



**HAL**  
open science

## Are behaviour and stress-related phenotypes in urban birds adaptive?

Aude Caizergues, Arnaud Grégoire, Rémi Choquet, Samuel Perret, Anne Charmantier

► **To cite this version:**

Aude Caizergues, Arnaud Grégoire, Rémi Choquet, Samuel Perret, Anne Charmantier. Are behaviour and stress-related phenotypes in urban birds adaptive?. *Journal of Animal Ecology*, 2022, 91 (8), pp.1627-1641. 10.1111/1365-2656.13740 . hal-03821524

**HAL Id: hal-03821524**

**<https://cnrs.hal.science/hal-03821524>**

Submitted on 19 Oct 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Are behaviour and stress-related phenotypes in urban**  
2 **birds adaptive?**

3

4

5 Aude E. Caizergues<sup>1\*</sup>, Arnaud Grégoire<sup>1</sup>, Rémi Choquet<sup>1</sup>, Samuel Perret<sup>1</sup> & Anne  
6 Charmantier<sup>1</sup>

7 <sup>1</sup>CEFE, Univ Montpellier, CNRS, EPHE, IRD, Univ Paul Valéry Montpellier 3, Montpellier, France

8

9 **\*Corresponding author:**

10 Aude E. Caizergues: audeemiliecaizergues@gmail.com

11

12 **ABSTRACT**

- 13 1. Urbanization is a worldwide phenomenon converting natural habitats into new artificial ones.  
14 Environmental conditions associated with urbanization represent great challenges for wildlife.  
15 Behaviour and stress tolerance are considered of major importance in the adaptation to novel  
16 urban habitats and numerous studies already reported behavioural and stress response phenotypes  
17 associated with urbanization, often suggesting they represented adaptations, while rarely  
18 demonstrating it.
- 19 2. The main goal of this study was to test the adaptive nature of urban shifts in behavioural and  
20 stress-related traits, and by adaptive we mean phenotypic change favouring traits in the same  
21 direction as selection.
- 22 3. Using seven years of monitoring of urban and forest great tits, we first tested for differences in  
23 exploratory behaviour, aggressiveness and breath rate, between both habitats. Second, we  
24 performed habitat-specific analyses of selection on the three former traits using (1) reproductive  
25 success and (2) survival estimated via capture-mark-recapture models, as fitness estimates, to  
26 determine whether shifts in these behavioural and stress-related traits were aligned with patterns of  
27 ongoing selection.
- 28 4. We found that urban birds displayed higher exploratory behaviour and aggressiveness, and higher  
29 breath rate, compared to forest birds. Selection analyses overall revealed that these shifts were not  
30 adaptive and could even be maladaptive. In particular, higher handling aggression and higher

31 breath rate in urban birds was associated with lower fitness. Higher exploration scores were  
32 correlated with lower survival in both habitats, but higher reproductive success only in forest  
33 males. Overall, differences in patterns of selection between habitats was not consistent with the  
34 phenotypic divergence observed.

35 5. Taken together, these results highlight that phenotypic shifts observed in cities do not necessarily  
36 result from new selection pressures and could be maladaptive. We hypothesize that divergences in  
37 behavioural traits for urban birds could result from the filtering of individuals settling in cities. We  
38 thus encourage urban evolutionary scientists to further explore the adaptive potential of  
39 behavioural traits measured in urban habitats i) by replicating this type of study in multiple cities  
40 and species, ii) by implementing studies focusing on immigrant phenotypes, and iii) by measuring  
41 selection at multiple life stages.

## 42 **KEYWORDS**

43 adaptation ; behaviour ; maladaptation ; natural selection ; urbanization

44

45

## 46 **1. INTRODUCTION**

47 Human induced environmental change is a worldwide phenomenon taking place at an unprecedented  
48 rate. These changes include habitat degradation, climate change, increased presence of invasive  
49 species, pollution, and over-exploitation of resources, and affect biodiversity in a pervasive way  
50 (Pelletier & Coltman, 2018). All species living on our planet have faced environmental changes during  
51 their past evolutionary history, yet the present extent and rate of human-induced alterations represent  
52 unprecedented novel challenges. Although a wide range of traits can be concerned, the first response  
53 of animals towards rapid environmental changes often implies behavioural shifts on a multitude of  
54 facets in response to different stimuli (reviewed in Tuomainen & Candolin 2011). For instance,  
55 behavioural shifts can affect foraging strategies, mating success, or predator avoidance behaviours  
56 (Lowry, Lill, & Wong, 2013), hence are closely linked to individual fitness. Consequently, behaviour  
57 is pervasively considered as a crucial factor determining how animals cope with environmental  
58 changes and new selective pressures (Baldwin, 1896; Mayr, 1963). In addition to behaviour, traits that  
59 are related to stress responses may play an equally important role in wild animal tolerance and  
60 adaptation to human-induced environmental changes. Indeed, anthropogenic environmental change  
61 often results in highly altered new habitats with high levels of perturbation (Sih, Ferrari, & Harris,  
62 2011) and living in such habitats might thus require higher stress tolerance or else induce higher levels  
63 of chronic stress that can have deleterious effects on fitness. For instance, vertebrates can cope with  
64 environmental challenges by way of activation of the HPA axis which regulates the secretion of

65 glucocorticoid hormones (Sapolsky et al., 2000). However, while some acute stress response occurring  
66 immediately after exposure to a stress stimuli (e.g. acute release of glucocorticoid) can promote  
67 survival (Landys et al., 2006; Wingfield, 2006), prolonged or repeated exposure to stressors can  
68 induce chronic stress that greatly impact fitness (French et al., 2007; Lendvai et al., 2007). Hence, a  
69 shift in how organisms handle acute and/or chronic stressors could be of major importance in  
70 facilitating adaptation to novel environmental stressors.

71 Urbanization is one of the most extreme human induced environmental change for biodiversity  
72 (Newbold et al., 2015). Urbanized areas combine multiple perturbations on relatively limited areas of  
73 land, including deforestation, fragmentation, artificialization, pollutions and high level of human  
74 disturbance (Grimm et al., 2008). While some species are unable to persist in these deeply modified  
75 habitats, others manage to tolerate the new conditions or even to thrive on the new resources.  
76 Behavioural plasticity has been identified as a key predictor of the successfulness of a species in urban  
77 habitats, in particular the capacity to innovate when facing new opportunities (Lowry et al., 2013).  
78 Furthermore, at an intra-specific level, some behavioural profiles (also referred as personalities, coping  
79 styles or temperament, Réale et al. 2007) can be better suited to cope with novel environmental  
80 challenges than others. In particular, it has been suggested that bolder, more exploratory and more  
81 aggressive individuals are particularly well suited to the urban habitat (Møller, 2008). In line with  
82 these hypotheses, numerous studies in mammals and birds have found that urban individuals indeed,  
83 displayed on average bolder (Evans, Boudreau, & Hyman, 2010; Prosser, Hudson, & Thompson,  
84 2006; Uchida et al., 2016), less careful (Chapman, Rymer, & Pillay, 2012), more exploratory (Martin  
85 & Réale, 2008), and more aggressive (Evans et al., 2010; Scales, Hyman, & Hughes, 2011) behaviours  
86 than their forest counterparts. Regarding stress-related traits (i.e. traits reflecting a physiological  
87 response to stress), patterns of responses are less consistent across species and traits (Sepp et al. 2018).  
88 Indeed, some studies found higher (e.g. Torné-Noguera et al. 2014), lower (e.g. Abolins- Abols et al.  
89 2016) or equal (e.g. Senar et al. 2017) levels of acute response (using breath rate as a proxy) to an  
90 induced stress in urban individuals, and this is also the case for other measures of stress response  
91 (heterocytes to lymphocytes ratio: Powell et al. , 2013; basal corticosterone level: Fokidis et al., 2009;  
92 Sepp et al., 2018).

93 Despite numerous evidence in vertebrates for divergence in behavioural traits and stress responses  
94 between urban and rural populations, little is known about the evolutionary implications of such  
95 urban-linked shifts (but see Lambert et al., 2021, Table 1 for a list of studies “suggesting” behavioural  
96 adaptations to urbanization). In particular, to our knowledge no study ever investigated if urban linked  
97 shifts in aggressiveness and exploration behaviour or in breath rate, emerged in response to habitat  
98 specific selective pressures, even though they are largely assumed adaptive in the urban habitat (e.g.  
99 Lowry et al. 2013). Behavioural particularities observed in urban habitats could result from adaptive  
100 responses to new environmental conditions, such as accepting new food resources or avoiding human

101 disturbance, but they also could be maladaptive and not confer any advantage to individuals. The  
102 majority of urban environmental conditions are relatively recent compared to environmental  
103 conditions experienced during a species evolutionary history. As a result, phenotypic reaction norms  
104 of individuals might not be suited respond in an optimal way to these environmental conditions that  
105 were never encountered during species evolutionary histories, and shifts might result in maladaptation  
106 (Sih, 2013; Tuomainen & Candolin, 2011). In addition, in such artificial environments cues exploited  
107 could be decoupled from the true quality, reinforcing these maladaptive responses (potentially leading  
108 to ecological/evolutionary traps, Demeyrier et al., 2016). Maladaptive responses might be particularly  
109 common for stress-related traits in urban dwellers. For instance, in waterbirds, fear of humans  
110 perceived as predators can result in temporary or permanent desertion of the nest that can heavily  
111 impact individual fitness (Carney & Sydeman, 1999). In the current context of ongoing massive  
112 erosion of biodiversity, it is now more than ever necessary to understand demographic and  
113 evolutionary dynamics associated with these phenotypic shifts.

114 Our study takes place in a context where differences in behaviour and stress response between urban  
115 and forest populations have been previously reported for the focal species, but where eco-evolutionary  
116 implications of these differences remain mostly speculative. Our main objective is to understand to  
117 which extent urban shifts in behavioural and stress-related traits are adaptive. In this study, we focus  
118 on three personality traits: handling aggression, exploration behaviour and breath rate under constraint.  
119 Handling aggression reflects aggressive behaviour in response to manipulation by humans and distress  
120 behaviour (Senar et al., 2017). Exploratory behaviour measures how individuals explore a novel  
121 environment and is often used as a proxy for risk-taking behaviour (e.g. Nicolaus et al. 2012). Breath  
122 rate is a non-invasive measure of primary stress response whereby rapid breath rate is indicative of  
123 higher stress response (Carere & van Oers, 2004; Krams et al., 2014). These three traits are expected  
124 to be correlated with each other following the pace-of-life syndrome hypothesis postulating that  
125 personality and physiological traits might have coevolved with life history traits (Réale et al., 2010).  
126 Individuals are positioned along a slow-fast pace-of-life gradient with fast individuals (displaying  
127 higher aggressiveness, more exploratory behaviour and higher breath rate) at one end and slow  
128 individuals (displaying lower aggressiveness, lower exploratory behaviour and lower breath rate) at  
129 the other.

