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

Author Names:

Thompson, M.J.[1,2], Réale, D.[1], Chenet, B.[3], Delaitre, S.[2], Fargevieille, A.[2], Romans, M.[3], Caro, S.P.[2]*, Charmantier, A.[2]*

*Caro and Charmantier should be considered joint last authors

[1] Département des sciences biologiques, Université du Québec à Montréal, 141 Avenue du Président-Kennedy, Montréal, QC H2X 1Y4, Canada

[2] CEFE, Univ Montpellier, CNRS, EPHE, IRD, Montpellier, FRANCE

[3] Parc de Lunaret, Ville de Montpellier, 50 Avenue Agropolis, 34090, Montpellier, France

Corresponding author: Megan J. Thompson (thompsonjoymegan@gmail.com)

Abstract

Urban phenotypic divergences are documented across diverse taxa and are commonly assumed to result from microevolution, but the underlying genetic and environmental drivers behind these phenotypic changes are unknown in most wild urban systems. We censused urban common garden studies in the literature (N = 77) across a range of taxa. Collectively, these studies showed that both genetic and plastic responses can contribute to urban phenotypic divergences, while revealing a lack of studies with vertebrates. We conducted our own common garden experiment using great tit (*Parus major*) eggs collected along an urbanization gradient in Montpellier, France 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, suggesting genetic differentiation has driven these trait differences. Conversely, wild differences in aggression and exploration were not maintained in the common garden, indicating that plasticity to urban conditions likely drives the documented behavioural shifts. Differences between individuals (genetic and environmentally induced) explained the most trait variation in the experiment, while variation among foster nests and captive social groups was limited. Among-individual variation in size and stress response was similar between common garden and wild contexts, whereas among-individual behavioural variation was lower in the common garden than the wild. Our results provide trait-specific evidence of evolution in an urban species where genetic change likely underlies urban shifts in morphology and stress physiology, but that urban behavioural divergences are driven by plasticity.

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

Introduction

Phenotypic differences between wild populations often occur along diverse environmental gradients. Various evolutionary and ecological processes shape the phenotypic diversity we observe in nature and lead to such 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 environmental variation 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 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, 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 and traits is not as well known. 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, but also in others across the species distribution.

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.

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[1]	Urban results[2]	Conclusion[3]	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 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	Higher urban 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	(Brans and De Meester 2018)

						Faster pace of life in urban fleas Some evidence of G x E for fecundity	
		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	(Merckx et al. 2024)

						Divergence not evidence in larval stage	
		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 (*Gelsemium sempervirens*)	Wild	Morphology	F1 generation	Larger floral traits	G	Patterns in same and opposite direction to those observed in wild	(Irwin et al. 2014)
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)

[1]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.

[2]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.

[3]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.

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 urbanization at each nest box using impervious surface area (ISA; sealed non-natural surfaces) from the Copernicus imperviousness density raster data set (resolution 10 m, tiles: E38N22/E38N23, projection: LAEA EPSG 3035; European Environment Agency 2020). We calculated the proportion of ISA using a 100-meter radius circular buffer around each nest box in QGIS (v3.22.0; QGIS Development Team 2023) and calculated a ratio between the number of ISA pixels over the number of pixels contained in the buffer area (range = 0 – 1, where 1 is all ISA). We used the proportion ISA at each nest box as a continuous urbanization metric to characterize the territory of breeding wild birds (captured at nest boxes) and the territory of origin for the birds raised during the common garden experiment.

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 insured 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. Diet consisted of hand-rearing powder solution (Nutribird A21 and A19, Versele-Laga, Deinze, Belgium), alternating with dead wax moth larvae and mealworms. Once chicks were 15 days old, the diet was enriched with a cake made of eggs, sunflower margarine, sugar, wheat and protein-rich pellet flours (Country's Best Show1-2 Crumble, Versele-Laga, Deinze, Belgium). Cake was supplemented with commercial powders containing mostly vitamins and minerals (Nutribird A21, Versele-Laga; and Nekton-S, Nekton GmbH, Pforzheim, Germany). 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. Early June, we transferred individuals to large outdoor aviaries (N = 8; size = 2.2 x 4.4 – 5.5 meters; Figure 1C.iii) and individuals were randomly organized into groups blind of habitat of origin and sex (N = 6 – 10 individuals per group). At this stage birds were eating independently a diet made of cake (see above) and live mealworms. Food and water were provided *ad libitum*.

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 to determine sex and nest of origin to control for genetic relatedness and 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. We took blood samples the day before birds were transferred to outdoor aviaries and we extracted DNA from these samples using DNeasy blood and tissue kits. Extracted DNA was sent to the Montpellier GenomiX platform (MGX) for RAD sequencing following a similar protocol as described in (Caizergues et al. 2022). We had a total of N = 343 individual samples for sequencing (N = 270 wild and 73 common garden individuals), which generated 10.1 M reads

and an average depth of 19.8x per individual using paired-end RAD sequencing (2*150pb). We obtained 185321 SNPs on autosomal chromosome after filtering and we randomly subsampled 600 independent SNPs with a minor Allele Frequency (MAF) of 0.4 to reconstruct the genetic relatedness between all birds (using R package 'Sequoia'; Huisman 2017).

In one foster brood, we could identify relatedness between individuals, but not their origin nest as we were missing information on parental identity (Table S1; foster nest ZOO46 received nestlings from FAC2 and CEF7). As we could not identify the urbanization level of the origin habitat with certainty for these cases, we used the average proportion ISA between the possible origin nest boxes as their urbanization value. Genotyping revealed that the ZOO46 foster nest contained three pairs of siblings (i.e., 6 individuals with 3 unique mothers and fathers). This was presumably because the four eggs collected from origin nest CEF7 contained two separate pairs of siblings; most likely a case of egg dumping. Therefore, we coded individuals from foster nest ZOO46 as being from three different origin nests to account for relatedness between individuals and differences in maternal egg investment.

Phenotypic measurements

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). Here we examine five phenotypic traits: handling aggression, breath rate index, exploration in a novel environment, tarsus length, and body mass. 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. 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 in the wild, cage or aviary in common garden) 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). On average, we measured individuals 1.69 times in the wild (range: 1 – 8) and 3.89 times in the common garden (range: 1 – 4; approx. 44 to 264 days old; Table S2).

Breath rate index: We placed the bird in a cloth bag and allowed a five-minute standardized period of rest before measuring its stress response to handling. 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. On average, we measured individuals 1.32 times in the wild (range: 1 – 6) and 3.89 times in the common garden (range: 1 – 4; approx. 44 to 264 days old; Table S2).

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). On average, we measured

individuals 1.23 times in the wild (range: 1 – 4) and 2.89 times in the common garden (range: 1 – 3; approx. 74 to 264 days old; Table S2).

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). On average, we measured individuals 1.7 times in the wild (range: 1 – 8) and 2.92 times in the common garden (range: 1 – 3; approx. 44 to 264 days old; Table S2).

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. Although there was uncertainty around

these variance estimates (i.e., credible intervals close to zero), we present this approach in supplementary materials as it provides additional insights on the minimal contributions from early maternal effects in the experiment (i.e., low V_{NO} estimates, supplementary methods and Table S3).

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

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

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

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

where V_I is the among-individual variance that comprises variance across individuals (V_{ID}), nests of origin (V_{NO}), foster nests (V_{NF}), and aviaries (V_{AV}). V_P is the total phenotypic variance and includes sources of among-individual variance (V_I), residual variance (V_R) and fixed effect variance (V_F). We included fixed effect variance generated by non-experimental effects in the model that included habitat and sex (de Villemereuil et al. 2018). For comparison, we computed *R* in wild birds as:

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

$$\text{where } V_P = V_{IND} + V_{SITE} + V_{YEAR} + V_{OBS} + V_R + V_F \tag{5}$$

where V_{IND} is the among-individual variance estimated by individual ID in the model. V_P is the total phenotypic variance and includes variance among individuals (V_{IND}), study sites (V_{SITE}), years (V_{YEAR}), observers (V_{OBS}), and residual variance. We also include variance from habitat, sex, and age fixed effects (V_F) in the total phenotypic variance. For the Poisson models (i.e., exploration), 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). 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).

