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## How does urbanisation affect natural selection?

### Abstract

1. Urbanisation is one of the most significant contributors to the Anthropocene, and urban evolutionary ecology has become an important field of research. While it is commonly assumed that cities impose new and/or stronger selection, the contradictory assertion that selection may be relaxed in cities is also frequently mentioned, and overall our understanding of the effects of urbanisation on natural selection is incomplete.
2. In this review, we first conduct a literature search to find evidence for patterns of natural selection on phenotypic traits including morphology, physiology, behaviour and life history, in urban populations of animals and plants. This search reveals that coefficients of natural selection in the context of urbanisation are scarce ( $n = 8$  studies providing selection gradients/differentials that include a total of  $n = 200$  coefficients) and a lack of standardized methods hinders quantitative comparisons across studies (e.g., with meta-analysis). These studies, however, provide interesting insight on the agents shaping natural selection in cities and improve our mechanistic understanding of selection processes at different spatial scales.
3. We then perform a second literature search to review genomic studies assessing selection intensity in cities on the genome of non-human natural populations. While this search returned 412 articles, only 29 of these truly investigated footprints of selection associated with urbanisation, and only one study provided urban genetic selection coefficients. Here again, we found highly heterogeneous approaches, yet studies provide strong evidence of genomic footprints of urban adaptation.
4. In neither the phenotypic nor genomic literature review were we able to quantitatively assess natural selection across urban *versus* non-urban habitats. Thus, we propose a roadmap of how future studies should provide standardised metrics to facilitate mega- or meta-analyses and explore generalised effects of urbanisation on selection.

**Keywords: Urban adaptation, evolution, natural populations, literature review, selection coefficient, phenotypic, genomic, city**

## 34 1. Introduction

35

36 The Anthropocene is defined as the period during which human activity has demonstrably  
37 impacted the planet's geology, climate and ecosystems (Waters *et al.* 2016). The  
38 consequences of human impacts on the planet have included rapid shifts in the phenotypes of  
39 wild organisms in response to anthropogenic disturbances (Hendry, Farrugia & Kinnison  
40 2008), such as a reduction in the size of harvested (*i.e.* fished or hunted) animals (Darimont *et al.*  
41 2009) or the repeated evolution of extreme pollution tolerance in populations of Atlantic  
42 killifish (*Fundulus heteroclitus*) of urban estuaries (Whitehead *et al.* 2012). These human-  
43 induced phenotypic shifts are driven by both rapid plasticity and genetic responses (Palumbi  
44 2001). Pervasive ecological alterations such as habitat degradation, climate change, pollution,  
45 exotic species introduction, or over-exploitation of resources, may in turn influence  
46 evolutionary processes such as gene flow, mutation rate, genetic drift and natural selection.  
47 The related eco-evolutionary feedback loops impede analysis of these individual processes,  
48 and predicting the future eco-evolutionary consequences of the human footprint on  
49 biodiversity is challenging (Pelletier *et al.* 2007; Hendry, Gotanda & Svensson 2017; Otto  
50 2018; Wood *et al.* 2021).

51

52 Despite the difficulty in studying these processes independently, there is a general consensus  
53 that "human activities have reshaped selection pressures" (Otto 2018). Perhaps there is  
54 already a general agreement on this statement because of long-standing evidence that humans  
55 can intentionally initiate and control artificial selection such as during plant or animal  
56 domestication (Driscoll, Macdonald & O'Brien 2009). In fact, from Darwin to modern  
57 quantitative genetics, agricultural domestication has resulted in huge steps in our  
58 understanding of natural selection and adaptive evolution (e.g. Thompson 2008; Gregory  
59 2009). Humans can also unintentionally influence the direction, shape and strength of natural  
60 selection. Contrary to the common expectation that human activities will result in novel and  
61 strong selection (Pelletier & Coltman 2018), a review of phenotypic selection coefficients  
62 across 37 different species found no evidence for stronger selection on average in human-  
63 disturbed compared to natural habitats, with notable exceptions such as strong size-related  
64 selection in fisheries (Fugere & Hendry 2018). Despite these findings, there is still a pervasive  
65 assumption in the literature that human altered environments, and in particular urban habitats,  
66 are "a powerful selective force" (Grimm *et al.* 2008) that can "increase the total strength of  
67 selection" (Alberti, Marzluff & Hunt 2017).

68  
69 Cities are increasingly recognized as agents of evolutionary change that can provide unique  
70 insight on patterns of evolution, specifically rapid adaptation (Donihue & Lambert 2015;  
71 Johnson & Munshi-South 2017; Thompson, Rieseberg & Schluter 2018; Lambert *et al.* 2021).  
72 Cities occupy only 3% of Earth's landmass, while hosting more than half of the human  
73 population. The UN projects that cities will continue to grow, with 68% of the population  
74 living in cities by 2050 (United Nations 2019). The rapid pace of urbanisation creates many  
75 challenges for an ever-growing urban human population, as well as for preserving urban  
76 biodiversity. The nascent field of urban evolutionary biology studies adaptation in cities  
77 compared to less anthropogenic habitats, in order to predict how wildlife can cope with  
78 growing urbanisation, and using cities as laboratories to explore eco-evolutionary processes  
79 involved in species rapid adaptation (Szulkin, Munshi-South & Charmantier 2020).

80  
81 Theoretically, the many constraints associated with urban life, such as reduced access to high  
82 quality food resources or shelter and strong disturbances by humans, cars and domestic pets,  
83 could shift the fitness landscape, decreasing mean absolute fitness, and increasing  
84 maladaptation and the opportunity for selection (Fig. 1 in Fugere & Hendry 2018). In contrast,  
85 urban conditions could also lead to relaxed selection. First, this can happen if human habitat  
86 alteration reduces the fitness advantage of a trait. For example, eutrophication and algae  
87 invasion in the Baltic Sea have rendered the male red nuptial coloration of three-spined  
88 sticklebacks (*Gasterosteus aculeatus*) ineffective in sexual courtship due to poor visibility,  
89 resulting in weaker natural selection (Candolin, Salesto & Evers 2007). Second, urban  
90 environments can be more homogeneous than natural habitats for some species, buffering  
91 organisms from environmental variation. For instance, the urban heat island (UHI) effect  
92 keeps cities warmer during extreme cold (Yang & Bou-Zeid 2018). Such buffering could  
93 decrease among-individual variation in fitness and relax selection pressures (see e.g.  
94 Rodewald & Arcese 2017). Note that a given city characteristic such as the UHI can have  
95 both positive and negative influences on fitness depending on the species or the season (see  
96 e.g. in humans, Macintyre *et al.* 2021).

97  
98 Cronin *et al.* (2022) reviewed evidence for divergent sexual selection in urban habitats and  
99 drivers, such as pollution or resource availability, that shape selection pressures acting on  
100 sexual traits. They identified 104 studies published between 1980 and 2021, providing insight  
101 on urban influences on sexual signalling (Table S1 in Cronin *et al.*, 2022). For instance, trade-

102 offs between signal transmission and attractiveness lead to altered male songs in urban areas,  
103 with higher minimum frequencies for several bird species (Slabbekoorn & den Boer-Visser  
104 2006; Wood & Yezerinac 2006; Dowling, Luther & Marra 2012). Although interpreted in the  
105 light of new sexual selection pressures (e.g. selection for increased signal transmission in the  
106 above example of male bird song), the majority of these case studies provide no estimates of  
107 the strength of selection.

108  
109 Natural selection can be defined in “purely phenotypic terms” (Arnold & Wade 1984) because  
110 it involves differences in fitness resulting from differences in phenotypic traits. As such,  
111 selection has been historically approached by estimating the covariance between a phenotype  
112 and relative fitness (Price 1970). Another approach examining natural selection in urban  
113 habitats determines how genomes have been shaped by urban adaptation *versus* urban-specific  
114 demography, gene flow or drift (Johnson *et al.* 2018). While these attempts rarely produce  
115 robust conclusions on natural selection without associated fitness measures, studies providing  
116 genetic selection coefficients can compare the magnitude of natural selection acting on  
117 genetic variants in urban *versus* non-urban habitats. Thurman and Barrett (2016) gathered  
118 3416 genetic selection coefficients published in 79 studies across habitat types, providing  
119 fascinating insight into how selection shapes genomes, for instance revealing stronger  
120 selection over shorter timescales. They highlighted the limited data available compared to the  
121 huge potential to estimate similar genetic selection coefficients across taxa and contexts. This  
122 study inspired us to perform a similar literature search, restricted to urban *versus* non-urban  
123 contexts, with the hope that Thurman and Barrett’s call lead to numerous new estimates.

