Pollinator movement activity influences genetic diversity and differentiation of spatially isolated populations of clonal forest herbs

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In agricultural landscapes, forest herbs live in small, spatially isolated forest patches. For their long-term survival, their populations depend on animals as genetic linkers that provide pollen- or seed-mediated gene flow among different forest patches. However, whether insect pollinators serve as genetic linkers among spatially isolated forest herb populations in agricultural landscapes remains to be shown. Here, we used population genetic methods to analyze: (A) the genetic diversity and genetic differentiation of populations of two common, slow-colonizing temperate forest herb species [*Polygonatum multiflorum* (L.) All. and *Anemone nemorosa* L.] in spatially isolated populations within three agricultural landscapes in Germany and Sweden and (B) the movement activity of their most relevant associated pollinator species, i.e., the bumblebee *Bombus pascuorum* (Scopoli, 1,763) and the hoverfly *Melanostoma scalare* (Fabricus, 1,794), respectively, which differ in their mobility. We tested whether the indicated pollinator movement activity affected the genetic diversity and genetic differentiation of the forest herb populations. Bumblebee movement indicators that solely indicated movement activity between the forest patches affected both genetic diversity and genetic differentiation of the associated forest herb *P. multiflorum* in a way that can be explained by pollen-mediated gene flow among the forest herb populations. In contrast, movement indicators reflecting the total movement activity at a forest patch (including within-forest patch movement activity) showed unexpected effects for both plant-pollinator pairs that might be explained by accelerated genetic drift due to enhanced sexual reproduction. Our integrated approach revealed that bumblebees serve as genetic linkers of associated forest herb populations, even if they are more than 2 km apart from each other. No such evidence was found for the forest associated hoverfly species which showed significant genetic differentiation among

forest patches itself. Our approach also indicated that a higher within-forest patch movement activity of both pollinator species might enhance sexual recruitment and thus diminishes the temporal buffer that clonal growth provides against habitat fragmentation effects.

Introduction

Agriculture is one of the main drivers of habitat fragmentation, i.e., habitat loss and spatial isolation of sub-populations of plants and animals (FAO and UNEP, 2020). In Europe, agricultural history shaped former natural landscapes in such a way that seminatural habitats today mainly occur as small spatial units such as forest patches. Many species comprising the total biodiversity in such landscapes depend on those habitats (Billeter et al., 2008). A large number of plant species require animals that serve as genetic linkers between populations from different habitat patches by transporting seeds and pollen (Lundberg and Moberg, 2003). These genetic linkers are important for the long-term maintenance of the genetic diversity of plant populations under spatial isolation (Jeltsch et al., 2013). The identity, abundance, and behavior of these genetic linkers determine their movement activity, i.e., the frequency of movement events within and between habitat patches. This influences pollen-mediated gene flow, which consequently determines the relative isolation of specific plant populations (Auffret et al., 2017). With stronger isolation, plant populations are more genetically differentiated and have lower levels of genetic diversity (Auffret et al., 2017). Negative effects of fragmentation on the genetic diversity of insect-pollinated plants have been shown (Aguilar et al., 2019). If pollinator abundances and activities are reduced in response to habitat fragmentation, this directly influences the associated plant populations (Cheptou et al., 2017; Chen et al., 2019). For forest plants, however, it is a matter of debate in how far insect movement activity results in pollen-mediated gene flow among plant populations of different small and spatially isolated patches (Kramer et al., 2008; Lowe et al., 2016). This debate often focuses exclusively on woody plants (Kramer et al., 2008; Breed et al., 2015). Meanwhile, the forest herb layer is often ignored, even though this community comprises a particularly diverse group of organisms (Dobeš et al., 2017) and thus contributes substantively to the biodiversity and functioning of forest patches (Gilliam, 2007; Valdés et al., 2020). Many clonal forest herbs with persistent root organs have an overlap of generations and long lifespans (Whigham, 2004). Forest herbs show certain characteristics, which can

be interpreted as adaptations to long-term stable conditions (Honnay et al., 2005), namely, first flowering at a high age, few and heavy seeds and the absence of long-distance seed dispersal mechanisms. These traits make them especially vulnerable to habitat fragmentation (Whigham, 2004). In fact, several studies found reduced allelic richness in small forest herb populations (Van Rossum et al., 2002; Vellend, 2004; Naaf et al., 2021) and genetic differentiation among spatially isolated forest herb populations (Jacquemyn et al., 2006; Schmidt et al., 2009).

Pollen-mediated gene flow among forest patches is generally considered restricted (Honnay et al., 2005) because most forest herbs are pollinated by insects with low mobility (Wilcock and Neiland, 2002). These pollinators are expected to move even less between small patches if they are forest habitat specialists and thus avoid crossing the agricultural landscape matrix (Honnay et al., 2005). Forest-specialized hoverflies are an example of this pollinator type (Moquet et al., 2018; Proesmans et al., 2019b). However, there is also another type of insect pollinator with longer foraging distances and less habitat sensitivity, such as wild bees including bumblebees (Greenleaf, 2007; Redhead et al., 2016). These wild bees are pollinators of several forest herb species (Fussell and Corbet, 1991; Hasegawa and Kudo, 2005; Kolb, 2008), and bumblebees are expected to even increase their foraging distances in resource-poor agricultural landscapes (Persson and Smith, 2011). In theory, plant species should be more sensitive to habitat fragmentation if associated with less mobile pollinators, such as forest-specialized hoverflies, and less if associated with more mobile pollinators such as bumblebees (Breed et al., 2015).

Estimates of gene flow among spatially isolated populations of insect-pollinated forest herbs have been based on measures of genetic differentiation (Van Rossum et al., 2002; Jacquemyn et al., 2006). This approach allows limited conclusions about the contribution of pollen-mediated gene flow. To address the question of whether pollinators serve as genetic linkers for spatially isolated forest herb populations requires an integrated design that includes both the population genetics of the forest herb and the movement of its associated pollinators. Such a combined approach has been identified as an important research

field for some time (Sork et al., 1999; Breed et al., 2015). Studies addressing both the plants and their pollinators typically analyzed the population genetic structure of plants and then compared plant species associated with groups of pollinators with different traits. This has been reported for closely related plant species with different pollinator types (Kramer et al., 2011; Breed et al., 2015) and for plant species within the same landscape with different associated pollinators (Naaf et al., 2021). Previous research has mainly focused on mobility in terms of the potential geographic distance that a specific pollinator type covers in a particular habitat. By additionally specifying the movement activity of a pollinator species, we can integrate the variability of a species' movement behavior, which depends on a variety of factors, such as season and landscape context. The movement behavior (i.e., the pollinator's decision to move between two locations) is a matter of foraging economy, meaning a trade-off between resource availability in a habitat patch and energy costs to get there (Hadley and Betts, 2012). The chances of a pollinator visiting a plant population within the landscape are largely affected by the spatial arrangement of land-use types (Kamm et al., 2010; Cranmer et al., 2012). Thus, the actual movement activity of a pollinator at a specific habitat patch can better explain the genetic diversity and differentiation of associated plant populations than the geographic distances between patches or abstract estimates of pollinator mobility.

Movement activity of pollinators and pollen transport can be estimated directly by using pollen analogs (Van Rossum, 2009; Martínez-Bauer et al., 2021). However, with this method, the number of plant populations studied and the trackable pollination distances are limited. Other direct methods as observations are very work intensive or expensive as, for example, radar tracking (Pope and Jha, 2017). An alternative, indirect way to estimate the pollinator movement activity among multiple populations and over larger distances is the analysis of the genetic diversity and differentiation of pollinators using, for example, microsatellite markers (Osborne et al., 2002; Goulson, 2010). This is because the pollinators' genetic structure reflects their movement limitations and habitat dependencies (Cushman et al., 2015). By applying such methods, we can combine genetic and spatial information for a high number of individuals. If we link the population genetic data with information on sampling locations, we can estimate the movement activity involving one specific forest patch or among forest patches. Additionally, based on genetic information, we can assign bumblebee individuals to common nests to infer the nest density and the routes of foraging flights (Chapman et al., 2003; Darvill et al., 2004; Knight et al., 2005; Pope and Jha, 2017).

Here, we combine the population genetic analysis of two slow-colonizing forest herbs with the genetically inferred movement activity of their associated pollinators, one bumblebee and one hoverfly species. We conducted the research in three Central European agricultural landscapes. Our objective was to determine the influence of pollinator movement activity on forest herb genetic diversity and differentiation with the following hypotheses:

H1: The hoverfly species is less mobile and more strongly associated with forest habitats than the bumblebee species.

H2: A higher pollinator movement activity leads to a higher pollen-mediated gene flow among forest herb populations.

We tested these hypotheses by examining whether the following predictions hold true:

P1: If H1 is true, we would expect to find a more pronounced spatial genetic structure at the landscape scale for the hoverfly species than for the bumblebee species.

P2: If H2 is true, we would expect to find (a) a higher genetic diversity within forest herb populations, and (b) a lower genetic differentiation among forest herb populations when the pollinator movement activity is high.

Materials and methods

Landscape windows

We conducted this study in three 5 km × 5 km landscape windows within typical Central European agricultural landscapes in western Germany, eastern Germany and southern Sweden (**Figure 1**). The three landscape windows were similar in terms of landscape composition (**Table 1**). The landscape window in western Germany showed a higher proportion of forest and grassland cover and a lower proportion of crop cover than the other two landscape windows.

