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An Approach to Distinguish between Plasticity and Non-random Distributions of Behavioral Types Along Urban Gradients in a Wild Passerine Bird

Philipp Sprau [1]* **and *Niels J. Dingemanse*** [1, 2]

[1] Behavioral Ecology, Department of Biology, Ludwig-Maximilians University of Munich, Munich, Germany, [2] Research Group Evolutionary Ecology of Variation, Max Planck Institute for Ornithology, Seewiesen, Germany

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****Correspondence:***
Philipp Sprau
philippsprau@gmx.de

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The impact of urbanization has been widely studied in the context of species diversity and life history evolution. Behavioral adaptation, by contrast, remains poorly understood because empirical studies rarely investigate the relative importance of two key mechanisms: plastic responses vs. non-random distributions of behavioral types. We propose here an approach that enables the simultaneous estimation of the respective roles of these distinct mechanisms. We investigated why risky behaviors are often associated with urbanization, using an urban nest box population of great tits (*Parus major*) as a study system. We simultaneously and repeatedly quantified individual behavior (aggression and flight initiation distance) as well as environmental factors characterizing level of urbanization (numbers of pedestrians, cars and cyclists). This enabled us to statistically distinguish plastic responses from patterns of non-random distributions of behavioral types. Data analyses revealed that individuals did not plastically adjust their behavior to the level of urbanization. Behavioral types were instead non-randomly distributed: bold birds occurred more frequently in areas with more cars and fewer pedestrians while shy individuals were predominantly found in areas with fewer cars and more pedestrians. These novel findings imply a major role for behavioral types in the evolutionary ecology of urban environments and call for the full integration of among- and within-individual variation in urban ecological studies.

Keywords: among-individual variation, phenotypic plasticity, personality, urbanization, great tit, within-individual variation

INTRODUCTION

Urbanization represents one of the fastest environmental changes of our times (United Nations, Department of Economic and Social Affairs, and Population Division, 2017) and poses novel challenges to wildlife (Sih et al., 2011). Urban habitats are characterized by a loss of natural resources (e.g., food) and increased anthropogenic levels of disturbance (e.g., pedestrian and vehicular traffic, and industrial noise). There is a growing body of literature describing how animal populations are affected by urbanization (Marzluff, 2008). In birds, for instance, breeding densities are often higher in urban compared to natural habitats (Beissinger and Osborne, 1982). Furthermore, several studies have reported advanced lay dates, smaller clutch sizes and decreased offspring production in urban compared to rural habitats (reviewed in Chamberlain et al., 2009). More recently, research has focussed on behavioral modifications in urban wildlife (Lowry et al., 2013). Yet, the mechanisms responsible for associations between behavior and level of urbanization remain largely unknown.

Phenotypic and environmental variation are often associated (Stearns, 1989). Relationships between phenotypes and environments can be caused by various distinct mechanisms such as phenotypic plasticity, differential settlement, and/or the selective (dis)appearance of distinct types of individuals (Dingemanse et al., 2010). Several studies have demonstrated behavioral plasticity in response to urbanization (Lefebvre, 1995; Seferta et al., 2001; Bouchard et al., 2007; Levey et al., 2009). Song birds such as nightingales (*Luscinia megarhynchos*) and great tits (*Parus major*) have been shown to adjust song amplitude (Brumm, 2004) and frequency (Slabbekoorn and den Boer-Visser, 2006) to background noise. Phenotypes, however, also vary among individuals. Repeatable differences among individuals have been demonstrated for a variety of behaviors such as aggressiveness, boldness and exploration (Bell et al., 2009; Garamszegi et al., 2012; Holtmann et al., 2017). These particular types of behavioral traits have recently also been explicitly linked to urbanization. For instance, urban male song sparrows (*Melospiza melodia*) are bolder and more territorial than their rural conspecifics (Evans et al., 2010). Boldness reflects the tendency of an individual to take risks (Wilson et al., 1994; Réale et al., 2007). Accordingly, bold individuals are often considered to cope better with risky and novel situations and may therefore be favored in highly disturbed areas such as cities.