Aggression in hand: We fit aggression in hand scores as the response variable using Gaussian mixed-effect models. For the wild model, we also controlled for age category (adult vs. yearling), the time of day in continuous format (minutes divided by 60), and the Julian date of measure (days since Jan 1) as fixed effects. For the CG model, we fit the fixed effects of time of day in continuous format and the assay number. Assay number in the CG combines age effects, date or seasonal effects, and habituation effects simultaneously as these factors are correlated with repeated assays over time in our experiment. In the case of aggression in hand, assay number also accounted for observer effects as observer ID was confounded with assay number and could not be estimated for this trait (i.e., only a single observer scored first aggression assay). Results were qualitatively similar when accounting for observer ID as a fixed effect in these models instead of assay number.

Breath rate index: We fit breath rate index as the response variable in Gaussian mixed-effect models. For the wild model, we also fit age category, Julian date of measure, and protocol type (new vs. old) as fixed effects. Initially breath rate index was measured as the number of breaths for 30 seconds (between 2013 – 2016) and so we account for this difference in protocol since

these initial measures have been converted to approximate the amount of time for 30 breaths (Caizergues et al. 2022). As time of day and temperature were correlated in this data set (r^2 = 0.48, $P < 0.001$), we chose to only include temperature as an additional fixed effect since this variable explained more variation in this trait than time of day. For the CG model, we fit time of day (as temperature conditions were constant) and the assay number as fixed effects.

Exploration: The number of hops and flights were fit as the response variable in a Poisson generalized mixed-effect model. For the model on wild birds, we fit age category, Julian date of measure, time of day, and protocol type (old vs. new) as fixed effects. For the CG model, we fit time of day and assay number as fixed effects. We excluded three individuals from this analysis; one individual was injured before the exploration assay (forest female) and two individuals did not regrow all their wing feathers after moulting which affected movement during the exploration assay (one urban female and one forest male).

Tarsus length: Tarsus length was fit as the response variable in Gaussian mixed-effect models. As tarsus length is fixed early in life and should not be affected over time, we controlled for fewer confounding effects for this trait. In the wild models, we did not add additional fixed effects. In the CG model, we included observer as a fixed effect, which accounted for both differences between observer measures and differences between life stages as the first observer measured individuals earlier in life and the other observer past 152 days old. We excluded one urban female from this analysis that was a clear outlier for this trait; this individual was very small and undeveloped at 10 days post hatching when entering captivity (e.g., weight = 4.6 g vs. average weight = 13.6 g).

Body mass: Body mass was fit as the response variable in Gaussian mixed-effect models. For the wild model, we also controlled for age category, date of measure, and time of day as fixed

effects. For the CG model, we also included the time of day and assay number as fixed effects. Besides the individual outlier that we excluded for tarsus length, we also excluded a forest female that was injured before body mass measurements.

Results

Aggression in hand: We found clear evidence that wild urban birds were more aggressive than wild forest birds with this habitat difference being driven by higher aggression in urban males (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 effect; Table 3.2A; 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 the 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). These differences were not 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 as the wild where birds from urban origins tended to have shorter tarsi than birds from forest origins, but we find no clear evidence that tarsus length differed by habitat of origin (credible interval overlapping zero). The habitat effect size was slightly weaker in the common garden than the wild (i.e., CG: $\beta_{habitat}$ = - 0.22, Table 3.2D vs. wild: $\beta_{habitat}$ = - 0.29, Table 3.1D), but was stronger when examining how tarsus length decreased over the urban gradient (i.e., CG: β_{ISA} = - 0.29 vs. wild: β_{ISA} = - 0.20, Table S4). 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).

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.

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 and random effects whose credible intervals exclude zero (or 0.01 for random effects; Bonnet et al. 2022). Computed among-individual variance (V_{IND} & V_I) 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	-0.01	-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	0.00	0 - 0	0.00	-0.02 - 0.01	-0.01	-0.03 - 0.01			0.00	0 - 0
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 - 0.08	0.25	0 - 0.74	0.08	0 - 0.3	0.01	0 - 0.02	0.09	0 - 0.3
Year ID (V_{YEAR})	0.03	0 - 0.07	0.05	0 - 0.19	0.06	0 - 0.22	0.00	0 - 0	**0.06**	**0.01 - 0.13**
Observer ID (V_{OBS})	**0.04**	**0.01 - 0.08**	**0.52**	**0.1 - 1.26**			0.00	0 - 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**
R_{WILD}	**0.39**	**0.30 – 0.46**	**0.50**	**0.34 – 0.60**	**0.54**	**0.39 – 0.66**	**0.69**	**0.60 – 0.74**	**0.41**	**0.25 – 0.49**

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 - 0.98**	**0.18**	**0.11 - 0.27**	**0.26**	**0.13 - 0.41**
Origin nest ID (V_{ON})	0.02	0 - 0.06	0.58	0 - 1.66	0.20	0 - 0.71	0.10	0 - 0.22	0.12	0 - 0.3
Foster nest ID (V_{NF})	0.01	0 - 0.05	0.15	0 - 0.68	0.28	0 - 1.1	0.03	0 - 0.1	0.04	0 - 0.13
Aviary ID (V_{AV})	0.01	0 - 0.05	0.14	0 - 0.62	0.05	0 - 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(V_{ID} + V_{NO} + V_{NF}+ V_{AV} + V_F)	**0.22**	**0.11 – 0.41**	**2.92**	**1.95 – 4.68**	**0.91**	**0.33 – 2.18**	**0.30**	**0.20 – 0.49**	**0.40**	**0.26 – 0.68**
R_{CG}	**0.26**	**0.14 – 0.40**	**0.53**	**0.39 – 0.67**	**0.35**	**0.14 – 0.55**	**0.78**	**0.62 – 0.91**	**0.43**	**0.32– 0.60**