124  
125 Recent reflections on the field of urban evolutionary biology underscore the low number of  
126 studies that have documented cases of biological adaptation to urban environments (Lambert  
127 *et al.* 2021) and the difficulty in reaching general conclusions on how urbanisation affects  
128 natural selection (Verrelli *et al.* 2022). The answer to this latter question can depend on the  
129 target of selection, the spatial and temporal scales considered, the age and history of cities,  
130 and the numerous agents of selection in cities such as air, light and sound pollution (Verrelli  
131 *et al.* 2022; Winchell *et al.* 2022). The aim of our study is to focus on the fundamental process  
132 of natural selection which drives adaptation, and assess how urbanisation alters selection, *via*  
133 1. evaluating whether the strength of phenotypic selection is generally stronger or weaker in  
134 cities when compared to natural habitats and 2. measuring the force of urban-related selection  
135 on adaptive genomic variants. We reviewed the literature comparing coefficients of natural

136 selection in urban and non-urban habitats using both phenotypic and genomic approaches  
137 with the initial aim of conducting meta-analyses to quantitatively assess trends for given traits  
138 or taxa. The scarcity of studies prevented such an analysis, and thus we provide a qualitative  
139 assessment of how natural selection can differ in urban *versus* non-urban environments, and  
140 outline a roadmap for how future studies should provide standardised metrics to facilitate  
141 mega- or meta-analyses and explore generalised effects of urbanisation on selection.

## 142 2. Urban natural selection on wild phenotypes

143

144 Understanding how natural selection varies across wild populations, but also in time, is  
145 considered a central question in evolutionary ecology (Hoekstra *et al.* 2001; Siepielski,  
146 DiBattista & Carlson 2009; Bell 2010; Morrissey & Hadfield 2012). It has led to an abundant  
147 literature, in particular following the publication of Lande and Arnold's (1983) accessible  
148 introduction on how to measure linear and non-linear selection differentials/gradients using  
149 multivariate regression on individual phenotypic and fitness data (Svensson 2023). A  
150 selection differential reflects the total selection acting on a single trait regardless of other  
151 potentially selected traits, while a selection gradient accounts for several traits being involved  
152 in the selection episode using a multivariate analysis (Mittell & Morrissey 2024).

153 To determine whether urbanisation affects the strength of selection, we first synthesised  
154 studies on phenotypic traits that i) estimated selection coefficients *i.e.* selection gradients or  
155 differentials in urban and non-urban environments and ii) provided compelling evidence for  
156 altered urban selection. Studies reviewed in section 2.1. were found by searching Google  
157 Scholar for studies that cited Lande & Arnold (1983; following approach in Fugere & Hendry  
158 2018) and mentioned either urban\*, city\*, town\*, or metro\* (conducted 15 January 2024,  $n =$   
159 6 relevant studies out of 439 hits, see full list in Table S1). We also conducted a Web of  
160 Science Core collection search (CNRS institution access in Montpellier, France on 15 January  
161 2024, see Table S2 for full search terms) that included articles containing urban-related search  
162 terms (Topic = "urban\* OR city\* or town\* OR metro\*") and either "selection gradient\*" or  
163 "selection differential\*" across all fields, which did not return any new article. We also  
164 included two other relevant studies that did not appear in either of these searches and were  
165 identified by reviewing the reference lists of relevant studies ( $n = 2$ ; Badyaev *et al.* 2008;  
166 Branston *et al.* 2021). From the Google Scholar search, we identified eight studies that  
167 estimated coefficients of selection but did not provide a comparison to non-urban habitats  
168 (Sol, Jovani & Torres 2003; Gregoire *et al.* 2004; Yeh & Price 2004; Price, Yeh & Harr 2008;  
169 Ryder *et al.* 2012; Lambrecht, Mahieu & Cheptou 2016; Houle *et al.* 2020; Spear *et al.* 2023).  
170 For instance, larger size was under strong selection in holy hawksbeard (*Crepis sancta*) in  
171 urban environments (Lambrecht, Mahieu & Cheptou 2016), but it is unknown whether  
172 selection for plant size is stronger or weaker for this species outside the urban setting. While  
173 these studies may be important for future meta-analyses, they are not discussed further in our  
174 comparative approach.



175

176 Overall, studies estimating wild selection coefficients were all conducted in avian or plant  
177 taxa ( $n = 8$ , five studies on three avian species and three studies on three plant species; Figure  
178 1; Table 1 & Supporting information Table S3), with a particular focus on morphological and  
179 phenological traits. The modest number of studies found highlights a crucial gap of  
180 knowledge on the magnitude of urban/non-urban differences in selection, as well as on the  
181 specific urban drivers that modify the strength of selection, which are often speculated upon  
182 but very rarely demonstrated.

183 TABLE 1. Studies estimating selection gradients or differentials in urban and non-urban environments. Order follows discussion in the main text.

184 ISA= Impervious surface area, CMR= capture-mark-recapture.

Taxa	Species	Phenotypes	Fitness proxy	Results	Reference
Birds	House finch ( <i>Carpodacus mexicanus</i> )	Morphology: Bill length, width, depth	Survival: Survival to following year	Stronger urban directional selection	(Badyaev <i>et al.</i> 2008)
	Great tit ( <i>Parus major</i> ) Blue tit ( <i>Cyanistes caeruleus</i> )	Morphology: Body mass	Survival: Survival to fledging	Stronger urban selection in both species, but urban selection significantly higher only in great tits	(Corsini <i>et al.</i> 2021)
	Great tit ( <i>Parus major</i> )	Morphology: Body mass, wing length, tail length Life-history: Lay date, clutch size	Reproduction: Number of fledglings	Selection on traits overall weak in both habitats, but significantly weaker urban selection for lay date and body mass (males)	(Caizergues, Gregoire & Charmantier 2018)
	Great tit ( <i>Parus major</i> )	Behaviour: Exploration, aggression, stress response	Reproduction: Number of fledglings	Selection on traits overall weak in both habitats, but significantly weaker urban selection for exploration (males)	(Caizergues <i>et al.</i> 2022a)
	Great tit ( <i>Parus major</i> ) Blue tit ( <i>Cyanistes caeruleus</i> )	Life-history: Lay date, clutch size	Reproduction: Number of fledglings	Selection was significantly stronger in the forest for both traits only in blue tits, no significant difference in great tits.	(Branston <i>et al.</i> 2021)
Plants	Yellow jessamine ( <i>Gelsemium sempervirens</i> )	Morphology: Floral size, display size & shape Physiology: Chemical defence	Reproduction: Seeds per plant	Significantly stronger urban selection for larger floral display size	(Irwin, Warren & Adler 2018)
	Blue passionflower ( <i>Passiflora caerulea</i> )	Morphology: Fruit diameter, mean seed number, crop size, peel carbohydrate content	Reproduction: Fruit removal	Weaker urban and semiurban selection for fruit crop size, but not statistically compared	(Palacio & Ordano 2023)
	Common ragweed ( <i>Ambrosia artemisiifolia</i> )	Morphology: Plant height Phenology: Transition to reproduction, first open male flower, male to female flower	Reproduction: Number of flowers & fruits	Net selection favoured larger plants and earlier phenology overall, but stronger selection on foreign genotypes	(Gorton, Moeller & Tiffin 2018)

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## 186 **2.1. Phenotypic selection differentials and gradients**

### 187 **2.1.1. Stronger urban natural selection**

188

189 Stronger estimates of urban selection have so far been reported in bird and plant species  
190 (Table 1). The limited number of studies in Table 1 suggests that in birds, selection in urban  
191 habitats may more strongly act on morphological traits than behaviour or life-history traits,  
192 with the underlying drivers often linked to changes in urban diet. Urban bird feeding activities  
193 have shifted urban diets towards larger seed resources which has led to strong selection  
194 favouring longer bills in urban house finches (*Carpodacus mexicanus*) compared to desert  
195 finches (Badyaev *et al.* 2008). Another example from great tits (*Parus major*) along an  
196 urbanisation gradient in Warsaw revealed stronger selection in more urbanised areas  
197 favouring higher body mass at hatching (Corsini *et al.* 2021), most likely related to the  
198 reduction in food resources (Seress *et al.* 2018) .

199

200 As in birds, evidence for stronger urban selection in plants has been documented in  
201 morphology, related to increases in floral size. In the Yellow jessamine (*Gelsemium*  
202 *sempervirens*), selection for larger floral displays is significantly stronger in urban areas  
203 compared to non-urban areas, perhaps driven by reductions in urban pollinators (Irwin,  
204 Warren & Adler 2018) Although the study supports stronger urban selection for floral display  
205 size, the authors highlight that they find only modest selection estimates for the other traits  
206 considered, suggesting that urbanisation may not contribute to sweeping changes in  
207 phenotypic selection as commonly expected (Irwin, Warren & Adler 2018).