Importantly, behavioral traits vary simultaneously among and within individuals, due to individual variation (aka "personality") and plasticity (Dingemanse et al., 2010). Therefore, associations between behavior and urbanization can similarly originate from multiple processes. This can be usefully illustrated by viewing behavior in terms of reaction norms (Nussey et al., 2007; Dingemanse et al., 2010), where each individual is characterized by a reaction norm intercept (representing its average behavior in the average environmental condition, or behavioral "type"), and a reaction norm slope, representing its level of response (plasticity) to changes in urbanization (**Figure 1**). Viewing behavior in this way clarifies that the relationship between behavior and urbanization is the result of the combined effects of phenotypic plasticity and non-random distributions of behavioral types over urban gradients. Previous studies have not distinguished between these two mechanisms in

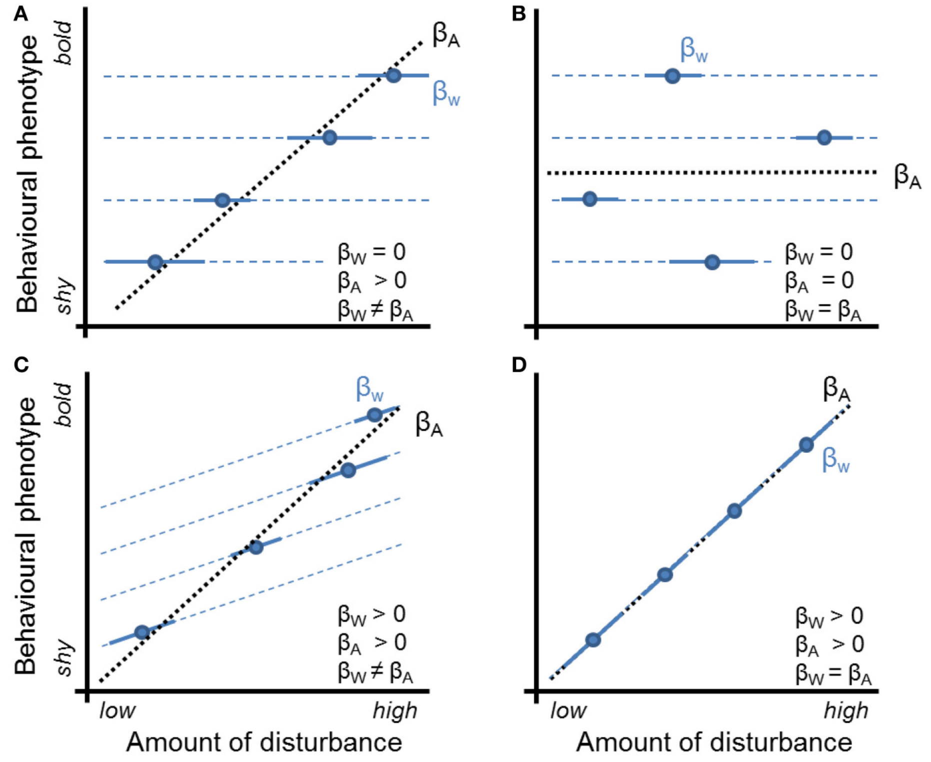

FIGURE 1 | Behavioral reaction norm plots, providing examples of possible relationships between anti-predator boldness (an exemplary behavioral phenotype) and an environmental gradient (e.g., amount of disturbance) among- vs. within-individuals. Each dot represents the average phenotype of an individual and each solid blue line represents its behavioral reaction norm over the range of environmental conditions (x-axis) that it occurred observationally. Among-individual variation in intercepts represents "personality variation" (*sensu* Dingemanse et al., 2010). Non-zero slopes represent within-individual plasticity (i.e., the observed relationship between y and x within the individual: β_W, blue lines). In contrast, blue dashed lines represent the hypothesized phenotype of each individual outside the range of environments in which it occurred observationally. The thick black dashed line represents the relationship between each individual's average behavior and average environment (β_A). Plots **(A–D)** show different scenarios for how behavior and the environment might be associated within and between individuals as detailed in the Introduction.

empirical demonstrations of relationships between behavior and urbanization.