Selection of forest herb species

We selected the forest herb species *Anemone nemorosa* L. and *Polygonatum multiflorum* (L.) All., two slow-colonizing forest specialists associated with different pollinator types (Naaf et al., 2021). The pollinator types differ in their habitat sensitivity and preferences, flight ranges, and movement behavior (**Supplementary Table 3**). For each of the two species, we studied one associated representative pollinator species, i.e., a bumblebee species for *P. multiflorum* and a hoverfly species for *A. nemorosa* (**Supplementary Table 3**). The corolla of *P. multiflorum* spezialises on elongated mouth organs, such as those of long-tongued bumblebees, whereas there is no such

FIGURE 1
Locations of landscape windows (red squares) in Germany and Sweden.
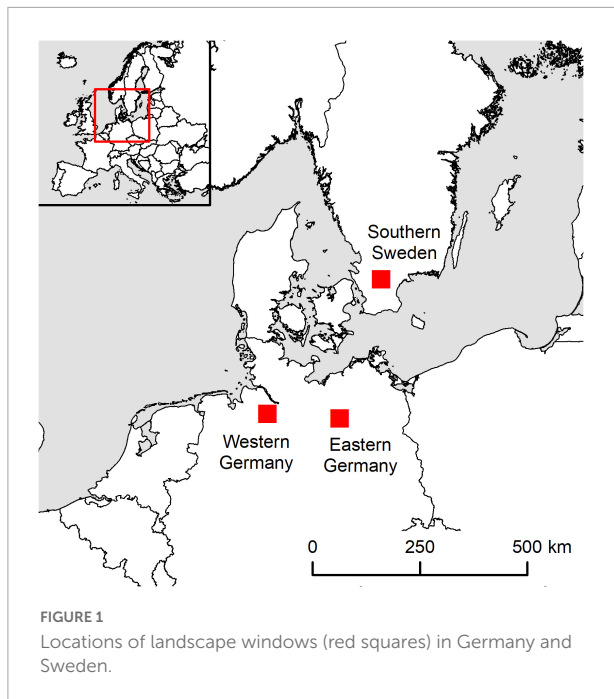
specialization in *A. nemorosa*, which is visited by a wider range of hoverflies, wild bees and other pollinators.

Sampling and identification of the most relevant pollinators

In each landscape window, we selected six deciduous forest patches, in which we collected leaf material of the forest herbs and pollinator individuals. Sampling of pollinators associated with *A. nemorosa* took place in April 2018 and 2019 and those pollinators associated with *P. multiflorum* were sampled in May 2018 and 2019.

The most relevant pollinator species should be common in all three landscape windows and show an important contribution to the pollination of the forest herb. To identify the most relevant pollinator species of each plant species and to capture a sufficient number of insect individuals for conducting population genetic analyses, we combined Malaise traps and observations, which included hand net catches of all flower visitors (**Supplementary material 1**). The Malaise trap sampling provided information about the pollinator species abundance and evenness. With the flower observations, we could quantify the proportion of flower visits per pollinator species. To increase the number of collected individuals per species and forest patch, we added further hand net catches in 2019.

The Malaise traps were installed in flowering stands of forest herbs (**Supplementary Figures 1B,D**) to passively catch pollinators that are attracted by the specific forest herb species. We standardized the insect visitation observations of the forest herbs at one transect of 25 m x 2 m per forest patch for *A. nemorosa* (**Supplementary Figure 1A**) and one patch of 2 m x 2 m per forest patch for *P. multiflorum* (**Supplementary Figure 1C**). The observations were conducted four to ten times per forest patch for 30 min between 9 a.m. and 8 p.m. and under appropriate weather conditions, i.e., no rain, temperatures > 10°C and low wind velocity (**Supplementary Tables 2.1, 2.2**). For southern Sweden, no observations were available as the preconditions were not met during the periods of field work. In total, we observed pollinators of *P. multiflorum* for 38.5 h with an average visitation rate of 3.2 visits/h per patch and of *A. nemorosa* for 36 h with an average visitation rate of 2.5 visits/h per transect (**Supplementary material 2**).

We evaluated the relevance of pollinator species for each herb species based on Malaise trap sampling, pollinator observations and literature information. According to the literature (Hasegawa and Kudo, 2005; Kosiński, 2012), long-tongued bumblebees are the most important

TABLE 1   Locations and land-use type composition of the three landscape windows (based on recent orthophotos).

Landscape window GPS-coordinates (centroids)	Western Germany 53.340226°N 9.430609°E	Eastern Germany 53.193325°N 11.978817°E	Southern Sweden 55.784102°N 13.628164°E
Total forest cover [%]	15.5	7.5	6.8
Deciduous forest cover [%]	8.8	6.0	5.7
Crop cover [%]	56.6	71.5	74.8
Grassland cover [%]	21.0	17.4	11.6
Settlement area cover [%]	4.3	2.5	5.2
Mean size of deciduous forest patches [ha]	1.56	1.63	2.00
Number of deciduous forest patches	141	92	71
Mean distance between centroids of analyzed plant populations [km]:			
Anemone nemorosa	2.6	2.0	2.4
Polygonatum multiflorum	2.8	3.1	2.3

pollinators of *P. multiflorum*, which was corroborated in our abundance and observation data. The species *Bombus pascuorum* (Scopoli, 1,763) was the most frequent flower visitor, with 57 visits out of 123 total observed visits (46%) (**Supplementary Figures 2.1A,B**). Moreover, from those bumblebee species that were recorded as flower visitors of *P. multiflorum*, *B. pascuorum* was the most abundant species in the Malaise trap samples, representing 97 out of 158 total individuals (61%) (**Supplementary Figures 2.1C–E**). Therefore, we chose *B. pascuorum* as the most relevant pollinator for *P. multiflorum*. Despite the fact that *A. nemorosa* is a generalist, hoverflies are especially important pollinators because of their very high local abundances in forests in spring (Strickmann, 2008). In our flower observations of *A. nemorosa*, we observed only nine individuals of four species of hoverflies (**Supplementary Figures 2.2A,B**), probably because of low temperatures in April 2018 and 2019. The mean temperature during *A. nemorosa* observations was approximately 15°C in 2018 and 16°C in 2019, which is low for hoverfly activities. The species *Melanostoma scalare* (Fabricus, 1,794) was observed as flower visitor of *A. nemorosa*, and in the Malaise traps, it had the highest abundance of all hoverflies that could be linked to *A. nemorosa*, representing 159 out of a total of 475 individuals that were caught (33%) (**Supplementary Figures 2.2C–E**). In a previous study in forest patches of the same landscape windows, relatively high numbers of *M. scalare* were observed (Proesmans et al., 2019a), and the species was reported to be a flower visitor of *A. nemorosa* (Strickmann, 2008). Consequently, we selected *M. scalare* as the associated pollinator for *A. nemorosa*. Different from *B. pascuorum*, the abundance of *M. scalare* in the Malaise trap samples (and of all other hoverfly species) was unevenly distributed among the patches (**Supplementary Table 4**). For the *P. multiflorum-B. pascuorum* pair 17 out of 18 forest patches and for the *A. nemorosa-M. scalare* pair 9 out of 18 forest patches had sufficient sampling and could thus be included in our analyses (**Supplementary Table 4**).

Pollinator species traits

Both selected species are typical representatives of their pollinator type. The species *B. pascuorum* is one of the most common bumblebee species in Central Europe (Goulson, 2010). Similar to other bumblebee species it is a central place forager with a queen establishing a nest in spring at a suitable position such as a field-forest boundary (Kells and Goulson, 2003). From this central position, workers fly to habitats with flower resources for foraging, which in the case of *B. pascuorum* includes forests. The workers establish stable traplines, which connect different foraging locations within one flight. The species *B. pascuorum* is considered a doorstep forager, with workers tending to forage as close as possible to their nests (Darvill et al., 2004). During flowering of a certain plant species,

B. pascuorum is relatively flower-constant, which means that it mainly collects pollen from one plant species (Chittka et al., 1999; Raine and Chittka, 2005).

The hoverfly *M. scalare* is a common species (Kormann, 2002) and both its larval and adult stages are associated with forest habitats (Speight, 2017). There is no available information regarding their flight ranges. An approximation can be derived first, from the related taxon *Melanostoma fasciatum*, of which individuals were caught at distances up to 180 m from an experimental pollen source (Wratten et al., 2003), and second, from the expectation that flight distances are short because of a small body size (Greenleaf, 2007). Since this species is not a nest-building species, it is expected to move in a linear manner through the landscape with an optimized balance of feeding and oviposition (Jauker, 2009). The species' general mobility within the landscape is considered low (Gatter et al., 1990). In addition, these two pollinator types also differ in their numbers of generations per year and hibernation stages (**Supplementary Table 3**).

