Title: The city and forest bird flock together in a common garden: genetic and environmental effects drive urban phenotypic divergence

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Abstract

Urban phenotypic divergences are documented across diverse taxa, but the underlying genetic and environmental drivers behind these phenotypic changes are unknown in most wild urban systems. We conduct a common garden experiment using great tit (*Parus major*) eggs collected along an urbanization gradient to: 1) determine whether documented morphological, physiological, and behavioural shifts in wild urban great tits are maintained in birds from urban and forest origins reared in a common garden (N = 73) and 2) evaluate how different sources of genetic, early maternal investment, and later environmental variation contributed to trait variation in the experiment. In line with the phenotypic divergence in the wild, common garden birds from urban origins had faster breath rates (i.e., higher stress response) and were smaller than birds from forest origins, while wild differences in aggression and exploration were not maintained in the experiment. Differences between individuals (genetic and environmentally induced) explained the most trait variation, while variation among foster nests and captive social groups was limited. Our results provide trait-specific evidence of evolution in an urban species where genetic change likely underlies urban differences in morphology and stress physiology, but that urban behavioural divergences are more strongly driven by plasticity.

Keywords: urbanization, urban gradient**,** common garden experiment, plasticity, genetic differentiation, great tits

Introduction

Various evolutionary and ecological processes shape the phenotypic diversity we observe in nature and lead to phenotypic divergences between populations (Mitchell-Olds et al. 2007; Hendry 2017). For instance, local selection pressures on heritable traits can lead to divergent adaptation to local environmental conditions (Kawecki and Ebert 2004). Developmental or reversible phenotypically plastic responses to local environments can also drive phenotypic adjustments (Ghalambor et al. 2007). Determining how local adaptation and plasticity interact to shape phenotypes is crucial as these processes can have different impacts on demographic and evolutionary trajectories of wild populations (Ghalambor et al. 2007; Snell-Rood 2013; Nicolaus and Edelaar 2018).

Some of the most striking examples of phenotypic diversity occur along urbanization gradients, such as urban shifts in multiple taxa towards smaller body sizes (Merckx et al. 2018). Such phenotypic shifts in urban populations are frequently documented across diverse taxa and traits (Szulkin et al. 2020; Diamond and Martin 2021), through changes in both phenotypic means (Miranda et al. 2013; Lambert et al. 2021) and more recently in phenotypic variation (Capilla-Lasheras et al. 2022; Thompson et al. 2022). Urban phenotypic divergences are commonly assumed to be driven by genetic change via selection, but there is still a lack of evidence that urban organisms are adapting to these novel urban conditions (Lambert et al. 2021) and plasticity could play a major role in urban phenotypic change (Yeh and Price 2004; Hendry et al. 2008). Determining the mechanisms behind phenotypic changes in urban organisms could importantly inform on whether urban populations will continue to adjust in pace with further environmental change. For these reasons, there have been several calls for research that disentangle the genetic and plastic contributions on urban phenotypes and, more specifically, calls for urban common garden experiments (Rivkin et al. 2019; Lambert et al. 2021; Sanderson et al. 2023).

Common garden experiments are a useful approach for exploring the genetic basis of phenotypic differences between populations. These manipulations rear individuals from different populations under the same environmental conditions from very early life stages, and ideally across several generations. As individuals develop and mature under common conditions, phenotypic differences that persist in this context should reflect underlying genetic differentiations rather than plastic responses to environmental conditions (Lambert et al. 2021). Thus, common gardens can help determine whether evolutionary (i.e., genetic) change drives documented phenotypic divergences in wild populations, and can be used to explore interactions between genetic and plastic changes acting in these systems (Conover et al. 2009). For these reasons, common garden approaches are needed in urban evolution research to explore the potential processes acting in these contexts (Alberti et al. 2017; Sanderson et al. 2023).

We censused 77 common garden studies with urban populations in the literature; an impressive number despite the effort and resources these experiments require (see synthesis in Table 1). Most of these studies have been published within the last ten years (83%, N = 64) and many support genetic divergence underlying shifts in urban phenotypes (86%, N = 66), which could indicate local adaptation to urban conditions via evolution. Fewer studies document plasticity to environmental conditions as a driver of phenotypic change (58%, N = 45), but this conclusion is especially common in multi-treatment common gardens where individuals are reared under multiple environmental treatments (e.g., temperature treatments). Experiments so far tend to use invertebrate or plant models (75%, N = 58), likely as these organisms are more easily reared, reproduced, and manipulated in captive environments. Many studies have focused on physiological phenotypes associated with tolerance to temperature as the urban heat island effect is known to increase temperature and heat stress in urban environments (Mohajerani et al. 2017). For example, urban damselflies (*Coenagrion puella*), water fleas (*Daphnia magna*), wood louse (*Oniscus asellus*), and acorn ants (*Temnothorax curvispinosus*) have higher heat tolerance compared to nonurban conspecifics when reared under common conditions, providing evidence that urban invertebrates have adapted to urban heat islands (Brans et al. 2017; Diamond et al. 2018; Tüzün and Stoks 2021; Yilmaz et al. 2021).

While common garden studies provide support in favour of urban evolution in some well-studied invertebrate and plant taxa, whether genetic change drives phenotypic shifts in other urban taxa is not as well known (e.g., Table 1: 10% or N = 8 common garden studies in birds). For instance, birds are one of the most studied taxa in urban ecology and evolution and there are growing generalizations on how urbanization impacts the traits of birds globally (e.g., earlier lay dates or smaller body sizes, Capilla-Lasheras et al. 2022; Hahs et al. 2023). Great tits (*Parus major*), specifically, have become a model species for studying urban evolution across Europe and Asia and, thus, research on this species is now contributing to large collaborative research efforts that evaluate trends across replicated urban gradients in continent-wide analyses (Vaugoyeau et al. 2016; Salmón et al. 2021; Thompson et al. 2022). Despite these exciting efforts, a fundamental gap exists about whether urban phenotypic shifts in this model species are driven by evolutionary change between populations or by plastic responses to urban conditions. Common garden experiments in urban dark-eyed juncos (*Junco hymemalis*) and European blackbirds (*Turdus merula*) suggest that genetic change could at least partially play a role in phenotypic differences across morphological, physiological, and behavioural traits (Table 1; Atwell et al. 2012; Miranda et al. 2013; Reichard et al. 2020), but it remains to be seen whether this holds for other urban bird species and, specifically, the great tit where urban phenotypic shifts have been well documented in the wild.

This study uses a common garden experiment to disentangle the mechanisms that shape urban phenotypes in populations of great tits in and around Montpellier, France. In this system, we have documented several phenotypic differences between urban and forest populations in life history, morphology, physiology, and behaviour (Charmantier et al. 2017; Caizergues et al. 2018; Caizergues et al. 2022); trends that tend to be consistent across other European populations (Vaugoyeau et al. 2016; Biard et al. 2017; Corsini et al. 2021; Thompson et al. 2022). In the wild, urban great tits are smaller, have faster breath rates under constraint, show higher aggressiveness when handled, and are faster explorers than their forest counterparts, although estimates of selection gradients suggest that these urban phenotypic shifts are not favoured by natural selection (Caizergues et al. 2018; Caizergues et al. 2022). Genomic studies have revealed that, despite evident gene flow in this system, a small but significant proportion of genetic variation is explained by urbanization. This result suggests some genetic divergence between the urban and forest populations (i.e., significant $F_{ST} = 0.006 – 0.009$ between urban and forest comparisons; Perrier et al. 2018; Caizergues et al. 2022). A common garden approach is the next logical step in deciphering the genetic and plastic influences on the phenotypic divergences documented in this urban system.

We reared great tits from eggs collected from urban and forest sites around Montpellier under common conditions to evaluate whether documented morphological, physiological, and behavioural differences persist under the same environment. We had two major aims. The first was to compare the phenotypes of birds from urban and forest origins reared in a common garden. We hypothesized stronger genetic change for highly heritable traits (e.g., morphology, physiology) than for lowly heritable ones (e.g., behaviour; Kinnison and Hendry 2001; Stirling et al. 2002) and, therefore, that morphological and physiological differences would be more likely to persist under common conditions. More specifically, we predicted that birds from urban origins would be smaller and more stressed (phenotypic difference persists), but not more aggressive or exploratory (phenotypic difference does not persist), than birds from forest origins. Our second aim was to evaluate how different sources of variation (i.e., genetic and environmental variation) shaped phenotypes in the experimental context. We present phenotypic estimates from wild populations alongside those from the common garden for comparison.

Methods

Study system and quantifying urbanization

Populations of urban and forest great tits have been monitored at nest boxes in and around the city of Montpellier, France as a part of a long-term study (Charmantier et al. 2017). The forest population has been monitored since 1991 in La Rouvière forest located 20 km north of Montpellier where the number of nest boxes of 32 mm diameter entrance ranged from 37 – 119 because of theft/replacement. The urban population has been monitored since 2011 throughout the city of Montpellier at study sites that differ in their degree of urbanization (163 – 208 urban nest boxes across 8 study areas, see Figure 1 in Caizergues et al. 2024). During each spring, nest boxes are visited once per week to follow the reproduction of breeding pairs. We catch adults at nest boxes when nestlings are around 12 days old, ring them with a unique metal band, age them based on plumage as either yearling (born previous year) or adult (born at least the year before last), take a blood sample, and measure several phenotypes (see section “phenotyping”; Caizergues et al. 2018; Caizergues et al. 2022). We quantified the proportion of impervious surface area (ISA; sealed non-natural surfaces) at each nest box to generate a continuous urbanization metric to characterise the territory of breeding wild birds (captured at nest boxes) and the territory of origin for the birds raised during the common garden experiment (see supplementary methods). We also categorized territories as urban or forest habitat types since phenotypic changes may not be consistent across continuous and categorical urbanization, which can provide additional insights. For example, in cases where a phenotypic change differs between habitat types but does not clearly change along the gradient, this could suggest that i) urban effects other than ISA explain phenotypic change or ii) phenotypic changes may be non-linear.