We conceive four scenarios for how behavior and level of urbanization may be associated, though we recognize that further scenarios could be proposed. In the first scenario, individuals are non-randomly distributed over an urban gradient with respect to their reaction norm intercept (i.e., average behavior, or behavioral type) while they simultaneously do not respond plastically to changes in urbanization. This scenario is illustrated in **Figure 1A**, where individuals with high intercept values for boldness are more likely to occur in more disturbed areas, leading to a positive among-individual effect of disturbance on behavior (i.e., $\beta_A > 0$, A for "among") and a zero within-individual effect (i.e., $\beta_W = 0$, W for "within"). In the second scenario, individuals are instead randomly distributed over an urban gradient with respect to their intercept (i.e., behavioral type) while simultaneously not responding plastically to changes in urbanization. This scenario is illustrated in **Figure 1B**, showing a zero among- ($\beta_A = 0$) and a zero within-individual effect ($\beta_W = 0$). In the third scenario, individuals are both non-randomly distributed over an urban gradient with respect to their intercept while also responding plastically to changes in urbanization. This scenario is illustrated in **Figure 1C**, where individuals with high intercept values for boldness (i.e., bold behavioral types) are more likely to occur in more disturbed areas, leading to a nonzero among-individual effect of disturbance on behavior (i.e., $\beta_A > 0$), while individuals also alter their phenotype in response to changes in level of disturbance, leading to a nonzero within-individual effect (i.e., $\beta_W > 0$). In both scenarios 1 and 3, importantly, the effect of non-random distributions of behavioral types causes a difference between the among- and within-individual effect of the environmental gradient (van de Pol and Verhulst, 2006) (specifically, $\beta_W < \beta_A$ in both examples). In the fourth scenario, individuals do not differ in intercept (i.e., all have the same behavioral type) but they do respond plastically to changes in urbanization. This scenario is illustrated in **Figure 1D**, where individuals up-regulate their boldness with increasing levels of disturbance (i.e., $\beta_W > 0$), and where among-individual relationships between boldness and disturbance exist solely because each individual happens to experience a narrow range of environmental conditions, causing among-individual variance in the average level of disturbance experienced (i.e., $\beta_A > 0$). As a single mechanism causes variation at both levels, environmental effects on behavior do not differ between hierarchical levels (van de Pol and Verhulst, 2006) (i.e., $\beta_A = \beta_w$ in **Figure 1D**). In summary, our sketch of alternative scenarios clarifies that conclusions regarding non-random distributions of behavioral phenotypes over urban gradients warrants repeated observations of both behavior and environmental factors such that level-specific effects of urbanization on behavior can be statistically teased apart (**Figure 1**).

We investigated the mechanisms causing relationships between urbanization and behavior in great tits. We simultaneously and repeatedly quantified an individual's behavior and environment. That is, we repeatedly exposed the same individual to territorial intrusion experiments to measure aggressiveness (Araya-Ajoy and Dingemanse, 2014) and to flight initiation distance experiments to measure boldness (Blumstein, 2003), while quantifying aspects of urbanization during each test (numbers of pedestrians, cars and cyclists). This enabled us to statistically distinguish within-individual plasticity from patterns of non-random distributions of behavioral types (**Figure 1**). Human activity is known to alter behavioral phenotypes in animals (Fernandez-Juricic and Schroeder, 2003; Slabbekoorn and Peet, 2003). Based on previous literature (Brumm, 2004; Evans et al., 2010), we expected that birds would plastically up-regulate aggressiveness and down-regulate flight initiation distance with increasing levels of disturbance, and that more aggressive and bolder individuals would occur predominantly in areas with higher levels of disturbance (**Figure 1C**).