### 2.1.2. Weaker urban natural selection

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209

210 Evidence in birds for weaker urban selection come from two tit species, but range across  
211 morphological, life-history, and behavioural traits. Urban great tits in the city of Montpellier  
212 tend to be smaller, faster explorers, more aggressive, and more stressed during handling, and  
213 tend to lay earlier and smaller clutches than tits living in a nearby forest habitat (Charmantier  
214 *et al.* 2017; Caizergues *et al.* 2022a). Selection gradients across these traits were overall weak  
215 in both habitats and, in some cases, patterns of selection were in the opposite direction to the  
216 documented phenotypic divergence (e.g. for breeding phenology, Caizergues, Gregoire &  
217 Charmantier 2018). In higher latitude populations of blue tits around Glasgow, there is  
218 selection for earlier lay dates and larger clutches in both urban and forest habitats but, again,  
219 the strength of selection on these traits was significantly weaker in urban areas (Branston *et*  
220 *al.* 2021). The authors show that their urban study sites possess fewer caterpillars and  
221 hypothesise that weaker environmental cues in urban areas could contribute to relaxed urban  
222 selection on phenology (Branston *et al.* 2021).

223

224 We describe above that urban selection can be stronger on floral display size, but we find  
225 support for weaker urban selection on other plant morphological traits. Bird visitation was  
226 found to exert selection on fruit crop size across populations of the blue passionflower  
227 (*Passiflora caerulea*), but this selection was weaker in the urban and semi-urban populations,  
228 and strongest in the rural population (Palacio & Ordano 2023). The authors suggest that this  
229 relaxed urban selection could be a result of either i) urban populations being closer to the  
230 phenotypic optimum as they tended to have larger average fruit crop sizes or ii) generalist bird  
231 species in urban areas being less selective of which fruits (and associated traits) they forage.  
232 This calls for an integration of species interactions to unravel the agents driving novel  
233 selection pressures in cities. In another example, selection favoured taller height and earlier  
234 phenology across common ragweeds (*Ambrosia artemisiifolia*) of urban and rural origins  
235 planted in both urban and rural study sites (Gorton, Moeller & Tiffin 2018). Genetic  
236 differentiation and stronger selection on foreign genotypes provide consistent support for  
237 local adaptation in ragweed, although plants from rural origins tended to have higher overall  
238 lifetime fitness when reared in both urban and rural study sites providing conflicting support  
239 (Gorton, Moeller & Tiffin 2018). The authors suggest this latter finding could result from  
240 weaker selection in the urban environment, potentially because urban habitats are more  
241 spatially heterogeneous (Gorton, Moeller & Tiffin 2018).

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### 2.1.3. Insight from urban phenotypic selection gradients

244

245 Across the studies in Table 1, no obvious generalisations have emerged that enable  
246 directional predictions on how urbanisation impacts selection across different traits and  
247 species. Our goal to conduct a formal quantitative analysis is not achievable given the few  
248 studies that have quantified selection coefficients ( $n = 8$ ). We list in the Supporting  
249 Information (Table S3) 200 effect sizes ( $n = 101$  urban and 99 non-urban) of linear selection  
250 gradients and differentials reported in these studies. We consider this a first step to inspire  
251 research interest in reporting these selection coefficients and their associated uncertainty, so  
252 that meta-analyses can be conducted in the future. Descriptively, the median (absolute) urban  
253 and non-urban selection gradients are similar ( $n$  urban = 70 with median: 0.091, range: 0.0001  
254 – 1.06;  $n$  non-urban = 68 with median: 0.10, range: 0.004 - 1.37), and within the range of  
255 previously reported summaries of selection in natural populations (see e.g. Kingsolver *et al.*  
256 2012; Morrissey & Hadfield 2012). Similar median strength of selection between urban and  
257 non-urban habitats is somewhat unsurprising given there is evidence of both stronger and  
258 weaker urban phenotypic selection (as summarized above) and selection estimation is  
259 classically associated with large measurement error (e.g. Dingemanse, Araya-Ajoy &  
260 Westneat 2021). Overall, our summary on phenotypic coefficients of selection implies that we  
261 so far are unable to generalise on how urbanisation impacts the strength of selection. Further  
262 comparative research of selection in and outside cities across diverse systems is needed before  
263 this conclusion can be re-addressed and any generalities can emerge.

264

265

### 2.2. Compelling evidence of modified selection

266

267 Since the literature search conducted above was not conclusive regarding differences in  
268 selection differentials or gradients between non-urban and urban areas, we further discuss  
269 studies that offer complementary insights on this topic. While these studies do not allow a  
270 quantitative comparison across species and contexts, they do provide unique insight into the  
271 mechanisms that may drive novel, stronger or weaker selection in cities. Demonstrating the  
272 causal agents of selection has always been a great challenge in evolutionary ecology, and it is  
273 usually not possible to firmly identify the drivers via a correlational approach (Mitchell-Olds  
274 & Shaw 1987; Svensson 2023). Cities are characterized by multifarious urban stressors such  
275 as multiple forms of pollution, heat, and altered and fragmented habitats (Diamond & Martin

276 2021). Only experimental manipulations such as alteration of the biotic (e.g. predation  
277 pressure) or abiotic (e.g. temperature) variables can clearly identify agents driving differences  
278 in selection in urban environments (Wade & Kalisz 1990; MacColl 2011).

### 2.2.1. Relaxed selection under altered resource and predator regimes

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In their extensive review on the effects of urbanisation on sexual selection, Cronin et al (Cronin *et al.* 2022) cited resource availability as one of the main biotic drivers influencing the strength and form of sexual selection in an urban context. While food is necessary for somatic growth and maintenance, it is also a key determinant of colourful ornaments. In the Northern Cardinal *Cardinalis cardinalis*, the red plumage of males is produced from carotenoid pigments that are found in their diet. A study of northern cardinals by Rodewald and colleagues (2011) across a rural-urban gradient in Ohio, USA, revealed that in more urban landscapes, brightness of male plumage no longer correlated with breeding phenology or reproductive success. The authors suggest that this relaxed selection for colour arises because of the over-abundance of carotenoid-rich exotic fruits in cities, such as honeysuckles. We find this example particularly striking because in other bird species with carotenoid-related colours, urban birds are generally paler (Janas, Gudowska & Drobniak 2024), although with strong variation across cities, and no insight yet on the force of natural or sexual selection on these colours (Salmón *et al.* 2023).

One of our favourite demonstrations of relaxed selection in cities was recently published and echoes the iconic study of industrial melanism in the peppered moth *Biston betularia* in industrial England (see e.g. Cook & Saccheri 2013). Following observations of parallel high prevalence of melanic eastern gray squirrels (*Sciurus carolinensis*) across 43 cities in North America, Cosentino and colleagues (2023) translocated 76 gray squirrels from urban areas in Syracuse to both urban and rural novel areas. The subsequent monitoring revealed that while gray squirrels had much higher survival than melanic squirrels in rural habitats, there was no such evidence for survival selection in the city. The authors favour the hypothesis that weaker selection against the conspicuous melanic morph in the city results from lower predation and human hunting pressure.

Finally, the role of altered predator communities is also cited as a possible agent of relaxed selection for fast growth rates in urban damselflies *Coenagrion puella* (Tüzün *et al.* 2017). Note that while experimental approaches such as this common garden on damselflies are very powerful to test for urban local adaptation (Lambert *et al.* 2021) they do not provide adequate quantitative measures of natural selection.

### 2.2.2. Reversed selection and identifying multiple agents of selection

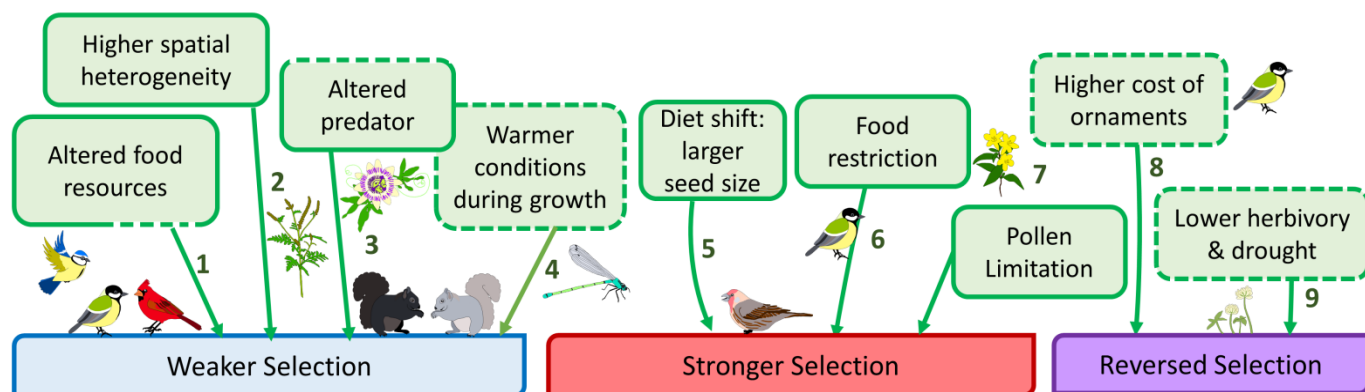
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Evidence for reversed selection in urban habitats is very scarce but it was shown in a common urban-dweller, the great tit. Using 17 years of capture-mark-recapture data and measures of the size of the black breast stripe of male great tits in and around Barcelona, Senar and colleagues (2014) found that forest males with larger stripes had higher survival while the reverse was true in urban males. While the size of male black ties has been positively associated with dominance status (e.g. Jarvi & Bakken 1984), tie size is also negatively correlated with exploration speed (Nicolaus *et al.* 2016). Senar thus hypothesizes that smaller ties in city birds is likely a by-product of selection on personality (Senar, pers.com.), which aligns with findings that urban great tits are bolder and faster explorers (e.g. Riyahi *et al.* 2017).

