



Phenotypic variation in urban environments: mechanisms and implications

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18 intraspecific variation; trait variation; diversity; heterogeneity; city

19 **Abstract**

20 In the past decade, numerous studies have explored how urbanization affects the mean phenotypes of
21 populations, but it remains unknown how urbanization impacts phenotypic variation, a key target of
22 selection that shapes, and is shaped by, eco-evolutionary processes. Our review suggests that
23 urbanization may often increase intraspecific phenotypic variation through several processes, a
24 conclusion aligned with results from our illustrative analysis on tit morphology across 13 European
25 city/forest population pairs. Urban-driven changes in phenotypic variation will have immense
26 implications for urban populations and communities, particularly through urbanization's effects on
27 individual fitness, species interactions, and conservation. We call here for studies that incorporate
28 phenotypic variation in urban eco-evo research alongside advances in theory.

29 **Urban phenotypic variation**

30 As **urbanization** (see Glossary) around the world has continued to grow over time, so too have
31 the fields of Urban Ecology and Evolution. Numerous studies have now examined ecological and
32 evolutionary questions across taxa that occupy urban habitats [1–3]. Urban organisms differ from their
33 non-urban conspecifics in many characteristics, and diverse examples show how urbanization affects the
34 mean phenotypes of populations [4]. However, we still know little about how urbanization shapes
35 **phenotypic variation**, the target of **selection** that will determine the ecology and future evolution of
36 urban populations. Few studies have compared phenotypic variation between urban and non-urban
37 populations [5,6,15,7–14](Table S1) and, to our knowledge, no studies have directly examined the
38 causal mechanisms and consequences of this variation.

39 Here we provide an overview of the mechanisms that shape phenotypic variation in urban
40 systems and synthesize potential implications of this variation (Figure 1). Our review focuses on
41 **intraspecific phenotypic variation** between urban and non-urban populations, while emphasizing the
42 value in examining phenotypic variation among urban subpopulations within cities alongside
43 **environmental heterogeneity** (Box 1). We discuss sampling considerations (Box 2) and show how
44 urbanization increases phenotypic variation in an illustrative analysis (Box 3). We note throughout
45 where knowledge is still lacking and recommend future research directions.

46 **Mechanisms shaping phenotypic variation in urban environments**

47 The eco-evolutionary processes that shape the expression of phenotypic variation, and thus
48 diversity, in natural populations have been well studied in a variety of systems [16]. We therefore only
49 provide a brief overview on how different processes such as dispersal, selection, plasticity, and
50 (epi)genetic mutations may shape phenotypic variation in an urban context (overview in Figure 1). We
51 do not provide a general rule for how different processes affect phenotypic variation in urban

environments as many factors likely contribute to variation in diverse and interactive ways. Overall, however, our synthesis suggests that urbanization has increased phenotypic variation in several urban systems (see also Box 3; Table S1).

Dispersal

Whether urbanization increases or decreases phenotypic variation will partially depend both on a species' **dispersal** abilities and on the direction of dispersal (e.g., **source-sink dynamics**)[17]. Dispersal has the potential to promote phenotypic variation within populations through the immigration of new individuals, phenotypes, and genotypes [18]. Non-urban populations might act as source populations [2] whereby constant movements of individuals into urban areas from more natural habitats results in higher phenotypic variation in urban populations. For less dispersive species, however, movements can be restricted by **habitat fragmentation** in urban habitats, which could contribute to declines in variation (via reductions in urban population sizes or increased **genetic drift**; Figure 1)[2], especially if new urban subpopulations are formed by **founder effects** [15,19]. As dispersal in urban environments is still not well studied, further work in this area will be conducive.

Selection

Phenotypic variation could be higher in many urban systems if both environmental heterogeneity (Box 1) and **relaxed selection** allow more diverse phenotypes to persist (Figure 1). Alternatively, by favouring adaptive phenotypes that provide a **fitness** advantage, both directional and stabilizing selection can deplete phenotypic variation in a population over time, via a reduction of the underlying genetic variance across generations or the selective disappearance of certain individuals within each generation. However, relaxed selection may be more pervasive in cities than previously thought (e.g., [13], including in humans [20]). A recent meta-analysis found that anthropogenic disturbances in non-urban habitats reduce the strength of selection [21]. On closer examination, the authors found that

75 absolute fitness has increased and variation in fitness decreased because of human disturbance, thus
76 weakening the opportunity for selection. Relaxed selection in urban environments may result from
77 reduced predation pressures, access to supplementary food [22], or a loss of fitness variation [13,21].
78 Novel and strong selection pressures might affect some urban populations [2,23], but phenotypic
79 variation could still increase in these populations if selection pressures vary with heterogeneity in cities
80 (see Box 1). A future focus on phenotypic variation and selection (direction and strength) in and outside
81 cities should provide new biological insights into the processes that affect urban phenotypes and
82 adaptation. These efforts will be especially meaningful as selection is still rarely estimated in urbanized
83 species [2].

84 *Plasticity*

85 **Phenotypic plasticity** (including **developmental plasticity**) promotes variation and
86 diversification within and between populations [24,25], and may be one of the most common
87 mechanisms allowing individuals to colonize and persist in urban environments [26]. Plasticity could
88 reduce intraspecific phenotypic variation in an urban population if most individuals are capable of
89 plastic shifts resulting in similar phenotypic expression (Figure 1). A well-studied urban trait that
90 demonstrates this trend is flight initiation distance (FID); the distance an individual allows before
91 retreating when approached by a risky stimulus. Most urban animals similarly reduce their phenotypic
92 mean, and thus variation, in FIDs if they can adjust their behaviours by habituating to non-threatening
93 stimuli like humans (via repeated exposures) [27], while non-urban individuals display more variable
94 responses (shown in blue-tailed skinks, *Emoia impar*)[12]. Similarly, a recent meta-analysis shows
95 declines in variation of antipredator behaviours following contact with humans in domesticated, captive,
96 and urban animals [28].

97 Conversely, phenotypic variation in urban populations could be higher than non-urban
98 populations because of impaired development and developmental plasticity (Figure 1; Box 3).
99 Developmental processes act to constrain phenotypic variation among and within individuals (e.g.,
100 **developmental canalization**;^[29]), and can be disrupted if the level of an environmental stressor passes
101 a threshold [30]. A recent meta-analysis found that developmental stress decreases the mean, but
102 increases the variation, in diverse phenotypic traits across several taxa [31]. Urban environments may
103 increase variation in populations if the many environmental stressors in these habitats (e.g., noise, light
104 pollution, chemicals, or increased temperature) disrupt developmental processes (Figure 1). For
105 example, impaired head shape development and higher phenotypic variation across head shape indices
106 was found in urban Common wall lizards (*Podarcis muralis*)[10]. Early life experiences or
107 environments (e.g., competition, diet, predation) contribute to phenotypic differences within populations
108 (e.g., [32,33]), but this is not well examined in an urban context.

109 *Mutation and epigenetics*

110 Pollution and environmental stress can increase rates of **genetic mutation**, hypermethylation, or
111 other epigenetic marks [34–37] suggesting that phenotypic variation could increase in urban populations
112 where these processes are occurring at higher rates (Figure 1; Box 3)[38–40]. **Epigenetics** may be an
113 important mechanism of adaptation for urban populations as it can move phenotypes closer to the fitness
114 optimum and increase mutation rates [41], but the role of (epi)genetic mutations in shaping urban
115 phenotypic variation remains unexplored.