130 Using a pair of forest and urban populations of great tits (*Parus major*), we first quantified divergence  
131 between urban and forest birds for the three studied traits and estimated survival in each habitat using  
132 Capture-Mark-Recapture (CMR) models. Since the focal traits can display between-individual  
133 covariance among behaviours – and thereby form so called “behavioural syndromes” (Sih, Bell, &  
134 Johnson, 2004) – we also investigated correlations between each pair of traits. We subsequently  
135 performed selection analyses to determine whether phenotypic divergences were aligned with patterns  
136 of ongoing linear and quadratic selection in each habitat. Since personality traits might both affect

137 reproduction and survival and not necessarily in the same direction (Dingemanse & Réale, 2005;  
138 Smith & Blumstein, 2008), we performed selection analyses using both survival (controlling for  
139 imperfect recapture rates using capture-mark-recapture modelling) and reproductive success as fitness  
140 proxies. Based on previously reported results (see above), we predicted that urban birds would be  
141 more aggressive, more exploratory and have a higher breath rate under constraint compared to forest  
142 birds. Following mainstream theories on urban behavioural adaptation (e.g. Lowry et al. 2013), we  
143 also predicted that high aggressiveness and more exploratory behaviour would be under positive –  
144 directional – selection in the urban habitat, but not higher breath rate.

145

## 146 **2. MATERIAL AND METHODS**

### 147 **2.2 Data collection**

#### 148 **2.2.1 Population monitoring**

149 A pair of forest and urban populations of great tits (*Parus major*) were monitored during the breeding  
150 period in two study sites in France. The forest of La Rouvière (43° 40' N, 3° 40' E) is a Mediterranean  
151 forest dominated by downy oaks (*Quercus pubescens*), and with 10% of holm oaks (*Quercus ilex*),  
152 located ca. 20km North-West of Montpellier city. Since 1991, great tit breeding events in nest-boxes  
153 have been monitored in this forest. Nest-box number has fluctuated between 51 and 92 across years. In  
154 the city of Montpellier (43° 36' N, 3° 52' E) 203 to 223 nest-boxes placed both in urban parks and on  
155 streets, have been monitored since 2012 with similar protocols as in the forest. For detailed maps of  
156 nest-box positions see Fig. S1.

157 During the breeding season, nest-boxes were visited at least weekly, to record brood development  
158 from nest building to fledging, providing records of reproductive success for each pair. Adults were  
159 captured inside nest-boxes when feeding 9 to 15 days old nestlings, measured and ringed with unique  
160 metal rings provided by the French CRBPO. Nestlings were measured and ringed at 15 days old (for  
161 more details on monitoring protocol see Caizergues et al. 2018).

162 All experimental protocols were approved by the local ethics committee for animal experimentation of  
163 Languedoc Roussillon (CEEA-LR, 05/06/2018) and regional institutions (bylaw issued by the  
164 Prefecture n°2012167-003). Captures were performed under personal ringing permits delivered by the  
165 CRBPO (Centre de Recherche par le Bagueage des Populations d'Oiseaux) for the Research Ringing  
166 Program number 369.

#### 167 **2.2.2 Behavioural assays**

168 While breeding monitoring and chick ringing started in 2012 in the city, parental captures started in  
169 2013, and behavioural trials were performed from 2014 onwards. In this study, the last year of data

170 included in the analyses is 2019. Following a parent capture in a nest-box, each breeding bird was  
171 submitted to this sequence of events: it was scored for handling aggression, isolated in a cloth bag for  
172 5 minutes (resting), measured for breath rate, kept in an acclimatization compartment adjacent to the  
173 main open-field cage, scored for exploration rate in the open-field during 4 minutes, extracted from the  
174 open-field cage, ringed (if not already ringed) and measured for morphological traits. Time between  
175 capture and release took on average 20 min, and in any case always less than 30 min.

176 First, right after capture, each bird was tested for handling aggression (HA) by the capturer (see  
177 detailed protocol in Dubuc-Messier et al. 2017 & ESM Table S1). The bird was handled with one  
178 hand by the capturer; while facing outward from the manipulator it was nagged at with a finger of the  
179 other hand (see Fig. S2A) following a standard procedure. After 15s of observation the capturer  
180 attributed a handling aggression score ranging from 0 (unreactive bird) to 3 (aggressive bird striking  
181 the handler each time and spreading its wings and tail) with increments of 0.5 (see detailed scoring in  
182 Supplementary Materials Table S1, Charmantier et al. 2017; note that the link between HA and  
183 aggressive behaviour in nature requires further exploration). Right after the HA test, the bird was put  
184 to rest in a cloth bag.

185 Second, after 5 minutes of resting, the bird was removed from the cloth bag, held still on its back by  
186 the handler (see Fig. S2B) who counted its breath rate. From 2013 to 2016 breath rate was estimated as  
187 the number of chest moves during 30s, while since 2017 the protocol was updated to measure the time  
188 to complete 30 chest moves. Measures from 2013-2016 were thus converted to approximate the time  
189 needed to complete 30 chests move to obtain the same scale measures. Each count was replicated  
190 twice and the handling breath rate was averaged over the two measures. The protocol had a small but  
191 significant effect on the breath rate measure (see Appendix 1) and was thus taken into account in the  
192 analyses by using residuals of the regression between breath rate and protocol, hereafter referred as  
193 Breath Rate Index (BRI). Breath rate is often used as a proxy of acute stress response (Carere & van  
194 Oers, 2004; Krams et al., 2014) whereby a smaller breath rate index (hence higher breath rate) reflects  
195 a stronger stress response.

196 Third, the bird was submitted to a “novel environment” test using an experimental open field cage  
197 built following Stuber and collaborators (2013; see Fig. S3). This experiment is classically used to  
198 estimate exploration behaviour in great and blue tits (Dubuc-Messier et al., 2017; Stuber et al., 2013).  
199 After measuring breath rate, the bird was placed in an acclimatization compartment right next to the  
200 main open-field cage (Fig. S3B, 3) for 2 minutes before being released in the exploration room (Fig.  
201 S3B, 1). The bird’s behaviour was recorded for 4 minutes. Videos were analysed with the software  
202 BORIS (Friard & Gamba, 2016) to count the number of flights and hops during the 4 minutes. The  
203 number of flights and hops r-squared transformed was used as a proxy of exploratory behaviour,  
204 hereafter called exploration score (ES), following previous studies (Dingemanse et al. 2002).

## 205 2.3 Statistical analyses

206 Data analyses were performed in four steps: (1) quantifying repeatabilities, between trait correlations  
207 and phenotypic divergence between forest and urban great tits for the three traits of interest; (2)  
208 estimating habitat-specific survival probability; and finally estimating the strength and direction of  
209 selection on the three studied traits via (3) reproductive success and (4) survival. Analyses of  
210 phenotypic divergence and reproductive selection analyses were performed using R software (R Core  
211 Team 2018). Selection analyses via survival were performed using E-SURGE (Choquet et al. 2009).

### 212 2.3.1 Phenotypic divergence, repeatability and between-individual correlations

213 Differences between urban and forest great tits in the three behavioural traits described above (HA,  
214 BRI, ES) were explored using linear mixed models (R package lme4, Bates et al. 2015). Fixed effects  
215 for the full model included: habitat (forest *versus* urban), year (categorical variable), sex, age (one year  
216 old (=yearling) *versus* 2+ years old (=adult)), rank of capture (*i.e.* how many times the focal bird was  
217 captured until the current capture, ranges from 1 (for first capture) to 6) and air temperature, as well as  
218 habitat by year and habitat by sex interactions. For BRI, body mass and hour of the day were also  
219 included in the full model since they are known to be highly linked with metabolism. Individual ring  
220 number was included as random effect to account for the non-independence of repeated individual  
221 measures, and manipulator identity was also included as random effect for handling aggression and  
222 breath rate models.

223 Model selection was performed using the *dredge()* function from the MuMIn R package (v1.43.17,  
224 Barton 2020) : starting from the complete model all possible models combining fixed effects were  
225 automatically run and ranked based on their quasi Akaike criterion corrected for sample size (AICc).  
226 Model averaging of the best models ( $\Delta AICc < 2$ ) where performed to obtain average estimate for each  
227 effect using the *model.avg()* function.

228 Repeatability of each trait was estimated using the *rpt()* function from the rptR R package  
229 (Stoffel, Nakagawa, & Schielzeth, 2017), with the best linear mixed model selected using the process  
230 described above.

231 To test for an association between traits forming a “syndrome” we estimated between-  
232 individual correlations across the three focal traits (Dingemanse & Wright, 2020) in each habitat. To  
233 measure the strength of a syndrome, it is important to correct for within-individual correlation or other  
234 sources of association between traits when estimating between-trait correlations (Cleasby et al., 2015).  
235 Hence, we estimated between-individual correlations using bivariate linear mixed models  
236 (Dingemanse et al. 2010) computed with MCMCglmm (Hadfield, 2010) and following Ferrari et al.  
237 (2013). Models included two of the traits as variable responses, significant fixed effects that were  
238 present in the best models selected in the between-habitat divergence test described above, as

239 explanatory variables, and individual ID as random effect. These models allowed to estimate the  
240 variance of each trait and the covariance between two traits '1' and '2', and to decompose  
241 (co)variances into individual ( $V_{I1}$ ,  $V_{I2}$  and  $Cov_{I1,2}$ ), and residual ( $V_{R1}$ ,  $V_{R2}$  and  $Cov_{R1,2}$ ) (co)variance  
242 components for the random intercepts. Correlations between two traits were calculated as  
243  $Cov_{X1,2}/\sqrt{(V_{X1} \times V_{X2})}$  (Dingemanse & Dochtermann, 2013), where X represents the component  
244 analysed (between-individual or residual) (for detailed protocol see Appendix 2).

245 Hence, we calculated two types of correlations. First, the between-individual correlation,  $R_{I1,2}$ ,  
246 represents effects responsible for the consistency in the correlation between the two traits across  
247 individuals and over time; strong  $R_{I1,2}$  across the three traits would be indicative of a behavioural  
248 syndrome. Second, the residual correlation  $R_{R1,2}$  represents the within-individual correlation  
249 influenced by the traits' phenotypic plasticity of the trait across an individual's lifetime, as well as by  
250 trade-off between traits (Dingemanse & Dochtermann, 2013). Finally, as it is usually reported in  
251 studies of behavioural syndrome, we calculated the total phenotypic correlation between each pair of  
252 traits to allow comparison with previous studies.