METHODS

Study Site

We studied an urban nest box population of great tits consisting of 157 nest boxes along urban-to-rural gradients in the city of Munich, Germany (48° 8′ 6.45" N 11° 34′ 55.132" E) during the breeding seasons of the years 2014 and 2015 (Sprau et al., 2016). Nest boxes were located in the entire city area of Munich (20 × 27 km^2) and covered a large range of human disturbance from highly disturbed habitats in the city center to relatively undisturbed habitats in sub-urban areas. All nest boxes were checked at least once per week from mid-March onwards and key fitness components quantified (e.g., lay date, clutch size, brood size, and number of fledged offspring). When the nestlings were 7–9 days old, parents were caught with a spring trap in the nest box, measured, and ringed if not previously captured.

Experimental Protocol

We quantified two behaviors both of which were assayed repeatedly for the same set of breeders: aggressiveness and boldness (measured as flight initiation distance; FID). Simulated territorial intrusions (i.e., aggression tests) were performed for all first broods found in our nest boxes by simultaneously presenting the male owner with a visual stimulus (a taxidermic mount of a male great tit) and an acoustic stimulus (a playback song) (as detailed in reference Araya-Ajoy and Dingemanse, 2014). In each year, each male was subjected to three aggression tests (between 7.30 and 15.00 h) when its mate was in the egg-laying phase (1, 3, and 5 days after its first egg was observed). The taxidermic mount was presented 1 m away from the subject's nest-box at 1.2 m height. We subsequently recorded the behavior of the subject for a period of 3 min after it had entered a 15-m radius around the nest box. Details of the experimental set up, and assayed behaviors, are provided in reference Araya-Ajoy and Dingemanse (2014). In short, an aggressive response was characterized by intensive alarm calling, approach to the stimulus, and, in the most extreme case, jumping and pecking of the cage that protected the mount. Here and elsewhere (Araya-Ajoy et al., 2016; Araya-Ajoy and Dingemanse, 2017) we used the subject's minimum approach distance to the mount as a measure of aggressiveness because previous work implied that this behavior represents a reliable predictor of the intensity of aggression. Subjects that did not arrive within 10 min were scored as non-responsive, and those data were thus not taken forward for analyses (Araya-Ajoy and Dingemanse, 2014, 2017). We used six mounts and 11 playback song stimuli (recorded from German great tits populations; sampling frequency: 44.1 kHz; resolution: 16 bit). One mount and one song stimulus (broadcasted with a Ligno Xtatic V2 Digital Soundsystem) were randomly allocated to each test (following Araya-Ajoy and Dingemanse, 2014). Songs were played back at 85 dB (measured at one meter from the sound source (Brumm, 2004). One of nine observers performed the experiment at a distance of 15 m. We performed 333 aggression tests with 107 unique males.

The occupants of each nest box were also subjected to three FID-tests during the nestling phase of their first brood (10, 12 and 14 days after the nestlings had hatched). FID-tests were conducted between 8:00 and 16:00 h. After identifying (by color ring combination) the focal individual as the male or female parent, FID was measured by walking at a constant speed toward a bird from a starting distance of 15 m away from the nest box (Blumstein, 2003). We used a laser distance meter (Bosch PLR 25) to quantify distance to the mount during the aggression tests, and start and flight initiation distance. Overall, we performed 308 flight initiation tests, on 59 females and 54 males. Note that because of nest failure prior to the onset of the FID-tests, the number of FID-tests is lower than the number of the aggression tests. Five and four individuals were assayed, respectively, for aggressiveness and flight initiation distance in both years of study. This study was carried out in accordance with the ethical guidelines of the Tierschutzgesetz (TierSchG, German animal protection law), and approved by the Regierung Oberbayern (55.2-1-54-2532.2-7-07).

The level of urbanization at each nest box was quantified by measuring human activity (the number of pedestrians, cyclists and cars, Table S1). Human activity was measured within a range of 15 m from the nest box for 2 min following each behavioral assay (detailed above).