116 **Implications of urban phenotypic variation**

117 As shown above, urban conditions can significantly impact phenotypic variation. These changes in
118 phenotypic variation can have immense implications for populations, communities, and ecosystems, as
119 well as for conservation programs (Figure 1).

120 *Implications for species interactions, communities, and ecosystem processes*

121 Species abundances and compositions are strongly modified during urbanization [3,42,43]. Although
122 largely ignored, intraspecific phenotypic variation and its effects on competition [44,45] may have
123 important implications for urban community compositions [46,47]. High trait variation associated with
124 niche expansion can reduce interspecific competition [44,48], potentially to a greater extent in urban
125 environments where individuals adopt novel resources and widen their niches [9,49,50]. Theory also
126 predicts that higher trait variation associated with competitive ability or niche differentiation can lead to
127 the exclusion of competitively inferior species [51]. If increases in intraspecific phenotypic variation in
128 urban-exploiters or urban-adapters contributes to competitive exclusions and declines in species richness
129 [50,52], we could see a parallel loss in unique **functional traits** in urban communities that contribute to
130 ecosystem services and functioning [53,54]. For these reasons, invasive species research would benefit
131 from approaches that consider phenotypic variation, particularly in an urban context where native
132 species are confronted with a high frequency of invasions [55,56]. One exemplar study quantified trait
133 variation associated with locomotor performance and temperature tolerance in invasive cane toads
134 (*Rhinella marina*) under lab conditions, and then modeled this variation alongside fine-scale climate and
135 landscape data to predict the cane toads fundamental niche and potential for expansion across Australia
136 [57]. Efforts that explore how intraspecific phenotypic variation shapes urban invasions and species
137 compositions would be useful additions to the urban literature, particularly in cases where competitive
138 exclusions disrupt urban ecosystem services [53,58].

139 Intraspecific phenotypic variation influences trophic interactions that promote ecological processes
140 and services like pollination or seed dispersal [53,59–61]. Wild urban bees, for example, have higher
141 intraspecific variation in functional foraging traits than non-urban bees, which may be driven by
142 introductions of non-native and diverse floral resources in urban gardens and parks [6]. Such higher

143 phenotypic variation within species of urban bees might widen their foraging niche and reduce the
144 amount of interactions they have with native flower species (i.e., decreased interaction strength [44,62]).
145 This would have cascading impacts on pollination and, thus, urban plant community composition. Multi-
146 species approaches are needed to explore eco-evolutionary and community dynamics [44,63], but these
147 approaches are still rare in urban research. We recommend future studies quantify and associate
148 phenotypic variation in multiple species to address the consequences of intraspecific phenotypic
149 variation on urban species interactions that are modified along urban gradients.

150 Urban species interactions can be modified or disrupted if the timing of key life cycle events (i.e.,
151 phenology) change, yet considering the role of phenotypic variation in these phenological mismatches
152 has been overlooked so far. Urbanization has been shown to affect both the peak and duration (i.e.,
153 variation) of many phenological events [64] and, sometimes, urbanization can cause asynchronous
154 phenological shifts in interacting species. For example, urban plant species tend to flower earlier, but
155 urban pollinators may not show a similar advance in diapause emergence and, thus, their foraging
156 activities can overlap less with key flowering resources [65]. As well, caterpillar emergence in urban
157 environments is more variable and has several small peaks instead of a single peak typical in natural
158 forest habitats [66]. This could have consequences for urban insectivorous birds such as great tits (*Parus*
159 *major*) who rely on caterpillar prey during nestling provisioning. Urban-modified phenological variation
160 could cause mismatches between interacting species on multiple trophic levels, which would have run-
161 off implications for selection and population dynamics [67].

162 *Implications for fitness, selection, and population dynamics*

163 Intraspecific phenotypic variation can also influence population dynamics [68–70]. For example,
164 higher intraspecific trait variation can promote diverse individual responses to environmental
165 fluctuations that buffer and stabilize population dynamics (i.e., portfolio effects)[44]; a process that is

166 especially applicable to urban populations undergoing rapid environmental change [46,71]. Phenotypic
167 variation can also drive population dynamics through selection [68,72], particularly during colonization
168 events [73]. Therefore, we anticipate that *a priori* knowledge on how phenotypic variation influences
169 fitness or performance metrics will be useful when exploring urban selection and population
170 implications.

171 Variation among individuals in their sexual signals can alter reproductive behaviours and selection
172 in urban populations. Higher variation in mate qualities can increase the benefits of choosiness, mate
173 searching behaviours, and intrasexual competition. These dynamics may be especially relevant in urban
174 environments where pollution (e.g., chemical, nutrient, noise, light) can disrupt the communication and
175 mating behaviours of animals [74,75]. For example, several species experience increased access to key
176 nutrients in urban environments which can reduce individual variation in the honesty of sexual signals
177 that indicate an individual's ability to acquire resources (see review [74]). Mate choice can also be
178 affected by urban pollution or stressors which can reduce the perceived variation in sexual signals. For
179 instance, the visual mating signals of three-spined sticklebacks (*Gasterosteus aculeatus*) have been
180 disrupted by human-induced algal blooms. As a result, females are unable to perceive variation among
181 males in their sexual signals and are, thus, more likely to choose lower quality mates that produce less
182 viable offspring [76]. Maladaptive mating in urban habitats could impede adaptation, contribute to
183 population declines, or divergences in mean phenotypes, potentially resulting in hybridization or
184 speciation [75,77].

185 Modified natural selection in urban environments could also alter phenotypic variation, potentially
186 resulting in life-history trade-offs or changes in subsequent selective processes. Urban Túngara frogs
187 (*Physalaemus pustulosus*), for example, experience reduced predation and parasitism risk while singing
188 (relaxed natural selection) and higher competition for mates (stronger sexual selection)[78]. As a result,

189 urban frogs call at higher rates, sing more complex and attractive songs, and plastically adjust their
190 songs to sound and light levels in the environment. The authors suggest that a broader range of sexual
191 signalling in urban frogs (i.e., higher phenotypic variation) afford them reproductive and survival
192 advantages over the more natural forest phenotype [78]. Phenotypic changes via plasticity can increase
193 fitness and promote adaptation in novel or stressful environments, but plasticity likely incurs significant
194 costs, for example by reducing growth rates, generation times, or fecundity [79]. Thus, populations that
195 show adaptive plastic responses or higher phenotypic variation in response to novel environments could
196 also shift toward slower life histories [79]. As many urban phenotypic changes may involve plasticity
197 [26], including the example above, it would be interesting to explore the role of urban-modified
198 phenotypic variation in life-history trade-offs and pace-of-life [80,81]. The selective and demographic
199 consequences of urban-modified life history variation are unexplored in most urban systems, hence
200 efforts tackling these ideas will provide timely insights into how sexual and natural selection shape
201 urban populations.

202 Beyond quantifying phenotypic variation in urban populations, urban studies should also aim to
203 determine to what extent a phenotypic trait is heritable [82]. This is especially important if the adaptive
204 or evolutionary implications of urban phenotypic variation are to be explored. While intraspecific
205 phenotypic variation has a key role in influencing urban eco-evolutionary dynamics, it is highly
206 informative to decipher between its genetic and environmental origins, and their interactions [63,83].
207 Exploring the origins of urban phenotypic variation calls on quantitative genetic approaches using long-
208 term data or experimental approaches like common gardens [4]. Fear of humans, for example, is
209 commonly thought to decrease in urban animals via habituation to humans, a form of phenotypic
210 plasticity. However, variation in behavioural responses to humans is more heritable than expected in
211 urban burrowing owls (*Athene cunicularia*) suggesting that a reduced fear of humans could also result

212 from an evolutionary response [84]. Efforts making these distinctions are not commonly applied as large
213 datasets or intensive experiments are required, but we emphasize here the value of these efforts in urban
214 research.