Common garden manipulation

Egg transfer to wild foster parents

Between April 5 – 22, 2022, we collected eggs from urban (N = 50 eggs from 4 sites) and forest (N = 40 eggs from 1 site) populations (Figure 1A; Table S1). We collected three to four unincubated eggs (cold to the touch) from each origin nest box. We ensured eggs were unincubated by collecting eggs from nest boxes where we were confident that females had initiated laying within the three to four days before collection and the collected eggs were still covered by nest material. We replaced collected eggs with false eggs to encourage the origin female to continue its reproduction and we moved collected eggs into foster nest boxes at our Montpellier Zoo study site where wild females had just commenced incubation (Figure 1B). The Montpellier Zoo is an intermediate site along our urban gradient because it is natural in its vegetative characteristics, but is exposed to humans and related stimuli (Demeyrier et al. 2016). We transferred eggs from their origin to foster nests within 6 hours. In one case, we transferred eggs two days later and we kept these clutches in a dark room and rotated eggs every 12 hours until their transfer (foster ZOO46; Table S1). On average, the collected urban eggs were significantly lighter than the collected forest eggs (urban: N = 34 eggs weighed, mean = 1.56 g, variance = 0.02; forest: N = 40 eggs weighed, mean = 1.70 g, variance = 0.0075; Welch's t-test: -4.87, df: 52.88, *P* < 0.001; Table S1).

Foster nest boxes contained eggs from two origin broods (N = 6 – 8 eggs total; Table S1). We did not mix urban and forest eggs in the same foster broods to ensure that we could confidently assign all nestlings a habitat of origin, even if biological parents abandoned or were not captured. Urban lay dates in our system were earlier than forest lay dates (urban origin nests laid on average 7.5 days earlier), but there was still overlap in reproductive phenology between habitats (Table S1). The percentage of unhatched eggs was similar across habitat of origin (18% urban and 20% forest). Unhatched forest eggs were all from one abandoned foster nest (invaded by hornets), whereas unhatched urban eggs were distributed across successful foster clutches (Table S1). Of the 90 eggs transferred, we had N = 73 nestlings hatch (41 urban and 32 forest; Table S1). We did not have mortality events so these sample sizes are representative of the number of individuals phenotyped after rearing (Table S2).

Captive rearing

Once nestlings could thermoregulate on their own at 10 days of age (Mertens 1977), we transferred nestlings to the Montpellier Zoo nursery between April 29 – May 16, 2022 (Figure 1C; Table S1). Due to advanced urban lay dates in our system, urban nestlings entered captivity on average 6 days before forest nestlings (urban mean = 126 Julian days, urban range = 119 – 135; forest mean = 132 Julian days, forest range = 128 – 136; Table S1). Upon arrival, we ringed and weighed nestlings before placing them into artificial nests with their foster broods. We kept them in incubators that mimicked a dim cavity and kept chicks in a quiet environment (1 – 3 broods per incubator; Figure 1C.i). At this stage, we hand-raised nestlings by feeding individuals every 30 minutes between 7:00 and 21:00 (see supplementary methods for "captive diet"). Individuals began to "fledge" their brood nests at an age of 18 days. We transferred these individuals in groups of 2-3 birds into small wire cages in the order of when they fledged (irrespective of sex, foster brood, or habitat of origin), where we trained them to feed by themselves (Figure 1C.ii). At this stage, we still fed individuals every 30 mins. Once birds were approximately 23 days old, we transferred them to larger cages (0.8 x 0.35 x 0.4 m) that allowed more movement (hops and flights) in groups of 2 individuals. At this stage their feeding schedule became less frequent, and birds were considered independent at an age of approximately 35 days when we transferred individuals to large outdoor aviaries (N = 8; size = 2.2 x 4.4 – 5.5 meters; Figure 1C.iii). Individuals were randomly organised into aviary groups blind of habitat of origin and sex (N = 6 – 10 individuals per group). All individuals were hand-reared by the same caretakers during the experiment who were blind to the origin of the birds and birds from both origins were mixed through all stages of the rearing protocol.

Blood sampling and genotyping

We took blood samples from individuals the day before birds were transferred to outdoor aviaries to determine i) sex and nest of origin to control for genetic relatedness and ii) assign each bird with an ISA of origin. For each foster brood, nestlings had two possible nests of origin from which eggs were collected so parents of nests of origin were also blood sampled and genotyped to assign nest of origin for each common garden bird (see supplementary methods).

Phenotypic measurements

Here we examine five phenotypic traits: handling aggression, breath rate index, exploration in a novel environment, tarsus length, and body mass. Phenotypic measurements of common garden birds (Figure 1D) followed the same protocols used to phenotype wild birds

(Caizergues et al. 2018; Caizergues et al. 2022). We took all phenotypic measures of common garden birds indoors at the nursery, so all individuals were phenotyped under similar conditions (i.e., constant temperature, noise, and light levels). We took repeated measures between 06 June 2022 – 31 January 2023 and all observers were blind to habitat of origin while phenotyping individuals during four separate phenotyping sessions. On average, we measured birds 1.8 times in the wild (range = 1 – 8) and 3.4 times in the common garden (range = 1 – 4; summary by trait in supplementary and Table S2). Following our phenotyping protocol in the wild where birds are mainly measured annually during the breeding season (02 April – 16 July), we measured phenotypes at the same time in the following order:

Handling aggression: We measured handling aggression immediately following capture (from nest box, cage, or aviary) by provoking the bird while holding it (Figure 1D.i). We scored their aggressive response between 0 (no reaction) to 3 (tail and wings extended, pecking, and vocalization) on a scale that increased in increments of 0.5 (see Dubuc-Messier et al. 2018; Caizergues et al. 2022 for further details).

Breath rate index: We placed the bird in a cloth bag and allowed a five-minute standardized period of rest. Once removing the bird from the bag and properly holding the bird (Figure 1D.ii), we recorded the time it took for a bird to take 30 breaths (i.e., movements of the breast). We took this measurement twice in immediate succession and took the average between these measures to represent an individual's breath rate index.

Exploration: We placed birds into a small compartment next to a novel environment arena where they had a standardized two-minute rest period. We then initiated the novel environment exploration trial by coaxing birds into the novel arena. We recorded their behaviours in this arena for 4 minutes on video then an observer later counted the number of hops and flights birds took while exploring this novel environment (Figure 1D.iii). Only one observer scored videos from the common garden (MJT) while multiple observers have scored videos from the wild populations (inter-observer reliability rho > 0.95, including MJT).

Tarsus length and body mass: Finally, we measured tarsus length (millimetres) with pliers to determine the length between the intertarsal notch and the end of the bent foot (i.e., Svensson's alternative method; Svensson 1992); Figure 1D.iv) and body mass (grams) using an electronic scale (Caizergues et al. 2021; Figure 1D.v).

Statistical analyses

We examined wild and common garden (CG) data using separate Bayesian mixed-effect models since model structures between contexts accounted for different fixed and random effects, while examining a similar main effect of interest (i.e., habitat type; aim 1). All models included habitat type (urban vs. forest) and sex (male and female), and their interaction, as fixed effects in the model to evaluate how phenotypic differences vary across habitats and sexes. If the interaction between habitat and sex was weak and largely overlapped zero, we dropped this effect and refitted the model to evaluate the phenotypic differences between habitats and sexes independently. In subsequent models, we replaced the habitat type effect with proportion ISA to further evaluate changes in the wild and CG phenotypes along a gradient of urbanization.

We have already published results on the phenotypic differences between wild urban and forest populations (Charmantier et al. 2017; Caizergues et al. 2018; Caizergues et al. 2022), but here we report estimates from wild populations that i) include more years of data (3 additional years, year range: 2011 – 2022) and ii) use data only from the study sites used in our common garden experiment (i.e., 1 forest and 4 urban study sites). All wild models included random effects that accounted for differences between individuals, study sites, and years, and we additionally accounted for differences between observers for handling aggression, breath rate index, and tarsus length (Table 2). In addition to examining habitat and sex differences in these models, we also accounted for fixed effects such as time of day, date of measurement, and protocol type following previously established model structures for these traits (Table 2; outlined by trait below; Caizergues et al. 2018; Caizergues et al. 2022).

Besides examining habitat of origin effects in common garden models (aim 1), we also explored how different components of genetic and environmental variation shaped traits in the common garden (aim 2). All common garden models included the same random effects (Table 2): Individual ID accounted for variance among individuals (V_{ID}), nest of origin ID accounted for variance among origin nests (V_{NO}), and foster nest ID accounted for variance among foster nests (V_{NF}). We also included aviary ID as a random effect that accounted for variation among social groups in the experiment (V_{AV}) for the behavioural and physiological traits considered (i.e., aggression, exploration, breath rate). Since individuals in the common garden experiment were genotyped, we conducted a complementary analysis of the common garden data using mixed-effect animal models (Charmantier et al. 2014) by fitting a genetic relatedness matrix (GRM) between individuals in our common garden context (de Villemereuil et al. 2018). Since we collected unincubated eggs for the experiment, the nest of origin random effect (V_{NO}) if fitted alone, may capture both genetic differences between individuals (i.e., whether they are siblings) and early environmental differences such as early maternal investment in the eggs. Therefore, the GRM approach allowed us to further evaluate how the variation of each trait was partitioned when accounting for individual genetic variation and nest of origin variation (V_A and V_{NO}, respectively) at the same time (see supplementary methods for model description).