### 253 2.3.2 Analyses of survival

254 Based on capture-recapture data collected during 7 breeding seasons (2013-2019), we estimated  
255 individual survival and recapture probabilities across habitats (urban *versus* forest), ages (yearling vs.  
256 adult) and sexes following Lebreton et al. (1992). Survival analyses were performed using a capture-  
257 mark-recapture (CMR) framework with the E-SURGE software. This framework is based on  
258 individual histories of capture (i.e. series of '1' and '0' indicating whether a focal bird was  
259 (re)captured or not each year) from which is estimated a survival probability ( $\Phi$ ) while taking into  
260 account imperfect detectability estimated *via* a recapture probability ( $p$ ). Prior to fitting the CMR  
261 model, we tested whether the data met the Cormack-Jolly-Seber's (CJS) assumptions (homogeneity of  
262 capture and survival probabilities and independence between individuals), and tested for over-  
263 dispersion with the goodness-of-fit tests using U-CARE (v2.3.2, Choquet et al. 2009). We initially  
264 tested for a time (*i.e.* year) effect on survival ( $\Phi$ ) and recapture ( $p$ ) probabilities, but since it was never  
265 retained in the best models, it was removed early on during analyses in order to limit over-  
266 parametrization.

267 Then, we included effects of habitat, age and sex, using the capture-recapture data set based on  
268 captures of parents between 2013 and 2019. All combinations of models including habitat $\times$ age,  
269 habitat $\times$ sex, habitat, age, sex or i (=constant) effects on the two parameters  $\Phi$  and  $p$  were tested, and  
270 model selection was performed using the quasi Akaike criterion (QAICc).

### 271 2.3.3 Selection analyses

#### 272 2.3.3.1 *Via reproductive success*

273 We estimated selection operating via reproductive success for the three focal traits. Since our study  
274 system offers little access to lifetime reproductive success, we opted to focus on annual reproductive  
275 success via the annual number of fledglings. The male and the female of each pair shared the same  
276 annual reproductive success, hence to avoid replicated measures within a single model, males and  
277 females were analysed separately.

278 Following the classic Lande and Arnold (1983) approach, first we quantified directional  
279 selection acting on each trait by calculating linear selection differentials. To identify the target of  
280 natural selection when studying multiple traits, we also estimated multivariate linear selection  
281 gradients  $\beta_i$  after controlling for indirect selection on the other traits. Linear selection differentials  
282 were estimated for each trait following equation (1), and linear selection gradients were estimated on  
283 all traits together following equation (2):

$$284 \quad \omega = \alpha + \text{habitat} + \beta x + \beta \text{habitat} \times x + \varepsilon \quad (1)$$

$$285 \quad \omega = \alpha + \text{habitat} + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_1' \text{habitat} \times x_1 + \beta_2' \text{habitat} \times x_2 + \beta_3' \text{habitat} \times x_3 + \varepsilon \quad (2)$$

286 In both equations, the strength and direction of selection is given by the slope ( $\beta$  or  $\beta_i$ ) of the  
287 regression between relative fitness ( $\omega$ ) and the standardized (per sex) value of a trait ( $x$  or  $x_i$ ),  $\alpha$   
288 represents the intercept and  $\varepsilon$  the error. Interaction terms ( $\text{habitat} \times x_i$ ) indicate whether selection differs  
289 between habitats.

290 Model selection was performed across all models, starting from the complete model described  
291 in equations 1 and 2 to a null model (=constant), using AICc. Once more, all models within two points  
292 of AICc were considered equivalent and estimates were obtained by a model averaging procedure. All  
293 linear mixed models included individual identity and year as random effects to control for repeated  
294 measurements on the same individuals and variation across years. To test for correlational selection  
295 (i.e. interaction term between traits), the null model of reference contained the additive effect of the  
296 three traits and habitat as fixed effect (see details in Appendix 3).

297 The aim of this study was to explore how directional, hence linear selection, was aligned with  
298 the documented phenotypic divergence between forest and city birds. However, non-linear selection  
299 could also differ between the forest and the urban habitats, and thereby shape differences in trait  
300 variances. Although differences in variance are not a focal objective here, we estimated quadratic  
301 selection differentials and gradients to present a complete picture on comparing selection. Quadratic  
302 selection differentials ( $\gamma$ ) and selection gradients ( $\gamma_i$  for each trait  $x_i$ ) as well as correlational selection  
303 gradients ( $\gamma_{ij}$  for traits  $x_i$  and  $x_j$ ) were estimated following these equations:

$$304 \quad \omega = \alpha + \text{habitat} + \beta x + (\gamma/2) \text{habitat} \times x^2 + \varepsilon \quad (3)$$

$$305 \quad \omega = \alpha + \text{habitat} + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3$$

$$\begin{aligned}
& + (\gamma_1/2)\text{habitat}\times x_1^2 + (\gamma_2/2)\text{habitat}\times x_2^2 + (\gamma_3/2)\text{habitat}\times x_3^2 \\
& + \gamma_{12}x_1x_2 + \gamma_{23}x_2x_3 + \gamma_{13}x_1x_3 + \varepsilon
\end{aligned}
\tag{4}$$

Where, similarly as in equations (1) and (2),  $\alpha$  represents the intercept,  $\beta$  and  $\beta_i$  represent respectively the linear selection differential and the linear selection gradient for each trait  $x_i$ , and  $\varepsilon$  represents the error. While a positive estimate of quadratic selection differential/gradient indicates disruptive selection, a negative estimate is indicative of stabilising selection. More details about methods and results on quadratic selection differentials can be found in Appendix 3.

### 2.3.3.2 Via survival

To estimate selection acting on behavioural traits via survival we tested whether an individual's probability of survival from one year to the next was associated with the value of the trait, based on capture-recapture data combined with behavioural data collected between 2014 and 2019. As previously described, we tested whether the data met CSJ assumptions, tested for over-dispersion with the goodness-of-fit tests using U-CARE and performed survival analyses with E-SURGE. We performed three sets of models, one on each of the traits studied in order to preserve statistical power. In our dataset, most individuals presented more than two measures for each trait. Hence, we used the Best Linear Unbiased Predictor (BLUP) for each individual as phenotypic value (e.g. Bergeron et al. 2013). BLUPs were extracted from linear mixed models on repeated measures of each trait, including age, year, hour of the day, air temperature and rank of capture as fixed effects. In the model for BRI, protocol of measure was also included as fixed effect to correct for the bias induced by the change in protocol since 2017 (see Appendix 1).

In this set of analyses, because the number of possible models highly increased with each added effect, we first selected the appropriate model structure for recapture probability ( $p$ ), starting from the full model  $p_{(\text{hab}\times\text{sex}+\text{trait}+\text{time})}$  and sequentially removing each effect from the full model. The model with the lowest AICc was selected (for a detailed list of the 5 bests models see Table S2). We then ran models with the same procedure for survival probabilities, starting from the full model  $\Phi_{(\text{time}+\text{habitat}\times\text{age}+\text{hab}\times\text{sex}+\text{hab}\times\text{sex}\times\text{trait})}$  with  $p$  following the best structure previously selected. A  $\text{habitat}\times\text{sex}\times\text{trait}$  interaction was interpreted as selection on the trait varying between habitats and sexes. Similarly, a  $\text{habitat}\times\text{trait}$  interaction indicated that selection on the trait varied between habitats. In addition, to test for potential quadratic selection acting on the traits we also added the  $\text{trait}^2$  and  $\text{habitat}\times\text{trait}^2$  effects in the best 10 models. In the end, models were ranked according to the AICc criterion and all models within a  $\Delta\text{AICc} < 2$  were considered equivalent.

337

## 3. RESULTS

### 339 **3.1 Repeatable non-correlated traits**

340 We confirmed that all three traits were repeatable (Table 1). Interestingly, almost all between-  
341 individual phenotypic correlations  $R_{1,2}$  between pairs of traits, measured in each habitat, had  
342 credibility intervals overlapping 0 (Table 2), suggesting that the three focal traits do not covary in a  
343 behavioural syndrome (detailed estimates of (co)variance components for each trait or pair of traits are  
344 available in Tables S4 and S5). Hence selection acting on one of the focal traits is unlikely to induce  
345 indirect selection on the other traits.

### 346 **3.2 Phenotypic divergence between urban and forest habitats**

347 When testing for phenotypic differences between habitats, model selection revealed a habitat effect for  
348 the three studied traits (Table 3). Urban individuals displayed on average higher handling aggression  
349 scores, and this difference was more pronounced in males ( $\text{mean}_{\text{urban-males}}=2.62 \pm 0.23$ ,  $\text{mean}_{\text{forest-}}$   
350  $\text{males}=1.74 \pm 0.11$ ; Fig. 1A) than in females ( $\text{mean}_{\text{urban-females}}=2.09 \pm 0.14$ ,  $\text{mean}_{\text{forest-females}}=1.67 \pm 0.12$ ;  
351 Fig. 1A).

352 Two equivalent models were selected when modelling BRI (Table 3), both including a habitat effect  
353 whereby urban individuals had higher breath rate (*i.e.* a lower index) than forest birds ( $\text{mean}_{\text{urban}}=-$   
354  $0.730 \pm 0.100$ ,  $\text{mean}_{\text{forest}}=-0.32 \pm 0.27$ ; Fig. 1B).

355 Model selection for ES revealed 12 equivalent models (Table 3) all including a habitat effect. Urban  
356 individuals were more active explorers than forest ones (ES  $\text{mean}_{\text{urban}}=5.88 \pm 0.37$ ,  $\text{mean}_{\text{forest}}=3.54 \pm$   
357  $0.72$ ; Fig. 1C). Sex, temperature, capture rank and habitat $\times$ sex effects were present in numerous  
358 models, suggesting they may affect ES. Since so many models were found equally supported, we do  
359 not draw any strong conclusion regarding these effects. Detailed effect sizes obtained using model  
360 averaging are presented in Supplementary Table S6.

### 361 **3.3 Habitat-specific survival**

362 On the overall capture-recapture dataset, a goodness-of-fit test conformed to the Cormack-Jolly-  
363 Seber's assumptions ( $\chi^2 = 30.6059$ ,  $P = 0.246$ ,  $\hat{c} = 1.177$ ; see Table S3 for detailed GOF test results).  
364 Deviances and AICc were corrected for the  $\hat{c}$  value in all models ( $\hat{c} = \chi^2 / \text{df}$ ).