215 *Implications for urban conservation management*

216 The advantages of incorporating intraspecific variation into urban conservation management has
217 been highlighted recently [71,85]. Increasing phenotypic variation in populations of conservation
218 concern has been suggested as an effective management approach in urban contexts. For example,
219 phenotypic restoration initiatives can help establish lost phenotypic variation through reintroductions of
220 missing phenotypes. Simulations have shown how reintroductions of larger seed types in human-
221 impacted forests can help restore seed profiles back to natural levels and maintain seed dispersal [60].
222 Efforts that increase phenological variation by extending the duration of key events in particular species,
223 like flowering time, might help interacting species, like specialist pollinators, that experience
224 asynchronous shifts due to environmental change [86]. Promoting trait diversity through management
225 programs has also been recognized in urban arboriculture where urban forests tend to consist of similar
226 species [54] or clones [87]. Low phenotypic variation among planted urban trees increases vulnerability
227 to drought or pests and can hamper the ecosystem services provided by trees in cities, hence increasing
228 trait diversity in trees can offer an easy urban management approach to counter these challenges [54].
229 Trait distributions likely have very different implications for population growth and stability than trait
230 means, and so including intraspecific phenotypic variation in population monitoring is warranted [45].

231 Phenotypic variation can also be used as a tool to regulate urban populations that cause problems for
232 native species and humans. Selective management approaches, for instance, decrease phenotypic
233 variation within a population by targeting “problem individuals” that possess certain phenotypes
234 associated with human impacts and conflicts [88]. For example, efforts evaluating personality variation

235 in deer populations show that deer with bolder personalities may be more likely to cause human harm
236 through vehicle collisions, crop damage, or disease transmission [89]. Simulations suggest that selective
237 harvesting of deer with bolder personality types could mitigate human-wildlife conflicts while sustaining
238 population sizes [89]. These management programs would be especially useful in urban environments at
239 the human-wildlife interface, but they require some knowledge of the phenotypic variation contained
240 within target populations. We expect that studies exploring the implications of phenotypic variation in
241 human-wildlife conflicts will help ensure that management interventions are successful and have longer
242 lasting impacts [88].

243 Conservation programs could aim to increase intraspecific variation in populations they want to
244 conserve and decrease variation in populations they want to mitigate [58,85]. This is because higher
245 intraspecific variation should have positive ecological effects on populations, in particular when the
246 population mean traits are not well matched to the fitness optimum of the environment (i.e., phenotype-
247 environment mismatch)[69], which might be more frequently observed in urban populations. However,
248 increasing phenotypic variation will not always benefit populations if individuals are already well
249 adapted to environmental conditions. A theoretical study demonstrates this and shows that high amounts
250 of phenotypic variation will have increasingly negative ecological consequences on populations as they
251 become better adapted to the local environmental optimum [90]. It will be imperative for urban
252 monitoring programs to evaluate how variation and means associate with local fitness optima to better
253 anticipate the implications of efforts that manage variation [69,85]. We suggest that these evaluations
254 occur on fine scales as phenotype-environment mismatches may differ substantially among urban
255 subpopulations due to within-city heterogeneity (Box 1). Considering phenotypic variation in urban
256 conservation has great promise and city municipalities could benefit from more focused research in this

257 area. We, however, recommend caution and prior investigations to comprehend the possible ecological
258 and evolutionary implications of such interventions.

259 **Concluding remarks**

260 Our synthesis suggests that urban conditions impact phenotypic variation through various processes,
261 and that urban effects on phenotypic variation have ecological, evolutionary, and management
262 implications. We expect phenotypic variation to increase in urban systems through dispersal, relaxed or
263 heterogenous selection, developmental plasticity, (epi)genetic mutations, or a combination of these
264 (Figure 1; Box 1; Box 3; Table S1). The synergistic and counteractive effects of these mechanisms could
265 shape variation in diverse and complex ways, and care will need to be taken to ensure appropriate
266 sampling design in urban studies (Box 2). Urbanization has significant impacts on species interactions
267 and individual fitness, which may exaggerate the effects of intraspecific phenotypic variation in urban
268 systems. Examining these hypotheses across cities, taxa, and traits will be important for further
269 generalizing how urbanization affects phenotypic variation, and in turn how variation affects
270 evolutionary and environmental change.

271 Most Urban Ecology/Evolution projects already have data on the variance around phenotypes in
272 their study populations and we, therefore, hope to encourage the comparison of variation, beside means,
273 of ecologically relevant traits in future work [31]. To this end, we show in a preliminary analysis that
274 urbanization increases the variation in morphological traits in tit species across Europe (Box 3). There is
275 a need to examine phenotypic variation both between and within cities (Box 1), and to examine
276 contributions of environmental features and heterogeneity to phenotypic variation at spatial and
277 temporal scales relevant to a species' biology (Box 2; see also Outstanding questions).

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287 **References**

- 288 1 Szulkin, M. *et al.* (2020) *Urban evolutionary biology*, Oxford University Press, USA.
- 289 2 Johnson, M.T.J. and Munshi-South, J. (2017) Evolution of life in urban environments. *Science*.
- 290 358, 1–11
- 291 3 Diamond, S.E. and Martin, R.A. (2021) Evolution in cities. *Annu. Rev. Ecol. Evol. Syst.* 52, 519–
- 292 540
- 293 4 Lambert, M.R. *et al.* (2021) Adaptive evolution in cities: progress and misconceptions. *Trends*
- 294 *Ecol. Evol.* 36, 239–257
- 295 5 Gorton, A.J. *et al.* (2018) Little plant, big city: a test of adaptation to urban environments in
- 296 common ragweed (*Ambrosia artemisiifolia*). *Proc. R. Soc. B Biol. Sci.* 285, 20180968
- 297 6 Eggenberger, H. *et al.* (2019) Urban bumblebees are smaller and more phenotypically diverse
- 298 than their rural counterparts. *J. Anim. Ecol.* 88, 1522–1533
- 299 7 Theodorou, P. *et al.* (2021) Urbanization is associated with shifts in bumblebee body size, with
- 300 cascading effects on pollination. *Evol. Appl.* 14, 53–68
- 301 8 Multini, L.C. *et al.* (2019) Urbanization as a driver for temporal wing-shape variation in
- 302 *Anopheles cruzii* (Diptera: Culicidae). *Acta Trop.* 190, 30–36
- 303 9 Falvey, C.H. *et al.* (2020) The finer points of urban adaptation: Intraspecific variation in lizard
- 304 claw morphology. *Biol. J. Linn. Soc.* 131, 304–318
- 305 10 Lazić, M.M. *et al.* (2015) Effects of environmental disturbance on phenotypic variation: an
- 306 integrated assessment of canalization, developmental stability, modularity, and allometry in lizard
- 307 head shape. *Am. Nat.* 185, 44–58
- 308 11 Gómez-Benítez, A. *et al.* (2021) The influence of urbanization on morphological traits in the
- 309 Balsas Basin Whiptail lizard (*Aspidoscelis costatus costatus*). *Urban Ecosyst.* 24, 327–333