We computed the repeatability (R) of each trait in the common garden experiment as:

$$R_{CG} = \frac{V_I}{V_P} \tag{1}$$

$$\text{where } V_I = V_{ID} + V_{NO} + V_{NF} + V_{AV} + V_{SEX} + V_{HAB} \tag{2}$$

$$\text{where } V_P = V_I + V_R \tag{3}$$

where V_I is the among-individual variance that comprises effects that vary consistently among individuals and drive biological differences between individuals including variance across individuals (V_{ID}), nests of origin (V_{NO}), foster nests (V_{NF}), aviaries or social groups (V_{AV}), and fixed effect variance among sexes and habitat types (V_{SEX} and V_{HAB}). We only chose to include fixed effect variance generated by biological effects in the model (i.e., habitat and sex), rather than experimental effects (e.g., time of day or observer), to quantify repeatability using natural sources of variation that may improve comparability to other studies (de Villemereuil, Morrissey, et al. 2018; Wilson 2018). V_P is the total phenotypic variance and includes sources of among-individual variance (V_I) and residual variance (V_R). For comparison, we computed R in wild birds as:

$$R_{WILD} = \frac{V_{IND}}{V_P} \tag{4}$$

$$V_{IND} = V_{ID} + V_{SITE} + V_{SEX} + V_{HAB} \tag{5}$$

$$\text{where } V_P = V_{IND} + V_{YEAR} + V_{AGE} + V_R \tag{6}$$

where V_{IND} is the among-individual variance and comprises variance across individuals (V_{ID}), study sites or local habitats (V_{SITE}), and sexes and habitat types (fixed effect variance: V_{SEX} and V_{HAB}). V_P is the total phenotypic variance and includes among-individual variance (V_{IND}) and residual variance (V_R). In the wild context, we also incorporated biological effects in V_P that vary within individuals including variance among years (V_{YEAR}) and fixed effect variance among ages (V_{AGE}). For Poisson models (i.e., exploration) that use a log link transformation, we used the QCglmm package (de Villemereuil et al. 2016) to convert the variance components and repeatability estimate from the latent scale to the data scale.

We conducted all analyses in R v4.3.3 (R Core Team 2024) using Bayesian mixed-effect models in the MCMCglmm package (Hadfield 2010) using a Gaussian error structure for all traits except exploration (number of hops) where we used a Poisson error structure. See supplementary methods and Table 2 for description of other fixed effects included for each model. We used weakly informative inverse-Gamma priors (V = 1, nu = 0.002) for fixed and random effects. We ran all models for 1000000 iterations, with a thinning of 500 and a burn-in period of 10000, which achieved effective sample sizes > 1000 across all estimates. We verified model fit by visually inspecting histograms and QQPlots of model residuals, and the relationship between the residuals and fitted values. We confirmed convergence of models by visually inspecting trace plots, verifying low autocorrelation, and by using Heidelberg stationary tests (de Villemereuil 2018).

Results

Aggression in hand: We found clear evidence that wild urban males were more aggressive than wild forest males, but no habitat difference for females (habitat*sex effect, Table 3.1A; Figure 2A). Results in the wild were qualitatively similar when examining how phenotypes changed along the urban gradient; there was clear evidence that wild males in habitats with higher proportion ISA had increased aggression (Table S4; Figure 3A). There was no clear evidence that this phenotypic difference was maintained in the common garden (weak habitat*sex or habitat effects overlapping zero; Table 3.2A; Table S5; Figure 2A), and no clear evidence that handling aggression increased with proportion ISA of the origin habitat of common garden birds (credible interval overlaps zero; Table S4). Individual ID explained 23% of the variation in aggression in the common garden, nest of origin explained 2%, foster nest explained 2%, and housing aviary explained 2% of the variation (Table 3.2A).

Breath rate index: We found weak evidence that wild urban birds had faster breath rates than wild forest birds (6% posterior distribution crossing zero, Table 3.1B; Figure 2B), but clear evidence that the amount of time to take 30 breaths decreased across the urbanization gradient in the wild populations (i.e., faster breath rates in more urbanized habitats, credible interval excludes zero, Table S4; Figure 3B). We found clear evidence that this phenotypic difference was maintained in common garden birds where birds from urban origins had faster breath rates than birds from forest origins (Table 3.2B; Figure 2B), but there was no clear change in breath rate across the urbanization gradient in the common garden (credible interval overlaps zero; Table S4; Figure 3B). Individual ID explained 40% of the variation in breath rates in the common garden, while origin nest (11%), foster nest (4%), and housing aviary (4%) explained less variation in this trait (Table 3.2B).

Exploration: We found clear evidence that exploration in the wild was higher in urban compared to forest birds (Table 3.1C; Figure 2C) and less clear evidence that exploration increased with increasing urbanization (5% posterior distribution crossing zero; Table S4; Figure 3C). There was no clear evidence that these differences were maintained in the common garden (credible overlapping zero; Table 3.2C; Figure 2C), and exploration of common garden birds did not increase clearly with proportion ISA of the origin habitat (Table S4; Figure 3C). Individual ID explained 17% of the variation in common garden exploration behaviours while origin nest (7%), foster nest (10%), and housing aviary (2%) explained less variation in this trait (Table 3.2C).

Tarsus length: We found clear evidence that tarsus length was significantly shorter in wild urban birds than wild forest conspecifics (Table 3.1D; Figure 2D) and with increasing urbanization in the wild (Table S4; Figure 3D). In the common garden experiment, this phenotypic divergence was in the same direction and had similar effect sizes as in the wild, with birds from urban origins tending to have shorter tarsi than birds from forest origins (CG: $\beta_{habitat} = -0.22$ vs. wild: $\beta_{habitat} = -0.29$; Table 3D) and tarsus length decreasing with increasing ISA (CG: $\beta_{ISA} = -0.29$ vs. wild: $\beta_{ISA} = -0.20$, Table S4). The large credible intervals in the common garden context however reduced certainty and suggests the smaller common garden sample size may limit the ability to detect statistical differences for this trait (CI overlapping zero, but note 91% of ISA CI excludes 0). Out of the random effects considered, individual ID explained 46% of the variation in tarsus in the common garden, followed by origin nest and foster nest which explained 25% and 7% of the variation, respectively (Table 3.2D).

Body mass: We found weak evidence that wild urban birds were lighter than wild forest birds (7% posterior crossing zero; Table 3.1E; Figure 2E), but clear evidence that body mass decreased with increasing urbanization (credible interval excluded zero, Table S4; Figure 3E). We found clear evidence that common garden birds from urban origins were lighter than birds from forest origins (Table 3.2E; Figure 2E). Results in the common garden across the urban gradient were consistent with this conclusion where the weight of common garden birds decreased with increasing urbanization of the origin habitat (Table S4; Figure 3E). Individual ID explained 28% variation in common garden body mass, followed by origin nest which explained 12% variation, and foster nest effects which explained 4% (Table 3.2E). On average common garden birds were significantly lighter than wild birds, reflecting an experimental effect on body mass (Welch's t-test: $t = -22.30$, $df = 272$, $P < 0.001$, Figure 2E; Figure 3E).

Additive genetic variance and heritability of common garden traits: The complementary quantitative genetic models (Table S3) revealed that breath rate index, tarsus length, and body mass measured in common garden birds were moderately heritable (breath rate: $V_A = 1.53$, $h^2 = 0.23$ [0.001 – 0.57]; tarsus length: $V_A = 0.16$, $h^2 = 0.33$ [0.003 – 0.72]; body mass: $V_A = 0.28$, $h^2 = 0.28$ [0.01 – 0.49]), while aggression in hand and exploration were lowly heritable (aggression: $V_A = 0.07$, $h^2 = 0.05$ [<0.001– 0.28]; exploration: $V_A = 0.43$, $h^2 = 0.02$ [<0.001 – 0.06]). Although there was uncertainty around variance estimates in these models (i.e., credible intervals close to zero), estimates of V_{NO} were low across all traits (Table S3) providing additional insight into the minimal contributions from early maternal effects in the experiment.

Discussion

We found evidence that both genetic and plastic changes have contributed to phenotypic shifts in wild urban tits, but the relative contributions of these drivers depend on the trait (aim 1). Specifically, our results provide evidence that genetic differences between populations have strongly driven the divergence observed in breath rate and body mass, while plasticity to urban conditions predominately contributes to divergences in aggression and exploration. Further, we found that individual differences tended to explain the most trait variation in the experiment, whereas nest of origin and foster nest variation had minimal contributions (aim 2).

We found support that genetic change or very early maternal investment in eggs has driven population divergences in breath rate index as phenotypic differences between birds from urban and forest origins were clearly maintained in our experiment (but note difference not statistically supported along gradient). In line with findings in the wild populations (Caizergues et al. 2022), birds from urban origins had faster breath rates than birds from forest origins. As breath rate index correlates with heart rate under constraint (Dubuc-Messier et al. 2016) and has previously been associated with physiological stress responses in this species (Carere and van Oers 2004; Krams et al. 2014), our results could indicate that genetic change in urban populations has contributed to a more proactive coping style in urban environments (Koolhaas et al. 2010; Koolhaas et al. 2011). Our results differ from those in urban European blackbirds (*Turdus merula*; Partecke et al. 2006) and juncos (*Junco hyemalis*; Atwell et al. 2012) where lower stress responses of urban birds were maintained when individuals were reared under common conditions. Indeed, there is no general consensus on how urbanization impacts stress responses in birds (reviewed in Bonier 2012) and so our results make a useful contribution towards understanding how physiology might impact adaptation to urban contexts.

We also found support that being smaller may have a genetic basis in cities. Specifically, birds from urban origins were lighter than birds from forest origins, despite being fed the same diet *ad libitum*. This habitat difference in body mass was statistically clear and higher in the common garden than the wild supporting a genetic basis for shifts to smaller urban body mass, rather than plasticity which could possibly reduce this wild phenotypic difference. Lighter urban body mass in the experiment could also be explained by early maternal investment in the egg, especially since the urban eggs collected for the experiment were on average lighter than forest eggs. Since egg size is highly heritable (e.g., egg volume $h^2 = 0.6 – 0.8$, Van Noordwijk et al. 1981; Hõrak et al. 1995; see also Christians 2002) and female body size can positively correlate with their egg size in this species (Hõrak et al. 1995) it is possible that genetic differences between females in maternal egg investment could shape body mass variation in the wild.