Insect DNA extraction and microsatellite genotyping

Total genomic DNA of the insects was extracted using the E.Z.N.A. Tissue DNA Kit (OMEGA Bio-Tek, United States) according to the manufacturer's protocol. We used the whole specimen for *M. scalare* and the right hind leg for *B. pascuorum*. For *B. pascuorum*, eight nuclear microsatellite markers were used, which have already been broadly applied in several bumblebee species, including *B. pascuorum* (Estoup et al., 1995, 1996; Dreier et al., 2014; **Supplementary Tables 5.4, 5.5**). For the hoverfly *M. scalare*, the company AllGenetics & Biology SL (Spain) developed a new set of 13 polymorphic microsatellite primers for our study (**Supplementary Tables 5.1–5.3**). The fragment length analyses were performed on a 3730xl DNA analyzer (Applied Biosystems, United States) by MACROGEN Europe (Amsterdam, Netherlands) with GeneScan ROX 350 as the size standard for *M. scalare* and GeneScan LIZ 500 for *B. pascuorum*. Alleles were called manually with the software GENEMAPPER 6 (Applied Biosystems). Individuals with > 20% missing values were omitted from further analyses. Ten percent of the individuals of each species were genotyped for a second time to quantify the error rate (Bonin et al., 2004; **Supplementary Tables 5.3, 5.5–5.7**). For all species the locus-specific error rate never exceeded 5% (mainly due to allelic dropout). We included all markers in further analyses after checking them for linkage disequilibrium. We used the function pair.ia() from the R package poppr 2.9.3 (Kamvar et al., 2014), which calculates the standardized index of association between the different microsatellite loci, including a permutation approach. For *B. pascuorum,* no pair of markers exceeded an index value of 0.1 (**Supplementary Figure 6C**), and

for *M. scalare*, no pair of markers exceeded an index value of 0.08 (**Supplementary Figure 6D**), which we considered sufficiently independent. Because the pollinator specimens originated from two different sampling years (2018 and 2019), we tested whether there was significant genetic differentiation between the sampling years with a hierarchical analysis of molecular variance (AMOVA). The AMOVA framework uses pairwise squared genetic distances between individuals to calculate the proportion of genetic variance that can be explained by different levels of hierarchical subdivision (Excoffier et al., 1992). Here, we used landscape windows and sampling years as the hierarchical levels. We implemented AMOVA with the function amova() from the R package pegas 1.1 (Paradis, 2010) with Euclidean distances and 1,000 permutations, which tested each hierarchical level against a random distribution. For the samples of *B. pascuorum*, the AMOVA found no significant differentiation between the sampling years (**Supplementary Table 7**). For *M. scalare*, the AMOVA found a significant differentiation between the sampling years, which explained 1.7% of the total variation (**Supplementary Table 7**). However, this was likely because the forest patches had been sampled unequally between the years. For *M. scalare*, we had samples from 2018 and 2019 only in southern Sweden. In a further hierarchical AMOVA for the samples from southern Sweden with the years as first and forest patches as second level, the years were not significant, but forest patches significantly explained 2.7% of the variation (**Supplementary Table 7**). Thus, we pooled individuals from both sampling years for both pollinator species for further analyses.

Genetic indicators of pollinator movement activity

The genetic structure of the pollinators reflects their movement activity (Loxdale and Lushai, 2001; Osborne et al., 2002; Mola and Williams, 2019), which we estimated based on their genetic information and sampling locations. We used six indicators to assess the among-forest patch movement activity, and four indicators to assess the total movement activity of each pollinator species (**Table 2**). We measured these indicators in three modes: for a single forest patch (1), for forest patch pairs (1:1), and for a focal forest patch in relation to all other forest patches in the landscape window (1:n).

We defined the among-forest patch movement activity as the cumulative frequency of movement events of a specific pollinator species among the sampled forest patches. For both pollinator species we estimated this movement activity between pairs of forest patches based on two measures of genetic differentiation as indicators (**Tables 2A,B**) according to Slatkin and Barton (1989) who showed that measures of genetic differentiation can be used to estimate the numbers of individuals that move among populations. For the bumblebee

species *B. pascuorum*, we also used four additional indicators of among-forest patch movement activity based on their nest-building behavior (**Tables 2C–F**). If individuals from common nests are found in multiple forest patches, we expect a higher rate of movement activity between these forest patches because bumblebees: are known to communicate about forage resources (Dornhaus and Chittka, 1999); are flower constant (Chittka et al., 1999; Goulson, 2010); and establish fixed traplines (Ohashi and Thomson, 2009). For the identification of nests of *B. pascuorum*, we applied the full-likelihood algorithm of the software COLONY 2.0., which assigns individuals to maternal and paternal families and links those individuals with identical parents to groups of full-sibship, i.e., common nests (Jones and Wang, 2010). We assumed that all our bumblebee samples were workers given that we sampled at a time of year when nests were already large and primarily comprised of workers (von Hagen and Aichhorn, 2003). We used the settings "monogamous mating" for both males and females and "medium long run" as published for *B. pascuorum* in Dreier et al. (2014). We analyzed combinations of landscape windows and years separately. Each run was repeated with a different random number of seeds. If the probability of individuals being full-siblings was larger than 80% in both runs we treated them as individuals from a shared nest.

We defined the total movement activity as the cumulative frequency of movement events of pollinator individuals collected in a forest patch. The total movement activity involves (A) movements among a focal forest patch and all other locations outside the forest patch (the across-landscape movement), and (B) the movements within the focal forest patch (within-forest patch movement). The across-landscape movement can include movements from forest patches which were not sampled. The number and origin of foraging pollinator individuals in a forest patch depends on the species' distribution in the landscape and on the landscape permeability. Thus, the relationship between the across-landscape and within-forest patch movement activity is specific for each pollinator species. For *B. pascuorum*, we expected the relative proportion of across-landscape movements and within-forest patch movements to be a trade-off between its long potential flight ranges and its doorstep-foraging behavior. For *M. scalare*, we expected the within-forest patch movement activity to be more dominant over the across-landscape movement activity given its low mobility. The indicators of total movement activity used here do not allow to differentiate between the across-landscape and the within-forest patch movement activity.

We used two measures of genetic diversity as indicators for the total movement activity which were applicable for both pollinator species (**Tables 2G,H**). In general low levels of genetic diversity can indicate low connectivity (Cushman et al., 2015) and small numbers of individuals (Frankham, 1996). Here, we interpret an increase of genetic diversity as an increase of the total movement activity which includes (A) a higher

TABLE 2 Indicators of pollinator movement activity that indicate the among-forest patch and the total movement activity.

Among-forest patch movement activity		
Indicator	**Measurement mode**	**Interpretation**
(A) G_{ST}/ $G"_{ST}$ Pairwise genetic differentiation (B) D_{PS} Pairwise genetic differentiation	1:1	Lower levels of genetic differentiation between pollinators caught in two different forest patches indicate higher levels of movement activity between those forest patches.
(C) $NESTS_{shared.01}$ Binomial variable if two forest patches shared at least one *B. pascuorum* nest or not	1:1	Shared nests between two forest patches indicate bumblebee movement activity between them.
(D) $FOREST\text{-}PATCHES_{shared}$ Sum of all forest patches with which one forest patch shared at least one *B. pascuorum* nest	1:n	The higher the sum of forest patches with which a forest patch shares nests of *B. pascuorum*, the more is this forest patch connected with other forest patches in terms of bumblebee movement activity.
(E) $NESTS_{shared.count}$ Number of shared nests of two forest patches	1:1	Higher numbers of shared nests between two forest patches indicate higher movement activity between them.
(F) $NESTS_{shared}$ Proportion of nests shared with any other forest patch	1:n	A higher proportion of shared nests indicates (A) this forest patch is involved in a higher rate of movement events among forest patches, (B) the contribution of among-forest patch movement to total movement activity is high for this forest patch.
Total movement activity		
Pairwise indicators	**Measurement mode**	**Interpretation**
(G) A_r Rarefied number of alleles (H) H_e Expected heterozygosity	1	Higher levels of genetic diversity indicate that the pollinators from the respective forest patch have a higher across-landscape or within-forest patch movement activity.
(I) $NESTS_{number}$* Number of nests that contribute to a forest patch	1	Higher numbers of nests contributing workers to a forest patch indicate an increase of the cross-landscape and within-forest patch movement activity.
(J) $NESTS_{sum}$* Sum of $NESTS_{number}$ of forest patch pairs	1:1	If the total movement activity of two forest patches is high, the chance for a more intense pairwise movement activity between them is higher.

Four indicators were calculated for both pollinator species (A,B,G,H) and six only for B. pascuorum (C–F,I,J). We used three measurement modes, measured for one forest patch (1), measured for forest patch pairs (1:1), and measured for a focal forest patch to all other forest patches in the landscape window (1:n).

*Rarefaction of $NESTS_{number}$ and $NESTS_{sum}$ was based on 14 individuals, i.e., the minimum number of individuals, with 100,000 random draws to compensate the varying number of sampled individuals among forest patches.

frequency of across-landscape movement events, i.e., higher connectivity, and (B) a higher frequency of within-forest patch movement events, i.e., larger numbers of individuals, which are active within the forest patch. We used allelic richness (A_r) and expected heterozygosity (H_e) as measures of genetic diversity because they can be used without any assumption about Hardy-Weinberg equilibrium, which we did not expect for a group of pollinators caught in one sampling site (forest patch). This should also be valid for the nest-building central-place foraging bumblebee (Woodard et al., 2015), for which higher levels of genetic diversity were shown to be correlated with larger colony sizes (Herrmann et al., 2007). Due to its central nest-building behavior, the prediction of the bumblebee movement behavior is complex because the contribution of different factors like nest size and nest density are not completely resolved yet (Knight et al., 2005). Thus, we added two further indicators of total movement activity for *B. pascuorum* that addressed the

number of nests which contributed workers to the forest patches (Tables 2I,J). These measures were based on the nest estimations described above.