A global study of the white clover (*Trifolium repens*) illustrates the difficult task of identifying agents of selection in a complex urban system. A large-scale study of 20 Canadian cities revealed parallel clines with decreased plant production of hydrogen cyanide (HCN) in response to urbanisation, indicating parallel evolution resulting from parallel selection favouring lower chemical defence in cities (Johnson *et al.* 2018). Despite the large number of cities in this study, agents of selection causing these evolved differences remained unclear. In an even larger scale study across a monumental 160 cities, Santangelo and colleagues analysed environmental predictors of HCN clines, concluding that herbivory selected for higher HCN in rural areas while lower drought selected for lower HCN in urban areas (Santangelo *et al.* 2022).

It is sobering to note that for most studies discussed in this section, even with extensive efforts across decades, agents of selection inducing new selective forces in urban environments remain hypothetical interpretations from field experts. We also note that, and again despite tremendous effort, most of these studies do not measure individual fitness and hence could not be included in a quantitative meta-analysis of standardised selection estimates. In comparison to the demanding challenge of collecting data that allow estimating phenotypic selection coefficients, selection coefficients from genomic data may be more tractable for many taxa.





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FIGURE 1. Illustrating some studies that have demonstrated divergent natural selection in urban habitats, with potential explanations regarding the agents that can lead to weaker, stronger, or reversed natural selection in cities. Studies either compared selection differentials / gradients between urban and non-urban habitats (solid lines, discussed in section 2.1), or used other approaches (dotted lines, section 2.2). List of species and references: 1: Great tit *Parus major* in (Caizergues, Gregoire & Charmantier 2018) ; Blue tit *Cyanistes caeruleus* in (Branston *et al.* 2021); Northern Cardinal *Cardinalis cardinalis* in (Rodewald, Shustack & Jones 2011); 2: Common ragweed *Ambrosia artemisiifolia* in (Gorton, Moeller & Tiffin 2018); 3: Blue passionflower *Passiflora caerulea* in (Palacio & Ordano 2023); Eastern gray squirrel *Sciurus carolinensis* in (Cosentino, Vanek & Gibbs 2023); 4: Damselfly *Coenagrion puella* in (Tüzün *et al.* 2017); 5: House finches *Carpodacus mexicanus* in (Badyaev *et al.* 2008); 6: Great tit in (Corsini *et al.* 2021); 7: Yellow jessamine *Gelsemium sempervirens* in (Irwin, Warren & Adler 2018); 8: Great tit in (Senar *et al.* 2014); 9: White clover *Trifolium repens* (Santangelo *et al.* 2022). All drawings from MJT.

### 3. Genomic insight on measuring urban selection coefficients

362  
363

364 Estimates of genetic selection coefficients can provide important knowledge on the nature of  
365 selection acting on adaptive traits and their underlying genetic architecture. These estimates  
366 can be complementary to selection coefficient estimates based on phenotypic and fitness data,  
367 depending on whether such phenotypic-based approaches have already been performed or are  
368 even possible. While linking phenotypes and fitness measured in the wild can provide insights  
369 into the functional significance of specific traits in a given environment, genomic selection  
370 coefficients quantify the overall past strength of natural selection on specific genetic variants,  
371 based on genomic data only. While genetic selection coefficients are often seen as analogous  
372 to phenotypic selection differentials, comparing discrepancies between them can inform on  
373 pleiotropic, epistatic, or linkage disequilibrium effects (Thurman & Barrett 2016). In addition,  
374 detecting stabilizing selection is a very challenging task when using genetic data because  
375 allele frequencies remain the same under such selection regimes.

376

377 Genetic selection coefficients for mono- or oligo-genic traits can be measured by genotyping  
378 one of a few candidate loci previously identified as being under selection and/or associated  
379 with phenotypic variation. Advances in genotyping methods, principally through  
380 democratization of high-throughput sequencing, present new opportunities to scan genomes,  
381 detect loci under selection and/or associated with phenotypic variation, and estimate genetic  
382 selection coefficients on polygenic traits for natural populations of non-model species  
383 (Nielsen 2005; Barrett & Hoekstra 2011; Bank *et al.* 2014; Manel *et al.* 2016; Matz 2018).  
384 Note that even with novel polygenic approaches, the genetic variation uncovered often  
385 explain only a small percentage of phenotypic variance. In the next section, we describe  
386 general methods for calculating genetic selection coefficients, then review their application in  
387 an urban context. Finally, we highlight important gaps in the current literature and we propose  
388 in the next section future steps that can be taken to help advance our knowledge of selection  
389 in urban environments.

390

#### 3.1. Overview of genetic approaches to measure selection coefficients

391  
392

393 As the scope of our article is to review the state of the literature, and not to describe in detail  
394 the methodology for calculating selection coefficients, we refer interested readers to  
395 comprehensive reviews of popular and useful methods for calculating genetic selection  
396 coefficients by Linnen & Hoekstra (2009) and Bank *et al* (2014).

397 In brief, the most straightforward strategies for measuring selection based on genomic data  
398 depend on the availability of measures of individual fitness (or fitness-related traits) and  
399 individual genotypes for causal loci or genome-wide variants (*e.g.*, SNPs). In the simplest  
400 cases, such as at a single Mendelian locus causing discrete polymorphic phenotypes, selection  
401 coefficients can be calculated from estimates of the relative fitness ( $w$ ) for a given genotype  
402 (Eanes 1999). Advances in genomic techniques now allow such estimates for quantitative trait  
403 loci and further, can aid in discovery of candidate loci, circumventing the need for prior  
404 knowledge of causal loci. One popular contemporary strategy is to use genome scans or  
405 Genome-Wide Association Study (GWAS) to identify loci underlying variation in fitness or  
406 fitness-related traits, and then measure selection at these candidate loci by associating allelic  
407 variation and individual fitness. For example, Béréños *et al.* (Berenos *et al.* 2015) combined  
408 genomic SNP data with fitness and phenotypic measures to investigate the genetic  
409 architecture of body size traits. The authors used a GWAS to identify SNPs associated with  
410 body size (*i.e.* hind length), tested the association between fitness and genotypes for outlier  
411 SNPs, then estimated selection coefficients at these SNPs. Their study illustrates how  
412 contemporary population genomics techniques paired with traditional phenotypic and fitness  
413 measures can both facilitate the discovery of candidate loci underlying quantitative traits and  
414 improve our understanding of how selection affects these loci in natural populations.

415

416 Many studies may lack fitness and phenotypic data. In these cases, selection  
417 coefficients can be calculated from changes in allelic frequencies over time (*i.e.*, multiple  
418 generations) or over geographic space (*i.e.*, clines). If the candidate gene is unknown, it can  
419 be analysed by applying first genome scans (methods reviewed in Hohenlohe *et al.* 2010) and  
420 then by estimating selection coefficient on identified outlier loci. Otherwise the selection  
421 coefficient for known candidate genes can be directly estimated. Selection coefficients are  
422 estimated by computing the probability of the underlying changes in allele frequencies over  
423 multiple generations or geographic locations, often using a likelihood-based approach (*e.g.*  
424 software package SelEstim, Vitalis *et al.* 2014) or approximate Bayesian computation (ABC)  
425 approaches (*e.g.* Bank *et al.* 2014; Stern, Wilton & Nielsen 2019). Importantly, these methods

426 are in general very sensitive to sample size (Pinsky *et al.* 2021) as well as the spatial and/or  
427 temporal distribution of sampling. As an example of this strategy relying on genomic data  
428 only, Walden and colleagues (2020) estimated selection coefficients at genes implicated in  
429 evolutionary response to spatially heterogeneous climatic conditions in *Arabidopsis lyrata*,  
430 first using GWAS and Environmental Association Analysis (EAA) to identify outlier SNPS  
431 and genes associated with local climatic variation, then estimating mean genomic selection  
432 coefficients for these loci using SelEstim (Vitalis *et al.* 2014). Their results revealed increased  
433 selection coefficients for environment-associated genes compared to coefficients for genes  
434 that were not associated with environment, suggesting that these genes have importance in  
435 climate adaptation. These methods could be easily extended to urban contexts, where  
436 temperatures persistently rise, and our comprehension of the selection pressures induced by  
437 these escalating temperatures remains constrained. Consequently, in the absence of accessible  
438 phenotype and fitness measures, this approach affords the potential to discern genes  
439 intricately linked to climate adaptation and facilitates the estimation and comparison of  
440 selection intensities between urban and natural environments.

