalba et al., 2017; Montesinos-Navarro et al., 2017, Andresen et al., 2018). This applies specifically to plant populations and communities that are now limited by low abundance in impoverished assemblies of parrots and other large-sized dispersers (Dirzo et al., 2007; Galetti et al., 2013; Hall and Walter, 2013). Unfortunately, most reviews on the loss of plant dispersal function in forests due to defaunation and other human activities have excluded parrots (Farwig and Berens, 2012; McConkey et al., 2012; Fleming and Kress, 2013; Sebastián-González et al., 2015; Peres et al., 2016), even though these key long-distance dispersers are among the vertebrates most threatened by habitat loss, hunting for bush meat and pet trade (Berkunsky et al., 2017).

We thus advocate the evaluation of historic and ongoing defaunation of key external dispersers to attempt to understand the influence of actual (rather than putative) dispersers on contemporary frugivore-plant mutualistic interactions.

## ETHICS STATEMENT

This study relies on observational data obtained in areas unrestricted to people and thus did not require special permits.

## AUTHOR CONTRIBUTIONS

GB, JT, JD-L, and FH designed and conducted field work. GB analyzed the data and wrote a first draft of the manuscript. All authors contributed to improve it.

## FUNDING

We acknowledge support for this research from Fundación Repsol and Loro Parque Fundación.

## ACKNOWLEDGMENTS

We thank L. Lima, M. de la Riva, A. Montesinos-Navarro, J. Salguero, D. Parada, T. Rojas, W. Montaño, and Ch. Dolan for their help in the fieldwork, P. Salinas and F. Dénes for help with the figures.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2019.00328/full#supplementary-material

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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