Sampling, genotyping, and population genetic structure of the forest herbs

In this study, we consider the forest herb individuals from each forest patch as a population. The analyzed populations were selected as representatives of old populations in ancient deciduous forest remnants to reduce signals of founder effects and to display gene flow effects. Old maps showed that those forest patches were forested in the eighteenth or nineteenth century. In spring 2018, we collected fresh leaf samples from up to 20 individuals. We used 10–20 individuals per population for analysis (Supplementary Tables 8.1, 8.3). Less than 20

individuals were used when population sizes were very small or genotyping failed (Naaf et al., 2021). For both forest herb species, we used six microsatellite markers for genotyping, which had been developed for congeneric species. We checked the allele data sets for repeated multilocus genotypes and excluded one sample of *P. multiflorum* with a repeated multilocus genotype within the same forest patch from further analysis. Further information regarding the sampling, estimation of population size and genotyping is given in **Supplementary Tables 5.6, 5.7** and in Naaf et al. (2021).

For both forest herb species, we calculated four measures of genetic diversity within populations, i.e., allelic richness (A_r), expected heterozygosity (H_e), observed heterozygosity (H_o), and the inbreeding coefficient $1\text{-}H_o/H_e$ (F) as well as three measures of pairwise genetic differentiation between populations, i.e., G_{ST}, $G"_{ST}$ and D_{PS} (1 minus the proportion of shared alleles). All these measures differ in their meaning and sensitivity (Waits and Storfer, 2016). For example, allelic richness is considered most sensitive for detecting recent losses of genetic diversity (Allendorf et al., 2012). While G_{ST} and $G"_{ST}$ are based on heterozygosity, D_{PS} quantifies the proportion of shared alleles among populations (Waits and Storfer, 2016). Because G_{ST} and $G"_{ST}$ were strongly correlated for all four species ($r \geq 0.99$) we only used $G"_{ST}$ in the downstream analysis as it is the recommended measure when analyzing small populations with microsatellites (Waits and Storfer, 2016). We tested the F-values for a significant deviation from zero using a two-sided permutation test with 999 permutations (Meirmans et al., 2018).

Data analysis

For different measurement modes we used specific types of models. For movement indicators that were measured for a single forest patch (1) or for a focal forest patch in relation to all other forest patches in the same landscape window (1:n), we used linear mixed models (LMM) (**Supplementary 11.3, 11.4**). This model type allowed us to integrate the different landscape windows as a random effect. We implemented the LMMs with the lme function from the R package nlme 3.1–155 (Pinheiro et al., 2019). For movement indicators that were measured for forest patch pairs (1:1), we used maximum-likelihood population-effects MLPE models (Clarke et al., 2002; **Supplementary materials 11.1, 11.5**). The MLPE models take the non-independence of population pairs sharing a common population into account. This correlation structure was implemented with the function corMLPE (Pope, 2020) and then used as an argument in the lme function.

We were interested in every combination of plant genetic diversity and differentiation measure and pollinator movement indicator. Thus, we performed multiple tests with the same set of movement indicators on each measure of genetic diversity or genetic differentiation. Such an approach can result in an increased probability of a type I error. To evaluate if a detected effect was potentially false positive, we also calculated corrected p-values (q) by controlling false discovery rate (FDR).

Genetic structuring of the pollinator species

Addressing our prediction 1, i.e., that the hoverfly shows a more pronounced spatial genetic structure than the bumblebee, we used hierarchical AMOVA to determine if and to what extent each pollinator species showed genetic structure according to the sampled forest patches. A significant differentiation among the forest patches would indicate that a species prefers movement within habitat patches over movement among them. We conducted this analysis per pollinator species with the landscape windows as the first level to address the nested sampling design and the forest patches as the second level. Each hierarchical level was tested against a random distribution. To test whether the pollinator populations showed effects of isolation-by-distance, we modeled the pairwise genetic differentiation of the pollinators as a function of geographic distance with MLPE models. We included the landscape window as a random effect in the models.

Linking the herbs' genetic diversity and differentiation to indicators of pollinator movement

To test our prediction 2a, i.e., that higher movement activities of the pollinators result in higher genetic diversity in the associated forest herb populations, we modeled the population genetic diversity of the forest herb populations as a function of the pollinator movement indicators (measurements modes: 1 and 1:n) using LMMs. Landscape window was treated as a random effect (random intercept models). Originally, we intended to include plant population size as a covariable in the models given that population size is a major determinant of genetic diversity within populations (Leimu et al., 2006). However, the small sample size of 17 for the *P. multiflorum-B. pascuorum* pairs, and 9 for the *A. nemorosa-M. scalare* pairs did not allow the inclusion of more than one predictor at a time. Instead, we tested, whether the population size of the plant species had an effect on the genetic indicators of pollinator movement using LMMs with landscape window as a random effect as above. We did not find any significant effect (**Supplementary material 11.3**).

For our prediction 2b, i.e., that higher pairwise movement activities of the pollinators between forest herb populations results in lower genetic differentiation between them, we applied MLPE models to model the pairwise genetic differentiation among the forest herb populations as a function of the pairwise

pollinator movement activity (measurement mode 1:1). The pairwise analysis with 40 *P. multiflorum-B. pascuorum* pairs and 18 *A. nemorosa-M. scalare* pairs allowed us to use geographic distance as a covariable in addition to the movement indicators. We used the landscape window as a random effect. The MLPE models were only reduced if a predictor was far from being significant ($p > 0.1$) based on a *t*-test.

Results

Genetic diversity and differentiation of forest herbs

The two forest herbs exhibited a similar degree of genetic diversity in terms of A_r and H_e (**Table 3**). The mean H_o of *P. multiflorum* was higher than its mean H_e, while for *A. nemorosa*, the mean H_o was lower than its mean H_e. This difference was also reflected in the distribution of *F*-values. Six out of 17 populations of *P. multiflorum* had an *F*-value significantly smaller than zero (**Supplementary Figure 8.1**), while all nine populations of *A. nemorosa* had an *F*-value significantly greater than zero (**Supplementary Figure 8.2**).

Indicators of pollinator movement

The number of sampled and genotyped individuals and, as a consequence, n_A of the two pollinator species differed strongly. All genetic diversity values were lower for *M. scalare* than for *B. pascuorum* (**Table 4**). For *B. pascuorum* G_{ST} ranged from 0 to 0.03, G''_{ST} from 0 to 0.1, and D_{PS} from 0.16 to 0.32. For *M. scalare*, G_{ST} ranged from 0 to 0.05, G''_{ST} from 0 to 0.09, and D_{PS} from 0.09 to 0.2.

TABLE 3 Number (N) of sampled and genotyped forest herb individuals, number of alleles (n_A), mean and standard deviation of allelic richness (A_r), expected heterozygosity (H_e), observed heterozygosity (H_o), inbreeding coefficient (*F*), and pairwise genetic differentiation (G_T, G''_{ST}, D_{PS}).

	Polygonatum multiflorum	*Anemone nemorosa*
N individuals	323	175
n_A	134	87
A_r (1)	8.38 ± 1.38[A]	8.55 ± 0.86[B]
H_e (1)	0.73 ± 0.03	0.75 ± 0.01
H_o (1)	0.8 ± 0.05	0.54 ± 0.04
F (1)	-0.09 ± 0.07	0.29 ± 0.05
G_{ST} (1:1)	0.06 ± 0.03	0.02 ± 0.01
G''_{ST} (1:1)	0.23 ± 0.01	0.06 ± 0.01
D_{PS} (1:1)	0.45 ± 0.01	0.25 ± 0.01

In brackets are the measurement modes: for a single forest patch (1), and for forest patch pairs (1:1).
[A]Rarefied at 19 (if N < 19 than A_r was estimated at N without rarefaction) N, [B]Rarefied at 18.

TABLE 4 Number (N) of sampled and genotyped pollinator individuals and number of alleles (n_A), mean and standard deviation of movement indicators within the forest patch: allelic richness (A_r), expected heterozygosity (H_e), NESTS$_{number}$, NESTS$_{shared}$, and FOREST-PATCHES$_{shared}$; movement indicators among the forest patches: G_{ST}, G''_T, D_{PS}, pairwise NESTS$_{shared.01}$, pairwise NESTS$_{shared.count}$, and pairwise NESTS$_{sum}$.

	B. pascuorum	*M. scalare*
N individuals	408	142
n_A	125	64
A_r (1)	6.99 ± 0.37[A]	2.78 ± 0.18[B]
H_e (1)	0.68 ± 0.03	0.44 ± 0.02
NESTS$_{number}$ (1)	13.32 ± 0.76[A]	X
NESTS$_{shared}$ (1:n)	0.10 ± 0.1	X
FOREST-PATCHES$_{shared}$ (1:n)	1.6 ± 1.06	X
G_{ST} (1:1)	0.00 ± 0.01	0.02 ± 0.02
G''_{ST} (1:1)	0.02 ± 0.02	0.04 ± 0.03
D_{PS} 1:1)	0.22 ± 0.04	0.16 ± 0.03
NESTS$_{shared.01}$ (1:1)	0.36 ± 0.49	X
NESTS$_{shared.count}$ (1:1)	0.58 ± 1	X
NESTS$_{sum}$ (1:1)	26.59 ± 1[A]	X

X, Indicator not applicable for 7 pollinator type. In brackets are the measurement modes: for a single forest patch (1), for a focal forest patch in relation to all other forest patches in the landscape window (1:n), and for forest patch pairs (1:1).
[A]Rarefied at 14, [B]Rarefied at 8.