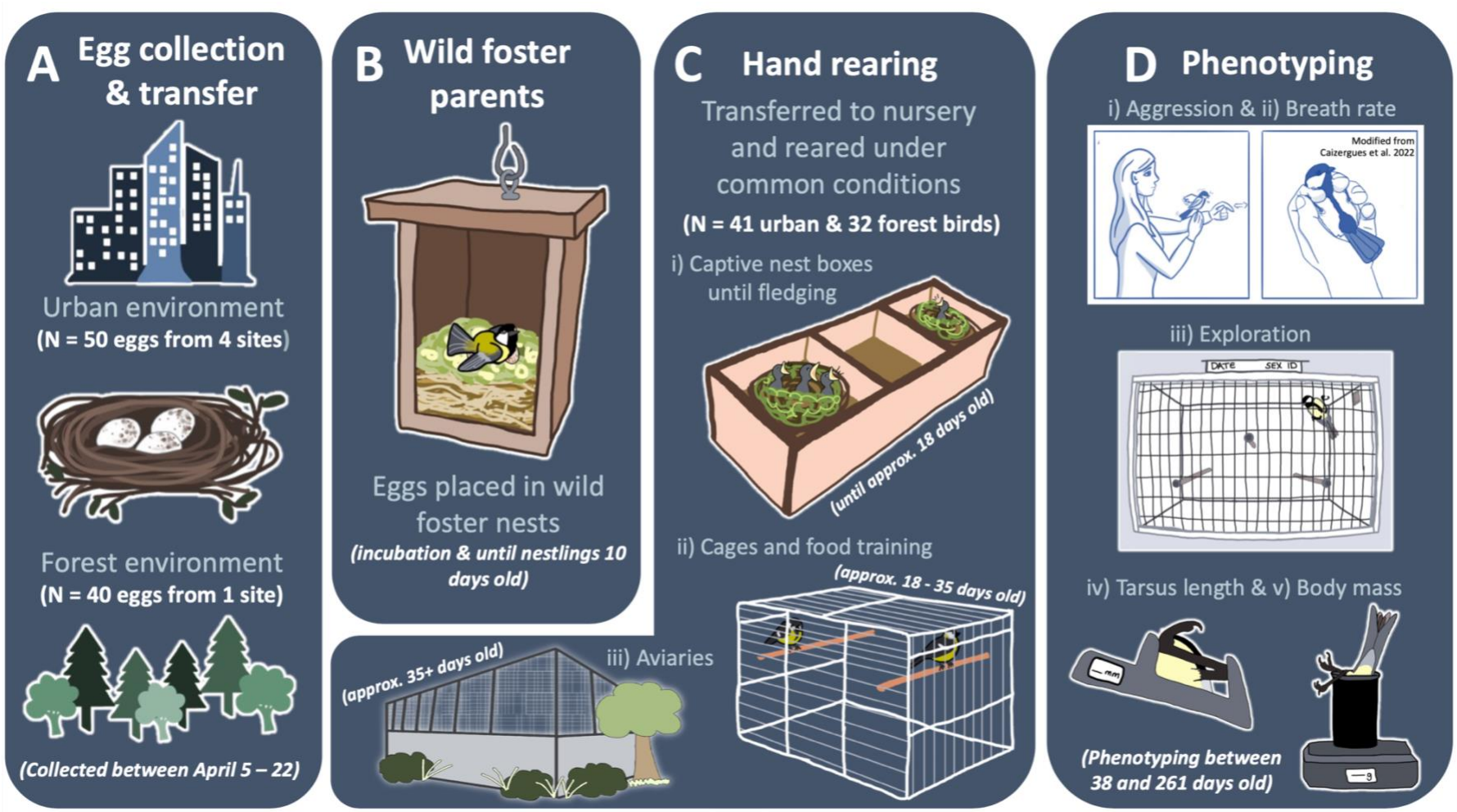

Figure 1 Procedure of common garden experiment including (A) egg collection, (B) transfer of eggs to wild foster parents, (C) transfer of nestlings to nursery for hand rearing under common conditions, and (D) phenotyping of common garden birds across i) handling aggression, ii) breath rate index, iii)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

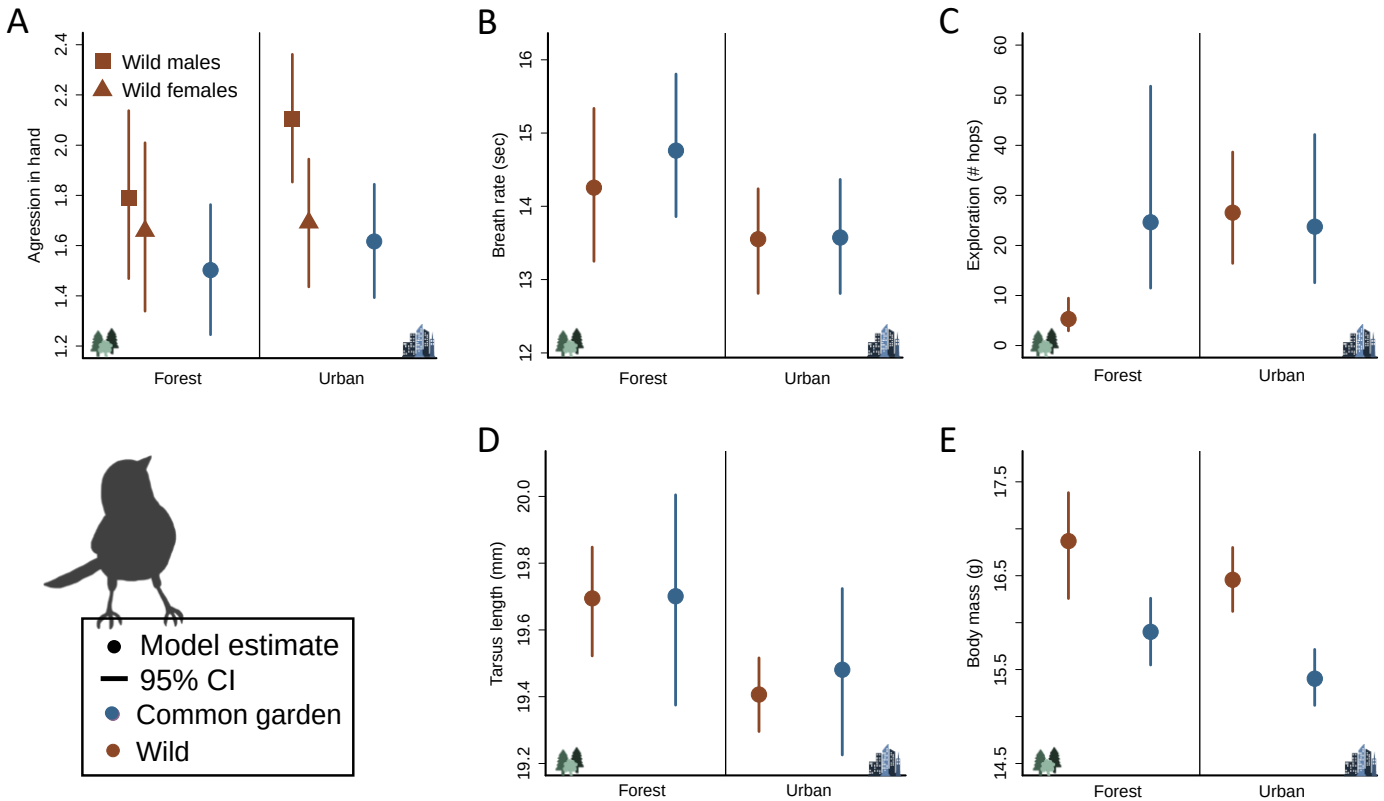

Figure 2 Habitat type model estimate and 95% credible intervals (CI) on phenotypic traits of wild (brown) and common garden birds (blue) across A) aggression in hand, B) breath rate index, C) exploration score, D) tarsus length, and E) body mass. Habitat differences varied clearly by sex only in one 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.