Statistical Analyses

We performed a principal component analysis (PCA) with varimax rotation ("prcomp" function of Package "stats" version 3.1.27 of R version 3.1.2) to ask whether our indexes of human activity (number of pedestrians, bikes and cars) could be summarized into a single axis (principal component) representing an urban gradient (Table S1).

We fitted univariate mixed-effect models to simultaneously estimate sources of variation in behavior within and among individuals (Dingemanse and Dochtermann, 2013) ("lmer" function of Package "lme4"version 1.1-10 of R). We investigated sources of variation in each of the two focal behaviors (aggressiveness and flight initiation distance) separately. Random intercepts were included for subject and observer identity, enabling us to partition the total variance into variance attributable to individual, observer, and within-individual-within-observer residual. Start distance (covariate: only for flight initiation distance; meter), year (factor: 2014 vs. 2015), time of day (factor: morning vs. afternoon trial), and test sequence (covariate; within-individual test-day number; first vs. second vs. third test day) were fitted as fixed effects. The PCA resulted in two components (PC1 and PC2) describing two orthogonal axes of human activity. As detailed in the Introduction (**Figure 1**), we considered that environmental effects on behavior could vary within and among individuals (van de Pol and Wright, 2009). Specifically, a within-individual effect of the environment on behavior represents evidence for within-individual phenotypic plasticity, while the difference between among- and within-individual effects represents statistical evidence for non-random distributions of behavioral types over environments (van de Pol and Wright, 2009; Dingemanse and Dochtermann, 2013). We thus calculated (1) each individual's average value ($\bar{x}_j$) for each of the two environmental variables (PC1 and PC2) as well as (2) each observation's deviation of these individual average values ($x_{ij} - \bar{x}_j$) effects, and fitted both as part of the statistical model detailed above (van de Pol and Verhulst, 2006; van de Pol and Wright, 2009). We then reformulated the model to test whether the effect of the focal environmental axis differed between the within- and among-individual levels. Therefore, instead of fitting $\bar{x}_j$ and ($x_{ij} - \bar{x}_j$) and each individual's average value ($\bar{x}_j$) instead, such that the former estimated the within-individual effect (β_W) (see formulae 3 in van de Pol and Wright, 2009). This enabled us to statistically assess the evidence for non-random distributions of behavioral types over environments (which would be the case provided that ($\beta_A - \beta_W$) $\neq 0$). We note that the within-subject centering approach used here has been criticized because values of $\bar{x}_j$ are estimated with error, which causes estimates of the among-individual slope (β_A) to be biased toward the within-individual slope (β_W) in datasets with a low numbers of repeats per individual (Ludtke et al., 2008), such as ours. This means that differences (Δ) between among- and within-individual effects (i.e., $\beta_A - \beta_W$), as well as associated levels of significance, represent conservative estimates. We assumed a Gaussian error distribution for aggression and boldness, which was confirmed by visual inspection of model residuals. All covariates were further centered on their mean value (Kreft et al., 1995). For each specified relationship, we calculated the parameter estimate with its associated 95% credible interval (calculated using the function "quantile"; Package "stats"version 3.1.2 in R). Credible intervals not including zero indicate statistical significance (i.e., $p < 0.05$) in the frequentist's sense.

RESULTS

Axes of Environmental Variation

PCA applied to summarize variation in the number of pedestrians, cyclists, and cars, resulted in two significant principle components (PCs) that jointly explained 81% of the variance (Table S2). PC1 (Eigenvalue: 1.40; explained variance: 47%) loaded negatively on the number of bikes, cars, and pedestrians (Table S1); high values of PC1 were thus indicative of lower levels of human activity in general. PC2 (Eigenvalue: 1.04; explained variance: 35%) loaded negatively on the number of cars (−0.76) but positively on the number of pedestrians (0.65), and thus seemed to differentiate between streets differing in the primary means of transportation (e.g., larger streets suitable for cars vs. smaller streets suitable for pedestrians).