The full-sibship analysis identified a total of 362 nests of *B. pascuorum* based on the genotypes of 408 individuals. Of these, 316 were singletons, i.e., nests with only one associated individual, leaving 46 nests with more than one associated individual. There was only one forest patch in southern Sweden that did not share any *B. pascuorum* nest with any other forest patch (**Figure 2**). The total number of nests shared by individuals from different forest patches per landscape window (**Table 5**) as well as the indicators for the among-forest patch movement activity (*NESTS$_{shared.01}$*, *FOREST-PATCHES$_{shared}$*, *NESTS$_{shared.count}$*, and *NESTS$_{shared}$*) (**Table 4**) were markedly higher in western Germany than in eastern Germany and southern Sweden. No nest was shared between more than two forest patches (**Figure 2**).

Genetic structuring of the pollinators

The hierarchical AMOVA revealed that both levels (1. landscape windows, 2. forest patches) contributed significantly to the genetic structure of both pollinator species. However, while the forest patches in which the individuals were sampled explained 2.4% of the hoverfly's genetic variance, they explained only 0.5% of the bumblebee's genetic variance (**Supplementary Table 11.2**). In the MLPE models, geographic distance had a significant effect on G''_{ST} but not on D_{PS} of *M. scalare* (**Figure 3** and **Supplementary Table 11.1**). With every kilometer, the G''_{ST} of *M. scalare* increased by 12%. For
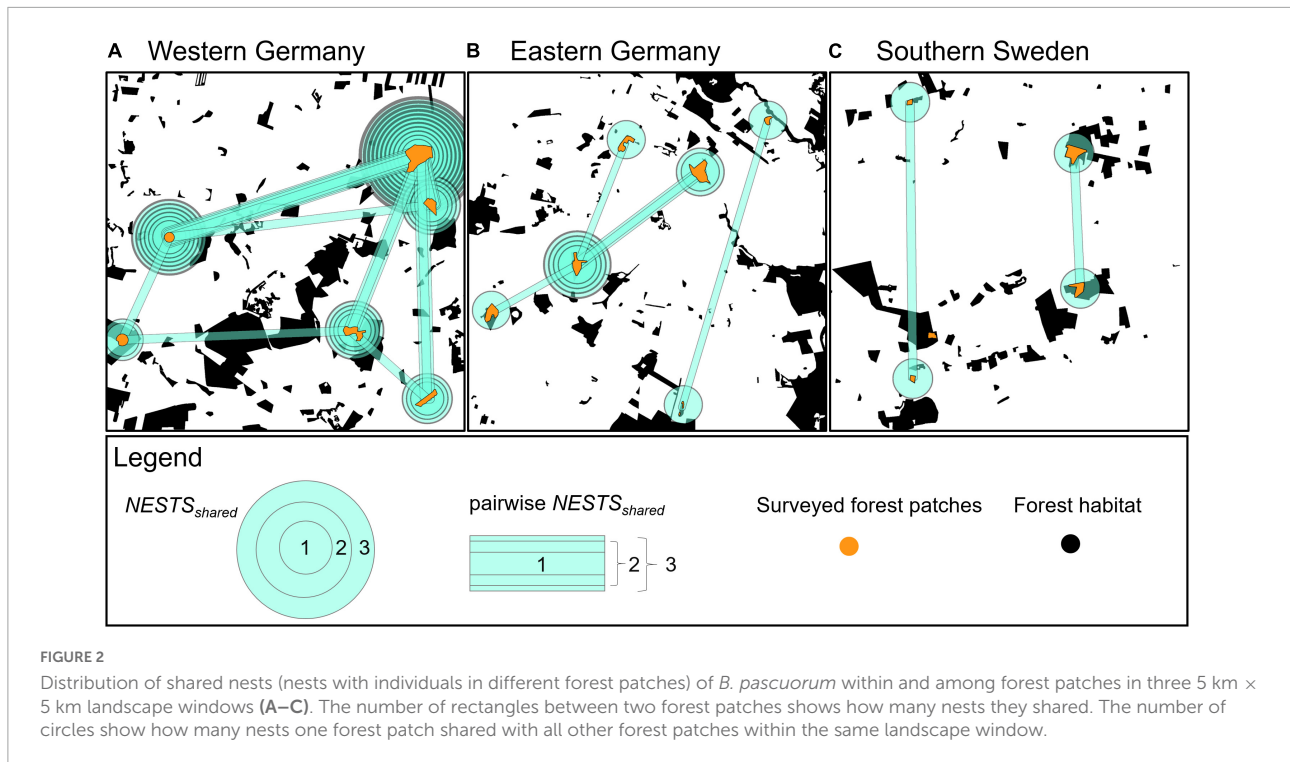
FIGURE 2

Distribution of shared nests (nests with individuals in different forest patches) of *B. pascuorum* within and among forest patches in three 5 km × 5 km landscape windows **(A–C)**. The number of rectangles between two forest patches shows how many nests they shared. The number of circles show how many nests one forest patch shared with all other forest patches within the same landscape window.

TABLE 5 Summary of full-sibship assignment (COLONY 2.0) for *B. pascuorum* for three landscape windows and 2 years.

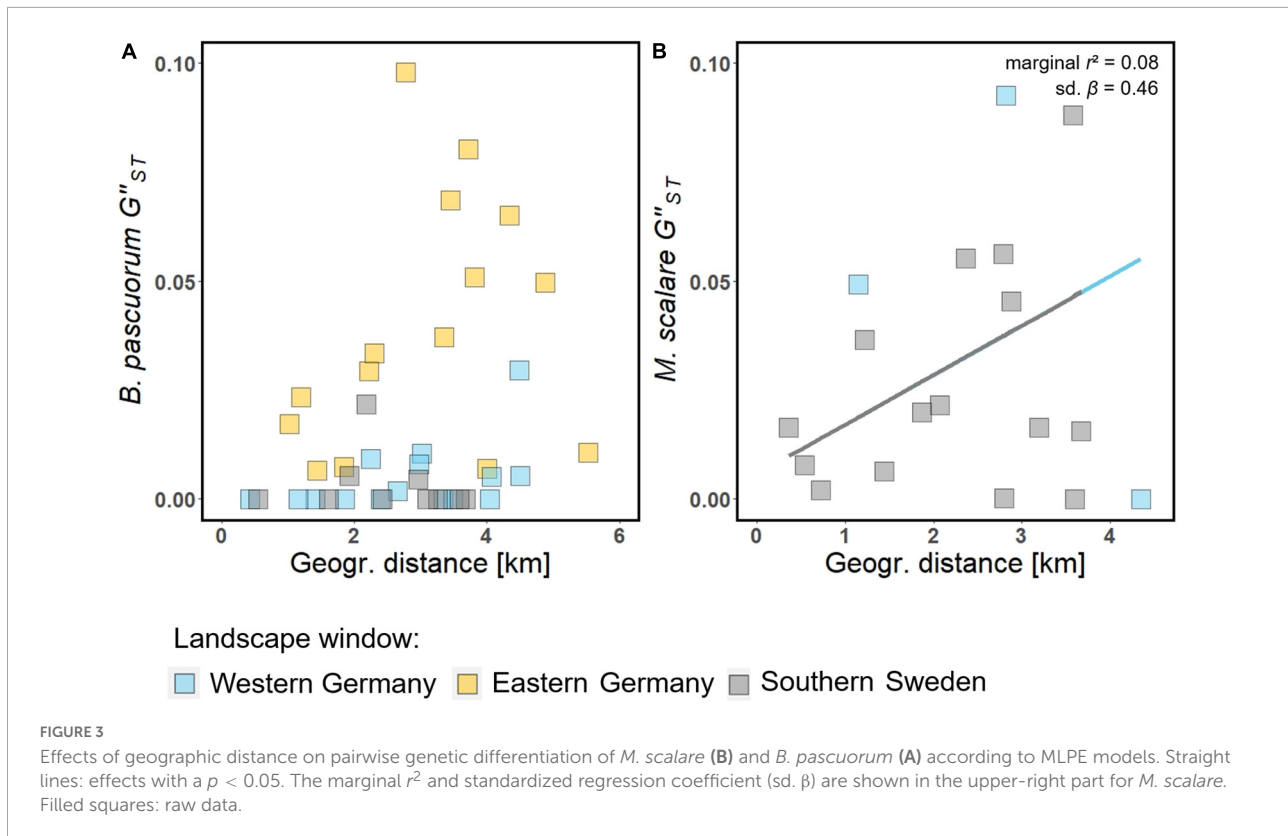| Landscape window and year		N individuals	N detected nests	N NESTS$_{within}$	N NESTS$_{shared}$
Western Germany	2018	58	53	3 (6%)	2 (4%)
	2019	119	97	9 (9%)	13 (13%)
	Both years	177	150	12 (8%)	15 (10%)
Eastern Germany	2018	35	33	1 (3%)	1 (3%)
	2019	104	91	9 (10%)	4 (4%)
	Both years	139	124	10 (8%)	5 (4%)
SouthernSweden	2018	43	42	0	1 (2%)
	2019	49	46	2 (4%)	1 (2%)
	Both years	92	88	2 (2%)	2 (2%)
Total		408	362	24 (7%)	22 (6%)

N NESTS$_{within}$, number of nests with more than one individual within a forest patch; N NESTS$_{shared}$, number of nests that were shared by at least two forest patches. In brackets percentage of N detected nests.