Birds from urban origins also tended to have smaller tarsi than birds from forest origins in line with the phenotypic shift from the wild (Caizergues et al. 2018) but this was not supported statistically. We could lack statistical power to make firm conclusions on whether this difference in tarsus length was maintained in the common garden, especially since the wild phenotypic difference in tarsus length is small and would require large samples to detect. The habitat difference for tarsus length was weaker in the common garden than the wild (i.e., difference of 0.22 mm in common garden vs 0.29 mm in wild) which may indicate that a combination of plastic and genetic effects explain the tarsus length difference. Tarsus length and body mass are heritable traits and tend to strongly correlate with each other in this species (Gebhardt-Henrich and Van Noordwijk 1991; Hõrak 1994; Gosler and Harper 2000; Young and Postma 2023), suggesting that parallel genetic change for tarsus alongside body mass is possible. However, tarsus development is also strongly influenced by environmental conditions in early life (Dhondt 1982; Merilä and Fry 1998; Talloen et al. 2010; Seress et al. 2020), and so we hypothesize that genetic and plastic effects both contribute to smaller tarsus lengths in urban birds. Further quantitative genetic approaches using long-term datasets on wild populations, and observed or genetically reconstructed pedigrees, will provide a useful complementary exploration on the underlying drivers behind shifts to smaller urban tarsus lengths in tits.

Decreases in traits associated with body size are documented across diverse taxa in cities (Merckx et al. 2018; Hahs et al. 2023) and this phenotypic shift is hypothesized to facilitate heat dissipation and be an adaptive response to rising global temperatures that are pronounced in urban areas via the heat island effect (Youngflesh et al. 2022; Sumasgutner et al. 2023). For example, *Daphnia* from urban origins had smaller body sizes and higher heat tolerance in a common garden experiment than those from nonurban origins, and there was evidence that smaller urban body sizes could indirectly increase heat tolerance in this species (Brans et al. 2017). Further, city great tits tend to be lighter than forest tits across Europe (Thompson et al. 2022) and, in Veszprém, reproduction of city tits is less affected by extreme temperature than their forest counterparts (Pipoly et al. 2022). These results suggest that city great tits could be adapted to warming conditions and our results could imply that decreases in urban body size are an adaptive response to heat island effects (but see Playà□Montmany et al. 2021). Smaller body size does not appear to afford urban great tits in Montpellier reproductive benefits (Caizergues et al. 2018), hence further work will be needed to evaluate whether smaller body sizes, or other correlated traits, are associated with higher survival in urban habitats.

Genetic change between populations that contribute to phenotypic differences can also arise via neutral evolutionary processes like genetic drift or founder effects (Leinonen et al. 2013), and differentiating these processes from local adaptation is informative to evaluate whether populations are adapting in pace with environmental change (de Villemereuil et al. 2020). We found evidence that breath rate and body mass differences are likely driven by genetic change or very early maternal investment, but we are unable to completely dismiss the role of neutral evolutionary processes towards genetic differences between populations. Using a complementary quantitative genetics approach, we estimated higher genetic differences underlying these traits (computed Q_{ST} values in Table S3: 0.06 and 0.08) than would be expected by neutral genetic variation between these populations (F_{ST} values between 0.006 – 0.008; Perrier et al. 2018). However, the uncertainty around these Q_{ST} estimates (credible intervals crossed 0.006) prevent us from completely excluding neutral evolutionary processes here. In future, rearing individuals from multiple city and forest comparisons in a common garden experiment would further strengthen our evidence against processes of neutral evolution and possibly demonstrate parallel evolutionary trajectories across multiple city populations.

We did not find evidence that genetic change has considerably contributed to urban behavioural shifts as birds from urban and forest origins did not clearly differ in their aggressive or exploratory behaviours in the experiment. It is commonly assumed that urban populations are evolving and adapting to novel urban conditions (Lambert et al. 2021), and behavioural adaptations may be particularly important in this process (Miranda et al. 2013). Alternatively, it has been argued that phenotypic adjustments through plasticity are probably more frequent (Hendry et al. 2008), especially for behavioural traits (Sol et al. 2013; Caspi et al. 2022). Our results provide support for the latter argument and contrast findings in urban blackbirds and juncos where behavioural differences were assumed to be a result of local adaptation (Atwell et al. 2012; Miranda et al. 2013). We therefore conclude that more aggressive and exploratory behaviours of wild urban great tits are most strongly driven by plastic adjustments to life in cities. Habitat matching behaviours (Edelaar et al. 2008; Camacho et al. 2020) could contribute to these urban differences in the wild, whereby more aggressive and exploratory individuals disperse and settle in more urbanized habitats, if these behaviours provide them an advantage in urban habitats. However, these behaviors were not found to covary at the individual level in an urban behavioral syndrome and do not seem to afford reproductive or survival benefits in the urban population (Caizergues, Grégoire, et al. 2022). Dispersal dynamics and habitat matching behaviours in an urban context are still poorly understood but, as these behaviors do not covary or improve fitness, we so far have limited evidence that habitat matching contributes to these urban phenotypic shifts.

Our second aim was to investigate how different sources of environmental and genetic variation contributed to repeatable individual differences across traits in our experimental context. The estimated among-individual variance and repeatability of traits in the common garden were remarkably similar to those estimated in the wild (i.e., similar estimates and overlapping credible intervals). Trait variation in the experiment tended to be shaped predominately by differences between individuals (i.e., Individual ID). Individual ID in the experiment could comprise both individual-specific genetic and environmentally induced individual differences, and our complementary quantitative genetic analysis suggested that individual genetic variation explains between 23 – 33% of the variation across breath rate, tarsus length and body mass, but only 2 – 5% for aggression and exploration behaviours (i.e., estimates of V_A in Table S3, but note wide credible intervals). Variation attributed to foster nest and aviary, likely related to brood and social environmental conditions respectively, remained low across traits (3 – 14%). Specifically, early environmental conditions can affect tarsus development and growth (e.g., Seress et al. 2020), but we found limited support that foster parents and nests contributed to individual differences in tarsus. Overall, estimated individual differences were similar between common garden and wild contexts, especially in those traits where we find evidence for underlying genetic differences between populations.

Finally, a few caveats should be considered when interpreting our results. First, we are unable to fully discount the contribution of very early maternal effects towards the maintained breath rate and body mass differences in the common garden. By collecting unincubated eggs we limited maternal contributions to egg investment, which could influence morphological traits like body mass (Hõrak et al. 1995). Although we found limited (but unclear) support for maternal effects in our experiment (i.e., negligible V_{NO}; Table S3), our results should be interpreted with this in mind. Second, birds in our experiment were assayed at a relatively young age (between 38 - 261 days old) compared to when they are usually assayed in the wild (73% observations at 1 year old), which may affect how our common garden estimates compare to our wild populations. However, measuring phenotypic traits earlier in our experiment seemed to have limited impact on results as most common garden phenotypes were similar to wild phenotypes. Body mass in the common garden was the only trait that seemed to differ from the wild. Wild juvenile birds (1 year of age) tend to be on average 0.3 g lighter than wild adults in this population, so this may indicate that age could at least partially contribute to the observed difference in mass between contexts. Third, we monitor forest great tits in one larger study site and, although this nonurban area contains different forest types, we lack replication to draw broad inferences on the phenotypes of forest great tits more generally. Fourth, although the effects of urbanization on wild trait divergences were in the same direction and of similar magnitude to what has been previously reported in these populations, there were three cases in this study where statistical support varied (statistically unclear effect of habitat on breath rate and body mass, and ISA on exploration). In all these cases >93% of the posterior was negative (positive for exploration) indicating weak tendencies, which were likely driven by methodological differences between this and previous studies (i.e., different subset of data, additional years of data, Bayesian approach). Finally, rearing individuals under the same restricted and benign conditions (e.g. *ad libitum* food supply) may have prevented us from detecting phenotypic differences if they are impacted by genetic and environmental interactions (G x E; Conover et al. 2009). Although difficult to conduct, multi-treatment common gardens where food or temperature are manipulated could be especially valuable for teasing apart genetic and environmental interactions acting on phenotypic shifts in urban great tits.

In conclusion, our survey of the literature for urban common garden experiments indicates that both plastic and genetic divergences between urban and nonurban populations are common. In our study we find evidence that urban phenotypic divergences in stress physiology and morphology are mainly driven by genetic change or very early maternal investment in eggs. Common gardens are not able to affirm local adaptation, unless realistic multi-treatment or reciprocal transplant approaches are used (e.g., Gorton et al. 2018; Tüzün and Stoks 2021), and evaluating reproductive and survival benefits of our common garden birds in aviaries would not be appropriate. Thus, investigating whether these genetic differences between populations are adaptive remains an avenue for future research. We did not find evidence that genetic change strongly drives urban behavioural shifts, which provides contrary evidence to urban common garden studies in other bird species (Atwell et al. 2012; Miranda et al. 2013). Further work will be needed to uncover whether plasticity predominantly drives other urban behaviours in great tits (e.g., neophilia or boldness) and determine the mechanisms underlying discrepancies with other studies. Our results highlight that phenotypic shifts in urban populations can be impacted by both genetic and plastic changes and make a valuable contribution in filling a fundamental gap concerning the urban evolution of a model species. Examining how evolutionary processes in urban contexts impact phenotypic and genetic variation will have important applications for conserving urban wildlife populations and their ecological roles in communities (Lambert and Donihue 2020; Des Roches et al. 2021), but will also improve our fundamental understanding of ecology and evolution in wild systems more broadly, especially in light of global environmental change.

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Table 1 Synthesis of studies (*N* = 77) that have used a common garden approach to compare the phenotypes of urban and nonurban populations (e.g., rural, forest, mountain, agricultural) across a variety of different groups (amphibians, birds, invertebrates, fish, plants, reptiles) and traits (physiology, behaviour, morphology, life history, phenology). Information concerning the traits measured, approach taken, results, conclusions, and other notes are shown for each study. Studies were collated from Lambert et al. 2021 (Table 1) and from a literature search using Google Scholar for articles since 2020 that included both "common garden" and "urban" (conducted March 22 2024).