365 In the analysis aiming to estimate survival and recapture for each sex, age and habitat, model selection  
366 revealed 2 models with substantial support ( $\Delta \text{AICc} < 2$ , Table 4). For both models, survival  
367 probability depended on habitat and age in interaction, with forest yearlings showing a substantially  
368 higher survival probability than the three other groups (Table 5).

### 369 **3.4 Reproductive Selection**

370 When estimating reproductive selection on HA, BRI and ES in females using number of fledglings as  
371 a fitness proxy, we found no evidence for linear selection on HA nor on ES (Table 6). BRI was under  
372 positive significant linear selection in females, *i.e.* females with a lower breath rate had a higher  
373 reproductive fitness. However, the difference in selection between urban and forest females for BRI  
374 was not significant, as suggested by the habitat×BRI interaction non included in any of the best  
375 models (Table S7).

376 In males, we found no evidence for directional reproductive selection on HA and BRI (Table 6) but  
377 positive reproductive selection favouring males with higher ES in the forest but not in the city. This  
378 difference in selection between forest and city was significant (habitat×ES interaction included in the  
379 two best models see Table S7).

380 When looking at non-linear selection gradients (see Appendix 3 and Table S8 for detailed results on  
381 selection differentials), quadratic terms were present in most of the best models (Table S9), however,  
382 estimates were significant only for BRI females and males and ES in males, suggesting slight  
383 stabilizing selection acting on female BRI (Table 7), diverging selection acting on male BRI  
384 ( $\gamma/2 = 0.070 \pm 0.031$ , Table 7), whereby fathers with low or high BRI produced more fledglings; as  
385 well as stabilising selection on male ES ( $\gamma/2 = -0.088 \pm 0.060$ , Table 7), suggesting that intermediate  
386 values of exploration scores were favoured. Finally, there was no evidence for correlational selection  
387 acting on the focal traits.

388

### 389 **3.5 Viability selection**

390 Goodness-of-fit tests conformed to the Cormack-Jolly-Seber's assumptions for each trait-specific  
391 dataset (ES data-set:  $\chi^2 = 29.222$ ,  $P = 0.084$ ,  $\hat{c} = 1.461$ ; BR:  $\chi^2 = 28.681$ ,  $P = 0.094$ ,  $\hat{c} = 1.434$ ;  
392 HA data-set:  $\chi^2 = 24.7$ ,  $P = 0.213$ ,  $\hat{c} = 1.235$ ). Deviances and AICc were corrected for the  $\hat{c}$  values  
393 in all sets of models ( $\hat{c} = \chi^2 / df$ ).

394 The best supported models for the recapture probability are presented in Table S2: in each case, the  
395 model with the lowest AICc estimated  $p$  constant. For the three traits, we found two equally  
396 performing models (Table 4,  $\Delta AICc < 2$ ). The habitat×age interaction was present in each set of  
397 selected models, in accordance with results from the global model (section 3.3).

398 For all three traits, the best model included a negative quadratic trait factor suggesting that all three  
399 traits were under stabilizing selection (Fig. 2A-C). When estimating viability selection on HA, the best  
400 model included HA as additive effect as well as habitat×HA<sup>2</sup> interaction; however, the second best and  
401 equivalent model (Table 4,  $\Delta AICc < 2$ ) did not include this interaction while it retained a quadratic HA<sup>2</sup>  
402 effect. Taken together these results suggest that high and low values of HA are counter-selected in

403 both habitats, yet possibly more so in the urban habitat (Fig. 2A). For breath rate,  $BRI^2$  effect was in  
404 interaction with habitat in the lowest AICc model (Table 4) providing again some evidence for  
405 stronger stabilizing selection in the city (Fig. 2B). Regarding exploration score, the linear and  
406 quadratic dimensions of selection were present in the best model but not in interaction with habitat,  
407 revealing that in both habitats birds were experiencing similar stabilizing selection, particularly  
408 counter-selecting low exploration behaviour (Fig. 2C). However, the quadratic term was not present in  
409 the second best selected model and Fig. 2C illustrates the weak stabilising force of selection.

410

#### 411 4. DISCUSSION

412 In this study, we investigated differences in behavioural traits and a stress-related trait, between  
413 great tits breeding in a forest *versus* an urban habitat, and explored whether the observed divergences  
414 were aligned with patterns of reproductive and viability selection ongoing in each habitat. The three  
415 traits studied were all repeatable yet not strongly correlated, suggesting they did not co-evolve in a  
416 behavioural “syndrome”. Overall, we confirmed previous findings that urban individuals were on  
417 average more aggressive, had a higher breath rate when handled by humans, and were faster explorers  
418 than their forest counterparts (Fig. 1 & Charmantier et al., 2017). In addition, urban yearling great tits  
419 had lower annual survival probability compared to forest yearlings (43% *versus* 62%), while adult  
420 birds had similar survival in both habitats (40% *versus* 41%, Table 5). Selection analyses provided  
421 evidence for stabilizing viability selection and both linear and quadratic reproductive selection acting  
422 on the three traits studied. Importantly in the context of divergent phenotypes, there was evidence for a  
423 divergence in selection between the forest and urban habitats in two cases only: a/ stabilising viability  
424 selection favouring intermediate levels of handling aggression and breath rate was stronger in the  
425 urban habitat (Fig. 2A & 2B), and b/ higher exploratory behaviour was associated with higher  
426 reproductive success in forest but not urban males (Table 6). Overall, the patterns of selection found  
427 here are not aligned with a divergence in phenotype.

428 As predicted, we found phenotypic divergence between urban and forest habitats for the three  
429 studied traits. Higher aggressiveness and exploratory behaviour are in line with previous results and  
430 studies in urban vertebrate populations (Charmantier et al., 2017; Evans et al., 2010; Martin & Réale,  
431 2008). Regarding breath rate, previously reported results were more contrasted. Our finding that urban  
432 individuals displayed higher breath rate is similar to some studies (Torné-Noguera et al., 2014) but  
433 contrasts with others (no difference: Senar et al., 2017 ; lower: Abolins- Abols et al., 2016),  
434 suggesting that the shift observed could be due to another factor than urbanization. Shifts in  
435 behavioural and stress-related traits in urban habitats are often regarded as evolutionary adaptations in  
436 response to novel urban environmental conditions (Lambert et al., 2021; Sepp et al., 2018), a  
437 hypothesis in line with findings of urban-rural genetic divergences associated with genes involved in

438 aggressiveness and exploratory behaviours (in particular SERT and DRD4, Van Dongen et al., 2015;  
439 Riyahi et al., 2017; Mueller et al., 2020). However, Lambert et al (2021) reported recently that only  
440 six urban studies convincingly demonstrated an evolutionary adaptation to urbanisation, and none of  
441 these six examples concerned animal behaviour. One of the key elements missing in most studies  
442 reviewed by Lambert and colleagues is the adaptive nature of the observed divergence in phenotype.  
443 Here we provide one of the first studies investigating the link between individual behaviour and fitness  
444 in an urban context to test for the adaptive nature of an urban-specific shift in behaviour (see also  
445 Halfwerk et al., 2019).

446 Correlations of behavioural traits are often observed in animals and referred as “behavioural  
447 syndromes” or even “pace-of-life syndromes” (POLS) if life history traits are involved (Réale et al.,  
448 2010). POLS represent (co)variation of personality and life history strategies across individuals. At  
449 two ends of a POLS continuum, *fast* individuals display low survival, high reproductive outputs,  
450 precocious reproduction, and bold behaviours, while *slow* individuals show high survival, low  
451 reproduction and shy personalities. In this study, urban birds displayed features of a *faster* pace-of-life:  
452 higher exploratory behaviour, higher aggressiveness, higher breath rate and lower survival at yearling  
453 stage. These signatures of a faster urban life contrast with previous findings on reproductive traits  
454 associated with a *slower* pace-of-life in the same population (i.e. smaller clutches, Caizergues et al.  
455 2018), and with a meta-analysis showing slower POLS in urban birds (Sepp et al., 2017, but note this  
456 meta-analysis did not consider behavioural traits). Note also that no correlation was found across the  
457 three studied traits, which contrasts with previous findings (Carere and van Oers 2004) and with the  
458 prediction that these traits co-evolve.

459 Our selection analyses revealed that shifts in avian behavioural and stress-related traits in the  
460 urban habitat were not aligned with patterns of ongoing directional or quadratic selection. Regarding  
461 behaviour, we detected stabilising selection acting on HA via survival and on ES via both survival and  
462 reproduction, counter-selecting extreme aggressive and exploratory behaviours in both habitats. While  
463 viability stabilizing selection acting on HA was stronger in the urban context (yet with a similar  
464 phenotypic optimum), reproductive linear selection acting on ES in urban males was relaxed compared  
465 to strong selection favouring faster forest explorers. Taken together, these results suggest that the  
466 phenotypic divergence between forest and city great tits in both HA and ES does not result from  
467 habitat-specific patterns of ongoing selection, and that the higher values of these traits observed in  
468 urban habitats are not adaptive. However, it is possible that spatio-temporal variation in natural  
469 selection within the city masks the complexity of selection on these characters. First, higher aggression  
470 or exploratory behaviour might be advantageous only in some parts of the city (for instance in highly  
471 urbanized areas where food is scarcer and competition potentially higher) but not in others. If there is  
472 such spatial variation in selection, estimating selection at a fine scale within the city might be  
473 necessary to apprehend the adaptive nature of high aggression and exploratory behaviour in urban

474 birds. Second, recent work by Corsini and collaborators (2020) suggests that selection on great tit  
475 morphology might act mainly during the early stages of life. If strong early-life selection also impacts  
476 behavioural traits, our analysis focused on adult behaviour could potentially miss an important stage of  
477 selection occurring before individuals start to reproduce.