B. pascuorum, we found no effect of geographic distance on D_{PS} or on $G"_{ST}$.

Effects of pollinator movement activity on the genetic diversity of the forest herb populations within forest patches

Indicators of pollinator movement activity had significant effects on the genetic diversity of both forest herb species according to linear mixed models (**Figure 4** and **Supplementary Table 11.4**). For the *P. multiflorum*-*B. pascuorum* pair, five out of 20 movement indicator-genetic diversity measure combinations were significant, with two additional combinations showing a trend ($p < 0.1$). The different indicators of movement activity showed partly contrasting effect directions. With higher $NESTS_{shared}$ and $FOREST\text{-}PATCHES_{shared}$, there were more alleles in a forest herb population (**Figures 4A,B**). In contrast, A_r of *B. pascuorum* had a negative effect on A_r of *P. multiflorum* (**Figure 4F**). F increased with $FOREST\text{-}PATCHES_{shared}$ (**Figure 4C**). A value of $FOREST\text{-}PATCHES_{shared}$ of approximately 4 was close to $F = 0$ for *P. multiflorum*. In contrast, H_e of *B. pascuorum* had a negative effect on F (**Figure 4G**). Similarly, the movement indicators had opposite effects on H_o of *P. multiflorum* (**Figures 4D,H**). The effects of movement indicators on H_o

FIGURE 3

Effects of geographic distance on pairwise genetic differentiation of *M. scalare* **(B)** and *B. pascuorum* **(A)** according to MLPE models. Straight lines: effects with a $p < 0.05$. The marginal r^2 and standardized regression coefficient (sd. β) are shown in the upper-right part for *M. scalare*. Filled squares: raw data.

of *P. multiflorum* were significant and borderline significant, respectively, only when not corrected for multiple testing (**Supplementary Table 11.4**).

For the *A. nemorosa-M. scalare* pair, we found one significant effect out of eight movement indicator-genetic diversity measure combinations, i.e., with a higher H_e of *M. scalare*, H_e of *A. nem*orosa decreased (**Figure 4E**).

Effects of pairwise pollinator movement activity on the genetic differentiation between forest herb populations

Two out of eight MLPE models showed significant effects of pairwise indicators of bumblebee pollinator movement activity on the genetic differentiation of *P. multiflorum* (**Figure 5** and **Supplementary Table 11.5**). The D_{PS} of *P. multiflorum* increased with increasing D_{PS} of *B. pascuorum*. There was no significant effect for *M. scalare-A. nemorosa* pair.
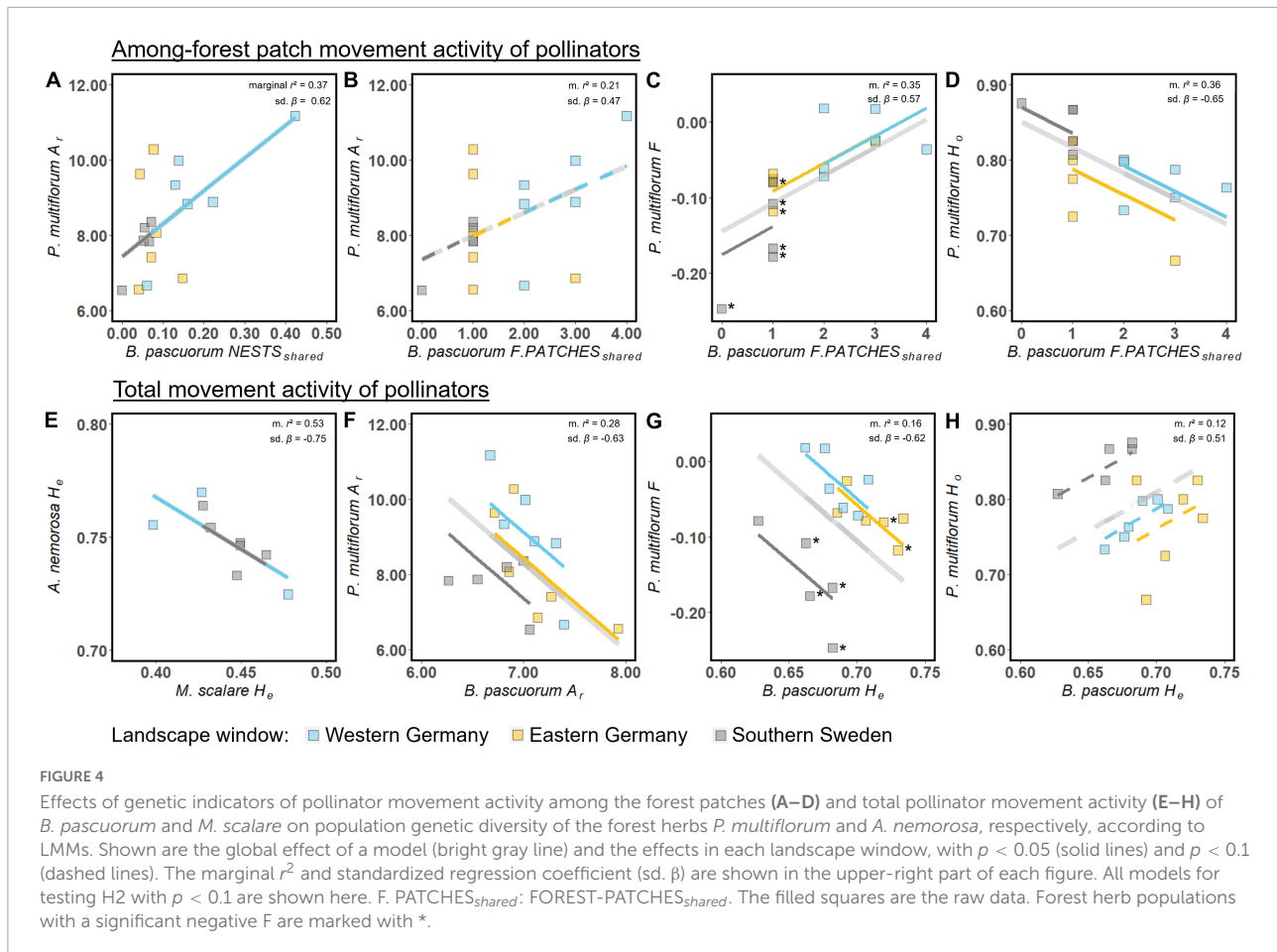
Discussion

Our integrated approach not only supported our assumptions about differences in flight distances and habitat

sensitivity between the two studied pollinator species (H1) but also demonstrated that the movement activity of the pollinators influences forest herb population genetics (H2). Different pollinator movement indicators showed contrasting effects on the forest herbs' genetic diversity and differentiation. The positive effects of movement indicators that solely indicated pairwise movement activity between forest patches (*B. pascuorum*: $NESTS_{shared}$, $FOREST\text{-}PATCHES_{shared}$, D_{PS}) revealed the presence of pollen-mediated gene flow among forest herb populations. This finding is noteworthy since no such effect of genetic linkers for spatially isolated forest herb populations has been shown before. In contrast, the unexpected, negative effects of movement indicators that indicated the total movement activity (*B. pascuorum*: A_r, H_e; *M. scalare*: H_e) could point to an acceleration of genetic drift within forest herb populations. We elaborate our interpretation of the findings in the following paragraphs.