Group	Urban vs.	Traits	Approach[A]	Urban results[B]	Conclusion[C]	Notes	Ref
AMPHIBIANS							
Agile frog (*Rana dalmatina*)	Woodland	Physiology	Eggs	Lower heat tolerance	E (& G)	Difference in common garden opposite to wild divergence	(Bókony et al. 2024)
Common toads (*Bufo bufo*)	Agricultural & natural	Physiology	F1 generation	No difference in toxicity and potency (or in opposite directions)	E	Urban and agricultural higher toxicity and potency in wild	(Bókony et al. 2019)
		Physiology	F1 generation	No difference in stress	E	Urban and agricultural higher stress response in wild	(Bókony et al. 2021)
Guttural Toad (*Sclerophrys gutturalis*)	Natural	Behaviour	F1 generation	No difference in boldness	E	Higher urban boldness in wild	(Mühlenhaupt et al. 2022)
BIRDS							
Dark-eyed juncos (*Junco hymemalis*)	Mountain	Behaviour Physiology	Fledglings	Higher boldness and lower stress response	G	Same results in wild	(Atwell et al. 2012)
		Morphology	Nestlings	Urban birds have shorter wing and tail lengths, and whiter tails	G	Same results in wild	(Rasner et al. 2004; Yeh 2004)
		Physiology	Fledglings	No difference in testosterone	E	Lower urban peak testosterone	(Atwell et al. 2014)
		Behaviour	Fledglings	Higher minimum frequency, no difference in peak or max frequency	G	Same results in wild	(Reichard et al. 2020)
		Physiology	Fledglings	Higher gonadal sensitivity	G	Advanced reproduction in wild	(Fudickar et al. 2017)
European blackbirds (*Turdus merula*)	Rural	Behaviour	Nestlings	More neophobic and seasonally less neophilic	G	Higher urban repeatability	(Miranda et al. 2013)
	Forest	Physiology	Nestlings	Attenuated stress responses	G	Trends varied seasonally	(Partecke et al. 2006)
		Behaviour Physiology	Nestlings	Lower migratory disposition and earlier gonadal development	G	Loss of migration in wild Observed in males only	(Partecke and Gwinner 2007)
INVERTEBRATES							
Water fleas (*Daphnia magna*)	Rural	Physiology	F2 generation 2 temperature treatments	Higher thermal tolerance, haemoglobin, and smaller body size	G & E	Higher thermal tolerance at warmer rearing temperature	(Brans et al. 2017)
		Morphology	F2 generation 2 temperature treatments	Smaller body size	G & E	Smaller body sizes at warmer rearing temperatures	(Brans et al. 2017)
		Physiology Life-history	F3 generation 2 temperature treatments	Higher energy reserves and lower enzyme activity	G & E	Rearing temperature affected some traits Pace of life syndrome in urban fleas	(Brans et al. 2018)
		Life-history Morphology	F2 generation 2 temperature treatments	Faster maturation, smaller, increased fecundity, early release of progeny	G & E	Trait changes in response to rearing temperature in same direction Faster pace of life in urban fleas Some evidence of G x E for fecundity	(Brans and De Meester 2018)

		Physiology	F2 generation 2 temperature treatments Toxicity treatment	Higher survival	G	No differential impacts of temperature	(Brans et al. 2021)
Water fleas (*Ceriodaphnia cornuta*)	River / Lake	Life-history Morphology	F2 generation Food treatments	Reduced dietary tolerance to cyanobacteria	G x E	Genomic differentiation between populations	(Zhang et al. 2022)
Mosquito (*Anopheles gambiae*)	Rural	Physiology	Larvae	Agricultural sites higher tolerance to carbamates	G		(Antonio-Nkondjio et al. 2011)
		Physiology	Larvae	Higher tolerance to ammonia	G		(Tene Fossog et al. 2013)
		Physiology	Larvae	Agricultural sites higher tolerance to DDT	G		(Tene Fossog et al. 2013)
Asian tiger mosquito (*Aedes albopictus*)	Rural	Phenology	F1 generation Light and dark treatment	No difference in diapause incidence	E	Diapause lower and more variable under light treatment	(Westby and Medley 2020)
Blowfly (*Calliphora vicina*)	Rural	Morphology	F2 and F3 generation 4 temperature treatments	Smaller and faster growth rates at higher temperatures	G x E	Sex difference across temperatures	(Hwang and Turner 2009)
Wormlion fly (*Vermileonidae sp.*)	Natural	Behaviour	Larvae	Higher foraging investment and more selective habitat choice	G		(Samocha and Scharf 2020)
Damselflies (*Coenagrion puella*)	Rural	Life-history Physiology	Larvae with immune challenge Control and heat wave treatments	No difference in survival, growth rates, immune response, or bioenergetics. No reduction in energy budgets when exposed to a simulated heat wave.	G x E	Heat wave experiment significantly influenced all traits measured	(Tüzün and Stoks 2021)
		Life-history Physiology	Larvae 5 temperature treatments	Lower growth rates and higher survival	G	No large difference across temperatures Some evidence for countergradient variation	(Tüzün et al. 2017)
		Behaviour	Larvae Control and pesticide treatments	Less active, bolder, and have slower growth rates when exposed to pesticides	G x E		(Tüzün et al. 2017)
		Behaviour	Larvae 2 temperature treatments Control and pesticide treatments	More active when exposed to pesticides, but no difference in exploration or food intake	G x E		(Tüzün et al. 2015)
(*Ischnura elegans*)		Life-history	F1 generation 3 temperature treatments 2 predator treatments	Lighter and slower growth rate	G x E	Central and high latitude origin populations Different responses across latitudes	(Palomar et al. 2023)
		Behaviour	F1 generation 2 temperature treatments	Higher encounter and predation rates, but only on prey from rural origins	G x E	Daphnia prey also reared in common garden	(Brans et al. 2022)
Spindle ermine moths (*Yponomeuta cagnagella*)	Pristine	Behaviour	Larvae	Urban moths less attracted to light	G	Males more likely to be attracted to light than females	(Altermatt and Ebert 2016)
		Morphology	Larvae	Smaller wings and less attracted to light	G		(Van de Schoot et al. 2024)
Latticed heath moth (*Chiasmia clathrate*)	Rural	Physiology	F2 and F3 generation	Higher heat tolerance in adults	G	Parallel patterns across three countries Divergence not evidence in larval stage	(Merckx et al. 2024)
		Phenology	F3 generation Light treatments	Reduced diapause induction	G & E	Light increases direct development and reduces development time	(Merckx et al. 2023)

& green-veined white butterfly (*Pieris napi*)		Life-history	F1 generation Photoperiod treatments	Lower day length threshold for direct development and reduced diapause induction	G x E	2 replicate cities Longer and later urban flight period in the wild	(Merckx et al. 2021)
Speckled wood (*Pararge aegeria*)	Rural	Behaviour	Eggs	No difference in personality	E	Urban males who were more active as larvae were more exploratory as adults	(Kaiser et al. 2018)
	Agricultural & woodland	Behaviour	F1 generation	No difference in boldness or activity	E	Covariation between boldness and activity existed in urban butterflies	(Kaiser et al. 2020)
Acorn ants (*Temnothorax curvispinosus*)	Rural	Phenology	F1 generation 5 temperature treatments	Urban earlier reproduction	G & E	Earlier reproduction in colonies from lower latitudes and those reared under warmer temperatures	(Chick et al. 2019)
		Physiology	F1 generations – 3 cities 5 temperature treatments	Higher heat tolerance (2 out of 3 cities)	G & E	Higher fitness under treatment like source environment Higher heat tolerance under warmer rearing temperatures	(Diamond et al. 2018)
		Physiology	F1 generation 2 temperature treatments	Higher plasticity in heat tolerance when reared under warmer temperature	G x E		(Diamond et al. 2018)
		Physiology	F1 generation 2 temperature treatments	Higher heat tolerance and lower cold tolerance	G x E		(Diamond et al. 2017)
		Physiology	F1 and F2 generations	Higher heat tolerance and lower cold tolerance	G	Maintained over F1 and F2 so likely not transgenerational plasticity	(Martin et al. 2019)
		Physiology Behaviour	F1 generation	Higher metabolism, and faster running speed at higher temperatures	G x E		(Chick et al. 2021)
Ant (*Temnothorax nylanderi*)	Forest	Life-history Morphology	F1 generation Cadmium treatment	Higher cadmium resistance	G & E	Difference not observed in field	(Jacquier et al. 2021)
		Life-history Morphology	F1 generation Cadmium treatment	Higher emergence rate and larger worker size when exposed do cadmium	G & E	Cadmium negatively affected life-history traits	(Jacquier et al. 2021)
Common woodlouse (*Oniscus asellus*)	Rural	Physiology	F1 generation 2 temperature treatments	Higher heat tolerance, but no difference in cold or desiccation tolerance	G & E	Tolerance varies between temperature treatments	(Yilmaz et al. 2021)
		Behaviour	F1 generation 2 temperature treatments	Higher running speed	G x E	Reduced running speed when reared at higher temperatures	(Yilmaz et al. 2021)
Grasshopper (*Chorthippus brunneus*)	Rural	Morphology Life-history	F1 generation 2 temperature treatments	Longer femur and wing lengths Higher body mass and growth rate in females only	G & E	Rearing temperature affected measured traits, but generally sex dependent	(y Gomez and Van Dyck 2012)
(*Chorthippus biguttulus*)	Non-roadside	Behaviour	Nymphs 2 noise treatments	Higher frequency vocal signals and increased syllable to pause ratio	G & E	Higher frequency signals in noisy treatment	(Lampe et al. 2014)
Bend-legged ground cricket (*Dianemobius nigrofasciatus*)	Rural	Life-history	F2 generation Light and noise treatments	Higher survival and smaller body size, but no other difference	G & E	Light and noise treatments affected growth, diapause, and number of progeny	(Ichikawa and Kuriwada 2023)
		Behaviour	F1 generation	Higher frequency and shorter chirp vocal signal	G	Vocal difference not more preferred by females	(Kuriwada 2023)
Synanthropic spider (*Steatoda triangulosa*)	Rural	Behaviour	Egg-sacs	Lower avoidance of light	G		(Czaczkes et al. 2018)