Phenotypic Plasticity and Non-random Distributions of Behavioral Types

Our analyses of the sources of variation in behavior, which focussed on the simultaneous estimation of within-individual (β_W) and among-individual (β_A) effects of environmental variables related to urbanization (**Figure 1**), demonstrated that individuals did not plastically adjust their aggressiveness nor their flight initiation distance (FID) in response to within-individual-among-day variation in PC1 or PC2 (Table 1). All models controlled for variation induced by aspects of the experimental design (starting distance, test sequence, time of day, and year), which were generally not of major importance (**Table 1**).

As a next step, we re-parameterised our models to directly estimate the difference (Δ) between the among- and within-individual effects ($\beta_A - \beta_W$) of each focal gradient as a test for non-random distributions of behavioral types (van de Pol and Verhulst, 2006; van de Pol and Wright, 2009). This analysis produced strong evidence for non-random distributions of behavioral types with respect to FID because the difference (Δ) in effect of PC2 among- vs. within-individuals was associated with 95% CIs that did not overlap zero (mode: 0.28; 95% CIs: 0.03, 0.56; **Table 1**). The analysis implied that individuals that allowed observers to approach closer (i.e., "bolder" birds) were overrepresented in areas with more cars and fewer pedestrians, whereas "shyer" birds were more likely found in areas with fewer cars and more pedestrians (**Figure 2**). By contrast, there was no strong evidence for non-random distributions of aggressiveness types as the 95% CIs overlapped zero for all tested differences (Δ) between the within- and among-individual levels for aggressive behavior (**Table 1**). Notably, there was some support for non-random distributions of aggressiveness types with respect to PC1 as the CIs associated with the difference (Δ) between levels for this gradient slightly overlapped zero (95% CIs: −0.001, 0.38) (**Table 1**), suggesting that aggressive types were perhaps overrepresented in areas with more cyclists, cars, and pedestrians (**Table 1**).

TABLE 1 | Sources of variation in boldness and aggressiveness.

	Boldness (Flight initiation distance)		**Aggressiveness (Minimal approach distance)**	
Fixed effects	**Estimate**	**95% CIs**	**Estimate**	**95% CIs**
Intercept	−0.57	−0.93, −0.19	0.05	−0.22, 0.34
PC1				
Within individuals	−0.14	−0.34, 0.06	−0.06	−0.23, 0.13
Among individuals	0.07	−0.08, 0.22	0.13	−0.02, 0.27
Δ (Among—within)	0.19	−0.01, 0.42	0.19	−0.001, 0.38
PC2				
Within individuals	−0.12	−0.41, 0.16	0.12	−0.23, 0.46
Among individuals	0.17	0.04, 0.30	−0.05	−0.22, 0.10
Δ (Among - within)	0.28	0.03, 0.56	−0.17	−0.55, 0.17
Start distance	−0.11	−0.22, 0.01	n.a.	n.a.
Year	−0.13	−0.57, 0.30	0.12	−0.15, 0.38
SEQUENCE (FIRST TEST AS REFERENCE)				
Second test	0.25	−0.04, 0.54	−0.05	−0.37, 0.23
Third test	0.23	−0.07, 0.56	−0.08	−0.4, 0.21
Time of day	−0.09	−0.26, 0.07	0.04	−0.09, 0.17
Random effects	**σ^2**	**95% CIs**	**σ^2**	**95% CIs**
Individual	0.013	0.01, 0.02	0.017	0.01, 0.02
Observer	0.20	0.12, 0.42	0.04	0.02, 0.08
Residual	0.65	0.56, 0.84	0.91	0.80, 1.14

We test here for within- and among individual effects of cyclists, cars, and pedestrians (summarized in PC1 and PC2, see Table S1). All models control for variation induced by various aspects of the experimental design (starting distance, test sequence, time of day, and year) and included random intercepts for subject individual and observer identity. We also present the difference (Δ) between among and within-individual effects derived from the same statistical model reformulated following reference (van de Pol and Wright, 2009). Parameter estimates and are provided with 95% credible intervals (CIs).