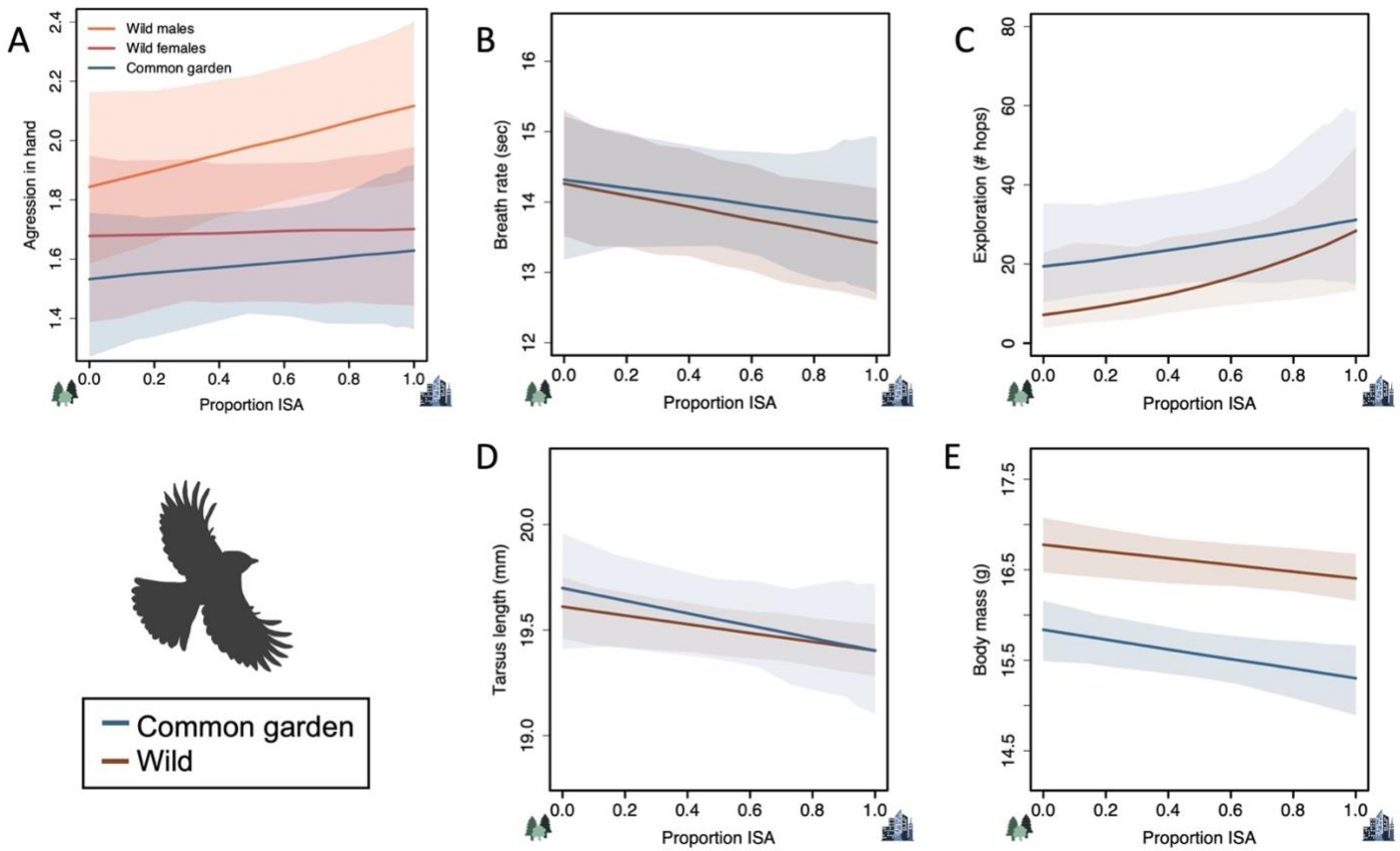

Figure 3 Proportion of impervious surface area **(**ISA) model estimate and 95% credible intervals (CI) on phenotypic traits of wild (brown) and common garden (blue) birds across A) aggression in hand, B) breath rate index, C) exploration score, D) tarsus length, and E) body mass. ISA effects varied 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.

Discussion

We found evidence that both genetic and plastic changes have contributed to the phenotypic shifts in wild urban tits, but the relative contributions of these drivers are trait specific (aim 1). Specifically, we found that genetic differences between populations likely contributes to breath rate and body mass divergences, and that plasticity to urban conditions 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 clear statistical 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 maintained in our experiment. 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, the wild habitat difference is small and we lack statistical power to make firm conclusions on whether this difference in tarsus length was maintained in the common garden. A power analysis suggests we had 48% power to detect the tarsus difference in the common garden and that future common garden efforts would need at least 75 individuals per urban and forest habitat group (cohen's d = 0.46 with 80% power, using R package "pwr"). 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. Our analysis showed that additive genetic variation (V_A estimate in Table S3) explains 33% of tarsus variation when individuals are reared under the same environmental conditions in our experiment. 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 Veszprem, 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 better adapted to warming conditions and since we find support that smaller urban body mass is driven by genetic change, 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, 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 could be explained by neutral genetic variation between these populations (F_{ST} values between 0.006 – 0.008; Perrier et al. 2018). However, the high 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 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 also 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 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 also explain these urban behavioural differences in the wild. Dispersal dynamics and habitat matching in an urban context are still poorly understood but, as more aggressive and exploratory phenotypes in this urban population do not afford survival or reproductive benefits (Caizergues et al. 2022), habitat matching may be less likely to contribute to these urban phenotypic shifts than plasticity.

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). Although credible intervals were overlapping between contexts, we estimated lower among-individual variance and repeatability for aggression and exploration in the common garden compared to the wild. Wild aggression and exploration divergences were not maintained in the common garden, and so it is likely that reduced environmental variation in the experiment decreased individual differences in these traits. 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). 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. 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 a result of 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 that genetic change has driven urban behavioural shifts (i.e., more labile traits), 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.

Acknowledgements

We would like to thank everyone who participated in the common garden and is not an author: Laura Gervais, Barbara Class, Dhanya Bharath, Christophe de Franceschi, Sam Perret from the CEFE and Vivian Espinasse, Thibault Pujol, Flavien Daunis, Cathie Troussier, Jérôme Brière, Laetitia Boscardin, Sébastien Pouvreau, Lucas Boussioux, Charlotte Gay, Marion Darde from the Montpellier Zoo. Thanks to Pierre de Villemereuil for useful discussions and coding help. MJT was supported by a Canadian Graduate Scholarship from the Natural Sciences and Engineering Research Council of Canada, a Fonds de recherche du Québec Nature et technologies PhD scholarship, and a mobility grant from le Centre Méditerranéen de l'Environnement et de la Biodiversité. This project was funded by the Agence Nationale de la Recherche (URBANTIT grant ANR-19-CE34-0008-05 to AC), the OSU-OREME, the long-term Studies in Ecology and Evolution (SEE-Life) program of the CNRS, and a Fonds de Recherche du Quebec Nature et Technologie to DR.

References:

Alberti M, Marzluff J, Hunt VM. 2017. Urban driven phenotypic changes: empirical observations and theoretical implications for eco-evolutionary feedback. Philosophical Transactions of the Royal Society B: Biological Sciences. 372(1712):20160029. doi:10.1098/rstb.2016.0029.

Altermatt F, Ebert D. 2016. Reduced flight-to-light behaviour of moth populations exposed to long-term urban light pollution. Biology letters. 12(4):20160111. doi:https://doi.org/10.1098/rsbl.2016.0111.

Antonio-Nkondjio C, Fossog BT, Ndo C, Djantio BM, Togouet SZ, Awono-Ambene P, Costantini C, Wondji CS, Ranson H. 2011. *Anopheles gambiae* distribution and insecticide resistance in the cities of Douala and Yaoundé (Cameroon): influence of urban agriculture and pollution. Malaria Journal. 10(1):1–13.

Atwell JW, Cardoso GC, Whittaker DJ, Campbell-Nelson S, Robertson KW, Ketterson ED. 2012. Boldness behavior and stress physiology in a novel urban environment suggest rapid correlated evolutionary adaptation. Behavioral Ecology. 23(5):960–969. doi:10.1093/beheco/ars059.

Atwell JW, Cardoso GC, Whittaker DJ, Price TD, Ketterson ED. 2014. Hormonal, behavioral, and life-history traits exhibit correlated shifts in relation to population establishment in a novel environment. The American Naturalist. 184(6):E147–E160. doi:10.1086/678398.

Baxter-Gilbert J, Riley JL, Whiting MJ. 2019. Bold new world: urbanization promotes an innate behavioral trait in a lizard. Behavioral Ecology and Sociobiology. 73(8):1–10.

Biard C, Brischoux F, Meillère A, Michaud B, Nivière M, Ruault S, Vaugoyeau M, Angelier F. 2017. Growing in cities: An urban penalty for wild birds? A study of phenotypic differences between urban and rural great tit chicks (*Parus major*). Frontiers in Ecology and Evolution. 5:1–14. doi:10.3389/fevo.2017.00079.

Bókony V, Balogh E, Ujszegi J, Ujhegyi N, Szederkényi M, Hettyey A. 2024. Tadpoles develop elevated heat tolerance in urban heat islands regardless of sex. Evol Biol. 51(1):209–216. doi:10.1007/s11692-024-09626-7.

Bókony V, Ujhegyi N, Hamow KÁ, Bosch J, Thumsová B, Vörös J, Aspbury AS, Gabor CR. 2021. Stressed tadpoles mount more efficient glucocorticoid negative feedback in anthropogenic habitats due to phenotypic plasticity. Science of the Total Environment. 753:141896. doi:10.1016/j.scitotenv.2020.141896.

Bókony V, Üveges B, Verebélyi V, Ujhegyi N, Móricz ÁM. 2019. Toads phenotypically adjust their chemical defences to anthropogenic habitat change. Scientific reports. 9(1):1–8.

Bonier F. 2012. Hormones in the city: Endocrine ecology of urban birds. Hormones and Behavior. 61(5):763–772. doi:10.1016/j.yhbeh.2012.03.016.

Bonnet T, Morrissey MB, De Villemereuil P, Alberts SC, Arcese P, Bailey LD, Boutin S, Brekke P, Brent LJN, Camenisch G, et al. 2022. Genetic variance in fitness indicates rapid contemporary adaptive evolution in wild animals. Science. 376(6596):1012–1016. doi:10.1126/science.abk0853.

Brans KI, Almeida RA, Fajgenblat M. 2021. Genetic differentiation in pesticide resistance between urban and rural populations of a nontarget freshwater keystone interactor, *Daphnia magna*. Evolutionary Applications. 14(10):2541–2552. doi:10.1111/eva.13293.

Brans KI, De Meester L. 2018. City life on fast lanes: Urbanization induces an evolutionary shift towards a faster lifestyle in the water flea *Daphnia*. Functional Ecology. 32(9):2225–2240.