310 12 Williams, D.M. *et al.* (2019) High human disturbance decreases individual variability in skink
311 escape behavior. *Curr. Zool.* 66, 63–70

312 13 Rodewald, A.D. and Arcese, P. (2017) Reproductive contributions of cardinals are consistent with
313 a hypothesis of relaxed selection in urban landscapes. *Front. Ecol. Evol.* 5, 1–7

314 14 Bókony, V. *et al.* (2012) Personality traits and behavioral syndromes in differently urbanized
315 populations of house sparrows (*Passer domesticus*). *PLoS One* 7, e36639

316 15 Littleford-Colquhoun, B.L. *et al.* (2017) Archipelagos of the Anthropocene: rapid and extensive
317 differentiation of native terrestrial vertebrates in a single metropolis. *Mol. Ecol.* 26, 2466–2481

318 16 Mitchell-Olds, T. *et al.* (2007) Which evolutionary processes influence natural genetic variation
319 for phenotypic traits? *Nat. Rev.* 8, 845–856

320 17 Lepczyk, C.A. *et al.* (2017) Biodiversity in the city: fundamental questions for understanding the
321 ecology of urban green spaces for biodiversity conservation. *Bioscience* 67, 799–807

322 18 Lenormand, T. (2002) Gene flow and the limits to natural selection. *Trends Ecol. Evol.* 17, 183–
323 189

324 19 Mueller, J.C. *et al.* (2018) Evolution of genomic variation in the burrowing owl in response to
325 recent colonization of urban areas. *Proc. R. Soc. B Biol. Sci.* 285, 20180206

326 20 You, W. and Henneberg, M. (2018) Relaxed natural selection contributes to global obesity
327 increase more in males than in females due to more environmental modifications in female body
328 mass. *PLoS One* 13, 1–20

329 21 Fugère, V. and Hendry, A.P. (2018) Human influences on the strength of phenotypic selection.
330 *Proc. Natl. Acad. Sci. U. S. A.* 115, 10070–10075

331 22 Lahti, D.C. *et al.* (2009) Relaxed selection in the wild. *Trends Ecol. Evol.* 24, 487–496

332 23 Rivkin, L.R. *et al.* (2019) A roadmap for urban evolutionary ecology. *Evol. Appl.* 12, 384–398

333 24 Pfennig, D.W. *et al.* (2010) Phenotypic plasticity's impacts on diversification and speciation.
334 *Trends Ecol. Evol.* 25, 459–467

335 25 Gilbert, S.F. *et al.* (2015) Eco-Evo-Devo: developmental symbiosis and developmental plasticity
336 as evolutionary agents. *Nat. Rev. Genet.* 16, 611–622

337 26 Hendry, A.P. *et al.* (2008) Human influences on rates of phenotypic change in wild animal
338 populations. *Mol. Ecol.* 17, 20–29

339 27 Blumstein, D.T. (2016) Habituation and sensitization: new thoughts about old ideas. *Anim. Behav.*
340 120, 255–262

341 28 Geffroy, B. *et al.* (2020) Evolutionary dynamics in the anthropocene: life history and intensity of
342 human contact shape antipredator responses. *PLoS Biol.* 18, e3000818

343 29 Willmore, K.E. *et al.* (2007) Phenotypic variability: Its components, measurement and underlying
344 developmental processes. *Evol. Biol.* 34, 99–120

345 30 Hoffman, A.A. and Hercus, M.J. (2000) Environmental stress as an evolutionary force.
346 *Bioscience* 50, 217–226

347 31 Sánchez-Tójar, A. *et al.* (2020) Illustrating the importance of meta-analysing variances alongside
348 means in ecology and evolution. *J. Evol. Biol.* 33, 1216–1223

349 32 Nicolaus, M. *et al.* (2016) Density fluctuations represent a key process maintaining personality
350 variation in a wild passerine bird. *Ecol. Lett.* 19, 478–486

351 33 Dirienzo, N. *et al.* (2019) Juvenile social experience generates differences in behavioral variation
352 but not averages. *Behav. Ecol.* 30, 455–464

353 34 Yauk, C. *et al.* (2008) Germ-line mutations, DNA damage, and global hypermethylation in mice
354 exposed to particulate air pollution in an urban/industrial location. *Proc. Natl. Acad. Sci. U. S. A.*
355 105, 605–610

356 35 Somers, C.M. *et al.* (2004) Reduction of particulate air pollution lowers the risk of heritable
357 mutations in mice. *Science*. 304, 1008–1010

358 36 Eeva, T. *et al.* (2006) Environmental pollution affects genetic diversity in wild bird populations.
359 *Mutat. Res.* 608, 8–15

360 37 Yauk, C.L. *et al.* (2000) Induced minisatellite germline mutations in herring gulls (*Larus*
361 *argentatus*) living near steel mills. *Mutat. Res. - Fundam. Mol. Mech. Mutagen.* 452, 211–218

362 38 Watson, H. *et al.* (2020) Urbanisation is associated with modifications in DNA methylation in a
363 small passerine bird. *Evol. Appl.* 14, 85–98

364 39 Riyahi, S. *et al.* (2015) Combined epigenetic and intraspecific variation of the DRD4 and SERT
365 genes influence novelty seeking behavior in great tit *Parus major*. *Epigenetics* 10, 516–525

366 40 McNew, S.M. *et al.* (2017) Epigenetic variation between urban and rural populations of Darwin's
367 finches. *BMC Evol. Biol.* 17, 1–14

368 41 Perrier, C. *et al.* (2020) Adaptation genomics in urban environments. In *Urban Evolutionary*
369 *Biology* (Szulkin, M. *et al.*, eds), pp. 74–90, Oxford University Press

370 42 Marques, P.S. *et al.* (2019) Intraspecific trait variation in urban stream ecosystems: toward
371 understanding the mechanisms shaping urban stream communities. *Freshw. Sci.* 38, 1–11

372 43 Faeth, S.H. *et al.* (2005) Trophic dynamics in urban communities. *Bioscience* 55, 399–407

373 44 Bolnick, D.I. *et al.* (2011) Why intraspecific trait variation matters in community ecology. *Trends*
374 *Ecol. Evol.* 26, 183–192

375 45 Moran, E. V. *et al.* (2016) Intraspecific trait variation across scales: Implications for
376 understanding global change responses. *Glob. Chang. Biol.* 22, 137–150

377 46 Alberti, M. *et al.* (2020) The complexity of urban eco-evolutionary dynamics. *Bioscience* 70,
378 772–793

379 47 Des Roches, S. *et al.* (2018) The ecological importance of intraspecific variation. *Nat. Ecol. Evol.*
380 2, 57–64

381 48 Violle, C. *et al.* (2012) The return of the variance: Intraspecific variability in community ecology.
382 *Trends Ecol. Evol.* 27, 244–252

383 49 Pagani-Núñez, E. *et al.* (2019) Niches in the anthropocene: passerine assemblages show niche
384 expansion from natural to urban habitats. *Ecography*. 42, 1360–1369

385 50 De León, L.F. *et al.* (2019) Urbanization erodes niche segregation in Darwin’s finches. *Evol.*
386 *Appl.* 12, 1329–1343

387 51 Hart, S.P. *et al.* (2016) How variation between individuals affects species coexistence. *Ecol. Lett.*
388 19, 825–838

389 52 Barabás, G. and D’Andrea, R. (2016) The effect of intraspecific variation and heritability on
390 community pattern and robustness. *Ecol. Lett.* 19, 977–986