478 Breath rate under constraint is linked to stress physiology and is used as a proxy of acute stress  
479 response, *i.e.* the short-term response to a stressful event, in contrast with chronic stress referring to a  
480 long term response (Dantzer et al., 2014). Higher breath rate shows higher short-term stress response  
481 to a stressor. In opposition to what is expected in case of adaptation to urban habitats, we found that  
482 urban birds displayed higher breath rate in response to handling and thus a higher acute stress response  
483 than forest individuals, while high breath rate was associated with reduced reproductive success in  
484 females and reduced survival in both sexes (Fig. 2B). Note however that we did not measure  
485 glucocorticoid secretion levels under stress, and therefore we cannot conclude with certitude that  
486 higher breath rate was associated with higher physiological stress response. In addition, we detected  
487 stabilizing viability selection acting on BRI that might be stronger in the urban habitat as suggested by  
488 the  $\text{habitat} \times \text{BRI}^2$  interaction present in one of the two best selected models (Table 4, Fig. 2B).  
489 Surprisingly, however, when considering reproductive success, while we detected slight stabilising  
490 selection on females BRI, we on the contrary found disruptive selection in males favouring extreme  
491 values of BRI in both habitats. Taken together these results suggest that higher breath rates in urban  
492 great tits are maladaptive. Note that previous studies found that acute stress responses associated with  
493 urbanization could differ depending on population, species and traits studied (Powell et al., 2013). In  
494 particular, it would be interesting to explore how birds handle chronic stressors present in urban  
495 habitats from a physiological point of view (Iglesias-Carrasco et al., 2020), especially because  
496 inadequate long-term physiological responses might have deleterious repercussions on the birds'  
497 quality of life and ultimately their fitness, and thus might be under stronger selection than acute-stress  
498 response.

499 Interestingly, across the three traits we found one case of relaxed selection (ES in males) but two  
500 instances of stronger stabilizing selection (HA and BRI) urban habitats. The later results contrast with  
501 recent meta-analyses pointing towards relaxed selection following anthropogenic disturbances (Fugère  
502 & Hendry, 2018; Lahti et al., 2009) and increased morphological variation in urban tit populations  
503 (Thompson et al., 2021). Whether urbanisation results generally in stronger or weaker selection is still  
504 highly debated and insufficiently explored empirically. While novel disturbances are typically  
505 expected to lead to stronger selection in the city (Alberti et al., 2017), urban features such as reduced  
506 predation or access to supplementary food (Lahti et al., 2009) could result in relaxed selection  
507 (Branston et al., 2021). It is likely that urban-related changes in selection will depend both on the trait  
508 studied as well as on the proxy of fitness used, especially since a trait can undergo opposite selection  
509 pressures across the life cycle (see e.g. BRI results with both disruptive and stabilizing selection

510 depending on the fitness parameter studied). Hence future research should aim at comprehensive work  
511 that will disentangle the different factors driving stronger or weaker selection pressures in the cities.

512 The result of the non-adaptive nature (*i.e.* not aligned with ongoing selection) of behavioural and  
513 stress response shifts linked with urbanization raises numerous questions regarding the origins of such  
514 shifts and the implications for populations. First, despite the fact that behavioural shifts in urban  
515 habitats do not confer better fitness outputs compared to forest habitats in this case study, they could  
516 be advantageous to colonise urban habitats or tolerate the new environmental conditions. Indeed,  
517 bolder great tits are known to disperse more and on longer distances than shyer ones (Dingemanse et  
518 al., 2003) and could thus be more prone to colonise urban habitats. Second, behavioural shifts can  
519 result from plasticity in response to the environmental conditions experienced by individuals. For  
520 example, some personality traits can emerge from endocrine stress physiology (Baugh et al., 2017)  
521 and experiments demonstrated that stress stimuli inflicted to individuals could induce a reduction of  
522 their neophobic behaviours in house sparrows (Gormally et al., 2018). Hence, some behavioural shifts  
523 can emerge from a habituation process, in contrast with other traits such as aggressive behaviour  
524 which are consistent across lifetime (Cavalli et al., 2018), and phenotypic flexibility could be  
525 responsible for phenotypic shifts between habitats without necessarily providing fitness benefits to  
526 individuals.

527 The plastic versus genetic origin of the behavioural and stress response differences between  
528 urban and rural environments remains largely debated (Minias et al., 2018; Riyahi et al., 2017). While  
529 we have shown here that the three focal traits are repeatable, repeatabilities of 38 to 52% leave an  
530 important margin for plastic responses in these traits. In particular, a plastic response in the focal traits  
531 could be initiated during early life stages. For instance, hormonal levels experienced in mothers and  
532 transmitted to eggs can affect offspring personality (Rokka et al. 2014). In addition, DNA methylation  
533 could also play a role in the behavioural shifts observed in urban habitats (Caizergues et al., 2022;  
534 Riyahi et al. 2015). In any case, determining the contribution of genetics and plasticity in behavioural  
535 and stress responses shifts observed in the urban habitat is an important future challenge, and would  
536 greatly benefit from experimental designs such as cross-fostering or common garden experiments  
537 (Diamond et al. 2017).

538 This study is we believe, the first to formally test for a link between avian behavioural as well  
539 as stress-related traits, and fitness components, in a context of urbanization. Our results suggest that, in  
540 opposition with expectations formulated in the literature, urban great tits display a faster pace-of-life  
541 with more exploratory behaviour and enhanced aggressiveness that may not be adaptive. In addition,  
542 urban individuals display higher breath rates even if this phenotype is counter-selected, revealing  
543 maladaptation. However, further investigations and complementary analyses will be necessary to  
544 conclude on general patterns of selection ongoing in urban compared to natural habitats. In particular,

545 replication in other populations will be a crucial step to understand whether the results found here are  
546 generalizable. In addition, selection analyses as performed here are data hungry while molecular tools  
547 can offer great opportunities to detect molecular adaptation with smaller datasets. We thus encourage  
548 urban evolutionary biologists to combine both quantitative genetic and genomic approaches to fully  
549 understand evolutionary processes ongoing in urban habitats (Perrier et al. 2020).

#### 550 **Acknowledgment**

551 We would like to thank everyone who participated in fieldwork, especially Marcel Lambrechts, the  
552 CEFE PLT platform (including Annick Lucas, Pablo Giovannini, Christophe de Franceschi), Baptiste  
553 Pépin, Arnaud Borne, the city Council of Montpellier and the Zoo du Lunaret. This project was funded  
554 by the European Research Council (grant no. ERC-2013-StG-337365-SHE) and the OSU-OREME.

#### 555 **Conflict of interest**

556 The authors declare no conflict of interest.

#### 557 **Authors' contributions**

558 Aude E. Caizergues collected the data, carried out the statistical analyses and wrote the manuscript.  
559 Arnaud Grégoire and Anne Charmantier conceived and funded the study, collected the data and  
560 contributed to the writing of the manuscript. Samuel Perret collected the data and Rémi Coquet  
561 participated in the survival data analyses. All authors gave approval for publication.

#### 562 **Data availability statement**

563 All data and custom codes used in this study are available on zenodo.org,  
564 <https://doi.org/10.5281/zenodo.6393083> (Caizergues et al., 2022)

#### 565 **REFERENCES**

- 566 Abolins- Abols, M., Hope, S. F., & Ketterson, E. D. (2016). Effect of acute stressor on reproductive  
567 behavior differs between urban and rural birds. *Ecology and Evolution*, 6(18), 6546–6555. doi:  
568 10.1002/ece3.2347
- 569 Alberti, M., Correa, C., Marzluff, J. M., Hendry, A. P., Palkovacs, E. P., Gotanda, K. M., ... Zhou, Y.  
570 (2017). Global urban signatures of phenotypic change in animal and plant populations.  
571 *Proceedings of the National Academy of Sciences of the United States of America*, 114(34),  
572 8951–8956. doi: 10.1073/pnas.1606034114
- 573 Baldwin, J. M. (1896). A new factor in evolution. *The American Naturalist*, 30(355), 536–553.
- 574 Barton, K. (2020). *MuMIn: Multi-Model Inference*. <https://CRAN.R-project.org/package=MuMIn>.  
575 Retrieved from <https://cran.r-project.org/package=MuMIn>

- 576 Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using  
577 **lme4**. *Journal of Statistical Software*, 67(1), 1–48. doi: 10.18637/jss.v067.i01
- 578 Baugh, A. T., Senft, R. A., Firke, M., Lauder, A., Schroeder, J., Meddle, S. L., ... Hau, M. (2017).  
579 Risk-averse personalities have a systemically potentiated neuroendocrine stress axis: A  
580 multilevel experiment in *Parus major*. *Hormones and Behavior*, 93, 99–108. doi:  
581 10.1016/j.yhbeh.2017.05.011
- 582 Bergeron, P., Montiglio, P. O., Réale, D., Humphries, M. M., Gimenez, O., & Garant, D. (2013).  
583 Disruptive viability selection on adult exploratory behaviour in eastern chipmunks. *Journal of*  
584 *Evolutionary Biology*, 26(4), 766–774. doi: 10.1111/jeb.12081
- 585 Branston, C. J., Capilla-Lasheras, P., Pollock, C. J., Griffiths, K., White, S., & Dominoni, D. M.  
586 (2021). Urbanisation weakens selection on the timing of breeding and clutch size in blue tits but  
587 not in great tits. *Behavioral Ecology and Sociobiology*, 75(11), 1–12. doi: 10.1007/S00265-021-  
588 03096-Z/FIGURES/4
- 589 Brommer, J. E., & Kluehn, E. (2012). Exploring the genetics of nestling personality traits in a wild  
590 passerine bird: testing the phenotypic gambit. *Ecology and Evolution*, 2(12), 3032–3044. doi:  
591 10.1002/ece3.412
- 592 Caizergues, Aude E., Grégoire, A., & Charmantier, A. (2018). Urban versus forest ecotypes are not  
593 explained by divergent reproductive selection. *Proceedings of the Royal Society B: Biological*  
594 *Sciences*, 285(1882), 20180261. doi: 10.1098/rspb.2018.0261
- 595 Caizergues, Aude E., Le Luyer, J., Grégoire, A., Szulkin, M., Senar, J., Charmantier, A., & Perrier, C.  
596 (2021). Epigenetics and the city: non- parallel DNA methylation modifications across pairs of  
597 urban- rural Great tit populations. *Evolutionary Applications*, 00, 1–17. doi: 10.1111/eva.13334
- 598 Caizergues, Aude E., Grégoire, A., Choquet, R., Perret, S., & Charmantier, A. (2022). *Are behaviour*  
599 *and stress-related phenotypes in urban birds adaptive? : Data and scripts*. doi:  
600 10.5281/ZENODO.6393083
- 601 Carere, C., & van Oers, K. (2004). Shy and bold great tits (*Parus major*): body temperature and breath  
602 rate in response to handling stress. *Physiology & Behavior*, 82(5), 905–912. doi:  
603 10.1016/J.PHYSBEH.2004.07.009
- 604 Carney, K. M., & Sydeman, W. J. (1999). A review of human disturbance effects on nesting colonial  
605 waterbirds. *Waterbirds*, Vol. 22, pp. 68–79. The Waterbird Society. doi: 10.2307/1521995
- 606 Cavalli, M., Baladrón, A. V., Isacch, J. P., Biondi, L. M., & Bó, M. S. (2018). The role of habituation  
607 in the adjustment to urban life: An experimental approach with burrowing owls. *Behavioural*  
608 *Processes*, 157, 250–255. doi: 10.1016/j.beproc.2018.10.011