Brans KI, Govaert L, Engelen JMT, Gianuca AT, Souffreau C, De Meester L. 2017. Eco-evolutionary dynamics in urbanized landscapes: evolution, species sorting and the change in zooplankton body size along urbanization gradients. Philosophical Transactions of the Royal Society B: Biological Sciences. 372(1712):20160030.

Brans KI, Jansen M, Vanoverbeke J, Tüzün N, Stoks R, De Meester L. 2017. The heat is on: Genetic adaptation to urbanization mediated by thermal tolerance and body size. Global change biology. 23(12):5218–5227.

Brans KI, Stoks R, De Meester L. 2018. Urbanization drives genetic differentiation in physiology and structures the evolution of pace-of-life syndromes in the water flea *Daphnia magna*. Proceedings of the Royal Society B: Biological Sciences. 285(1883). doi:10.1098/rspb.2018.0169.

Brans KI, Tüzün N, Sentis A, De Meester L, Stoks R. 2022. Cryptic eco-evolutionary feedback in the city: Urban evolution of prey dampens the effect of urban evolution of the predator. Journal of Animal Ecology. 91(3):514–526. doi:10.1111/1365-2656.13601.

Breitbart ST, Agrawal AA, Wagner HH, Johnson MT. 2023. Urbanization and a green corridor do not impact genetic divergence in common milkweed (*Asclepias syriaca L.*). Scientific Reports. 13(1):20437.

Caizergues AE, Charmantier A, Lambrechts MM, Perret S, Demeyrier V, Lucas A, Grégoire A. 2021. An avian urban morphotype: how the city environment shapes great tit morphology at different life stages. Urban Ecosystems. 45(5):929–941. doi:10.1007/s11252-020-01077-0.

Caizergues AE, Grégoire A, Charmantier A. 2018. Urban versus forest ecotypes are not explained by divergent reproductive selection. Proceedings of the Royal Society B: Biological Sciences. 285:20180261.

Caizergues AE, Grégoire A, Choquet R, Perret S, Charmantier A. 2022. Are behaviour and stress-related phenotypes in urban birds adaptive? Journal of Animal Ecology. 91(8):1627–1641.

Caizergues AE, Le Luyer J, Grégoire A, Szulkin M, Senar J, Charmantier A, Perrier C. 2022. Epigenetics and the city: Non-parallel DNA methylation modifications across pairs of urban-forest Great tit populations. Evolutionary Applications. 15(1):149–165. doi:10.1111/eva.13334.

Caizergues AE, Robira B, Perrier C, Jeanneau M, Berthomieu A, Perret S, Gandon S, Charmantier A. 2024. Cities as parasitic amplifiers? Malaria prevalence and diversity in great tits along an urbanization gradient. Peer Community Journal. 4:article no. e38. doi:10.24072/pcjournal.405.

Camacho C, Sanabria-Fernández A, Baños-Villalba A, Edelaar P. 2020. Experimental evidence that matching habitat choice drives local adaptation in a wild population. Proceedings of the Royal Society B: Biological Sciences. 287:20200721.

Campbell-Staton SC, Velotta JP, Winchell KM. 2021. Selection on adaptive and maladaptive gene expression plasticity during thermal adaptation to urban heat islands. Nature communications. 12(1):6195.

Capilla-Lasheras P, Thompson MJ, Sánchez-Tójar A, Haddou Y, Branston CJ, Réale D, Charmantier A, Dominoni DM. 2022. A global meta-analysis reveals higher variation in breeding phenology in urban birds than in their non-urban neighbours. Ecology Letters. 25(11):2552–2570.

Carere C, van Oers K. 2004. Shy and bold great tits (*Parus major*): Body temperature and breath rate in response to handling stress. Physiology and Behavior. 82(5):905–912. doi:10.1016/j.physbeh.2004.07.009.

Caspi T, Johnson JR, Lambert MR, Schell CJ, Sih A. 2022. Behavioral plasticity can facilitate evolution in urban environments. Trends in Ecology & Evolution. 37(12):1092–1103.

Charmantier A, Demeyrier V, Lambrechts M, Perret S, Grégoire A. 2017. Urbanization is associated with divergence in pace-of-life in great tits. Frontiers in Ecology and Evolution. 5:1–13. doi:10.3389/fevo.2017.00053.

Charmantier A, Garant D, Kruuk LE. 2014. Quantitative genetics in the wild. OUP Oxford.

Cheptou P-O, Carrue O, Rouifed S, Cantarel A. 2008. Rapid evolution of seed dispersal in an urban environment in the weed Crepis sancta. Proceedings of the National Academy of Sciences. 105(10):3796–3799.

Chick LD, Strickler SA, Perez A, Martin RA, Diamond SE. 2019. Urban heat islands advance the timing of reproduction in a social insect. Journal of Thermal Biology. 80:119–125. doi:10.1016/j.jtherbio.2019.01.004.

Chick LD, Waters JS, Diamond SE. 2021. Pedal to the metal: Cities power evolutionary divergence by accelerating metabolic rate and locomotor performance. Evolutionary Applications. 14(1):36–52. doi:10.1111/eva.13083.

Christians JK. 2002. Avian egg size: variation within species and inflexibility within individuals. Biological reviews. 77(1):1–26.

Conover DO, Duffy TA, Hice LA. 2009. The covariance between genetic and environmental influences across ecological gradients. The Year in Evolutionary Biology 2009, Volume 1168. 28:100.

Corsini M, Schöll EM, Di Lecce I, Chatelain M, Dubiec A, Szulkin M. 2021. Growing in the city: Urban evolutionary ecology of avian growth rates. Evolutionary Applications. 14(1):69–84. doi:10.1111/eva.13081.

Czaczkes TJ, Bastidas-Urrutia AM, Ghislandi P, Tuni C. 2018. Reduced light avoidance in spiders from populations in light-polluted urban environments. The Science of Nature. 105(11):1–5.

Demeyrier V, Lambrechts MM, Perret P, Grégoire A. 2016. Experimental demonstration of an ecological trap for a wild bird in a human-transformed environment. Animal Behaviour. 118:181–190. doi:10.1016/j.anbehav.2016.06.007.

Des Roches S, Pendleton LH, Shapiro B, Palkovacs EP. 2021. Conserving intraspecific variation for nature's contributions to people. Nature Ecology and Evolution. 5(5):574–582. doi:10.1038/s41559-021-01403-5.

Dhondt AA. 1982. Heritability of blue tit tarsus length from normal and cross-fostered broods. Evolution. 36(2):418–419.

Diamond SE, Chick L, Perez ABE, Strickler SA, Martin RA. 2017. Rapid evolution of ant thermal tolerance across an urban-rural temperature cline. Biological Journal of the Linnean Society. 121(2):248–257.

Diamond Sarah E., Chick LA, Perez A, Strickler SA, Zhao C. 2018. Evolution of plasticity in the city: Urban acorn ants can better tolerate more rapid increases in environmental temperature. Conservation Physiology. 6(1):1–12. doi:10.1093/conphys/coy030.

Diamond Sarah E, Chick LD, Perez A, Strickler SA, Martin RA. 2018. Evolution of thermal tolerance and its fitness consequences: parallel and non-parallel responses to urban heat islands across three cities. Proceedings of the Royal Society B: Biological Sciences. 285(1882):20180036.

Diamond SE, Martin RA. 2021. Evolution in cities. Annual Review of Ecology, Evolution, and Systematics. 52:519–540.

Diamond SE, Martin RA, Bellino G, Crown KN, Prileson EG. 2022. Urban evolution of thermal physiology in a range-expanding, mycophagous fruit fly, *Drosophila tripunctata*. Biological Journal of the Linnean Society. 137(3):409–420.

Dubuc-Messier G, Caro SP, Perrier C, van Oers K, Réale D, Charmantier A. 2018. Gene flow does not prevent personality and morphological differentiation between two blue tit populations. Journal of Evolutionary Biology. 31(8):1127–1137. doi:10.1111/jeb.13291.

Dubuc-Messier G, Réale D, Perret P, Charmantier A. 2016. Environmental heterogeneity and population differences in blue tits personality traits. Behavioral Ecology. 28:arw148. doi:10.1093/beheco/arw148.

Edelaar P, Siepielski AM, Clobert J. 2008. Matching habitat choice causes directed gene flow: A neglected dimension in evolution and ecology. Evolution. 62(10):2462–2472. doi:10.1111/j.1558-5646.2008.00459.x.