Fruit flies (*Drosophilia tripunctata*)	Rural	Physiology	F2+ generation	Higher heat tolerance and lower cold tolerance	G	Sex difference Compare mid and low urbanization	(Diamond et al. 2022)
(*Drosophilia suzukii*)	Rural	Physiology	F3 generation	Lower thermal limit tolerance, but no difference in heat tolerance	G	Difference not found in wild	(Sato and Takahashi 2022)
FISH							
Atlantic killifish (*Fundulus heteroclitus*)	Clean	Physiology	F2 generation	Higher tolerance to pollutants	G	Transcriptional mechanism underlies divergence	(Whitehead et al. 2012)
Creek chub (*Semotilus atromaculatus*)	Rural	Behaviour	Fry 2 water velocity treatments	Higher swimming efficiency	G & E	Same results in wild Marginal support for G x E for one urban location	(Kern and Langerhans 2019)
		Morphology	Juvenile 2 water velocity treatments	More streamlined body shape	G	Marginal support for G x E for one urban location	(Kern and Langerhans 2018)
MAMMALS							
Egyptian fruitbat (*Rousettus aegyptiacus*)	Rural	Behaviour	Pups	Bolder, faster learners, and less exploratory	E	Cross-fostering revealed that maternal rather than genetic effects may drive difference	(Harten et al. 2021)
PLANTS							
Holy hawksbeard (*Crepis sancta*)	Rural	Life-history	Seeds	Higher number of non-dispersing seeds	G	Same results in wild	(Cheptou et al. 2008)
		Phenology Morphology Physiology	Seeds	Later phenology, larger, and higher water-use efficiency and photosynthesis	G	Most patterns same direction as selection in urban field site	(Lambrecht et al. 2016)
Common ragweed (*Ambrosia artemisiifolia*)	Rural	Phenology Life-history	Seeds Urban and rural treatments	Earlier phenology and lower fitness	G & E	Reciprocal transplant Trend for G x E for life-history traits, but not phenology	(Gorton et al. 2018)
		Phenology Morphology	Seeds Germination experiment	Higher percent germination, smaller height, and higher leaf desiccation index	G & E	Other traits, including flowering time, showed no difference	(Kostanecki et al. 2021)
Common milkweed (*Asclepias syriaca*)	Rural	Life-history Phenology	Seeds	Limited support for difference	E	Heritable genetic variation and weak phenotypic divergences in wild	(Breitbart et al. 2023)
White clover (*Trifolium repens*)	Nonurban	Phenology Morphology Life-history	F1 generation	Later phenology, larger flowers and biomass, thinner stolons, reduced cyanogenesis, and greater seed set	G	Some difference may be driven by pollinators along urban gradient	(Thompson et al. 2016; Santangelo et al. 2020)
Virginia pepperweed (*Lepidium virginicum*)	Rural	Phenology Morphology Fecundity	Seeds 5 cities	Earlier phenology, taller, and higher fecundity	G	City populations more genetically related	(Yakub and Tiffin 2017)
Chitinolytic fungi (*Chrysosporium pannorum, Trichoderma koningii, Torulmocyes lagena, Penicillium bilaii*)	Rural	Physiology	F1 generation 5 temperature treatments	Higher growth rate at higher temperatures	G x E	Evidence for cogredient or countergradient variation in growth rate	(McLean et al. 2005)
Southern crabgrass (*digitaria ciliaris*)	Farmland	Life-history	Seeds 2 competition treatments	More shoots, shorter, and higher growth rates in high competition treatment	G x E		(Fukano et al. 2020)
Yellow jessamine	Wild	Morphology	F1 generation	Larger floral traits	G	Patterns in same and opposite	(Irwin et al. 2014)

(*Gelsemium sempervirens*)						direction to those observed in wild	
Asian dayflower (*Commelina communis*)	Rural	Morphology	Seedlings	Lower leaf number and specific leaf area, no difference in other traits	G & E	Different patterns observed in the wild	(Taichi and Ushimaru 2024)
Pineappleweed (*matricaria discoidea*)	Rural	Life-history Morphology	Seeds Temperature treatments Soil treatments	Limited support for differences	E	Reciprocal common garden as treatments mimicked urban conditions	(Géron et al. 2022)
Common dandelion (*taraxacum officinale*)	Suburban Rural	Phenology Morphology Physiology	Seeds Herbivore treatment	Reduced early seed production following herbivory	G x E	Increased herbivory resistance after exposure to herbivores	(Pisman et al. 2020)
Thale cress (*Arabidopsis thaliana*)		Morphology Physiology	Seeds & F1 generation Herbivory treatments	Larger and higher tolerance to caterpillars, but not aphids	G	No wild size difference and higher urban aphid herbivory	(Qu et al. 2022)
		Phenology Life-history	F1 generation Growth treatments Indoor & Outdoor	Several differences including earlier flowering time	G	Genomics identifies set of clonal lineages	(Schmitz et al. 2024)
Reed canary grass (*Phalaris arundinacea*)	Natural	Physiology Morphology	Seeds Stress treatments	Greater stress tolerance characteristics	G x E	Some traits adaptive in treatments	(Weston et al. 2021)
REPTILES							
Australian water dragons (*Intellagama lesueurii*)	Semi-natural & natural	Behaviour	F1 generation	Higher boldness, but no difference in exploration or neophilia	G	Boldness only repeatable trait	(Baxter-Gilbert et al. 2019)
Crested anole (*Anolis cristatellus*)	Forest	Physiology	F1 generation 3 temperature treatments	No difference in heat tolerance	E	Higher urban heat tolerance in wild Higher urban gene expression change after exposed to heat challenge	(Campbell-Staton et al. 2021)
	Natural	Morphology	F1 generation	Longer limbs and more lamellae	G	Same results in wild	(Winchell et al. 2016)

[A] Generation measured in the common garden (F1 – F3) and, in cases where the F0 generation was used, the life stage individuals were brought to common conditions is shown. In the case of multi-treatment common garden approaches, details on the rearing treatments are also shown.

[B] Comparitive results using the urban group as the reference category. In the study listed last, urban anoles were found to have longer limbs and more lamellae than natural anoles.

[C] Conclusion of underlying mechanisms responsible for phenotypic difference between populations recorded as either G = genetic differentiation between populations, E = plasticity to environment, G & E = both genetic differentiation and plasticity, and G x E = genetic differentiation in plastic responses to environment.

Table 2 Summary of 1) wild and 2) common garden model structures that account for different fixed and random effects. Ranges for continuous fixed effects and number (N) of random effect levels for each trait are shown. Note that urbanization was also examined as a continuous effect in additional models (see model results in Table S4).

1) WILD MODELS					
	A) Aggression Range: 0 – 3 score	B) Breath rate Range: 7.7 – 21.7 sec	C) Exploration Range: 0 – 320 hops	D) Tarsus length Range: 17.32 – 21.70 mm	E) Body mass Range: 14 – 20.4 g
Fixed effects					
Habitat	forest vs. urban	forest vs. urban	forest vs. urban	forest vs. urban	forest vs. urban
Sex	female vs. male	female vs. male	female vs. male	female vs. male	female vs. male
Age	adult vs. yearling	adult vs. yearling	adult vs. yearling	-	adult vs. yearling
Time of day	Range: 7.16 – 17.13	-	Range: 6.47 – 16.67	-	Range: 7.17 – 17.13
Date of measure	Range: 101 – 196	Range: 108 – 190	Range: 109 – 190	-	Range: 101 – 197
Protocol	-	new vs. old	new vs. old	-	-
Temperature	-	Range: 8 – 33.7	-	-	-
Random effects					
Individual ID (V_{IND})	N = 772	N = 531	N = 472	N = 837	N = 810
Site ID (V_{SITE})	N = 5	N = 5	N = 5	N = 5	N = 5
Year ID (V_{YEAR})	N = 12	N = 9	N = 9	N = 12	N = 12
Observer ID (V_{OBS})	N = 29	N = 12	-	N = 27	-
2) COMMON GARDEN MODELS					
	A) Aggression Range= 0 – 3 score	B) Breath rate Range: 8.7 – 22.3 sec	C) Exploration Range: 0 – 253 hops	D) Tarsus length Range: 17.99 – 21 mm	E) Body mass Range: 12.9 – 19.3 g
Fixed effects					
Habitat	forest vs. urban	forest vs. urban	forest vs. urban	forest vs. urban	forest vs. urban
Sex	female vs. male	female vs. male	female vs. male	female vs. male	female vs. male
Time of day	Range: 7.42 – 11.67	Range: 7.60 – 11.97	Range: 7.67 – 12.02	-	Range: 8.22 – 11.53
Assay number	1 – 4 (categorical)	1 – 4 (categorical)	1 – 3 (categorical)	-	1 – 3 (categorical)
Observer ID		-	-	1 – 2 (categorical)	-
Random effects					
Individual ID (V_{ID})	N = 73	N = 73	N = 70	N = 72	N = 71
Origin nest ID (V_{ON})	N = 23	N = 23	N = 23	N = 23	N = 23
Foster nest ID (V_{NF})	N = 11	N = 11	N = 11	N = 11	N = 11
Aviary ID (V_{AV})	N = 8	N = 8	N = 8	N = 8	N = 8

Table 3 Fixed and random effect estimates and credible intervals (CI) for 1) wild and 2) common garden contexts across phenotypic traits (A: handling aggression, B: breath rate index, C: exploration, D: tarsus length, and E: body mass). Exploration estimates are from a Poisson generalized mixed-effect model, while all other traits were fit with Gaussian mixed-effect models. Common garden models estimated Individual ID (V_{ID}), origin nest ID (V_{NO}), foster nest ID (V_{NF}), aviary ID (V_{AV}), and residual variance (V_R). The number of observations (obs) and individuals (ind) for each trait and context are shown. Shown in bold are fixed effects whose credible intervals exclude zero or random effects whose lower CI is ≥0.001. Computed among-individual variance ($V_{IND}= V_{ID}+V_{SITE}+V_{SEX}+V_{HAB}$, $V_I =V_{ID}+V_{NO}+V_{NF}+V_{AV}+V_{SEX}+V_{HAB}$; equations 2&5) and repeatability (R) are shown for both contexts for comparison.