- 609 Chapman, T., Rymer, T., & Pillay, N. (2012). Behavioural correlates of urbanisation in the Cape  
610 ground squirrel *Xerus inauris*. *Naturwissenschaften*, 99(11), 893–902.
- 611 Charmantier, A., Demeyrier, V., Lambrechts, M., Perret, S., & Grégoire, A. (2017). Urbanization Is  
612 Associated with Divergence in Pace-of-Life in Great Tits. *Frontiers in Ecology and Evolution*, 5,  
613 53. doi: 10.3389/fevo.2017.00053
- 614 Choquet, R., Lebreton, J.-D., Gimenez, O., Reboulet, A.-M., & Pradel, R. (2009). U-CARE: Utilities  
615 for performing goodness of fit tests and manipulating Capture&#128;&#147;REcapture data.  
616 *Ecography*, 32(6), 1071–1074. doi: 10.1111/j.1600-0587.2009.05968.x
- 617 Choquet, R., Rouan, L., & Pradel, R. (2009). Program E-Surge: A Software Application for Fitting  
618 Multievent Models. In *Modeling Demographic Processes In Marked Populations* (pp. 845–865).  
619 Springer US. doi: 10.1007/978-0-387-78151-8\_39
- 620 Cleasby, I. R., Nakagawa, S., & Schielzeth, H. (2015). Quantifying the predictability of behaviour:  
621 Statistical approaches for the study of between-individual variation in the within-individual  
622 variance. *Methods in Ecology and Evolution*, 6(1), 27–37. doi: 10.1111/2041-210X.12281
- 623 Corsini, M., Schöll, E. M., Di Lecce, I., Chatelain, M., Dubiec, A., & Szulkin, M. (2020). Growing in  
624 the city: Urban evolutionary ecology of avian growth rates. *Evolutionary Applications*. doi:  
625 10.1111/eva.13081
- 626 Dantzer, B., Fletcher, Q. E., Boonstra, R., & Sheriff, M. J. (2014, January 1). Measures of  
627 physiological stress: A transparent or opaque window into the status, management and  
628 conservation of species? *Conservation Physiology*, Vol. 2, p. 2014. Oxford University Press. doi:  
629 10.1093/conphys/cou023
- 630 Demeyrier, V., Lambrechts, M. M., Perret, P., & Grégoire, A. (2016). Experimental demonstration of  
631 an ecological trap for a wild bird in a human-transformed environment. *Animal Behaviour*, 118,  
632 181–190. doi: 10.1016/J.ANBEHAV.2016.06.007
- 633 Diamond, S. E., Chick, L., Perez, A., Strickler, S. A., & Martin, R. A. (2017). Rapid evolution of ant  
634 thermal tolerance across an urban-rural temperature cline. *Biological Journal of the Linnean  
635 Society*, 121(2), 248–257. doi: 10.1093/BIOLINNEAN/BLW047
- 636 Dingemanse, N. J., Both, C., Drent, P. J., van Oers, K., & van Noordwijk, A. J. (2002). Repeatability  
637 and heritability of exploratory behaviour in great tits from the wild. *Animal Behaviour*, 64(6),  
638 929–938. doi: 10.1006/ANBE.2002.2006
- 639 Dingemanse, N. J., Both, C., van Noordwijk, A. J., Rutten, A. L., & Drent, P. J. (2003). Natal  
640 dispersal and personalities in great tits ( *Parus major* ). *Proceedings of the Royal Society of  
641 London. Series B: Biological Sciences*, 270(1516), 741–747. doi: 10.1098/rspb.2002.2300

- 642 Dingemans, N. J., & Dochtermann, N. A. (2013). Quantifying individual variation in behaviour:  
643 mixed-effect modelling approaches. *Journal of Animal Ecology*, 82(1), 39–54. doi:  
644 10.1111/1365-2656.12013
- 645 Dingemans, N. J., Dochtermann, N., & Wright, J. (2010). A method for exploring the structure of  
646 behavioural syndromes to allow formal comparison within and between data sets. *Animal*  
647 *Behaviour*, 79(2), 439–450. doi: 10.1016/j.anbehav.2009.11.024
- 648 Dingemans, N. J., & Réale, D. (2005). Natural selection and animal personality. *Behaviour*, 142(9),  
649 1159–1184. doi: 10.1163/156853905774539445
- 650 Dingemans, N. J., & Wright, J. (2020). Criteria for acceptable studies of animal personality and  
651 behavioural syndromes. *Ethology*, 126(9), 865–869. doi: 10.1111/eth.13082
- 652 Dubuc-Messier, G., Réale, D., Perret, P., & Charmantier, A. (2017). Environmental heterogeneity and  
653 population differences in blue tits personality traits. *Behavioral Ecology*, 28(2), 448–459. doi:  
654 10.1093/beheco/arw148
- 655 Evans, J., Boudreau, K., & Hyman, J. (2010). Behavioural Syndromes in Urban and Rural Populations  
656 of Song Sparrows. *Ethology*, 116(7), 588–595. doi: 10.1111/j.1439-0310.2010.01771.x
- 657 Ferrari, C., Pasquaretta, C., Carere, C., Cavallone, E., von Hardenberg, A., & Réale, D. (2013).  
658 Testing for the presence of coping styles in a wild mammal. *Animal Behaviour*, 85(6), 1385–  
659 1396. doi: 10.1016/j.anbehav.2013.03.030
- 660 Fokidis, H. B., Orchinik, M., & Deviche, P. (2009). Corticosterone and corticosteroid binding globulin  
661 in birds: Relation to urbanization in a desert city. *General and Comparative Endocrinology*,  
662 160(3), 259–270. doi: 10.1016/J.YGCEN.2008.12.005
- 663 French, S. S., McLemore, R., Vernon, B., Johnston, G. I. H., & Moore, M. C. (2007). Corticosterone  
664 modulation of reproductive and immune systems trade-offs in female tree lizards: Long-term  
665 corticosterone manipulations via injectable gelling material. *Journal of Experimental Biology*,  
666 210(16), 2859–2865. doi: 10.1242/jeb.005348
- 667 Friard, O., & Gamba, M. (2016). BORIS : a free, versatile open- source event- logging software for  
668 video/audio coding and live observations. *Methods in Ecology and Evolution*, 7(11), 1325–1330.  
669 doi: 10.1111/2041-210X.12584
- 670 Fugère, V., & Hendry, A. P. (2018). Human influences on the strength of phenotypic selection.  
671 *Proceedings of the National Academy of Sciences of the United States of America*, 115(40),  
672 10070–10075. doi: 10.1073/PNAS.1806013115/-/DCSUPPLEMENTAL
- 673 Gormally, B. M. G., Wright-Lichter, J., Reed, J. M., & Romero, L. M. (2018). Physiological and

674 behavioral responses of house sparrows to repeated stressors. *PeerJ*, 2018(6), e4961. doi:  
675 10.7717/peerj.4961

676 Grimm, N. B., Faeth, S. H., Golubiewski, N. E., Redman, C. L., Wu, J., Bai, X., & Briggs, J. M.  
677 (2008). Global Change and the Ecology of Cities. *Science*, 319(5864), 756–760. doi:  
678 10.1126/science.1150195

679 Hadfield, J. D. (2010). MCMC methods for multi-response generalized linear mixed models: The  
680 MCMCglmm R package. *Journal of Statistical Software*, 33(2), 1–22. doi: 10.18637/jss.v033.i02

681 Halfwerk, W., Blaas, M., Kramer, L., Hijner, N., Trillo, P. A., Bernal, X. E., ... Ellers, J. (2019).  
682 Adaptive changes in sexual signalling in response to urbanization. *Nature Ecology and*  
683 *Evolution*, 3(3), 374–380. doi: 10.1038/s41559-018-0751-8

684 Iglesias-Carrasco, M., Aich, U., Jennions, M. D., & Head, M. L. (2020). Stress in the city: meta-  
685 analysis indicates no overall evidence for stress in urban vertebrates. *Proceedings of the Royal*  
686 *Society B: Biological Sciences*, 287(1936). Royal Society Publishing. doi:  
687 10.1098/rspb.2020.1754

688 Krams, I. A., Vrublevska, J., Sepp, T., Abolins-Abols, M., Rantala, M. J., Mierauskas, P., & Krama, T.  
689 (2014). *Sex-Specific Associations Between Nest Defence, Exploration and Breathing Rate in*  
690 *Breeding Pied Flycatchers*. doi: 10.1111/eth.12222

691 Lahti, D. C., Johnson, N. A., Ajie, B. C., Otto, S. P., Hendry, A. P., Blumstein, D. T., ... Foster, S. A.  
692 (2009). Relaxed selection in the wild. *Trends in Ecology & Evolution*, 24(9), 487–496. doi:  
693 10.1016/J.TREE.2009.03.010

694 Lambert, M. R., Brans, K. I., Des Roches, S., Donihue, C. M., & Diamond, S. E. (2021, December  
695 17). Adaptive Evolution in Cities: Progress and Misconceptions. *Trends in Ecology and*  
696 *Evolution*, Vol. 36, pp. 239–257. Elsevier Current Trends. doi: 10.1016/j.tree.2020.11.002

697 Lande, R., & Arnold, S. J. (1983). The Measurement of Selection on Correlated Characters. *Evolution*,  
698 37(6), 1210–1226. doi: 10.1111/j.1558-5646.1983.tb00236.x

699 Landys, M. M., Ramenofsky, M., & Wingfield, J. C. (2006, September 1). Actions of glucocorticoids  
700 at a seasonal baseline as compared to stress-related levels in the regulation of periodic life  
701 processes. *General and Comparative Endocrinology*, Vol. 148, pp. 132–149. Academic Press  
702 Inc. doi: 10.1016/j.ygcen.2006.02.013

703 Lebreton, J. D., Burnham, K. P., Clobert, J., & Anderson, D. R. (1992). Modeling survival and testing  
704 biological hypotheses using marked animals: a unified approach with case studies. *Ecological*  
705 *Monographs*, 62(1), 67–118. doi: 10.2307/2937171