European Environment Agency. 2020. Imperviousness Density 2018. https://land.copernicus.eu/user-corner/technical-library/imperviousness-2018-user-manual.pdf.

Fudickar AM, Greives TJ, Abolins-Abols M, Atwell JW, Meddle SL, Friis G, Stricker CA, Ketterson ED. 2017. Mechanisms associated with an advance in the timing of seasonal reproduction in an urban songbird. Frontiers in Ecology and Evolution. 5:85.

Fukano Y, Guo W, Uchida K, Tachiki Y. 2020. Contemporary adaptive divergence of plant competitive traits in urban and rural populations and its implication for weed management. Journal of Ecology. 108(6):2521–2530.

Gebhardt-Henrich SG, Van Noordwijk AJ. 1991. Nestling growth in the great tit I. Heritability estimates under different environmental conditions. Journal of Evolutionary Biology. 4(3):341–362.

Géron C, Lembrechts JJ, Hamdi R, Berckmans J, Nijs I, Monty A. 2022. Phenotypic variation along urban-to-rural gradients: an attempt to disentangle the mechanisms at play using the alien species Matricaria discoidea (Asteraceae). Plant Ecology. 223(10):1219–1231.

Ghalambor CK, McKay JK, Carroll SP, Reznick DN. 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. Functional Ecology. 21(3):394–407. doi:10.1111/j.1365-2435.2007.01283.x.

y Gomez GSM, Van Dyck H. 2012. Ecotypic differentiation between urban and rural populations of the grasshopper Chorthippus brunneus relative to climate and habitat fragmentation. Oecologia. 169(1):125–133.

Gorton AJ, Moeller DA, Tiffin P. 2018. Little plant, big city: a test of adaptation to urban environments in common ragweed (*Ambrosia artemisiifolia*). Proceedings of the Royal Society B: Biological Sciences. 285(1881):20180968.

Gosler AG, Harper DGC. 2000. Assessing the heritability of body condition in birds: a challenge exemplified by the great tit Parus major L.(Aves). Biological Journal of the Linnean Society. 71(1):103–117.

Hadfield JD. 2010. MCMC Methods for Multi-Response Generalized Linear Mixed Models: The {MCMCglmm} {R} Package. Journal of Statistical Software. 33(2):1–22.

Hahs AK, Fournier B, Aronson MF, Nilon CH, Herrera-Montes A, Salisbury AB, Threlfall CG, Rega-Brodsky CC, Lepczyk CA, La Sorte FA. 2023. Urbanisation generates multiple trait syndromes for terrestrial animal taxa worldwide. Nature communications. 14(1):4751.

Harten L, Gonceer N, Handel M, Dash O, Fokidis HB, Yovel Y. 2021. Urban bat pups take after their mothers and are bolder and faster learners than rural pups. BMC Biol. 19(1):190. doi:10.1186/s12915-021-01131-z.

Hendry AP. 2017. Eco-evolutionary Dynamics. Princeton: Princeton University Press. [accessed 2024 Apr 12]. https://www.degruyter.com/document/doi/10.1515/9781400883080/html.

Hendry AP, Farrugia TJ, Kinnison MT. 2008. Human influences on rates of phenotypic change in wild animal populations. Molecular Ecology. 17(1):20–29. doi:10.1111/j.1365-294X.2007.03428.x.

Hõrak P. 1994. Effect of nestling history on adult size and reproduction in the Great Tit. Ornis fennica. 71(2):47–54.

Hõrak P, Mänd R, Ots I, Leivits A. 1995. Egg size in the Great Tit Parus major: individual, habitat and geographic differences. Ornis fennica. 72(3):97–114.

Huisman J. 2017. Pedigree reconstruction from SNP data: parentage assignment, sibship clustering and beyond. Molecular Ecology Resources. 17(5):1009–1024. doi:10.1111/1755-0998.12665.

Hwang CC, Turner BD. 2009. Small-scaled geographical variation in life-history traits of the blowfly *Calliphora vicina* between rural and urban populations. Entomologia Experimentalis et Applicata. 132(3):218–224.

Ichikawa I, Kuriwada T. 2023. The combined effects of artificial light at night and anthropogenic noise on life history traits in ground crickets. Ecological Research. 38(3):446–454. doi:10.1111/1440-1703.12380.

Irwin RE, Warren PS, Carper AL, Adler LS. 2014. Plant–animal interactions in suburban environments: implications for floral evolution. Oecologia. 174:803–815.

Jacquier L., Doums C, Four-Chaboussant A, Peronnet R, Tirard C, Molet M. 2021. Urban colonies are more resistant to a trace metal than their forest counterparts in the ant *Temnothorax nylanderi*. Urban Ecosystems. 24:561–570.

Jacquier Lauren, Molet M, Bocquet C, Doums C. 2021. Hibernation conditions contribute to the differential resistance to cadmium between urban and forest ant colonies. Animals. 11(4):1050.

Kaiser A, Merckx T, Van Dyck H. 2018. Urbanisation and sex affect the consistency of butterfly personality across metamorphosis. Behavioral Ecology and Sociobiology. 72(12):1–11.

Kaiser A, Merckx T, Van Dyck H. 2020. An experimental test of changed personality in butterflies from anthropogenic landscapes. Behavioral Ecology and Sociobiology. 74(7):1–8.

Kawecki TJ, Ebert D. 2004. Conceptual issues in local adaptation. Ecology letters. 7(12):1225–1241.

Kern E, Langerhans RB. 2019. Urbanization alters swimming performance of a stream fish. Frontiers in Ecology and Evolution. 6:229.

Kern EMA, Langerhans RB. 2018. Urbanization drives contemporary evolution in stream fish. Global Change Biology. 24(8):3791–3803.

Kinnison MT, Hendry AP. 2001. The pace of modern life II: From rates of contemporary microevolution to pattern and process. Genetica. 112–113:145–164. doi:10.1007/978-94-010-0585-2_10.

Koolhaas JM, Bartolomucci A, Buwalda B, de Boer SF, Flügge G, Korte SM, Meerlo P, Murison R, Olivier B, Palanza P. 2011. Stress revisited: a critical evaluation of the stress concept. Neuroscience & Biobehavioral Reviews. 35(5):1291–1301.

Koolhaas JM, De Boer SF, Coppens CM, Buwalda B. 2010. Neuroendocrinology of coping styles: towards understanding the biology of individual variation. Frontiers in neuroendocrinology. 31(3):307–321.

Kostanecki A, Gorton AJ, Moeller DA. 2021. An urban–rural spotlight: Evolution at small spatial scales among urban and rural populations of common ragweed. Journal of Urban Ecology. 7(1):juab004.

Krams IA, Vrublevska J, Sepp T, Abolins-Abols M, Rantala MJ, Mierauskas P, Krama T. 2014. Sex-specific associations between nest defence, exploration and breathing rate in breeding pied flycatchers. Wright J, editor. Ethology. 120(5):492–501. doi:10.1111/eth.12222.

Kuriwada T. 2023. Differences in male calling song and female mate location behaviour between urban and rural crickets. Biological Journal of the Linnean Society. 139(3):275–285.

Lambert MR, Brans KI, Roches SD, Donihue CM, Diamond SE. 2021. Adaptive evolution in cities: progress and misconceptions. Trends in Ecology & Evolution. 36(3):239–257.

Lambert MR, Donihue CM. 2020. Urban biodiversity management using evolutionary tools. Nature Ecology and Evolution. 4(7):903–910. doi:10.1038/s41559-020-1193-7.

Lambrecht SC, Mahieu S, Cheptou P-O. 2016. Natural selection on plant physiological traits in an urban environment. Acta Oecologica. 77:67–74.

Lampe U, Reinhold K, Schmoll T. 2014. How grasshoppers respond to road noise: Developmental plasticity and population differentiation in acoustic signalling. Functional Ecology. 28(3):660–668. doi:10.1111/1365-2435.12215.

Leinonen T, Mccairns RJS, Hara RBO, Merilä J. 2013. QST – FST comparisons : evolutionary and ecological insights from genomic heterogeneity. Nature Reviews Genetics. doi:10.1038/nrg3395.