441

### 442 **3.2. A review of genomic selection coefficients in the wild urban context**

443

444 While genomic selection coefficients can provide valuable insight to understanding basic  
445 questions in evolutionary biology, there remains limited knowledge about the distribution of  
446 selection coefficients ( $s$ ) in natural populations. A meta-analysis by Thurman and Barrett  
447 (2016) aimed to quantify natural selection at the genetic level, reviewing over 2000 papers  
448 and ultimately extracting ~3000 estimates from 79 studies. While their analysis provided  
449 valuable insight into the magnitude and tempo of selection in natural environments, for  
450 example, suggesting that selection is stronger over shorter timescales, their study also  
451 underscored that a critical lack of published selection coefficients constrained their ability to  
452 conclusively address these topics. We anticipated that in the years since Thurman and  
453 Barrett's review, an increased number of studies would report genomic selection coefficients,  
454 including studies set in an urban context. Thus, our objective was to replicate their meta-  
455 analysis and literature search to retrieve and analyse estimates of selection, with a focus on  
456 estimates of selection coefficients in urban contexts.

457

### 3.2.1. Systematic review and data extraction

458  
459  
460 We identified relevant papers with literature searches using the Web of Science Core  
461 Collection (conducted 8 November 2023, see Table S2 for full search terms), filtering results  
462 to include only primary articles in evolutionary biology containing urban-related search terms  
463 (“Document Types: Article; Research Areas: Evolutionary Biology; Topic = “urban\* OR  
464 city\* or town\* OR metro\*”). We conducted three independent searches using different sets of  
465 search terms. In Search 1, we specified key terms used by Thurman and Barrett (2016) to find  
466 published estimates of natural selection acting at the genetic level: Topic = (“selection  
467 coefficient\* OR genotyp\* selection OR adapt\* gene”). In Search 2, we broadened our criteria  
468 to include selection gradients: Topic = (“selection coefficient OR selection gradient”). In  
469 Search 3, we targeted potentially relevant papers by specifying popular approaches for  
470 quantifying the strength of selection from genomic data, as identified from Linnen &  
471 Hoekstra’s (2009) review: Topic = (“MK test OR McDonald Kreitman test OR McDonald-  
472 Kreitman test OR dn/ds test OR nonsynonymous substitutions synonymous substitutions OR  
473 allele frequenc\* ecotone OR allele frequenc\* cline width OR CLR test OR composite-  
474 likelihood-ratio test”). These searches returned 264, 15, and 133 articles respectively, for a  
475 total of 412 papers (see Table S4).

476  
477 De-duplication resulted in 355 articles that we screened for inclusion. Our initial  
478 criteria for inclusion in our review were that each study: 1) provided a selection coefficient or  
479 selection gradient for a genetic unit (allele, SNP, QTL, chromosome, etc); 2) provided the  
480 selection statistic for both an urban and a non-urban population; and 3) focused on natural  
481 populations (*e.g.*, not laboratory or domesticated plants and animals). No paper met all  
482 criteria, however, so we relaxed our criteria to encompass studies that 1) provided genomic  
483 evidence for selection or selection coefficients/gradients estimated from genomic data; 2)  
484 provided this genomic evidence for at least one urban population; and 3) focused on natural  
485 populations.

486

### 3.2.2. Insights from the literature review and discussion

488

489 Of the 355 articles that we reviewed, no papers met all initial criteria for inclusion, and only  
490 34 (9.6%) met our relaxed criteria for inclusion. Many of the remaining 321 articles were  
491 irrelevant to our focus despite our specified criteria (*e.g.*, they concerned agricultural  
492 populations, lacked urban context, or did not report genetic data). Our difficulty in identifying  
493 relevant studies mirrors challenges reported by Thurman & Barrett in their meta-analysis  
494 (Thurman & Barrett 2016): of the 2200 studies reviewed for inclusion, they were able to  
495 accept only 79 (3.5%). Anticipating this challenge, we sought to improve the relevancy of our  
496 results and the efficacy of our search by complementing Thurman & Barrett's key terms  
497 (Search 1) with independent searches for more broad key terms (Search 2) and for key terms  
498 explicitly targeting well known and long-used methods for calculating genetic selection  
499 coefficients (Search 3). Still, we retrieved few relevant papers. In fact, Search 2 and Search 3  
500 combined were less successful than Search 1: while Search 1 returned 29 relevant papers out  
501 of 264 total papers (11.0%), Search 2 returned only 1 relevant paper out of 15 total papers  
502 (6.7%) while Search 3 returned only 2 relevant papers out of 133 total studies (1.5%).  
503 Together, however, these three searches incorporated a range of both broad and targeted  
504 keywords. We therefore suggest that our detection of few relevant papers reflects an apparent  
505 lack of genetic selection coefficients reported in the urban eco-evolutionary literature.

506

507 **Box 1. A single study to date reporting urban genomic selection coefficient**

508 Baltzegar et al (2021) studied the evolution of a knockdown resistance (*kdr* haplotype) in the  
509 mosquito *Aedes aegypti* in response to insecticide use in the city of Iquitos (Peru). The  
510 frequency of resistant alleles was expected to increase over time, with positive selection  
511 coefficients induced by insecticide exposure. The authors genotyped the *kdr* mutations  
512 associated with pyrethroid resistance, in 9882 mosquitoes sampled in several locations in  
513 Iquitos City before/during/after the use of insecticides. They then estimated selection  
514 coefficients of the resistance alleles at each locus using the Wright-Fisher approximate  
515 Bayesian computation method for temporally sampled data (Foll, Shim & Jensen 2015). The  
516 frequency of *kdr* resistance mutations increased rapidly after insecticides exposure, with very  
517 large selection coefficients of 0.313 (95% CI : 0.007, 0.821) and 0.485 (95% CI : 0.145,  
518 0.969) for the two resistance mutations. The authors discuss that these selection coefficients  
519 may be underestimated because of several violations of the model's assumptions, including  
520 non-independence of the mutations and spatio-temporal heterogeneity of the selection  
521 pressure. The authors also note that, although frequencies of resistant alleles shifted quickly

522 during the study period, genetic heterogeneity existed not only at the citywide scale but also  
523 on a very fine scale within the city. In the scope of our review, we note that this example does  
524 not address the question of whether urbanisation leads to stronger, weaker or reversed  
525 selection but that a comparison with similar estimates outside the urban context would allow  
526 conclusions on urban-specific selection.

527

528 The 34 papers we retained for our study (Table S5) included urban-associated  
529 genomic “signatures of selection”, *e.g.*, using genomic scans to identify candidate SNPs for  
530 divergent selection between urban and non-urban environments (Nielsen 2005; Hohenlohe *et*  
531 *al.* 2010). Six papers of these 34 papers presented genomic data that could be used to estimate  
532 the strength of selection. Of these, five studies reported urban-non-urban genetic clines;  
533 however, none of these papers used the documented genetic clines to calculate selection  
534 coefficients (Linnen & Hoekstra 2009). Only 1 study of the 34 reported selection coefficients;  
535 however, this was for a single urban population without a non-urban statistical complement  
536 (see box 1).

537

538

#### 539 **4. Roadmap for future phenotypic and genomic studies**

540

541 This review has revealed how difficult it is to presently conduct a quantitative comparative  
542 analysis of studies exploring urban versus non-urban natural selection, both at the phenotypic  
543 and the genomic levels, using a systematic literature review and meta-analytical approach.  
544 What would it take for future studies to allow such a quantitative approach and derive more  
545 general interpretations on which traits in which taxa can be expected to be under stronger,  
546 weaker or reversed selection in the urban context?