- 706 Lendvai, Á. Z., Giraudeau, M., & Chastel, O. (2007). Reproduction and modulation of the stress  
707 response: an experimental test in the house sparrow. *Proceedings of the Royal Society B:*  
708 *Biological Sciences*, 274(1608), 391–397. doi: 10.1098/rspb.2006.3735
- 709 Lowry, H., Lill, A., & Wong, B. B. M. (2013). Behavioural responses of wildlife to urban  
710 environments. *Biological Reviews*, 88(3), 537–549. doi: 10.1111/brv.12012
- 711 Martin, J. G. A., & Réale, D. (2008). Animal temperament and human disturbance: Implications for  
712 the response of wildlife to tourism. *Behavioural Processes*, 77(1), 66–72. doi:  
713 10.1016/j.beproc.2007.06.004
- 714 Mayr, E. (1963). *Animal species and evolution* (Harvard Uni).
- 715 Minias, P., Jedlikowski, J., & Włodarczyk, R. (2018). Development of urban behaviour is associated  
716 with time since urbanization in a reed-nesting waterbird. *Urban Ecosystems*, 21(6). doi:  
717 10.1007/s11252-018-0781-7
- 718 Møller, A. P. (2008). Flight distance of urban birds, predation, and selection for urban life. *Behavioral*  
719 *Ecology and Sociobiology*, 63(1), 63–75. doi: 10.1007/s00265-008-0636-y
- 720 Mueller, J. C., Carrete, M., Boerno, S., Kuhl, H., Tella, J. L., & Kempnaers, B. (2020). Genes acting  
721 in synapses and neuron projections are early targets of selection during urban colonization.  
722 *Molecular Ecology*, 29(18), 3403–3412. doi: 10.1111/mec.15451
- 723 Newbold, T., Hudson, L. N., Hill, S. L. L., Contu, S., Lysenko, I., Senior, R. A., ... Day, J. (2015).  
724 Global effects of land use on local terrestrial biodiversity. *Nature Biotechnology*, 520(7545), 45–  
725 50.
- 726 Nicolaus, M., Tinbergen, J. M., Bouwman, K. M., Michler, S. P. M., Ubels, R., Both, C., ...  
727 Dingemans, N. J. (2012). Experimental evidence for adaptive personalities in a wild passerine  
728 bird. *Proceedings of the Royal Society B: Biological Sciences*, 279(1749), 4885–4892. doi:  
729 10.1098/rspb.2012.1936
- 730 Pelletier, F., & Coltman, D. W. (2018, January 15). Will human influences on evolutionary dynamics  
731 in the wild pervade the Anthropocene? *BMC Biology*, Vol. 16, pp. 1–10. BioMed Central Ltd.  
732 doi: 10.1186/s12915-017-0476-1
- 733 Perrier, C., Caizergues, A. E., & Charmantier, A. (2020). Adaptation Genomics in Urban  
734 Environments. In M. Szulkin, J. Munshi-South, & A. Charmantier (Eds.), *Urban Evolutionary*  
735 *Biology* (pp. 72–88). Oxford University Press. doi: 10.1093/oso/9780198836841.003.0005
- 736 Powell, C., Lill, A., & Johnstone, C. P. (2013). Body Condition and Chronic stress in Urban and Rural  
737 Noisy Miners. *The Open Ornithology Journal*, 6(1), 25–31. doi: 10.2174/1874453201306010025

- 738 Prosser, C., Hudson, S., & Thompson, M. B. (2006). Effects of Urbanization on Behavior,  
739 Performance, and Morphology of the Garden Skink, *Lampropholis guichenoti*. *Journal of*  
740 *Herpetology*, *40*(2), 151–159. doi: 10.1670/38-05A.1
- 741 R Core Team. (2018). *R: A language and environment for statistical computing*. Vienna, Austria: R  
742 Foudation for Statistical Computing.
- 743 Réale, D., Garant, D., Humphries, M. M., Bergeron, P., Careau, V., & Montiglio, P. O. (2010,  
744 December 27). Personality and the emergence of the pace-of-life syndrome concept at the  
745 population level. *Philosophical Transactions of the Royal Society B: Biological Sciences*, Vol.  
746 365, pp. 4051–4063. Royal Society. doi: 10.1098/rstb.2010.0208
- 747 Réale, D., Reader, S. M., Sol, D., McDougall, P. T., & Dingemanse, N. J. (2007, May 1). Integrating  
748 animal temperament within ecology and evolution. *Biological Reviews*, Vol. 82, pp. 291–318.  
749 John Wiley & Sons, Ltd. doi: 10.1111/j.1469-185X.2007.00010.x
- 750 Riyahi, S., Björklund, M., Mateos-Gonzalez, F., & Senar, J. C. (2017). Personality and urbanization:  
751 behavioural traits and DRD4 SNP830 polymorphisms in great tits in Barcelona city. *Journal of*  
752 *Ethology*, *35*(1), 101–108. doi: 10.1007/s10164-016-0496-2
- 753 Riyahi, S., Sánchez-Delgado, M., Calafell, F., Monk, D., & Senar, J. C. (2015). Combined epigenetic  
754 and intraspecific variation of the *DRD4* and *SERT* genes influence novelty seeking behavior in  
755 great tit *Parus major*. *Epigenetics*, *10*(6), 516–525. doi: 10.1080/15592294.2015.1046027
- 756 Rokka, K., Pihlaja, M., Siitari, H., & Soulsbury, C. D. (2014). Sex-specific differences in offspring  
757 personalities across the laying order in magpies *Pica pica*. *Behavioural Processes*, *107*, 79–87.  
758 doi: 10.1016/j.beproc.2014.07.019
- 759 Sapolsky, R. M., Romero, L. M., & Munck, A. U. (2000). How Do Glucocorticoids Influence Stress  
760 Responses? Integrating Permissive, Suppressive, Stimulatory, and Preparative Actions\*.  
761 *Endocrine Reviews*, *21*(1), 55–89. doi: 10.1210/edrv.21.1.0389
- 762 Scales, J., Hyman, J., & Hughes, M. (2011). Behavioral Syndromes Break Down in Urban Song  
763 Sparrow Populations. *Ethology*, *117*(10), 887–895. doi: 10.1111/j.1439-0310.2011.01943.x
- 764 Senar, J. C., Garamszegi, L. Z., Tilgar, V., Biard, C., Moreno-Rueda, G., Salmón, P., ... Isaksson, C.  
765 (2017). Urban Great Tits (*Parus major*) Show Higher Distress Calling and Pecking Rates than  
766 Rural Birds across Europe. *Frontiers in Ecology and Evolution*, *5*, 163. doi:  
767 10.3389/fevo.2017.00163
- 768 Sepp, T., McGraw, K. J., Kaasik, A., & Giraudeau, M. (2018). A review of urban impacts on avian  
769 life-history evolution: Does city living lead to slower pace of life? *Global Change Biology*, 1–18.  
770 doi: 10.1111/gcb.13969

- 771 Sih, A. (2013). Understanding variation in behavioural responses to human-induced rapid  
772 environmental change: A conceptual overview. *Animal Behaviour*, 85(5), 1077–1088. doi:  
773 10.1016/j.anbehav.2013.02.017
- 774 Sih, A., Bell, A., & Johnson, J. C. (2004). Behavioral syndromes: an ecological and evolutionary  
775 overview. *Trends in Ecology & Evolution*, 19(7), 372–378. doi: 10.1016/j.tree.2004.04.009
- 776 Sih, A., Ferrari, M. C. O., & Harris, D. J. (2011). Evolution and behavioural responses to human-  
777 induced rapid environmental change. *Evolutionary Applications*, 4(2), 367–387. doi:  
778 10.1111/j.1752-4571.2010.00166.x
- 779 Smith, B. R., & Blumstein, D. T. (2008). Fitness consequences of personality: a meta-analysis.  
780 *Behavioral Ecology*, 19(2), 448–455. doi: 10.1093/beheco/arm144
- 781 Stoffel, M. A., Nakagawa, S., & Schielzeth, H. (2017). rptR: repeatability estimation and variance  
782 decomposition by generalized linear mixed-effects models. *Methods in Ecology and Evolution*, 8,  
783 1639–1644. doi: doi:10.1111/2041-210X.12797
- 784 Stuber, E. F., Araya-Ajoy, Y. G., Mathot, K. J., Mutzel, A., Nicolaus, M., Wijmenga, J. J., ...  
785 Dingemans, N. J. (2013). Slow explorers take less risk: a problem of sampling bias in ecological  
786 studies. *Behavioral Ecology*, 24(5), 1092–1098. doi: 10.1093/beheco/art035
- 787 Thompson, M. J., Capilla-Lasheras, P., Dominoni, D. M., Réale, D., & Charmantier, A. (2021).  
788 Phenotypic variation in urban environments: mechanisms and implications. *Trends in Ecology &*  
789 *Evolution*. doi: 10.1016/J.TREE.2021.09.009
- 790 Torné-Noguera, A., Pagani-Núñez, E., & Senar, J. C. (2014). Great Tit (*Parus major*) breath rate in  
791 response to handling stress: urban and forest birds differ. *Journal of Ornithology*, 155(1), 315–  
792 318. doi: 10.1007/s10336-013-1025-5
- 793 Tuomainen, U., & Candolin, U. (2011). Behavioural responses to human-induced environmental  
794 change. *Biological Reviews*, 86(3), 640–657. doi: 10.1111/j.1469-185X.2010.00164.x
- 795 Uchida, K., Suzuki, K., Shimamoto, T., Yanagawa, H., & Koizumi, I. (2016). Seasonal variation of  
796 flight initiation distance in Eurasian red squirrels in urban versus rural habitat. *Journal of*  
797 *Zoology*, 298(3), 225–231. doi: 10.1111/jzo.12306
- 798 Van Dongen, W. F. D., Robinson, R. W., Weston, M. A., Mulder, R. A., & Guay, P. J. (2015).  
799 Variation at the DRD4 locus is associated with wariness and local site selection in urban black  
800 swans. *BMC Evolutionary Biology*, 15(1), 253. doi: 10.1186/s12862-015-0533-8
- 801 Wingfield, J. C. (2006). Control of physiological and behavioral responses to capricious environments.  
802 *Journal of Experimental Zoology*, 305, 192.

803  
804  
805  
806  
807  
808  
809  
810  
811  
812

### TABLES

813 **Table 1:** Sample sizes and repeatability of handling aggression, breath rate and exploration  
814 score, estimated with rpt() R function using the best model selected (see Table 3), with  
815 associated standard error (SE), 95% confidence intervals (CI) and P-value.