Martin RA, Chick LD, Yilmaz AR, Diamond SE. 2019. Evolution, not transgenerational plasticity, explains the adaptive divergence of acorn ant thermal tolerance across an urban–rural temperature cline. Evolutionary applications. 12(8):1678–1687.

McLean MA, Angilletta Jr MJ, Williams KS. 2005. If you can't stand the heat, stay out of the city: thermal reaction norms of chitinolytic fungi in an urban heat island. Journal of Thermal Biology. 30(5):384–391.

Merckx T, Nielsen ME, Heliölä J, Kuussaari M, Pettersson LB, Pöyry J, Tiainen J, Gotthard K, Kivelä SM. 2021. Urbanization extends flight phenology and leads to local adaptation of seasonal plasticity in Lepidoptera. Proc Natl Acad Sci USA. 118(40):e2106006118. doi:10.1073/pnas.2106006118.

Merckx T, Nielsen ME, Kankaanpää T, Kadlec T, Yazdanian M, Kivelä SM. 2023. Dim light pollution prevents diapause induction in urban and rural moths. Journal of Applied Ecology. 60(6):1022–1031. doi:10.1111/1365-2664.14373.

Merckx T, Nielsen ME, Kankaanpää T, Kadlec T, Yazdanian M, Kivelä SM. 2024. Continent-wide parallel urban evolution of increased heat tolerance in a common moth. Evolutionary Applications. 17(1):e13636. doi:10.1111/eva.13636.

Merckx T, Souffreau C, Kaiser A, Baardsen LF, Backeljau T, Bonte D, Brans KI, Cours M, Dahirel M, Debortoli N, et al. 2018. Body-size shifts in aquatic and terrestrial urban communities. Nature. 558(7708):113–116. doi:10.1038/s41586-018-0140-0.

Merilä J, Fry JD. 1998. Genetic variation and causes of genotype-environment interaction in the body size of blue tit (*Parus caeruleus*). Genetics. 148(3):1233–1244.

Mertens JAL. 1977. Thermal conditions for successful breeding in great tits (Parus major L.) I. Relation of growth and development of temperature regulation in nestling great tits. Oecologia. 28(1):1–29.

Miranda AC, Schielzeth H, Sonntag T, Partecke J. 2013. Urbanization and its effects on personality traits: A result of microevolution or phenotypic plasticity? Global Change Biology. 19(9):2634–2644. doi:10.1111/gcb.12258.

Mitchell-Olds T, Willis JH, Goldstein DB. 2007. Which evolutionary processes influence natural genetic variation for phenotypic traits? Nature Reviews. 8:845–856. doi:10.1038/nrg2207.

Mohajerani A, Bakaric J, Jeffrey-Bailey T. 2017. The urban heat island effect, its causes, and mitigation, with reference to the thermal properties of asphalt concrete. Journal of environmental management. 197:522–538.

Mühlenhaupt M, Baxter-Gilbert J, Makhubo BG, Riley JL, Measey J. 2022. No evidence for innate differences in tadpole behavior between natural, urbanized, and invasive populations. Behavioral Ecology Sociobiology. 76(1):11. doi:10.1007/s00265-021-03121-1.

Nicolaus M, Edelaar P. 2018. Comparing the consequences of natural selection, adaptive phenotypic plasticity, and matching habitat choice for phenotype–environment matching, population genetic structure, and reproductive isolation in meta-populations. Ecology and Evolution. 8(8):3815–3827. doi:10.1002/ece3.3816.

Palomar G, Wos G, Stoks R, Sniegula S. 2023. Latitude-specific urbanization effects on life history traits in the damselfly *Ischnura elegans*. Evolutionary Applications. 16(8):1503–1515. doi:10.1111/eva.13583.

Partecke J, Gwinner E. 2007. Increased sedentariness in European blackbirds following urbanization: a consequence of local adaptation? Ecology. 88(4):882–890.

Partecke J, Schwabl I, Gwinner E. 2006. Stress and the city: Urbanization and its effects on the stress physiology in European blackbirds. Ecology. 87(8):1945–1952. doi:10.1890/0012-9658(2006)87[1945:SATCUA]2.0.CO;2.

Perrier C, Lozano del Campo A, Szulkin M, Demeyrier V, Gregoire A, Charmantier A. 2018. Great tits and the city: Distribution of genomic diversity and gene–environment associations along an urbanization gradient. Evolutionary Applications. 11(5):593–613. doi:10.1111/eva.12580.

Pipoly I, Preiszner B, Sándor K, Sinkovics C, Seress G, Vincze E, Bókony V, Liker A. 2022. Extreme hot weather has stronger impacts on Avian reproduction in forests than in cities. Frontiers in Ecology and Evolution. 10.

Pisman M, Bonte D, De La Peña E. 2020. Urbanization alters plastic responses in the common dandelion *Taraxacum officinale*. Ecology and Evolution. 10(9):4082–4090. doi:10.1002/ece3.6176.

Playà-Montmany N, González-Medina E, Cabello-Vergel J, Parejo M, Abad-Gómez JM, Sánchez-Guzmán JM, Villegas A, Masero JA. 2021. The thermoregulatory role of relative bill and leg surface areas in a Mediterranean population of Great tit (*Parus major*). Ecology and Evolution. 11(22):15936–15946. doi:10.1002/ece3.8263.

QGIS Development Team. 2023. QGIS Geographic Information System. https://www.qgis.org.

Qu J, Bonte D, Vandegehuchte ML. 2022. Phenotypic and genotypic divergence of plant–herbivore interactions along an urbanization gradient. Evolutionary Applications. 15(5):865–877. doi:10.1111/eva.13376.

R Core Team. 2024. R: A language and environment for statistical computing.

Rasner CA, Yeh P, Eggert LS, Hunt KE, Woodruff DS, Price TD. 2004. Genetic and morphological evolution following a founder event in the dark-eyed junco, *Junco hyemalis thurberi*. Molecular Ecology. 13(3):671–681.

Reichard DG, Atwell JW, Pandit MM, Cardoso GC, Price TD, Ketterson ED. 2020. Urban birdsongs: higher minimum song frequency of an urban colonist persists in a common garden experiment. Animal Behaviour. 170:33–41. doi:10.1016/j.anbehav.2020.10.007.

Rivkin LR, Santangelo JS, Alberti M, Aronson MFJ, de Keyzer CW, Diamond SE, Fortin MJ, Frazee LJ, Gorton AJ, Hendry AP, et al. 2019. A roadmap for urban evolutionary ecology. Evolutionary Applications. 12(3):384–398. doi:10.1111/eva.12734.

Salmón P, Jacobs A, Ahrén D, Biard C, Dingemanse NJ, Dominoni DM, Helm B, Lundberg M, Senar JC, Sprau P, et al. 2021. Continent-wide genomic signatures of adaptation to urbanisation in a songbird across Europe. Nature Communications. 12(1):2983. doi:10.1038/s41467-021-23027-w.

Samocha Y, Scharf I. 2020. Comparison of wormlion behavior under man-made and natural shelters: urban wormlions more strongly prefer shaded, fine-sand microhabitats, construct larger pits and respond faster to prey. Current Zoology. 66(1):91–98.

Sanderson S, Bolnick DI, Kinnison MT, O'Dea RE, Gorné LD, Hendry AP, Gotanda KM. 2023. Contemporary changes in phenotypic variation, and the potential consequences for eco-evolutionary dynamics. Ecology Letters. 26(S1). doi:10.1111/ele.14186.

Santangelo JS, Miles LS, Breitbart ST, Murray-Stoker D, Rivkin LR, Johnson MTJ, Ness RW. 2020. Urban environments as a framework to study parallel evolution. In: Szulkin M, Munshi-South J, Charmantier A, editors. Urban Evolutionary Biology. USA: Oxford University Press. p. 36–53.

Sato A, Takahashi Y. 2022. Responses in thermal tolerance and daily activity rhythm to urban stress in *Drosophila suzukii*. Ecology and Evolution. 12(12):e9616. doi:10.1002/ece3.9616.

Schmitz G, Linstädter A, Frank ASK, Dittberner H, Thome J, Schrader A, Linne von Berg K, Fulgione A, Coupland G, De Meaux J. 2024. Environmental filtering of life-history trait diversity in urban populations of *Arabidopsis thaliana*. Journal of Ecology. 112(1):14–27. doi:10.1111/1365-2745.14211.