547

##### 548 **4.1. Facing the challenge of standardised estimations of natural selection**

549

550 For meta-analysts to combine summary statistics on urban and non-urban natural selection,  
551 the primary studies estimating selection should ideally integrate all insight from the vast  
552 literature that delivers guidance for adequate statistical approaches, and provide coefficients  
553 with their error estimation informing on accuracy. Our objective here is not to provide an  
554 exhaustive insight on how to measure natural selection in the wild as others have covered this  
555 extensively (Brodie, Moore & Janzen 1995; Linnen & Hoekstra 2009; Stinchcombe, Kelley &  
556 Conner 2017). Table 2 provides a synthesised list of questions and challenges that must be  
557 addressed when estimating natural selection based on wild phenotypic and fitness data. These  
558 challenges are split in three categories: 1. Questions that need addressing at the start of any  
559 project measuring coefficients of natural selection, 2. Outstanding biological questions  
560 regarding what aspect of the trait-fitness relationship we want to measure, with specific  
561 questionings in the urban context, 3. Statistical challenges that make biological questions  
562 more difficult to answer. Note that there is subjectivity in whether a challenge is placed in the  
563 second or third category as many challenges in the third section can be considered outstanding  
564 questions of focal interest (e.g. spatio-temporal patterns of selection).

565

566 *In fine*, is it possible to gather comparable estimates of natural selection in the urban context  
567 despite “the tremendous quantitative and statistical sophistication that is being brought to  
568 measuring selection on phenotypes and genomes” (Stinchcombe, Kelley & Conner 2017)?  
569 The long list and associated (non-exhaustive) references in Table 2 are sobering and many of  
570 the issues raised here could be broken-down into a number of sub-considerations. Since long-



571 term monitorings of urban populations are often much shorter than comparable datasets in  
 572 natural settings, power issues will likely be one of the top challenges to face.

573

574 TABLE 2. The challenges of estimating natural selection in wild urban and non-urban  
 575 populations and associated literature.

The challenge	References addressing it
<b>1. Initial fundamental considerations:</b>	
How to estimate total selection over a life cycle?	(Lande 1982; Arnold & Wade 1984; Charlesworth 1993; Shaw & Geyer 2010)
What is the best estimate of fitness?	(Arnold 1983; Franklin & Morrissey 2017)
Do we have sufficient power to detect selection?	(Mitchell-Olds & Shaw 1987; Hersch & Phillips 2004)
Which statistical methodology to implement depending on the trait-fitness relationship we aim to measure?	(Janzen & Stern 1998; Morrissey & Sakrejda 2013; Morrissey 2014a; Morrissey & Goudie 2022)
How to standardize fitness and traits?	(Lande & Arnold 1983; Hereford, Hansen & Houle 2004)
<b>2. How to best estimate:</b>	
- Direct and indirect selection, and causal pathways <i>e.g. What is the shape and force of natural selection acting on a focal trait in cities versus non-urban habitats?</i>	(Lande & Arnold 1983; Arnold & Wade 1984; Scheiner, Mitchell & Callahan 2000; Morrissey 2014b; Henshaw, Morrissey & Jones 2020)
- Linear and non-linear selection <i>e.g. Is there an optimum phenotype in the urban environment?</i>	(Lande & Arnold 1983; Stinchcombe <i>et al.</i> 2008; Henshaw & Zemel 2017)
- Environmental sensitivity of selection <i>e.g. What causes novel selection in the city?</i>	(Wade & Kalisz 1990; MacColl 2011; Chevin, Visser & Tufto 2015)
<b>3. How to best account for:</b>	
- Multicollinearity	(Lande & Arnold 1983; Mitchell-Olds & Shaw 1987; Morrissey 2014a; Chong, Fung & Stinchcombe 2018; Morrissey & Ruxton 2018)
- Non-Gaussian trait distributions	(de Villemereuil <i>et al.</i> 2016; Bonamour <i>et al.</i> 2017; de Villemereuil 2018; Pick <i>et al.</i> 2022)
- Non-Gaussian fitness distribution	(Shaw & Geyer 2010)
- Uncertainty and sampling error (in particular for meta-analyses)	(Morrissey & Hadfield 2012; Stinchcombe, Simonsen & Blows 2014; Morrissey 2016)
- The problem of environmental covariance between trait and fitness	(Rauscher 1992; Kruuk, Merila & Sheldon 2003; Morrissey, Kruuk & Wilson 2010; Morrissey & Henshaw 2022)
- Individual interactions	(Hadfield & Thomson 2017)
- Spatial autocorrelation in fitness	(Marrot, Garant & Charmantier 2015)
- Temporal autocorrelation and fluctuating selection	(Chevin, Visser & Tufto 2015; de Villemereuil <i>et al.</i> 2020)
- The missing traits and the invisible fraction	(Grafen 1988; Hadfield 2008; Mittell & Morrissey 2024)
- Trait variation across lifetime ( <i>i.e.</i> labile traits)	(Dingemans, Araya-Ajoy & Westneat 2021; Araya-Ajoy <i>et al.</i> 2023)

576

577       **4.2. Towards more selection coefficient estimations in genomic studies of urban**  
578       **adaptation**

579

580 Many challenges outlined in Table 2 are related to measuring and analysing fitness data, and  
581 hence will not apply when adopting a genomic perspective on selection. While population  
582 genomic studies performed in urban contexts did not report selection coefficients at loci  
583 underlying fitness variation, many studies did identify putative genomic footprints of  
584 selection. These studies mostly followed a similar strategy, comparing populations in urban  
585 *versus* non-urban environments to 1) identify candidate SNPs under divergent selection  
586 through multiple approaches, and 2) identify biological pathways and functions involved in  
587 adaptation to urban environments (Harris & Munshi-South 2017; Theodorou *et al.* 2018;  
588 Caizergues *et al.* 2022b). To provide more selection coefficients, a simple first step will be to  
589 extend studies that have already identified potential genomic footprints of selection and/or  
590 loci associated to phenotypic variations to include estimates of selection coefficients, for  
591 example, using the SelEstim software (Vitalis *et al.* 2014) and other aforementioned methods  
592 (section 3.1). Second, the statistical methods for calculating selection coefficients usually  
593 require large sample sizes, hence more comprehensive sampling—of large populations,  
594 multiple spatial and/or temporal points, and ideally, with phenotypic and fitness measures—  
595 would allow more precise estimates of genetic selection coefficients.

596

597 While genomic studies usually lack fitness estimation, thus limiting the scope for adaptive  
598 interpretations of signatures of selection, they have the great advantage of providing insight  
599 on historical patterns of selection that have shaped current phenotypic divergence and clines  
600 across urban gradients. In contrast, phenotypic approaches reviewed in section II provide  
601 insight on current patterns of natural selection with often strong variation across years and  
602 study areas for a given trait and taxa. Phenotypic and genomic approaches should therefore be  
603 used as complementary approaches to study urban-related natural selection at micro and  
604 macro-temporal scales.

605

606 **4.3. Final considerations: fitness landscapes, opportunity for selection and mega-**  
607 **analyses**

608

609 As a final look toward the future, we outline three different approaches that provide a  
610 different perspective on natural selection in urban areas.

611

612 First, rather than aiming to model a fitness-trait relationship using constraining parametric  
613 approaches, a more flexible approach could be to model the urban and non-urban fitness  
614 landscapes using nonparametric approaches such as cubic splines (Schluter 1988; Morrissey  
615 & Sakrejda 2013). Alternatively, one can use parametric approaches that relate more  
616 explicitly to theoretical predictions than do classic selection gradients from Lande & Arnold  
617 (1983). In particular, models of moving optimum are popular in theory on adaptation to  
618 changing environments (e.g. Kopp & Matuszewski 2014), and can be inferred empirically  
619 (Chevin, Visser & Tufto 2015). Using such models, one could compare the location, width  
620 and height of the fitness peak between urban and non-urban populations in a comparable way  
621 to studies that have used this approach to investigate temporal fluctuations of selection  
622 (Chevin, Visser & Tufto 2015; de Villemereuil *et al.* 2020). Note that this fitness landscape  
623 approach also bears methodological limitations, e.g. it requires strong assumptions but also  
624 large sample sizes, to test how both the height and width of the fitness optimum may change,  
625 and as such it may not facilitate a quantitative comparison across urbanisation gradients. It  
626 could however provide a powerful tool to partition selection episodes and to identify  
627 environmental drivers of urban-specific selection (Gamelon *et al.* 2018), and relate to the  
628 abundant literature on local adaptation involving quantitative traits (e.g. Kawecki & Ebert  
629 2004; Yeaman 2015; Yeaman 2022). Understanding how natural selection on a given  
630 character changes across different episodes of selection (e.g. viability selection and fertility  
631 selection, Walsh & Lynch 2018) and different landscapes will provide crucial insight to  
632 understand how the trait may evolve in our ever changing city landscapes.