391 53 Sol, D. *et al.* (2020) The worldwide impact of urbanisation on avian functional diversity. *Ecol.*
392 *Lett.* 23, 962–972

393 54 Paquette, A. *et al.* (2021) Praise for diversity: A functional approach to reduce risks in urban
394 forests. *Urban For. Urban Green.* 62, 127157

395 55 Gaertner, M. *et al.* (2017) Non-native species in urban environments: patterns, processes, impacts
396 and challenges. *Biol. Invasions* 19, 3461–3469

397 56 Forsman, A. (2014) Effects of genotypic and phenotypic variation on establishment are important
398 for conservation, invasion, and infection biology. *Proc. Natl. Acad. Sci. U. S. A.* 111, 302–307

399 57 Kolbe, J.J. *et al.* (2010) Modeling the consequences of thermal trait variation for the cane toad
400 invasion of Australia. *Ecol. Appl.* 20, 2273–2285

401 58 Des Roches, S. *et al.* (2021) Conserving intraspecific variation for nature’s contributions to

402 people. *Nat. Ecol. Evol.* 5, 574–582

403 59 Zwolak, R. (2018) How intraspecific variation in seed-dispersing animals matters for plants. *Biol.*
404 *Rev.* 93, 897–913

405 60 Carvalho, C. da S. *et al.* (2021) Rescuing intraspecific variation in human-impacted
406 environments. *J. Appl. Ecol.* 58, 350–359

407 61 Snell, R.S. *et al.* (2019) Consequences of intraspecific variation in seed dispersal for plant
408 demography, communities, evolution and global change. *AoB Plants* 11, 1–19

409 62 Gibert, J.P. and Brassil, C.E. (2014) Individual phenotypic variation reduces interaction strengths
410 in a consumer-resource system. *Ecol. Evol.* 4, 3703–3713

411 63 De Meester, L. *et al.* (2019) Analysing eco-evolutionary dynamics—The challenging complexity
412 of the real world. *Funct. Ecol.* 33, 43–59

413 64 Jochner, S. and Menzel, A. (2015) Urban phenological studies - Past, present, future. *Environ.*
414 *Pollut.* 203, 250–261

415 65 Fisogni, A. *et al.* (2020) Urbanization drives an early spring for plants but not for pollinators.
416 *Oikos* 129, 1681–1691

417 66 Pollock, C.J. *et al.* (2017) Integrated behavioural and stable isotope data reveal altered diet linked
418 to low breeding success in urban-dwelling blue tits (*Cyanistes caeruleus*). *Sci. Rep.* 7, 1–14

419 67 Visser, M.E. and Gienapp, P. (2019) Evolutionary and demographic consequences of
420 phenological mismatches. *Nat. Ecol. Evol.* 3, 879–885

421 68 Pelletier, F. *et al.* (2007) The evolutionary demography of ecological change: linking trait
422 variation and population growth. *Science.* 315, 1571–1574

423 69 Gibert, J.P. (2016) The effect of phenotypic variation on metapopulation persistence. *Popul. Ecol.*
424 58, 345–355

425 70 Wright, J.P. *et al.* (2016) The more things change, the more they stay the same? When is trait
426 variability important for stability of ecosystem function in a changing environment. *Philos. Trans.*
427 *R. Soc. B Biol. Sci.* 371, 20150272

428 71 Mimura, M. *et al.* (2017) Understanding and monitoring the consequences of human impacts on
429 intraspecific variation. *Evol. Appl.* 10, 121–139

430 72 Farine, D.R. *et al.* (2015) From individuals to groups and back: The evolutionary implications of
431 group phenotypic composition. *Trends Ecol. Evol.* 30, 609–621

432 73 Duckworth, R.A. and Aguillon, S.M. (2015) Eco-evolutionary dynamics: investigating multiple
433 causal pathways linking changes in behavior, population density and natural selection. *J.*
434 *Ornithol.* 156, 115–124

435 74 Snell-Rood, E. *et al.* (2015) Life-history evolution in the anthropocene: Effects of increasing
436 nutrients on traits and trade-offs. *Evol. Appl.* 8, 635–649

437 75 Candolin, U. and Wong, B.B.M. (2019) Mate choice in a polluted world: Consequences for
438 individuals, populations and communities. *Philos. Trans. R. Soc. B Biol. Sci.* 374, 20180055

439 76 Candolin, U. *et al.* (2016) Environmental change disrupts communication and sexual selection in
440 a stickleback population. *Ecology* 97, 969–979

441 77 Thompson, K.A. *et al.* (2018) Speciation and the city. *Trends Ecol. Evol.* 33, 815–826

442 78 Halfwerk, W. *et al.* (2019) Adaptive changes in sexual signalling in response to urbanization. *Nat.*
443 *Ecol. Evol.* 3, 374–380

444 79 Snell-Rood, E.C. *et al.* (2018) Mechanisms of plastic rescue in novel environments. *Annu. Rev.*
445 *Ecol. Evol. Syst.* 49, 331–354

446 80 Sepp, T. *et al.* (2017) A review of urban impacts on avian life-history evolution: does city living
447 lead to slower pace of life? *Glob. Chang. Biol.* 24, 1452–1469

448 81 Brans, K.I. and De Meester, L. (2018) City life on fast lanes: Urbanization induces an
449 evolutionary shift towards a faster lifestyle in the water flea *Daphnia*. *Funct. Ecol.* 32, 2225–2240

450 82 Hoffmann, A.A. and Merilä, J. (1999) Heritable variation and evolution under favourable and
451 unfavourable conditions. *Trends Ecol. Evol.* 14, 96–101

452 83 Brans, K.I. *et al.* (2017) Eco-evolutionary dynamics in urbanized landscapes: evolution, species
453 sorting and the change in zooplankton body size along urbanization gradients. *Philos. Trans. R.*
454 *Soc. B Biol. Sci.* 372, 20160030

455 84 Carrete, M. *et al.* (2016) Heritability of fear of humans in urban and rural populations of a bird
456 species. *Sci. Rep.* 6, 1–6

457 85 Lambert, M.R. and Donihue, C.M. (2020) Urban biodiversity management using evolutionary
458 tools. *Nat. Ecol. Evol.* 4, 903–910

459 86 Olliff-Yang, R.L. *et al.* (2020) Mismatch managed? Phenological phase extension as a strategy to
460 manage phenological asynchrony in plant–animal mutualisms. *Restor. Ecol.* 28, 498–505

461 87 Vanden Broeck, A. *et al.* (2018) Genetic diversity loss and homogenization in urban trees: the
462 case of *Tilia × europaea* in Belgium and the Netherlands. *Biodivers. Conserv.* 27, 3777–3792

463 88 Swan, G.J.F. *et al.* (2017) Ecology of problem individuals and the efficacy of selective wildlife
464 management. *Trends Ecol. Evol.* 32, 518–530

465 89 Honda, T. *et al.* (2018) A review of urban wildlife management from the animal personality
466 perspective: The case of urban deer. *Sci. Total Environ.* 644, 576–582

467 90 Dibble, C.J. and Rudolf, V.H.W. (2019) Phenotype-environment matching predicts both positive
468 and negative effects of intraspecific variation. *Am. Nat.* 194, 47–58

469 91 Miles, L.S. *et al.* (2019) Gene flow and genetic drift in urban environments. *Mol. Ecol.* 28, 4138–
470 4151