Seress G, Sándor K, Evans KL, Liker A. 2020. Food availability limits avian reproduction in the city: An experimental study on great tits *Parus major*. Journal of Animal Ecology. 89(7):1570–1580. doi:10.1111/1365-2656.13211.

Snell-Rood EC. 2013. An overview of the evolutionary causes and consequences of behavioural plasticity. Animal Behaviour. 85(5):1004–1011. doi:10.1016/j.anbehav.2012.12.031.

Sol D, Lapiedra O, González-Lagos C. 2013. Behavioural adjustments for a life in the city. Animal Behaviour. 85(5):1101–1112. doi:10.1016/j.anbehav.2013.01.023.

Stirling DG, Réale D, Roff DA. 2002. Selection, structure and the heritability of behaviour. Journal of Evolutionary Biology. 15(2):277–289.

Sumasgutner P, Cunningham SJ, Hegemann A, Amar A, Watson H, Nilsson JF, Andersson MN, Isaksson C. 2023. Interactive effects of rising temperatures and urbanisation on birds across different climate zones: A mechanistic perspective. Global Change Biology. 29(9):2399–2420. doi:10.1111/gcb.16645.

Svensson L. 1992. Identification guide to European passerines. The author.

Szulkin M, Munshi-South J, Charmantier A. 2020. Urban evolutionary biology. Oxford University Press, USA.

Taichi N, Ushimaru A. 2024. Trait variation along an urban–rural gradient in Asian dayflower: the contribution of phenotypic plasticity and genetic divergence. Plant Biol J. 26(1):74–81. doi:10.1111/plb.13595.

Talloen W, Lens LUC, Van Dongen S, Adriaensen F, Matthysen E. 2010. Mild stress during development affects the phenotype of great tit *Parus major* nestlings: a challenge experiment. Biological Journal of the Linnean society. 100(1):103–110.

Tene Fossog B, Antonio-Nkondjio C, Kengne P, Njiokou F, Besansky NJ, Costantini C. 2013. Physiological correlates of ecological divergence along an urbanization gradient: differential tolerance to ammonia among molecular forms of the malaria mosquito *Anopheles gambiae*. BMC ecology. 13(1):1–12.

Tene Fossog B, Poupardin R, Costantini C, Awono-Ambene P, Wondji CS, Ranson H, Antonio-Nkondjio C. 2013. Resistance to DDT in an urban setting: common mechanisms implicated in both M and S forms of *Anopheles gambiae* in the city of Yaoundé Cameroon. PloS one. 8(4):e61408.

Thompson KA, Renaudin M, Johnson MTJ. 2016. Urbanization drives the evolution of parallel clines in plant populations. Proceedings of the Royal Society B: Biological Sciences. 283(1845). doi:10.1098/rspb.2016.2180.

Thompson MJ, Capilla-Lasheras P, Dominoni DM, Réale D, Charmantier A. 2022. Phentoypic variation in urban environments: mechanisms and implications. Trends in Ecology & Evolution. 37:171–182.

Tüzün N, Debecker S, de Beeck LO, Stoks R. 2015. Urbanisation shapes behavioural responses to a pesticide. Aquatic Toxicology. 163:81–88.

Tüzün N, Mueller S, Koch K, Stoks R. 2017. Pesticide-induced changes in personality depend on the urbanization level. Animal behaviour. 134:45–55.

Tüzün N, Op de Beeck L, Brans KI, Janssens L, Stoks R. 2017. Microgeographic differentiation in thermal performance curves between rural and urban populations of an aquatic insect. Evolutionary applications. 10(10):1067–1075.

Tüzün N, Stoks R. 2021. Lower bioenergetic costs but similar immune responsiveness under a heat wave in urban compared to rural damselflies. Evolutionary applications. 14(1):24–35.

Van de Schoot E, Merckx T, Ebert D, Wesselingh RA, Altermatt F, Van Dyck H. 2024. Evolutionary change in flight-to-light response in urban moths comes with changes in wing morphology. Biol Lett. 20(3):20230486. doi:10.1098/rsbl.2023.0486.

Van Noordwijk AJ, Keizer LCP, Van Balen JH, Scharloo W. 1981. Genetic variation in egg dimensions in natural populations of the Great Tit. Genetica. 55:221–232.

Vaugoyeau M, Adriaensen F, Artemyev A, Bańbura J, Barba E, Biard C, Blondel J, Bouslama Z, Bouvier J-C, Camprodon J, et al. 2016. Interspecific variation in the relationship between clutch size, laying date and intensity of urbanization in four species of hole-nesting birds. Ecology and Evolution. 6(16):5907–5920. doi:10.1002/ece3.2335.

de Villemereuil P. 2018. Quantitative genetic methods depending on the nature of the phenotypic trait. Annals of the New York Academy of Sciences. 1422(1):29–47. doi:10.1111/nyas.13571.

de Villemereuil P, Gaggiotti OE, Goudet J. 2020. Common garden experiments to study local adaptation need to account for population structure. Journal of Ecology. 00.

de Villemereuil P, Morrissey MB, Nakagawa S, Schielzeth H. 2018. Fixed-effect variance and the estimation of repeatabilities and heritabilities: Issues and solutions. Journal of Evolutionary Biology. 31(4):621–632.

de Villemereuil P, Mouterde M, Gaggiotti OE, Till-Bottraud I. 2018. Patterns of phenotypic plasticity and local adaptation in the wide elevation range of the alpine plant *Arabis alpina*. Jacquemyn H, editor. Journal of Ecology. 106(5):1952–1971. doi:10.1111/1365-2745.12955.

de Villemereuil P, Schielzeth H, Nakagawa S, Morrissey M. 2016. General methods for evolutionary quantitative genetic inference from generalized mixed models. Genetics. 204(3):1281–1294. doi:10.1534/genetics.115.186536.

Westby KM, Medley KA. 2020. Cold nights, city lights: artificial light at night reduces photoperiodically induced diapause in urban and rural populations of *aedes albopictus* (Diptera: *Culicidae*). Journal of Medical Entomology. 57(6):1694–1699.

Weston LM, Mattingly KZ, Day CTC, Hovick SM. 2021. Potential local adaptation in populations of invasive reed canary grass (*Phalaris arundinacea*) across an urbanization gradient. Ecology and Evolution. 11(16):11457–11476. doi:10.1002/ece3.7938.

Whitehead A, Pilcher W, Champlin D, Nacci D. 2012. Common mechanism underlies repeated evolution of extreme pollution tolerance. Proceedings of the Royal Society B: Biological Sciences. 279(1728):427–433.

Winchell KM, Reynolds RG, Prado-Irwin SR, Puente-Rolón AR, Revell LJ. 2016. Phenotypic shifts in urban areas in the tropical lizard Anolis cristatellus. Evolution. 70(5):1009–1022.

Yakub M, Tiffin P. 2017. Living in the city: urban environments shape the evolution of a native annual plant. Global change biology. 23(5):2082–2089.

Yeh PJ. 2004. Rapid evolution of a sexually selected trait following population establishment in a novel habitat. Evolution. 58(1):166–174.

Yeh PJ, Price TD. 2004. Adaptive Phenotypic Plasticity and the Successful Colonization of a Novel Environment. American Naturalist. 164(4):531–542.

Yilmaz AR, Diamond SE, Martin RA. 2021. Evidence for the evolution of thermal tolerance, but not desiccation tolerance, in response to hotter, drier city conditions in a cosmopolitan, terrestrial isopod. Evolutionary applications. 14(1):12–23.

Young EA, Postma E. 2023. Low interspecific variation and no phylogenetic signal in additive genetic variance in wild bird and mammal populations. Ecology and Evolution. 13(11):e10693. doi:10.1002/ece3.10693.

Youngflesh C, Saracco JF, Siegel RB, Tingley MW. 2022. Abiotic conditions shape spatial and temporal morphological variation in North American birds. Nature Ecology & Evolution. 6(12):1860–1870.

Zhang H, He Y, Yang J, Mao H, Jiang X. 2022. Contemporary adaptive evolution in fragmenting river landscapes: evidence from the native waterflea Ceriodaphnia cornuta. Journal of Plankton Research. 44(1):88–98.