1) WILD

	A) Aggression N = 1308 obs, 772 ind		B) Breath rate N = 702 obs, 531 ind		C) Exploration N = 581 obs, 472 ind		D) Tarsus length N = 1437 obs, 837 ind		E) Body mass N = 1375 obs, 810 ind	
Fixed effects	Est	CI	Est	CI	Est	CI	Est	CI	Est	CI
Intercept	2.29	1.68 - 2.84	12.74	10.53 - 15.19	3.32	0.78 - 5.97	19.42	19.25 - 19.58	15.99	15.35 - 16.65
Habitat (urban)	0.03	-0.3 - 0.46	-0.70	-1.71 - 0.35	**1.60**	**0.84 - 2.2**	**-0.29**	**-0.48 - -0.11**	-0.41	-1.14 - 0.18
Sex (male)	0.13	-0.04 - 0.3	0.06	-0.3 - 0.4	-0.06	-0.47 - 0.32	**0.54**	**0.48 - 0.62**	**0.60**	**0.5 - 0.7**
Age (yearling)	-0.09	-0.2 - 0.02	-0.008	-0.32 - 0.29	0.13	-0.19 - 0.48			**-0.31**	**-0.39 - -0.24**
Time of day	**-0.04**	**-0.06 - -0.01**			-0.01	-0.1 - 0.07			**0.04**	**0.02 - 0.06**
Date of measure	-0.001	-0.003 - 0.001	-0.003	-0.02 - 0.01	-0.01	-0.03 - 0.01			0.003	0.001 – 0.005
Protocol (old)			0.16	-0.39 - 0.71	0.16	-0.35 - 0.63				
Temperature			**0.09**	**0.06 - 0.13**						
Habitat * Sex	**0.28**	**0.03 - 0.53**								
Random effects										
Individual ID (V_{IND})	**0.43**	**0.34 - 0.52**	**2.61**	**2.06 - 3.19**	**2.73**	**1.99 - 3.5**	**0.28**	**0.25 - 0.31**	**0.39**	**0.33 - 0.45**
Site ID (V_{SITE})	0.02	<0.001 - 0.08	0.25	<0.001 - 0.74	0.08	<0.001 - 0.3	0.008	<0.001 - 0.02	0.09	0.001 - 0.3
Year ID (V_{YEAR})	0.03	<0.001 - 0.07	0.05	<0.001 - 0.19	0.06	<0.001 - 0.22	0.002	<0.001 - 0.004	**0.06**	**0.01 - 0.13**
Observer ID (V_{OBS})	**0.04**	**0.01 - 0.08**	**0.52**	**0.1 - 1.26**			0.005	0.001 - 0.01		
Residual variance (V_R)	**0.55**	**0.48 - 0.62**	**1.75**	**1.4 - 2.1**	**1.61**	**1.08 - 2.14**	**0.02**	**0.02 - 0.02**	**0.27**	**0.24 - 0.31**
V_{IND}	**0.48**	**0.38 – 0.70**	**2.89**	**2.28 – 4.36**	**3.44**	**2.57 – 4.61**	**0.38**	**0.34 – 0.44**	**0.57**	**0.47 – 1.15**
R_{WILD}	**0.45**	**0.37 – 0.56**	**0.62**	**0.52 – 0.73**	**0.13**	**0.06 – 0.20**	**0.94**	**0.93 – 0.95**	**0.62**	**0.53 – 0.78**

2) COMMON GARDEN

	A) Aggression N = 285 obs, 73 ind		B) Breath rate N = 283 obs, 73 ind		C) Exploration N = 203 obs, 70 ind		D) Tarsus length N = 211 obs, 72 ind		E) Body mass N = 210 obs, 71 ind	
Fixed effects	Est	CI	Est	CI	Est	CI	Est	CI	Est	CI
Intercept	1.40	0.23 - 2.59	9.43	7.21 - 11.62	4.02	1.91 - 6.07	19.43	19.1-19.76	15.84	14.71 - 16.98
Habitat (urban)	0.12	-0.2 - 0.45	**-1.16**	**-2.32 - -0.02**	-0.05	-1.06 - 0.81	-0.22	-0.64 - 0.17	**-0.50**	**-0.96 - -0.03**
Sex (male)	-0.23	-0.5 - 0.05	**0.81**	**0.03 - 1.6**	-0.36	-0.84 - 0.14	**0.46**	**0.24 - 0.67**	**1.03**	**0.75 - 1.33**
Time of day	0.04	-0.07 - 0.16	**0.47**	**0.27 - 0.67**	-0.05	-0.24 - 0.12			-0.10	-0.2 - 0.02
Measurement (2)	-0.17	-0.44 - 0.12	**0.94**	**0.5 - 1.37**	-0.18	-0.67 - 0.24			0.12	-0.04 - 0.28
Measurement (3)	-0.25	-0.52 - 0.01	**0.50**	**0.04 - 0.9**	-0.13	-0.58 - 0.3			**0.50**	**0.37 - 0.67**
Measurement (4)	-0.38	-0.62 - -0.12	-0.38	-0.8 - 0.02						
Observer (2)							**0.07**	**0.04 - 0.11**		
Random effects										
Individual ID (V_{ID})	**0.19**	**0.08 - 0.32**	**2.15**	**1.1 - 3.18**	**0.47**	**<0.001 - 0.98**	**0.18**	**0.11 - 0.27**	**0.26**	**0.13 - 0.41**
Origin nest ID (V_{NO})	0.02	<0.001 - 0.06	0.58	<0.001 - 1.66	0.20	<0.001 - 0.71	0.10	<0.001 - 0.22	0.12	<0.001 - 0.3
Foster nest ID (V_{NF})	0.01	<0.001 - 0.05	0.15	<0.001 - 0.68	0.28	<0.001 - 1.1	0.03	<0.001 - 0.1	0.04	<0.001 - 0.13
Aviary ID (V_{AV})	0.01	<0.001 - 0.05	0.14	<0.001 - 0.62	0.05	<0.001 - 0.21				
Residual variance (V_R)	**0.56**	**0.46 - 0.67**	**1.58**	**1.29 - 1.87**	**1.66**	**1.22 - 2.17**	**0.01**	**0.01-0.02**	**0.18**	**0.14 - 0.23**
V_I	**0.25**	**0.14 – 0.45**	**3.74**	**2.54 – 5.83**	**1.01**	**0.39 – 2.49**	**0.38**	**0.26 – 0.60**	**0.74**	**0.53 – 1.11**
R_{CG}	**0.30**	**0.17 – 0.44**	**0.68**	**0.54 – 0.78**	**0.10**	**0.03 – 0.17**	**0.97**	**0.95 – 0.98**	**0.80**	**0.73– 0.87**

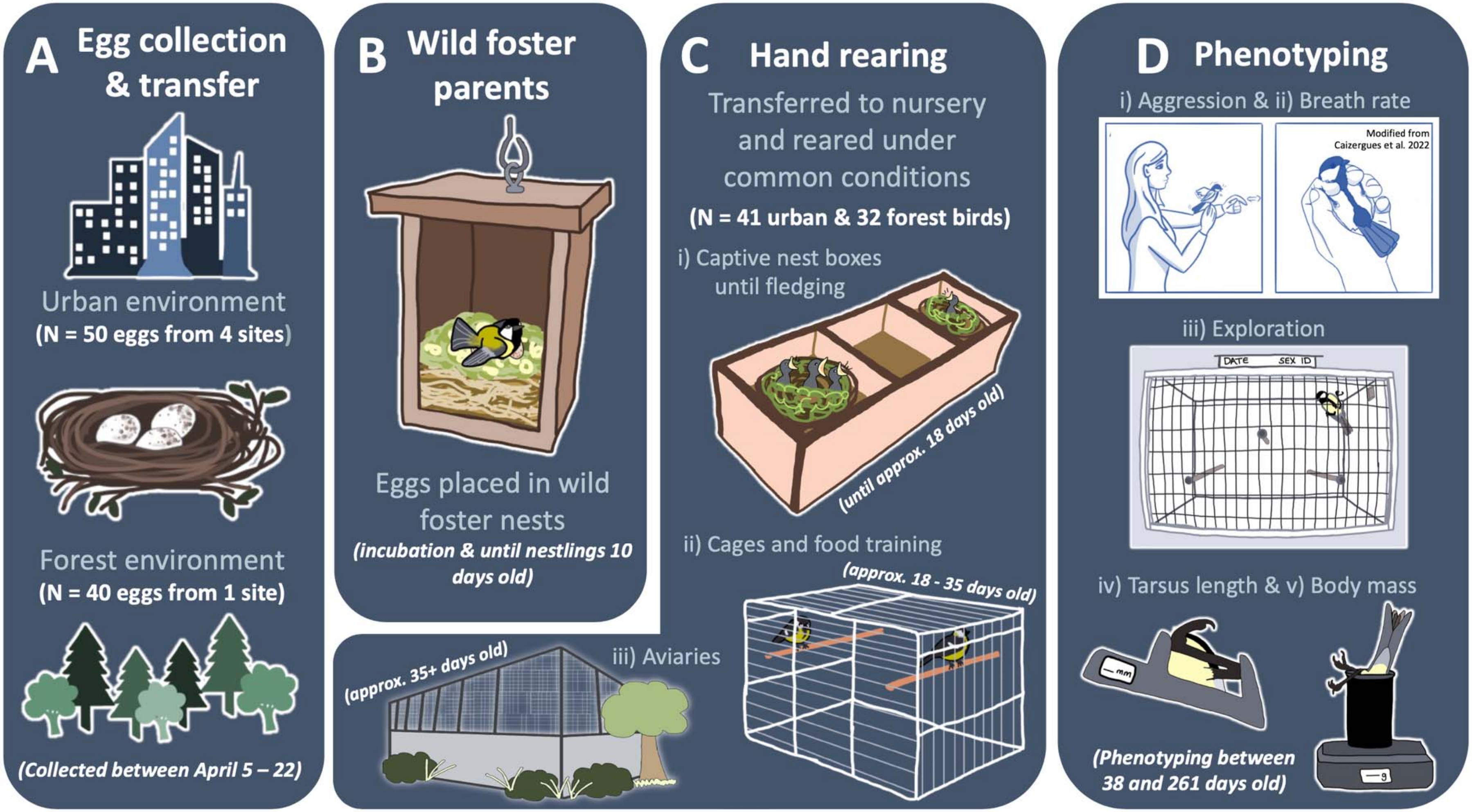

Figure 1 Procedure of common garden experiment including (A) egg collection, (B) transfer of eggs to wild foster parents, (C) transfer of nestlings to nursery for hand rearing under common conditions, and (D) phenotyping of common garden birds across i) handling aggression, ii) breath rate index, iii) exploration in a novel environment, iv) tarsus length, and v) body mass traits.