471 92 Edelaar, P. and Bolnick, D.I. (2012) Non-random gene flow: An underappreciated force in
472 evolution and ecology. *Trends Ecol. Evol.* 27, 659–665

473 93 van Dongen, W.F.D. *et al.* (2015) Variation at the DRD4 locus is associated with wariness and
474 local site selection in urban black swans. *BMC Evol. Biol.* 15, 1–11

475 94 Camacho, C. *et al.* (2020) Experimental evidence that matching habitat choice drives local
476 adaptation in a wild population. *Proc. R. Soc. B Biol. Sci.* 287, 20200721

477 95 Merckx, T. *et al.* (2018) Body-size shifts in aquatic and terrestrial urban communities. *Nature*
478 558, 113–116

479 96 Oliveira Hagen, E. *et al.* (2017) Impacts of urban areas and their characteristics on avian
480 functional diversity. *Front. Ecol. Evol.* 5, 1–15

481 97 Uchida, K. *et al.* (2021) Urban biodiversity and the importance of scale. *Trends Ecol. Evol.* 36,
482 123–131

483 98 Pickett, S.T.A. *et al.* (2017) Dynamic heterogeneity: a framework to promote ecological
484 integration and hypothesis generation in urban systems. *Urban Ecosyst.* 20, 1–14

485 99 Shochat, E. *et al.* (2006) From patterns to emerging processes in mechanistic urban ecology.
486 *Trends Ecol. Evol.* 21, 186–191

487 100 Stofberg, M. *et al.* (2019) Juggling a “junk-food” diet: responses of an urban bird to fluctuating
488 anthropogenic-food availability. *Urban Ecosyst.* 22, 1019–1026

489 101 Tryjanowski, P. *et al.* (2015) Urban and rural habitats differ in number and type of bird feeders
490 and in bird species consuming supplementary food. *Environ. Sci. Pollut. Res.* 22, 15097–15103

491 102 Qiu, T. *et al.* (2017) Impacts of urbanization on vegetation phenology over the past three decades
492 in Shanghai, China. *Remote Sens.* 9, 1–16

493 103 Marko, P.B. and Hart, M.W. (2011) The complex analytical landscape of gene flow inference.

494 *Trends Ecol. Evol.* 26, 448–456

495 104 Pélabon, C. *et al.* (2020) On the use of the coefficient of variation to quantify and compare trait
496 variation. *Evol. Lett.* 4, 180–188

497 105 Sprau, P. *et al.* (2017) Multidimensional environmental predictors of variation in avian forest and
498 city life histories. *Behav. Ecol.* 28, 59–68

499 106 Szulkin, M. *et al.* (2020) How to Quantify Urbanization When Testing for Urban Evolution? In
500 *Urban Evolutionary Biology* (Szulkin, M. *et al.*, eds), pp. 13, Oxford University Press, USA

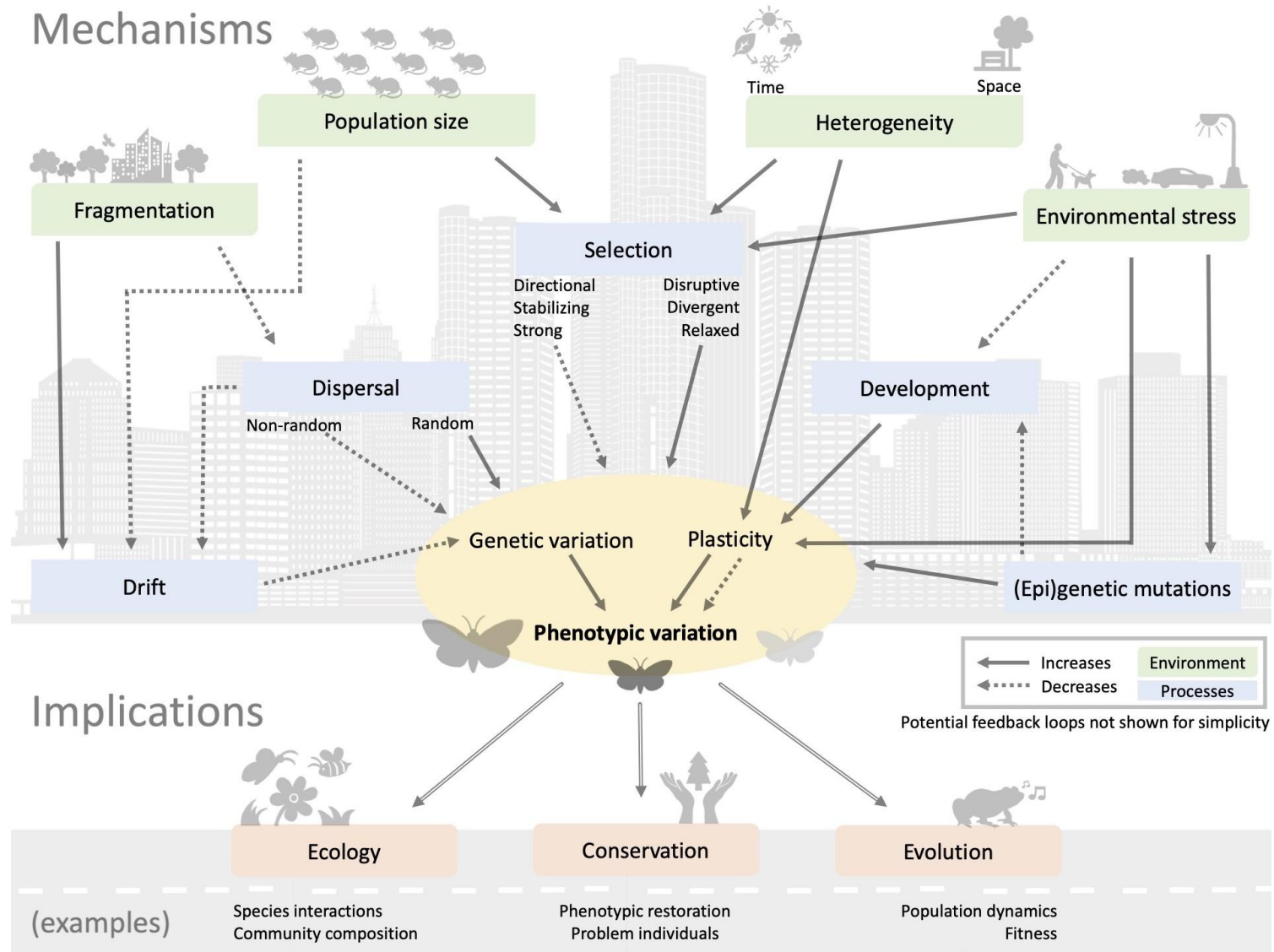
501 107 Caizergues, A.E. *et al.* (2021) An avian urban morphotype: how the city environment shapes
502 great tit morphology at different life stages. *Urban Ecosyst.* DOI: 10.1007/s11252-020-01077-0

503 108 Senar, J.C. and Björklund, M. (2021) Recent spread of blue tits into the Barcelona urban
504 environment : morphological differences and the role of balanced dispersal. *Evol. Ecol.* 35, 83–99

505 109 Salmón, P. *et al.* (2021) Continent-wide genomic signatures of adaptation to urbanisation in a
506 songbird across Europe. *Nat. Commun.* 12, 2983

507 110 Nakagawa, S. *et al.* (2015) Meta-analysis of variation: Ecological and evolutionary applications
508 and beyond. *Methods Ecol. Evol.* 6, 143–152

509



510

511 Figure 1 Hypothesized mechanisms that affect intraspecific phenotypic variation within an urban population, and examples of
 512 ecological, evolutionary, and conservation management implications of this variation.