DISCUSSION

Our study revealed that behavioral types, with respect to flight initiation distance, were non-randomly distributed over an urban gradient while individuals did not plastically adjust their behavior in response to changes in urban gradients experienced across repeated observations (days). Bolder birds (i.e., birds that could be approached by humans closely) were overrepresented in areas with more cars and fewer pedestrians, whereas shyer birds were more likely found in areas with fewer cars and more pedestrians (**Figure 2**). These findings imply that associations between behavior and urban gradients vary across hierarchical levels, in this case within and among individuals, and that meaningful conclusions regarding non-random distributions of "personality" types over urban environments thus require repeated measures study designs and variance partitioning approaches, as applied in this study.

Human activity is known to affect behavior and abundance of animals (Gill et al., 1996; Fernandez-Juricic, 2000); there is considerable evidence that birds living in highly disturbed areas are more tolerant of humans than their conspecifics living in less disturbed areas (Moller, 2008; Evans et al., 2010; Scales et al., 2011; Clucas and Marzluff, 2012). In a similar vein, recent studies have shown that birds in more disturbed environments may display higher levels of territorial and defensive behaviors (Cilento and Jones, 1999; Evans et al., 2010; Fokidis et al., 2011; Scales et al., 2011). Most of these studies, however, have solely focused on differences in behavioral phenotypes at the population level. Researchers have only recently begun to assess individual variation in the context of urban ecology (Miranda et al., 2013). In this study we shed new light on relationships between urbanization and behavioral phenotypes by partitioning variation in behavioral phenotypes into within- and among-individual components; this enabled us to investigate the relative roles of distinct mechanisms causing such associations. The applied approach allowed us to simultaneously assess whether individuals responded plastically to urban gradients and whether behavioral types were non-randomly distributed over urban gradients (**Figure 1**). Our findings reveal that behavioral types were indeed non-randomly distributed along a key axis of

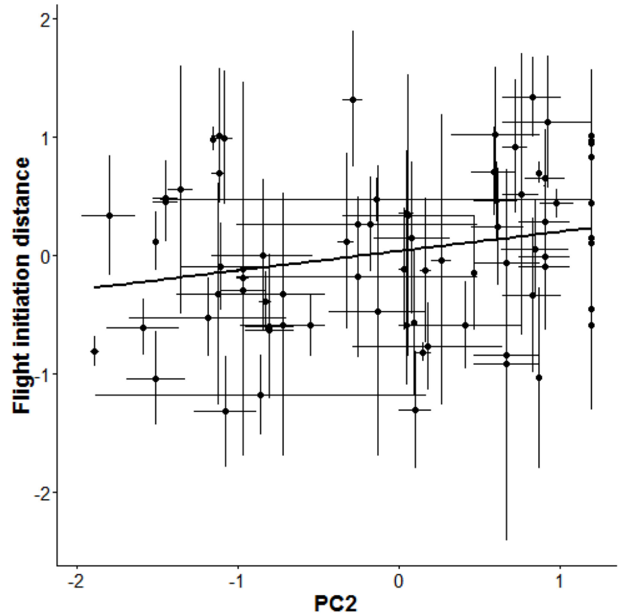

FIGURE 2 | Among-individual variation in flight initiation distance (FID) as a function of the compound environmental gradient PC2 that combines number of pedestrians and cars (more positive loadings represent more pedestrians and lower loadings more cars). As individuals do not plastically respond to changes in PC2 (**Table 1**), the relationship implies a non-random distribution of individual-level behavioral type along this urban gradient. The thick black line presents the regression line between FID and PC2 based on the fitted GLMM (**Table 1**). Shown are average scores ± SE.

urbanization (**Figure 2**): bolder individuals, i.e., birds that could be approached by humans closely, were predominantly found in areas with more cars, whereas shyer individuals were found more often in areas with more pedestrians. The documented effect might be explained by sensory constraints caused by traffic noise. We tested this *post-hoc* explanation by analyzing noise measurements that were taken during each test (detailed in the Supplementary Material), which demonstrated that noise neither affected FID nor aggression (Table S3).