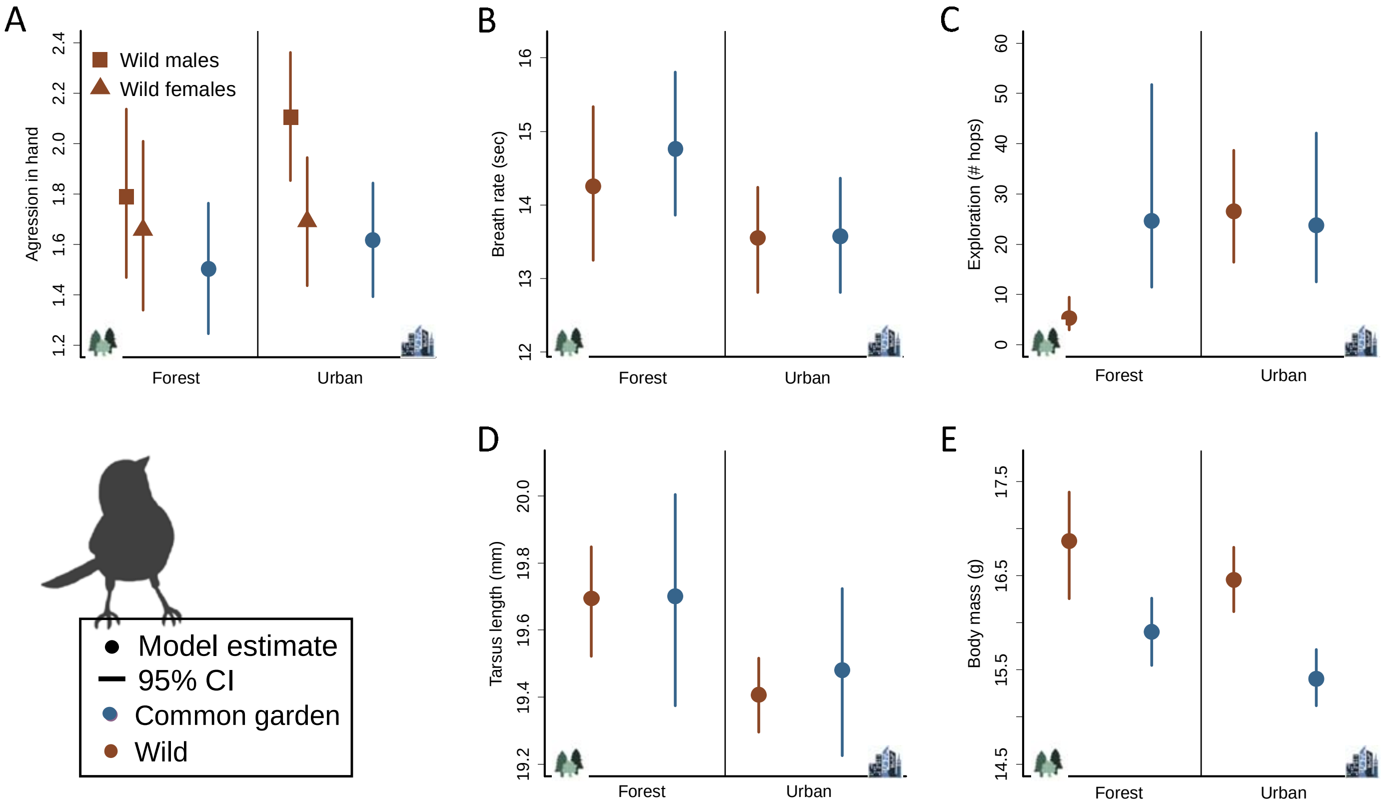

Figure 2 Habitat type model estimate and 95% credible intervals (CI) on phenotypic traits of wild (brown) and common garden birds (blue) across A) aggression in hand, B) breath rate index, C) exploration score, D) tarsus length, and E) body mass. Habitat differences varied clearly by sex only in one case (A: aggression in wild birds) and these sex differences are shown (wild males: squares, wild females: triangles); aggression did not differ clearly by sex in the common garden. Phenotypes were measured in the wild annually during the breeding season between 2011– 2022, whereas we measured phenotypes in the common garden between 06 June 2022 – 31 January 2023. See also Figure S1 for plots that show raw data.

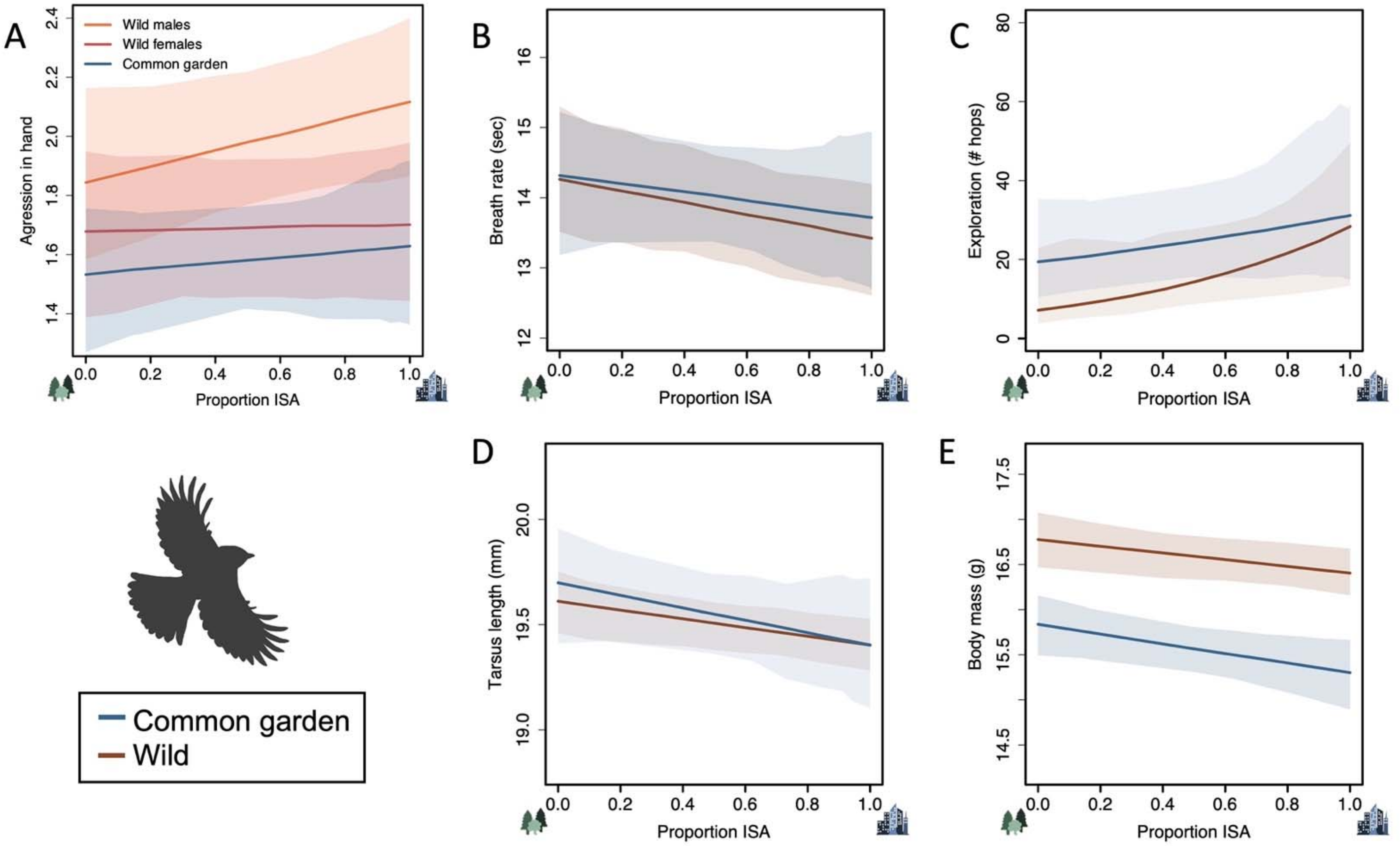

Figure 3 Proportion of impervious surface area **(**ISA) model estimate and 95% credible intervals (CI) on phenotypic traits of wild (brown) and common garden (blue) birds across A) aggression in hand, B) breath rate index, C) exploration score, D) tarsus length, and E) body mass. ISA effects varied clearly by sex only in one case (A: aggression in wild birds) and these sex differences are shown (wild males: orange, wild females: red); aggression over the ISA gradient in the common garden did not differ clearly by sex. Phenotypes were measured in the wild annually during the breeding season between 2011– 2022, whereas we measured phenotypes in the common garden between 06 June 2022 – 31 January 2023. See also Figure S2 for plots that show raw data.

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