513 **Box 1: Environmental heterogeneity and within-city comparisons**

514 Urbanization could affect intraspecific phenotypic variation differently across subpopulations within
515 a city, and these differences might be in part explained by variable contributions from environmental
516 heterogeneity. Environmental heterogeneity likely plays a large role in urban evolution where
517 interactions between natural and anthropogenic features affect processes such as selection and dispersal
518 [23]. For example, higher environmental heterogeneity within cities compared to natural habitats expose
519 organisms to diverse local conditions or selective pressures that vary in space and time and can
520 contribute to higher phenotypic variation at the city level if trait data are pooled across field sites within
521 the city or across years [8]. **Fluctuating selection** or differential plasticity in response to heterogeneity
522 may also explain why urban subpopulations of common ragweed (*Ambroisa artemisiifolia*)[5] and easter
523 water dragons (*Intellagama lesueurii*)[15] have higher **phenotypic differentiation** than non-urban
524 subpopulations; a pattern shown in a variety of systems at the genetic level [91].

525 Dispersal could also influence how phenotypic variation is quantified over space if individuals
526 choose environments that best match their phenotype (i.e., **matching-habitat choice**)[92]. Although this
527 is still a fairly unexplored idea in the urban context, there is evidence for habitat matching in urban
528 swans [93] and grasshoppers [94]. Non-random dispersal could reduce phenotypic variation within
529 urban subpopulations if like-individuals settle in similar urban habitat types, but increase city-level
530 phenotypic variation and differentiation among urban subpopulations (Figure 1). Within-city
531 comparisons are needed alongside urban *versus* non-urban comparisons to disentangle the complex
532 interactions that exist between urban phenotypic variation and heterogeneity at different scales [95,96].

533 There remains no consensus on whether urban habitats are more environmentally heterogenous, and
534 this is likely because scale is an important, but overlooked factor [46,97]. Urban habitats are known as
535 more spatially heterogenous [98], but less temporally variable [99]. In Table I (extended version in

536 Table S2), we provide examples from the literature that illustrate how urban environmental features may
537 increase or decrease environmental heterogeneity depending on the spatial or temporal scale in
538 consideration. Due to these discrepancies, it will be important for urban studies to report local scale
539 environmental data alongside phenotypic data, so that future work can begin to account for the role of
540 urban heterogeneity at multiple scales.

541 Table I Examples demonstrating how environmental features can increase or decrease environmental heterogeneity in urban habitats
 542 depending on the scale considered. See also Online Supplemental Information Table S2 for more examples.

Environmental feature	↑ Heterogeneity vs. ↓ Heterogeneity	Scale		References
		Spatial ^a	Temporal ^b	
Anthropogenic food sources	Anthropogenic food availability fluctuates over a week.	Small	Short	[100]
	Anthropogenic food sources are more predictable and stable over seasons or years.		Long	[101]
Land cover and vegetation	Land cover fragmentation in urban areas increases spatial heterogeneity.	Small, Large	Long	[98]
	Urban trees have lower species and genetic diversity.	Large		[54,87]
	Higher primary productivity in urban areas, which is more seasonally and annually stable.		Long	[43]
	Local land cover types increase vegetative growing seasons in urban areas.	Small	Long	[102]

543 ^a Environmental heterogeneity affected at small (local or home range level) or large (city or regional level) spatial scales.

544 ^b Environmental heterogeneity affected at short (within a day or week) or long (between seasons or years) temporal scales.

545 **Box 2: Sampling considerations**

546 As phenotypic variation is a population attribute, it will be important for authors to define what
547 they mean by “population” when comparing phenotypic variation. A population is often defined in
548 relation to gene flow and drift, but directly calculating population size in many wild species is not
549 feasible [103]. We use the term population loosely in this review to refer to groups of urban and non-
550 urban samples that are spatially close to one another (e.g., individuals in a city vs. surrounding area).
551 The type of measurement, size of geographic range, and conspecific density are important sampling
552 factors that could directly affect the amount of phenotypic variation estimated in a population.
553 Therefore, the scale considered (see also Box 1) and the sampling design used might affect the amount
554 of phenotypic variation measured, particularly in cross-sectional studies. Comparing variation between
555 two samples requires standardized measures of variation (e.g., coefficient of variation) that consider the
556 scale of the trait measured and the mean-standard deviation relationship (further discussion in [104]).

557 Many studies, including meta-analyses [21], focus on comparing two contrasting populations,
558 one urban and one non-urban. This may create biases in comparative analyses or review syntheses if the
559 definition of urban and rural sites differs between studies. For instance, sites that are defined as urban
560 can differ in size or location within a city. Small green areas in city centres, and large parks or
561 cemeteries, can equally be considered as urban, but they are likely to be different ecologically.
562 Sometimes, the urban environment is sampled in a more heterogenous way than in more classic urban
563 studies, which includes randomly selecting sampling locations [105] or using hierarchical designs [95].
564 It is worth noting that such alternative designs could lead to larger phenotypic variation in the urban
565 population, because they are likely to sample a larger array of microhabitats.

566 We wish to both point out these sampling considerations and acknowledge that dealing with
567 these issues uniformly across studies in free-ranging populations can be challenging. We recommend

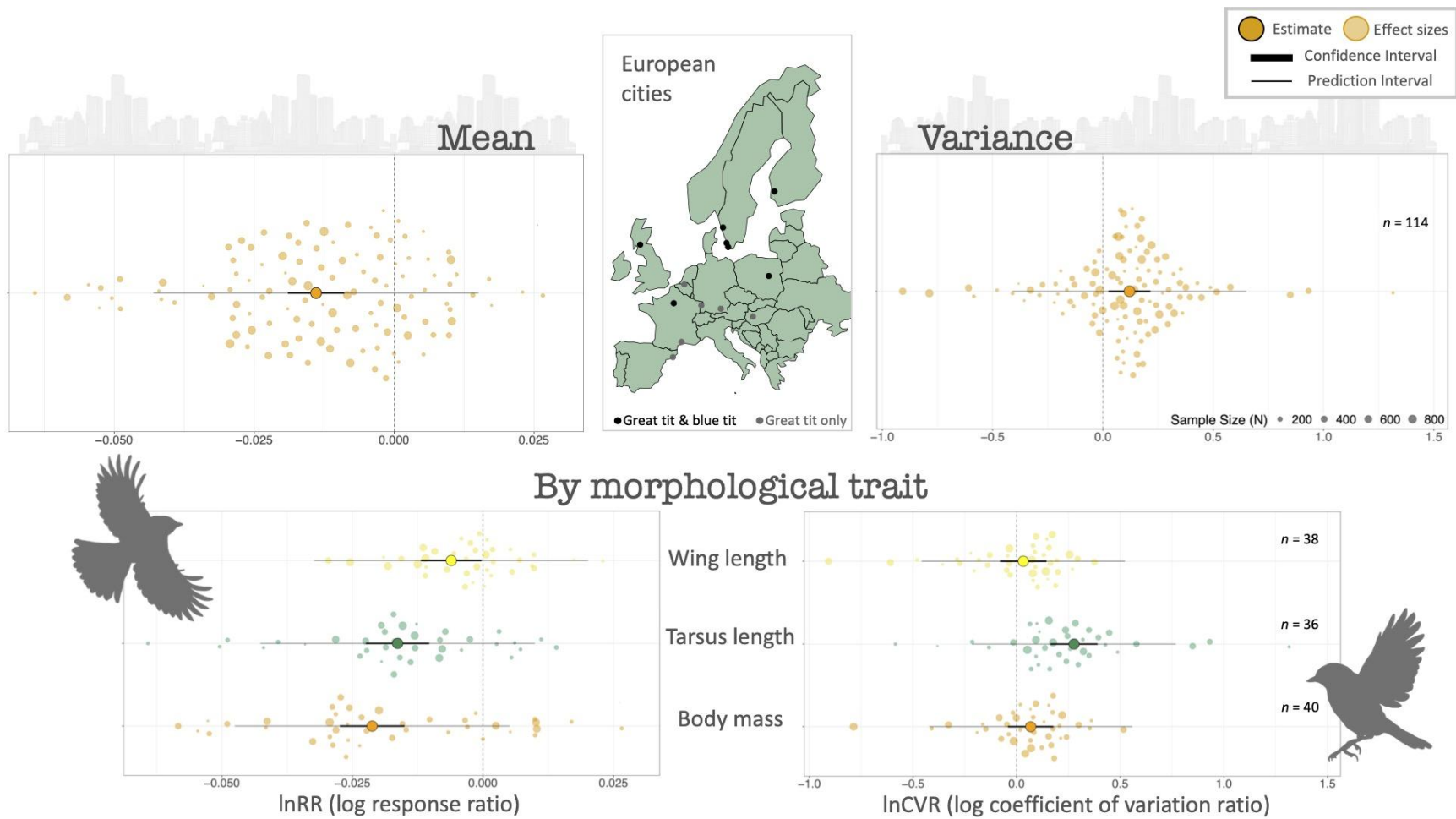
568 researchers control for unbalanced sampling and report relevant information about their study
569 populations when comparing phenotypic variation, for instance by explicitly quantifying the level of
570 urbanization at study sites. Urbanization or urban environmental features are still not well quantified in
571 many studies that examine phenotypic shifts. In particular, environmental measures are often
572 anthropomorphically biased and may not represent the environmental scales that urban organisms
573 occupy [106].