Non-random distributions of behavioral types along environmental gradients have previously been documented in eastern chipmunks (*Tamias striatus*) where more explorative and docile individuals occupy habitats that experience the highest rates of human disturbance (Martin and Réale, 2008). Accordingly, bold animals might have an innately higher disturbance tolerance level than shy individuals. In urban environments, cars usually impose high risks as evident from high numbers of road kills (Spellerberg, 1998; Benitez-Lopez et al., 2010). In contrast, pedestrians may impose lower disturbance levels because birds quickly habituate to humans. Because bold individuals are often considered to cope better with risky situations (Smith and Blumstein, 2008), bold phenotypes in our study may be selected for in high-traffic environments because bolder individuals are more successful in colonizing such environments. Alternatively, bold phenotypes might be outcompeted by shy phenotypes for preferred types of territories and hence settle in these types of areas. It is also possible that prolonged exposure to particular environments permanently affects an individual's behavioral type, resulting in birds becoming bolder in high-traffic environments by means of developmental or other forms of plasticity with permanent effects. Experimental tests are therefore now required to address whether the non-random distributions of behavioral phenotypes documented in our study were caused by non-random settlement, habitat- and type-specific survival, or irreversible plasticity in response to long-term exposure to urban environmental effects. Similarly, we studied a very specific component of the urban environment, focusing on human traffic. Whether the relationships between behavior and aspects of urbanization shown in this paper apply generally to other components of urbanization remains to be evaluated by future studies.

Surprisingly, great tits did not show any sign of a plastic response to day-to-day variation in urban environmental gradients. It is possible that exposure to high levels of human disturbance for prolonged periods triggers habituation (see above) and consequently reduces short-term plastic responses. Such effects may suggest that birds experience only minor fluctuations in environmental conditions, and that each bird's characteristic level of urbanization is relatively stable. In urban environments, such a scenario seems unlikely as the numbers of cars, pedestrians and cyclists, in fact, varied substantially, for instance, between workdays and weekends. Temporal variation between workdays and weekends has in fact previously been shown to cause plastic adjustments in other behaviors (Brumm, 2004). We therefore conclude that phenotypic adjustments to day-to-day variation in human disturbance might well differ between behavioral traits, perhaps because the costs or limits associated with phenotypic plasticity are trait-specific (DeWitt et al., 1998; Auld et al., 2010). Our recent studies on aggressiveness, for example, demonstrated that this particular behavior (which birds did not plastically adjust to changes in human disturbance; **Table 1**) is also not plastically adjusted to population density (Araya-Ajoy and Dingemanse, 2017) or perceived predation risk (Abbey-Lee et al., 2016). Overall, the lack of evidence for within-individual plasticity suggests that its role in urban ecology may be more modest than previously anticipated (Lowry et al., 2013). At the same time, urbanization seems to drive non-random distributions of behavioral types via mechanisms yet to be revealed. Our study thereby demonstrates the importance of partitioning behavioral variation across hierarchical levels (Han et al., 2016; Moirón et al., 2016; Nicolaus et al., 2016), both in urban and other behavioral ecological studies, and the novel insights that may be gained by doing so.

In conclusion, we showed for great tits breeding in the city that behavioral types were non-randomly distributed over an urban environmental gradient. Based on these findings, future research should investigate whether non-random distribution of types is caused by selective appearance (i.e., differential settlement), selective disappearance (i.e., natural selection) or urbanization-related behavioral modification (i.e., developmental or other forms of irreversible plasticity).

AUTHOR CONTRIBUTIONS

PS and ND conceived and designed the study, conceived the statistical approach, and wrote the manuscript

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fevo.2017.00092/full#supplementary-material

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