633

634 Second, while this review has covered studies that compare the relative strength of selection  
635 for a particular trait between urban and non-urban areas, we can also compare overall  
636 selection intensity between habitats. A comparison of overall selection between the two  
637 habitats could be better obtained using measures of the opportunity for selection  $I$ , or the

638 variance in relative fitness (Crow 1958; Arnold & Wade 1984). Note that while  $I$  reflects the  
639 upper limit of the intensity of natural selection, recent findings show that it can be highly  
640 influenced by demographic stochasticity (Reed, Visser & Waples 2023), and it is likely that  
641 the continuously changing urban landscape where temporary construction sites are numerous,  
642 entails higher demographic stochasticity. Ultimately, we should be able to link  $I$  with  
643 population demography, and determine the overall link with selection as we classically  
644 measure it (*i.e.* on specific traits and at specific times in the life cycle), and total fitness. This  
645 is already an achievable goal in all studies that include fitness measures such as survival  
646 and/or reproductive success (e.g. number of flowers, fruits and seeds produced per plants in  
647 (Irwin, Warren & Adler 2018).

648

649 Third and finally, a promising solution for future quantitative approaches aiming at comparing  
650 urban and non-urban natural selection is to conduct mega-analyses on individual based data  
651 for phenotypes, genomic data, and fitness measures (Eisenhauer 2021) rather than meta-  
652 analyses on heterogeneous non comparable estimations. Mega-analyses pool raw data from  
653 multiple populations in order to use the same statistical treatment rather than gather estimates  
654 from different studies in meta-analyses. The success of such mega-analytical approaches rely  
655 heavily on data platforms and repositories that allow large-scale sharing of standardized data  
656 (such as SPI-birds, see Culina *et al.* 2021) following FAIR principles (aka Findability,  
657 Accessibility, Interoperability and Reusability, Wilkinson *et al.* 2016), while also promoting  
658 interactions and collaborations between data holders. A mega-analytic approach will not only  
659 address the challenges of estimating natural selection (Table 2) in a standardised way across  
660 all data, but will also allow the measurement of urban gradients in a homogeneous way rather  
661 than rely on heterogeneous measures of urbanisation gathered from studies combined in a  
662 meta-analysis approach (Szulkin *et al.* 2020). We call here for use of mega-analyses in the  
663 near future as we envisage it will be a powerful way to assess how different natural selection  
664 can be in cities in a wide array of taxa and traits.

665

666

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## **Authors' anonymous responses following revision of “How does urbanisation affect natural selection?”**

### REVIEWER REPORTS & AUTHORS RESPONSES

#### **Associate Editor comments:**

Your manuscript has been evaluated by two reviewers with expertise in the area of urban ecology and evolution. Both reviewers are positive about your manuscript and its relevance. The need for a focus on studies that quantify, or attempt to quantify, selection in urban environments is a clear and relevant message. I generally agree with their assessment. I think the topic is very relevant, and your thorough literature review is a valuable contribution that may help move the field forward. The two reviewers have some critical comments that I also agree with. The manuscript is too lengthy and could benefit from streamlining some of the background material and providing more synthesis directed specifically toward urban environments. Reviewer one provides detailed comments in this regard. Given that addressing the comments from reviewers may take some extensive edits to the manuscript I cannot recommend acceptance, but I encourage you to address the comments and suggestions carefully and resubmit your manuscript.

Response: We thank the AE and the reviewers for the useful comments. In the revised version of the manuscript that we hereby submit, we have mainly

1. Reduced section 2 substantially (19% reduction of this section with nearly 700 words removed) and streamlined the background material,
2. Focused more on the urban context and challenges across all sections, this was done in particular in sections 4.1 (which was the least specific to the urban context, and we believe that the full table 2 is still useful as no such summary can be presently found in the literature) and 4.3.

#### **Reviewer comments:**

Reviewer: 1

Comments to the Corresponding author  
I have mixed thoughts on this paper.

On the good side, the idea to undertake a rigorous examination of data supporting claims for urban environments having altered selection was a good one. Despite the number of studies with some relationship to urban evolution, there appear to be surprisingly few that provide estimates (or needed data) to robustly evaluate urban vs. non-urban selection. This result has some value in drawing attention to the lack of data supporting claims about urban selection and adaptation. Putting those results in context and providing insightful guidance on moving forward would be useful.

Response: We thank the reviewer for these appreciative comments on the scope of our review.

Unfortunately, given that the data to rigorously examine differences in selection between urban and non-urban environments, I find the paper to be unnecessarily long (e.g. section 2.1, 2.2). I also would like to see more insight into the strengths and weaknesses of the studies and approaches, rather than a retelling, summarizing of results. Expanding the scope of the search to evaluate the number of studies that have shown adaptive differences between urban and non-urban populations (even if there are no estimates of selection) would be a valuable addition, and worthwhile given the limited number of studies with selection estimates.

Response: We have reduced the manuscript, in particular sections 2.1 and 2.2 as suggested by the reviewer. Sentences and sections removed will be obvious using the track-change version, and also

how we have attempted to remove specifically result summaries and replace them by comments on strengths and weaknesses.

Regarding the comment on expanding the search to all studies showing adaptive differences, we feel this goes a long way outside the aim of our study, and also, it's pretty much what Lambert et al (2021) have done already. We paste here a comment from reviewer 2 which we find explains our choice well: "What I appreciate about this manuscript is circling back to the fundamental process - natural selection - rather than just on adaptation or adaptive plasticity."

Specific comments:

1.1. Line 93: "urban conditions could also lead to relaxed selection. First, this can happen if human habitat alteration makes a costly trait no longer adaptative." But, if the trait is costly but is not adaptive in a specific environment selection will not be "relaxed" in that environment, but rather that trait will bear a fitness cost and selection will act against it.

Response: Thank you for spotting this, indeed we wanted here to highlight cases of reduced fitness advantage but not cases of maladaptation, we have rephrased accordingly.

1.2. Line 163: the terms used to search for relevant studies seem reasonable. However, it is a bit concerning that the authors "were aware of two other relevant studies that did not appear in any of these searches." How is it that these studies were not found in the search? Does them not being found lead the reader to expect there are many other studies that were not found by the searches?

Response: This is a good question from the reviewer that we also investigated during these searches. Unfortunately, there is increasing evidence that search engine algorithms (including Google scholar and Web of Science) can miss relevant articles and may return different literature lists depending on, for example, institution subscriptions, search location, field of study, or publishing country (see further discussions in Pozsgai et al. 2021, Mongeon & Paul-Hus 2016, Gusenbauer 2022, and Tennant 2020). These reasons may explain why our search did not identify these two additional articles even though both unidentified studies including the terms "urban" (multiple times) and either "selection differential" (for Badyaev et al 2008) or "selection gradient" (for Branston et al 2021). We instead identified these two additional studies by reviewing the reference lists of the identified relevant articles in our searches; we have now added this additional information to the text (L163). We are hopeful that we have identified most of the relevant articles in these searches and importantly outline important information related to our searches, including information on which institution access was used to conduct our searches (L160).

References (we are happy to add some of these in the ms if you feel it is useful):

-Pozsgai, G., Lövei, G. L., Vasseur, L., Gurr, G., Batáry, P., Korponai, J., ... & You, M. (2021). Irreproducibility in searches of scientific literature: A comparative analysis. *Ecology and Evolution*, 11(21), 14658-14668.

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1.3. Line 167: Agricultural environments are certainly relevant to selection in human altered landscapes, but their relevance to selection in urban settings? A thorough search of the literature with agricultural and selection would also be daunting, and it doesn't appear this is something the authors are done.

Response: We agree with this comment, this is why we only cite these 8 papers that came out with the



search but in the end were not relevant in our review, and do not interpret their results further. We have rephrased this sentence to clarify.

1.4. Line 175: “we include a non-exhaustive list of studies that illustrate how urbanisation can strengthen or weaken selection” “how it can” or studies that provide examples?

Response: In the effort to reduce section 2 this sentence was removed.

1.5. Line 178: not only a crucial gap of knowledge of the drivers of selection, but a gap in knowledge in the magnitude of urban/non-urban differences in selection.

Response: We agree and have rephrased accordingly.

1.6. Line 184: The difference between selection gradients and selection differentials is likely not one all readers will be familiar with. I think it is also worth pointing out that selection estimates based on phenotypes of individuals versus those based on breeding values / genetic families (the Rausher study that is cited) might be informative.

Response: We agree that a clear explanation on how selection differentials and gradients differ was needed and we have added this in the first paragraph of section 2 (L149-152).

1.7. Line 314: I don't understand why the challenge of identifying drivers of selection “is particularly salient in the context of cities” – it's not like this is easily done in non-urban environments and also not like non-urban environments are not complex.

Response: We have now removed this mention.

1.8. Line 340. The squirrel example is really nice. thanks.

Response: Thank you, we really enjoy it too!