Pollinator types differ in their potential to function as genetic linkers (H1)

Our results confirm that the genetic structuring of the two pollinator species differs. As predicted (P1), the genetic differentiation of the hoverfly, *Melanostoma scalare*, was sensitive to geographic distance on the studied scale and showed

FIGURE 4

Effects of genetic indicators of pollinator movement activity among the forest patches **(A–D)** and total pollinator movement activity **(E–H)** of *B. pascuorum* and *M. scalare* on population genetic diversity of the forest herbs *P. multiflorum* and *A. nemorosa*, respectively, according to LMMs. Shown are the global effect of a model (bright gray line) and the effects in each landscape window, with $p < 0.05$ (solid lines) and $p < 0.1$ (dashed lines). The marginal r^2 and standardized regression coefficient (sd. β) are shown in the upper-right part of each figure. All models for testing H2 with $p < 0.1$ are shown here. F. PATCHES$_{shared}$: FOREST-PATCHES$_{shared}$. The filled squares are the raw data. Forest herb populations with a significant negative F are marked with *.

structuring according to the forest patches. In contrast, we found no such effect of geographic distance on the genetic differentiation of the bumblebee, *Bombus pascuorum*, of which the genetic structure was hardly explained by the forest patches (**Figure 3** and **Supplementary Table 11.2**). This is likely because *M. scalare* covers shorter flight distances than *B. pascuorum* and is more strongly associated with forest habitat (**Supplementary Table 3**). These differences should be reflected in the effects of their movement activity on forest herb population genetics (Breed et al., 2015).
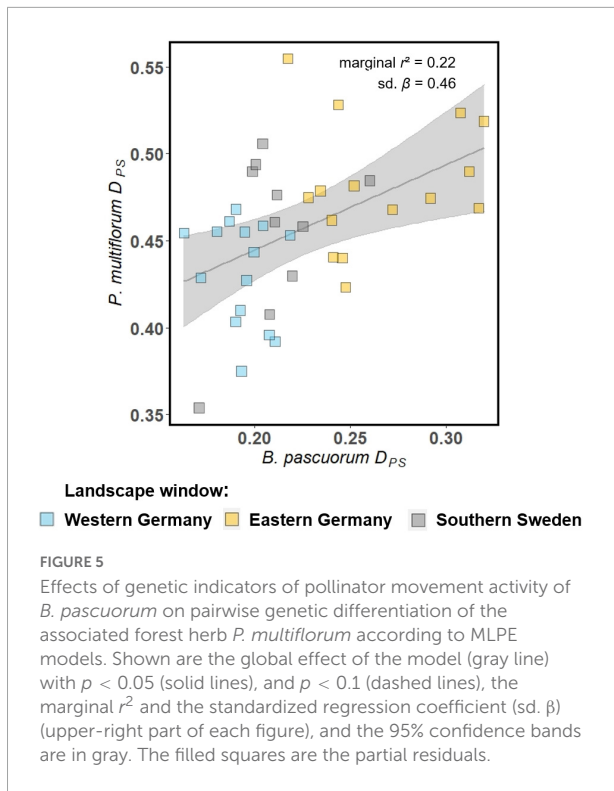
The results for *B. pascuorum* are in line with previous landscape-scale population genetic research on the same species, which showed low genetic differentiation (Chapman et al., 2003) and a weak signal of isolation by distance (Dreier et al., 2014). However, Chapman et al. (2003) only used measures based on heterozygosity, which also showed a weaker signal of differentiation than D_{PS} in our study. In contrast to G_{ST} or G''_{ST}, D_{PS} does not assume any population genetic preconditions such as Hardy–Weinberg equilibrium. Thus, our approach indicates that D_{PS} should be included in future research addressing genetic differentiation among sampling sites within a population of highly mobile organisms on a landscape scale.

Population genetic studies of hoverflies are rare and focused on migratory species on a European scale (Francuski et al., 2013; Raymond et al., 2013). The comparability between these species and *M. scalare* is limited because the migration behavior of hoverfly species (migratory vs. resident) is of central importance for their population structure (Luder et al., 2018).

Bombus pascuorum is a genetic linker of *Polygonatum multiflorum* populations (H2)

Our results indicate that *B. pascuorum* serves as a genetic linker among *P. multiflorum* populations in different forest patches. This is supported by (A) the finding of an unexpectedly high number of forest patches that shared nests of *B. pascuorum*, (B) positive effects of among-forest patch movement indicators of *B. pascuorum* on genetic diversity (A_r) and differentiation (D_{PS}) of *P. multiflorum*, and (C) positive effects of among-forest patch movement indicators of *B. pascuorum* on the *F*-value of *P. multiflorum*.

FIGURE 5

Effects of genetic indicators of pollinator movement activity of *B. pascuorum* on pairwise genetic differentiation of the associated forest herb *P. multiflorum* according to MLPE models. Shown are the global effect of the model (gray line) with $p < 0.05$ (solid lines), and $p < 0.1$ (dashed lines), the marginal r^2 and the standardized regression coefficient (sd. β) (upper-right part of each figure), and the 95% confidence bands are in gray. The filled squares are the partial residuals.

More forests shared nests than expected

We found that 16 out of 17 forest patches shared at least one *B. pascuorum* nest (**Figure 2** and **Supplementary Figure 10**), despite the high percentage of singletons (**Table 5**). This is a higher proportion (94%) than expected as it indicates that the pollinators cover larger distances among forest patches than previously reported forage distances. We only included nests that could be detected in multiple runs. This is a conservative approach. Thus the real number of shared nests might be even higher. The mean distance between all forest patches was 2.83 km ($\pm$ 1.22 km), and the mean distance between forest patches sharing a nest was still 2.42 km ($\pm$ 1.20 km) (**Figure 2**, **Table 5**, and **Supplementary Table 9.3**). These distances appear large in comparison to reported foraging distances of *B. pascuorum*, where distances larger than 1 km are considered rare (Darvill et al., 2004; Knight et al., 2005; Westphal et al., 2006; Redhead et al., 2016). The mentioned studies describe the movement behavior of *B. pascuorum* workers in summer with a high availability of flower resources, while we analyzed their movement behavior in spring. Plants in most other habitats do not flower that early in the year (Proesmans et al., 2019b). Thus, forest herbs are especially important as nectar resources for insect species active early in the year, including *B. pascuorum* (Goulson et al., 2010). Our study indicates that spatially isolated forest herb populations in different forest patches are visited by *B. pascuorum* workers from a shared nest even if geographic distances are over 2 km.

Movement activity of *Bombus pascuorum* indicates inflow of alleles (P2a) and a reduction in genetic differentiation among *Polygonatum multiflorum* populations (P2b)

As predicted (P2a), we found a positive effect of the among-forest patch movement activity of *B. pascuorum* ($NESTS_{shared}$ and $FOREST\text{-}PATCHES_{shared}$) on the A_r of *P. multiflorum* (**Figures 4A,B**). Consistently with prediction 2b, we found that, the pairwise movement indicator (D_{PS}) of *B. pascuorum* positively affected the pairwise genetic differentiation of *P. multiflorum* (D_{PS}). This means that a higher bumblebee movement activity decreases the genetic differentiation of the forest herb. Higher gene flow is considered to homogenize populations (Ellstrand, 2014). Previous research has shown that plant populations associated with more mobile pollinator species are less differentiated (Kramer et al., 2011) and have more diverse pollen pools (Breed et al., 2015; Torres-Vanegas et al., 2019). Our findings add that a higher movement activity of a single pollinator species also results in an increase in the genetic diversity and genetic connectivity of the associated plant.

Bumblebee movement reduces heterozygote excess (P2a)

For the interpretation of the effects of $FOREST\text{-}PATCHES_{shared}$ on the F-value and H_o (**Figures 4C,D**), we have to consider that most populations (15 out of 17) of *P. multiflorum* had a negative F-value, which indicates heterozygote excess. Therefore, the F-value cannot be interpreted as a measure for inbreeding in this case. Reasons for heterozygote excess are diverse and can be of either a methodological or biological nature (Stoeckel et al., 2006). For *P. multiflorum*, there are two possible biological reasons. First, previous work has shown that a negative F-value occurs in populations of clonal species (Reichel et al., 2016), such as *P. multiflorum*. If the populations have a low rate of individuals reaching sexual maturity (Kosiński, 2015), many sampled genotypes reflect a former population structure, when the forest habitat was less fragmented and hosted a higher number of individuals. With a high proportion of clonal reproduction within the population, approaching Hardy-Weinberg equilibrium will take a long time which is reflected in the negative F-value in our populations (Reichel et al., 2016). With a longer time of clonal reproduction the heterozygote excess will even increase by the accumulation of mutations (Stoeckel et al., 2006). Second, the number of inflorescences and the number of flowers within a florescence differs tremendously among *P. multiflorum* individuals within a forest patch. If a low number of individuals dominates recruitment within a forest patch, this will also result in heterozygote excess (Pudovkin et al., 1996; Stoeckel et al., 2006). We found that a higher bumblebee movement activity among the forest patches resulted in F-values of *P. multifl*orum closer to 0, while with a lower movement activity among the forest

patches, F-values became more negative. This means that with a higher pollen-mediated gene flow, the populations approach Hardy-Weinberg-equilibrium faster. This is because pollen influx increases the number of homozygous offspring through sexual recruitment by involving more different pollen donors from more forest patches. This also explains the decrease in H_o of *P. multiflorum* with more pollen-mediated gene flow among forest patches (**Figure 4D**). This dynamic phenomenon counterbalances both the effects of prolonged clonal growth and the effects of a low number of breeders within a forest patch. In our model, we found such a balanced situation, when one forest patch shared *B. pascuorum* nests with four other forest patches reaching Hardy-Weinberg equilibrium ($F = 0$) (**Figure 4C**).

High total pollinator movement activity reduces genetic diversity and increases differentiation of the associated forest herb

The movement indicators representing the total movement activity, including the within-forest patch movement activity (*B. pascuorum*: A_r, H_e; *M. scalare*: H_e), had unexpected effects on the genetic diversity and differentiation of both forest herbs: With a higher total movement activity (A_r, H_e) of the pollinators, the genetic diversity of forest herb populations decreased (**Figures 4E–H**). Theoretically, these patterns could represent non-causal relationships due to the correlation with another, not tested factor, such as a forest patch or landscape attribute. However, we lack any candidate variable that would influence all four species in the observed way particularly, because the two pollinator types had been selected for their contrasting traits concerning their mobility and sensitivity to the agricultural matrix. Thus, we consider it more convincing to interpret the observed effects as signs of accelerated genetic drift in forest herb populations receiving a more intense pollination service. There are two arguments for this interpretation.