Box 3: Morphological variation in urban *versus* forest tits

We conducted an illustrative analysis to examine how urbanization may affect phenotypic variation of morphological traits using data on great and blue tits (*Parus major*, *Cyanistes caeruleus*) from a collaborative network of researchers across 13 different European forest and city pairs (see Figure I; Table S3; Figure S1 and S2). First, we expected urbanization to decrease the mean of morphological traits based on previous findings [107,108]. Second, we hypothesized an increase in phenotypic variation for morphology in urban tits because a) environmental stress can increase (epi)genetic mutations or disrupt developmental mechanisms, b) fluctuating selection pressures via environmental heterogeneity might increase morphological variation within urban tit populations, and c) European tits are good dispersers [109], which should reduce the effects of fragmentation that act to decrease phenotypic variation in urban populations for other less-dispersive taxa. We used $\ln RR$ ($\ln \frac{\text{Mean}_{\text{urban}}}{\text{Mean}_{\text{non-urban}}}$) and $\ln CVR$ ($\approx \ln \frac{CV_{\text{urban}}}{CV_{\text{non-urban}}}$) to compare morphological mean and variance, respectively, between urban and non-urban tits from multiple systems (see supplementary for details) [110].

Urbanization tended to decrease the mean ($\ln RR$), but increase the variation ($\ln CVR$), in morphology as predicted. Urban birds tended to be smaller, with this effect being stronger in mass and tarsus length than wing length (Figure I; Table S4). Interestingly, tits tended to have more variable body sizes in cities, a trend driven mainly by tarsus length (Figure I; Table S4). Estimates of mean and variance effect sizes were similar for the two species, and slightly stronger in females than males (see supplementary, Figure S3; Table S4). Multiple of the above hypotheses may explain this increased morphological variation in urban tits. For example, the morphological traits we examine have different developmental trajectories where the tarsus develops early in life and remains fixed, wing metrics can vary annually with moults, and body mass can fluctuate continuously. Disruptions in development could

597 then only have observable effects on variation for early developing and constant traits, like tarsus length.
598 Fluctuating selection and high heterogeneity among urban habitats could also increase morphological
599 variation in tits at the city level (Box 1). This analysis reveals that urbanization increases phenotypic
600 variation in tit morphology. Further work is needed to determine the mechanisms that interact to affect
601 shifts in phenotypic variation in urban environments, as well as the consequences of higher phenotypic
602 variation in cities.



603

604 Figure I Urbanization increases the variance (lnCVR; right), but decreases the mean (lnRR; left), in European tit morphology.

605 Models evaluating the overall effect of urbanization (top) and contributions from morphological traits (bottom) are shown. Individual

606 effect sizes ($n = 114$) are shown and scaled by their sample size. See also Online Supplemental Information Table S3, Table S4, and

607 Figure S1-S3 for detailed information and results.

608 **Glossary**

609 Developmental canalization: a developmental process that constrains phenotypic variation by buffering
610 variation from genetic and/or environmental sources.

611 Developmental plasticity: the capacity of a genotype to alter its phenotype depending on environmental
612 conditions during ontogeny.

613 Dispersal: the movement of individuals between geographical areas or habitats.

614 Environmental heterogeneity: diversity in the presence and arrangement of biotic and abiotic features
615 over space and time.

616 Epigenetics: the study of any process that alters gene activity (e.g., gene expression) without changing
617 the DNA sequence. These alterations can be heritable and reversible.

618 Fitness: the contribution of an individual to the gene pool of the next generation, relative to other
619 individuals within a population. Fitness metrics are quantitative measures associated with survival or
620 reproductive output.

621 Fluctuating selection: changes in the strength or shape of selection pressures through space or time.

622 Functional traits: Phenotypic traits of an individual that influence their fitness or performance and can
623 affect ecological processes and functions.

624 Founder effect: a reduction in genetic variation because a population is established by only a few
625 individuals from an ancestral population.

626 Genetic drift: changes in the frequency of gene variants in a population due to random sampling of
627 individuals.

628 Genetic mutation: Permanent alteration of a DNA sequence that results in a genetic variant that may be
629 passed to future offspring.

630 Habitat fragmentation: landscape-level process that leads to a habitat becoming discontinued.

631 Intraspecific phenotypic variation: the measurable or observable phenotypic variation within a species.

632 Matching-habitat choice: the tendency for individuals to settle in a habitat that improves their fitness
633 according to their phenotype, thereby promoting local adaptation.

634 Phenotypic variation: the measurable or observable variation in a trait.

635 Phenotypic plasticity: the capacity of a genotype to express different phenotypes depending on
636 environmental conditions.

637 Phenotypic differentiation: phenotypic differences between two or more (sub)populations.

638 Relaxed selection: reduction in the strength of the association between fitness and a given phenotype.

639 Selection: the relationship between fitness and a phenotypic trait. Directional or stabilizing selection can
640 decrease phenotypic variation while divergent or disruptive selection can increase phenotypic variation
641 within a population.

642 Source-sink dynamics: a model that links variation in habitat quality to population dynamics where
643 population growth is expected in high quality or source habitats and population declines are expected in
644 low quality or sink habitats.

645 Species interactions: Interactions between individuals of different species which broadly include
646 interspecific competition, predation, herbivory, parasitism, mutualism, and commensalism.

647 Urbanization: a process of environmental change resulting from dense human presence and occupancy.