1.9. Line 384: why “contexts” and not “environments”?

Response: We have reworded as suggested.

1.10. Line 385 “despite tremendous effort, most of these studies would be unable to provide standardized selection differentials or gradients...” Were these studies trying to provide those estimates and for some reason unable to? Or was it not their intent? As written, it seems they were trying but somehow not able to, but I doubt that is the case.

Response: Indeed, these studies didn't attempt to provide these estimates, but we wanted to say that even if they did, they couldn't have because of lack of fitness estimates. We have now clarified this by rewording the sentence.

1.11. The authors' use of “genomic selection” seems to include anything that involves sequence data. However, genomic selection has a specific meaning (as stated on the NIH website “Genomic selection is a form of marker-assisted selection in which genetic markers covering the whole genome are used so that all quantitative trait loci (QTL) are in linkage disequilibrium with at least one marker.”). Referring to any estimates of selection that involve sequence data, and not differentiating among the different analyses and what they tell us, as genomic selection is problematic.

I think it is important to point out sequence-based estimates of selection are very different from organismal-based estimates: most sequence-based approaches integrate selection across long time periods (although estimating changes in allele frequencies do not), with the exception of mendelian traits estimates are on genes that are responsible for only a small portion of phenotypic variation for any particular trait (or fitness), and the potential for false positives (particularly with small datasets) is high.

Response: To address this concern, we have changed references of “genomic selection” to “genetic selection” throughout the manuscript, so as to encompass both multiple loci and single alleles and markers. Note that the comparison between the different types of selection estimates was already developed at the end of section 4.2, but we have now added more comments on this at the start of section 3 (see comment 1.12).

1.12. Line 390: Maybe one can get estimates of selection – genome wide or on some targeted genomic regions – more easily than estimating selection through field experiments. But I would contend they are far from equivalent. A discussion of the relative merits (i.e. what each approach tells us and does not) would seem helpful. I really don’t think that estimates based on genomic data are a “substitute” (line 413) for those based on organismal phenotypes.

Response: Thank you for pointing this out, we agree with this comment and have reworded this sentence accordingly as well as added a short discussion on relative merits (L371).

1.13. Figure 1 is nice, and informative. It seems a much more efficient approach for presenting results than the discussion in sections 2.1 and 2.2.

Response: Thank you for this positive comment. We hope that it will indeed balance the reduction in length of these sections by providing a synthetic view of the literature.

1.14. Line 419. If only “one or a few” loci are being examined, then they are going to account for only a small percentage of phenotypic variance (with the exception of Mendelian traits, which represent only a small percentage of traits of ecological interest).

Response: We have modified the sentence to state that this applies only to mono- and oligogenic traits. It is exceedingly challenging to apply this method to polygenic traits but we mention this L384.

1.15. Line 439 “it is possible to directly link fitness to underlying genetic architecture” hmm... has there ever been a robust GWAS of fitness that has been able to explain more than a small percentage of variation?

Response: This sentence has now been removed.

1.16. Lines 510 – 516. The list of tests used in these searches seem a bit dated. More importantly, the tests are expected to provide insight into very different time scales, can detect very different types of selection (e.g. MK and dN:dS only identify coding variants, whereas CLR can also detect selection that affect expression, dN:dS will likely only identify variants that have experience repeated bouts of selection....).

Response: We have updated the explanation and justification for this search 3 in section 3.2.2 “...we sought to improve... and for key terms explicitly targeting well known and long-used methods.. (Search 3).” The “well known” aspect and large net approach targeting different types of selection is important because we did not want to do a search targeting every single method, obscure or not. If this third search is not satisfactory to the Editor we can also remove it altogether, this would not alter our conclusions.

1.17. Probably something that happened during submission (and not the authors fault), but Table S3 in the review pdf is not in a useable form.

Response: We apologize for this issue and will make sure the table reads well during this new submission

1.18. I was expecting to see a supplemental table with a list of all studies that were returned by the search terms and for those that were not included, the reason that they were excluded.

Response: We have now included two new supplementary tables that provide both lists of papers from

the phenotypic and genetic searches: these are the new Tables S1 and S4. Regarding the criteria for retaining the studies in the main manuscript tables, they are all detailed in the Methods section.

1.19. I can't make sense of Table S3 so maybe the information is there, but if not, for the sequence-based estimates information on tests and criteria for concluding selection should be shown.

Response: Only one study in this table provides an estimate for a genetic selection coefficient and the interpretation of this study is detailed in Box 1. We hope that Table S5 (previously Table S3) is readable in our new submission.

Reviewer: 2

Comments to the Corresponding author

This is a compelling and comprehensive review of evidence for the process of natural selection in urban environments. The fields of urban evolutionary biology and evolutionary ecology have exploded in popularity over the past decade. What I appreciate about this manuscript is circling back to the fundamental process - natural selection - rather than just on adaptation or adaptive plasticity. As has been long noted in evolutionary-centered fields, there is a strong compulsion to create stronger narratives about selection and adaptation than the evidence can show us. This compulsion has embedded itself in urban-focused fields now. This review, I believe, will help temper those feelings and help researchers gather the evidence they need to actually advance our understanding of an increasingly urban world and to tell more honest stories.

In general, the conceptual background of this manuscript is solid and the literature review methodology is adequate. From my perspective, the language and writing is clear and the authors. I really have only two critiques to improve this work.

Response: We thank the reviewer for these appreciative comments on the usefulness of our review and its methodology and clarity.

2.1. First, you reference the Lambert et al. (2021) paper in TREE only once, and in a very general way about urban evolutionary ecology. But the Lambert et al synthesis paper is only a few years old and was pretty critical of the field of urban evolutionary biology. Their review paper underscored how few studies actually documented cases of biological adaptation to urban environments. Of course your study is broader in that it looks at evolutionary ecology and selection rather than focused narrowly on adaptation per se. Even so, a key message from the Lambert et al.(2021) piece was that biologists had become overly enthusiastic about the potency of urban selective pressures and adaptive responses to cities without actually showing evidence of it. I strongly encourage you to more explicitly build on that paper further because it helps readers connect related messages in the literature. In particular, the field of urban evolutionary biology and urban evolutionary ecology cannot advance unless they can be critical of themselves. It would be good scholarship to build off this work to understand what was learned from the conclusions of that review and where you are aiming to advance the conversation.

Response: Our manuscript refers to many reviews and opinion papers published in the field of urban evolutionary ecology, as in fact it is a field where opinion papers have flourished in the last decade, if we may say so, in a somewhat disproportionate measure compared to empirical findings. This means that some of the shortcomings of the field that we discuss in this paper, related to limited evidence for urban-specific natural selection, have been mentioned previously in these reviews. This is true for the Lambert et al 2021 paper cited by the reviewer, but it is also true for other articles such as Donihue & Lambert 2015 (where they called for a better exploration on natural selection and its drivers in urban ecosystems) Johnson & Munshi-South 2017 (e.g. their table S1 illustrated their conclusion that “the influence of urbanization on genetic drift and gene flow has been investigated more extensively than mutation or selection”) or Winchell et al 2022 (e.g. they explain how estimating selection gradients raises specific challenges in urban landscapes). All these papers were cited only once in our review,

and because one priority of this revision was to reduce rather than lengthen our manuscript, we did not opt for reciting these more extensively. In an attempt to help the reader connect messages as stated by the reviewer we have however rephrased the paragraph in the introduction which presents past reflections on these issues (L125 and onwards) citing Lambert et al there. See our next response for another addition of a Lambert et al citation.

2.2. The Lambert et al. (2021) study also provided a methodological framework for studying urban adaptation. Your study starts to dig into that conversation some as well and it would be beneficial if you actively built on recommendations from the prior review when contextualizing observational and experimental needs for understanding natural selection more broadly.

Response: At the end of section 2.2.1, we now cite Lambert et al (2021) to build on their methodological advice to measure urban adaptation and how it has its limitation when the aim is to estimate the force of natural selection. We believe this also helps in our objective to streamline background material as suggested by the AE.

2.3. Second, the concluding paragraphs of your manuscript provide some guiding suggestions to the research community. However, your suggestions aren't particularly urban-centric. That may be OK. But I would challenge you to provide richer examples of how to apply methods to urban-specific instances. Perhaps by taking a few case studies of urban study systems that haven't quite demonstrated natural selection yet and suggesting how they might do so. A common message is that urban environments are hard to work in and so the methods used to study evolutionary ecology don't always apply to cities or that we should be more flexible. A big contribution of your could be to show when and how to apply methods for studying natural selection specifically to urban environments.

Response: Thank you for this suggestion, we have aimed at making the concluding section 4.3. more specific to the urban context and challenges, and we have given as advised examples of already existing data that could be used to provide the suggested standardised estimations (L645-648).