First, for a doorstep forager species such as *B. pascuorum*, the within-forest patch movement might constitute a large part of its total movement activity because the forest offers both potential nesting (Svensson et al., 2000) and foraging (Kreyer et al., 2004) habitat (**Supplementary Table 3**). Therefore, high values of the movement indicators A_r and H_e may reflect the within-forest patch rather than the across-landscape movement activity. This interpretation is supported by the additional finding that the A_r of *B. pascuorum* was correlated (r = 0.63 and p = 0.006) with the number of nests with multiple individuals caught within a forest patch (**Table 5** and **Supplementary Table 9.2**). If it is true, high indicator values should point to a high local pollination service rather than a long-distance pollen-mediated gene flow. Previous research showed for *Bombus impatiens* that the abundance of nests per hectare could predict the bumblebees' pollination service for

pumpkins (McGrady et al., 2021), and that the abundance of bumblebee individuals was positively correlated with the seed set of *Trifolium* species as host plants (Geib et al., 2015).

Second, long-living plants can store their genetic diversity over a longer time even under habitat fragmentation (Fuller and Doyle, 2018). This applies in particular to clonal forest herbs, such as *P. multiflorum* (Honnay et al., 2005). If sexual recruitment in these species increases, genetic diversity erodes faster (Honnay et al., 2005; Van Rossum, 2008). In this respect, previous studies have shown this dynamic for small fragmented plant populations (Van Rossum, 2008; Meloni et al., 2013).

Combining both arguments, our results might indicate that a higher within-forest patch movement activity, implying a higher pollination service, leads to higher sexual recruitment (Aguilar et al., 2006). This higher sexual recruitment might cause a faster loss of alleles thus far conserved *via* clonal growth (**Figure 4F**). A higher within-forest patch movement activity of *B. pascuorum* and an increased sexual recruitment should also result in a reduction of heterozygote excess, i.e., a higher F-value. However, as discussed above, it is likely that populations of *P. multiflorum* are dominated by a few larger clones, which serve as pollen donors. In this case, an increase in sexual recruitment would intensify the heterozygote excess. This would be an explanation for the negative effect of H_e of *B. pascuorum* on the F-values of *P. multiflorum* (**Figure 4G**).

Following the same rationale, we may interpret the negative effect of H_e of *M. scalare* on the H_e of *A. nemorosa* (**Figure 4E**) as follows: a high within-forest patch movement activity/pollination service of *M. scalare* indicated by H_e leads to more sexual recruitment of *A. nemorosa* within a forest patch. This accelerates genetic drift and reduces the long-term conservation of alleles through clonal reproduction.

The long-term survival of genotypes and thus populations by clonal reproduction has been discussed as a plant strategy to survive long enough to bridge periods of unfavorable conditions (Eriksson, 1996; Piessens et al., 2005). One example of such unfavorable conditions is the lack of pollinators (Eriksson, 1996). Our findings suggest that this strategy might not be successful under habitat fragmentation if the pollinator movement activity within a habitat patch is high, but an adequate amount of among-habitat patch movement is missing.

Melanostoma scalare is not a genetic linker of *Anemone nemorosa* populations

With our linear mixed models, we found no effect of the movement activity of *M. scalare* that could be linked to pollen-mediated gene flow among *A. nemorosa* populations. This was an expected result since *M. scalare* is a habitat specialist that flies over short distances. We showed, however, that the hoverfly's movement activity within the forest patch

reduced the genetic diversity of *A. nemorosa* (**Figure 4E**). This finding supports the argumentation that *M. scalare* is a relevant pollinator of *A. nemorosa* populations but not a relevant genetic linker among them. Nevertheless, it is important to consider three factors for interpreting this outcome. First, the sample size of *M. scalare* individuals per forest patch was too small for population genetic analysis in half of our forest patches (**Supplementary materials 2, 4**). Therefore, the number of forest patches that could be included in the models for the *M. scalare-A. nemorosa* pair was rather low. Second, our pollinator observations could not definitively confirm a strong association between *A. nemorosa* and *M. scalare* in the forest patches of this study (**Supplementary Figure 2.2**; Naaf et al., 2021). Identifying the most important pollinator of *A. nemorosa* is challenging because its flowering period is very early in the year when the temperatures are still (too) low for most insect pollinators. The number and type of pollinators that visit *A. nemorosa* can vary strongly with the weather conditions in a specific year, especially because the species is a generalist that accepts many different visitor types (see section "Materials and methods": Sampling and identification of the associated pollinators; **Supplementary materials 1–3**). Third, the mean population size of *A. nemorosa* (1,923,304 individuals) was large in comparison to the mean population size of *P. multiflorum* (1,204 individuals). Nevertheless, its mean visitation rate of 2.5 visits/h per transect was lower than the 3.2 visits/h of *P. multiflorum*. In a larger population, the effect of gene flow on genetic diversity and genetic differentiation requires a longer time to become apparent (Varvio et al., 1986). If there are indeed few events in which *M. scalare* transports pollen of *A. nemorosa* among forest patches, the signal may be below the detectability of our sampling design.

Landscape composition might influence the genetic linkage of forest herb populations

The level of movement activity of *B. pascuorum* as well as the levels of genetic diversity and differentiation of *P. multiflorum* populations differed among the agricultural landscape windows. In western Germany, the observed effects indicated the highest level of pollen-mediated gene flow due to bumblebee movement (**Figures 4A–D**, **5**). Western Germany also had the highest total forest and grassland cover, the highest number of forest patches and the lowest crop cover (**Table 1**). These differences in landscape composition may explain the different levels of pollen-mediated gene flow. The foraging behavior of bumblebees is very sensitive to the quality of foraging resources and the available types of habitats (Carvell et al., 2011, 2012). If the landscape composition influences pollinator movement this also affects the population dynamics of the associated plant species (Cranmer et al., 2012). For example, the amount

of fake pollen transported by bumblebees among populations of *Primula vulgaris* was higher above grassland than above cropland (Van Geert et al., 2014). Woodland and grassland, at the expense of cropland in the landscape surrounding *P. multiflorum* populations increased allelic richness and reduced heterozygote excess (Naaf et al., 2021, 2022). It is also likely that our movement indicators reflect a certain landscape composition. Bumblebee nesting and foraging behavior is influenced by the arrangement and distribution of specific land-use types (Cranmer et al., 2012; Kallioniemi et al., 2017). Even though we found no evidence for *M. scalare* being a genetic linker of *A. nemorosa* populations among forest patches, the landscape composition still affects the hoverfly's movement activity. With a very high number of hedgerows, there might also be more movement of forest-specialized hoverflies between the forest patches in spring (Haenke et al., 2014).

Considering landscape effects, we have to take into account that the agricultural landscape itself has been changing over time (Skokanová et al., 2016). This is also true for our three landscape windows (unpublished data). Within the temporal scale applied for population genetic analyses and depicted by microsatellite markers A_r and H_e are assumed to have slightly different reaction times (Epps and Keyghobadi, 2015). Thus, A_r of *P. multiflorum* might reflect a more recent landscape composition than H_e of *A. nemorosa*. A deeper understanding of how different land-use types affect the movement activity of pollinators is crucial if we want to ensure the long-term survival of isolated forest herb populations in agricultural landscapes that depend on pollinators as genetic linkers.

Conclusion

Our results revealed that the bumblebee *B. pascuorum* serves as a genetic linker for the forest herb *P. multiflorum*, while we found no such evidence for the forest-specialized hoverfly *M. scalare,* which is an important pollinator of *A. nemorosa*. For spatially isolated forest herb populations that are connected by bumblebee movement, pollen-mediated gene flow in agricultural landscapes may be present even over distances > 2 km. Genetic links are apparently affected by the landscape composition, which needs further study. We also found hints for both forest herb-pollinator pairs that a higher total pollinator activity might accelerate genetic drift through enhanced sexual recruitment and thus might diminish the temporal buffer that clonal growth provides against habitat fragmentation effects. This should be considered in landscape planning with the aim of increasing pollinator abundance. Conservation measures such as wild flower strips in proximity to forest patches, which aim to increase local pollinator activities would counterintuitively increase the loss of genetic diversity in forest herb populations if it is not counterbalanced by sufficient functional connectivity and by preserving larger forest

habitats. A deeper and more comprehensive understanding of the behavior of genetic linkers will help to manage agricultural landscapes in a way that allows genetic exchange between (semi-)natural habitats.

Data availability statement

The allele tables of the forest herbs are accessible on DRYAD *via* https://doi.org/10.5061/dryad.tb2rbp00k. The allele tables of the pollinators are also accessible on DRYAD *via* doi: 10.5061/dryad.sf7m0cg8w. The r-script for the analysis is stored as **Supplementary material**.

Author contributions

SIJH, TN, and KK conceived and designed the study. JTF, SIJH, TN, SH, and KK performed the molecular lab work. JTF, TN, and SH did the allele calling. JTF analyzed the data and wrote the first draft of the manuscript with contributions of TN, SH, SIJH, and KK. All authors contributed to revisions and involved *in site* selection, field work, and sampling.

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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.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.908258/full#supplementary-material

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