Trait	Number of measures	Number of individuals with replicated measures (at least 2)	Repeatability	SE	CI	P
Handling aggression	851	138	0.383	0.056	0.276-0.495	$7.2 \times 10^{-10}$
Breath rate index	874	146	0.440	0.055	0.34-0.547	$2.06 \times 10^{-14}$
Exploration score	850	139	0.523	0.050	0.428-0.627	$2.98 \times 10^{-13}$

816  
817  
818

819 **Table 2:** Between individual ( $R_{I,2}$ ), residual ( $R_{R,2}$ ) (above the diagonal) and total (below the  
820 diagonal) correlations between behavioural and stress-related traits with their lower and upper  
821 credibility intervals ( $R_{X1,2} = \text{Cov}_{X1,2} / \sqrt{(V_{X1} \times V_{X2})}$ ) in urban and forest environments.

Urban					
	Handling aggression	Breath rate index		Exploration score	
		$R_{II,2}$	$R_{RI,2}$	$R_{II,2}$	
				$R_{RI,2}$	
Handling aggression		0.158 (-0.178 ; 0.491)	-0.274 (-0.390 ; -0.145)	-0.193 (-0.385 ; 0.040)	0.024 (-0.143 ; 0.185)
Breath rate index	-0.403 (-0.463 ; -0.331)			-0.544 (-0.804 ; -0.194)	-0.036 (-0.173 ; 0.092)
Exploration score	-0.095 (-0.215 ; 0.022)		-0.175 (-0.296 ; 0.049)		
Forest					
	Handling aggression	Breath rate index		Exploration score	
		$R_{II,2}$	$R_{RI,2}$	$R_{II,2}$	
				$R_{RI,2}$	
Handling aggression		-0.583 (-0.814 ; 0.005)	-0.171 (-0.375 ; 0.043)	0.155 (-0.307 ; 0.529)	0.030 (-0.250 ; 0.297)
Breath rate index	-0.243 (-0.382 ; -0.110)			0.080 (-0.291 ; 0.418)	-0.055 (-0.292 ; 0.231)
Exploration score	0.054 (-0.106 ; 0.225)		-0.218 (-0.387 ; -0.063)		

822

823

824

825

826

827 **Table 3:** Model selection for handling aggression, breath rate index and exploration scores as  
828 a function of habitat, year, sex, age, capture rank, temperature, habitat×year, habitat×sex and  
829 habitat×age. All models included individual as random effect and HA and BRI also included  
830 manipulator identity as random effect. Models were ranked according to their AICc values,  
831 models in black are equally supported ( $\Delta AICc < 2$ ), detailed effects sizes are presented in  
832 Table S6.

	Models	d.f.	Deviance	AICc	$\Delta$ AICc
	<b>Handling Aggression</b>				
	habitat+year+sex+habitat×sex	12	3599.54	3623.80	0.00
	habitat+year+sex+temperature+habitat×sex	13	3599.46	3625.70	1.96
	habitat+year+sex+capture rank+habitat×sex	13	3599.54	3625.80	2.04
	habitat+sex+habitat×sex	7	3612.68	3626.20	2.37
	habitat+year+sex+temperature+capture rank+habitat×sex	14	3599.46	3627.80	4.00
	<b>Breath Rate</b>				
	habitat+year+temperature+capture rank	12	2797.74	2822.10	0.00
	habitat+year+temperature+capture rank+habitat×an	17	2789.33	2824.10	1.97
	habitat+year+sex+temperature+capture rank	13	2797.73	2824.10	2.05
	habitat+year+age+temperature+capture rank	13	2797.73	2824.20	2.05
	habitat+year+sex+temperature+capture rank +habitat×sex	14	2797.06	2825.60	3.45
	<b>Exploration Score</b>				
	habitat+temperature+capture rank	6	4803.23	4815.30	0.00
	habitat+capture rank	5	4805.64	4815.70	0.38
	habitat+sex+temperature+capture rank	7	4801.96	4816.10	0.76
	habitat+temperature+capture rank	5	4806.03	4816.10	0.77
	habitat+temperature+capture rank+habitat×sex	8	4799.95	4816.10	0.79
	habitat+sex+capture rank	6	4804.33	4816.40	1.10
	habitat+sex+capture rank+habitat×sex	7	4802.34	4816.50	1.14
	habitat+sex+temperature+habitat×sex	7	4802.78	4816.90	1.58
	habitat+sex+temperature	6	4804.82	4816.90	1.59
	habitat+age+temperature+capture rank	7	4802.85	4817.00	1.65
	habitat	4	4802.97	4817.00	1.68
833	habitat+age+capture rank	6	4805.20	4817.30	1.96

834

835

836

837

838

839

840

841

842 **Table 4:** Model selection for survival probability in relation to habitat, age, sex (N=938, full  
843 dataset from 2013 to 2019) and Handling Aggression (N=881) or Breath Rate Index (N=668)  
844 or Exploration Score (N=661) measured between 2013 and 2019 for the overall dataset and  
845 2014 and 2019 for other datasets. Models are ranked according to QAICc values, and only the

846 best models (i.e. lowest QAICc) are presented for each trait. Equally supported models  
 847 ( $\Delta QAICc < 2$ ) are represented in bold. For all models (except in the global analysis), the  
 848 probability of recapture is considered constant (see Table S2).

Models	# Par.	Deviance	QAICc	$\Delta QAICc$
<b>Global dataset</b>				
$\Phi_{\text{hab.age}} \mathbf{p}$ .	<b>5</b>	<b>1689,36</b>	<b>1441,71</b>	<b>0</b>
$\Phi_{\text{hab.age}} \mathbf{P}_{\text{hab}}$	<b>6</b>	<b>1689,16</b>	<b>1443,55</b>	<b>1,85</b>
$\Phi_{\text{hab.age+sex}} \mathbf{p}$ .	6	1689,35	1443,72	2,01
$\Phi_{\text{hab.age}} \mathbf{p}_{\text{sex}}$	6	1689,36	1443,73	2,02
$\Phi_{\text{hab.age}} \mathbf{P}_{\text{habitat+sex}}$	7	1689,15	1445,57	3,86
<b>Handling aggression</b>				
$\Phi_{\text{HA+hab.age+hab.HA}^2} \mathbf{p}$ .	<b>8</b>	<b>1410,61</b>	<b>1426,61</b>	<b>0</b>
$\Phi_{\text{HA+HA}^2} \mathbf{+hab.age} \mathbf{p}$ .	<b>7</b>	<b>1412,80</b>	<b>1426,80</b>	<b>0,16</b>
$\Phi_{\text{hab+HA+HA}^2} \mathbf{p}$ .	5	1419,33	1429,39	2,64
$\Phi_{\text{age+HA+HA}^2} \mathbf{p}$ .	5	1421,68	1431,73	5,00
$\Phi_{\text{age+HA+hab.HA}^2} \mathbf{p}$ .	4	1421,66	1433,73	6,99
<b>Breath rate index</b>				
$\Phi_{\text{hab.age+hab.BRI}^2} \mathbf{p}$ .	<b>7</b>	<b>1251,68</b>	<b>1265,79</b>	<b>0</b>
$\Phi_{\text{hab.age+BRI+BRI}^2} \mathbf{p}$ .	<b>7</b>	<b>1252,52</b>	<b>1266,63</b>	<b>0,84</b>
$\Phi_{\text{hab.age+BRI+hab.BRI}^2} \mathbf{p}$ .	8	1251,66	1267,80	2,01
$\Phi_{\text{hab+BRI+BRI}^2} \mathbf{p}$ .	5	1260,52	1270,58	4,78
$\Phi_{\text{hab.age}} \mathbf{p}$ .	5	1265,65	1275,71	9,92
<b>Exploration score</b>				
$\Phi_{\text{ES+ES}^2} \mathbf{+hab.age} \mathbf{p}$ .	<b>7</b>	<b>1255,10</b>	<b>1269,22</b>	<b>0</b>
$\Phi_{\text{ES+hab.age}} \mathbf{p}$ .	<b>7</b>	<b>1257,55</b>	<b>1269,63</b>	<b>0,41</b>
$\Phi_{\text{hab+age}} \mathbf{p}$ .	5	1261,50	1271,56	2,34
$\Phi_{\text{hab+ES+ES}^2} \mathbf{p}$ .	5	1262,93	1272,99	3,78
$\Phi_{\text{hab+ES}} \mathbf{p}$ .	4	1264,99	1273,03	3,82

849

850

851

852

853

854

855

856

857

858 **Table 5:** Estimates of survival ( $\Phi$ ) and recapture ( $p$ ) probabilities in forest and urban great tits  
 859 (yearlings = one year olds, adults = two years and older). Estimates are obtained from model  
 860 averaging of the equally supported best models presented Table 4.

Parameter	Age class	Forest		Urban	
		est.	95% CI	est.	95% CI
$\phi$	Yearling	0.616	0.488-0.731	0.435	0.355-0.523
	Adult	0.413	0.333-0.497	0.454	0.403-0.505
$p$	All	0.727	0.632-0.786	0.716	0.637-0.786

861

862 **Table 6:** Reproductive linear selection gradients acting on handling aggression (HA), breath  
 863 rate index (BRI) and exploration score (ES) in urban and forest great tits, estimated using  
 864 model averaging for the best equally supported models presented in Table S7.

Sex	Habitat	Sample size	Linear selection gradients		
			HA	BRI	ES
females	forest	103	-0.022 ± 0.095	<b>0.132 ± 0.046</b>	0.077 ± 0.103
	city	276	0.078 ± 0.116		0.011 ± 0.119
males	forest	93	-0.060 ± 0.050	0.021 ± 0.064	<b>0.230 ± 0.100</b>
	city	247		0.011 ± 0.058	-0.034 ± 0.117

865

866

867 **Table 7:** Reproductive quadratic selection gradients and correlational selection acting on  
 868 handling aggression (HA), breath rate index (BRI) and exploration score (ES) in urban and  
 869 forest great tits, estimated using model averaging for the best equally supported models  
 870 presented in Table S9.

Sex	Habitat	Sample size	Quadratic selection gradients ( $g/2$ )			Correlational selection		
			HA <sup>2</sup>	BRI <sup>2</sup>	ES <sup>2</sup>	HA*BRI	HA*ES	BRI*ES
females	forest	103	0.030 ± 0.055	<b>-0.051 ± 0.038</b>	0.015 ± 0.067	-0.098 ± 0.046	0.081 ± 0.045	-0.044 ± 0.046
	city	276		<b>-0.056 ± 0.031</b>	-0.015 ± 0.069			
males	forest	93	0.018 ± 0.064	<b>0.070 ± 0.031</b>	<b>-0.088 ± 0.060</b>	-0.018 ± 0.048	0.033 ± 0.051	-0.006 ± 0.052
	city	247						

871

872

873

874

875

876

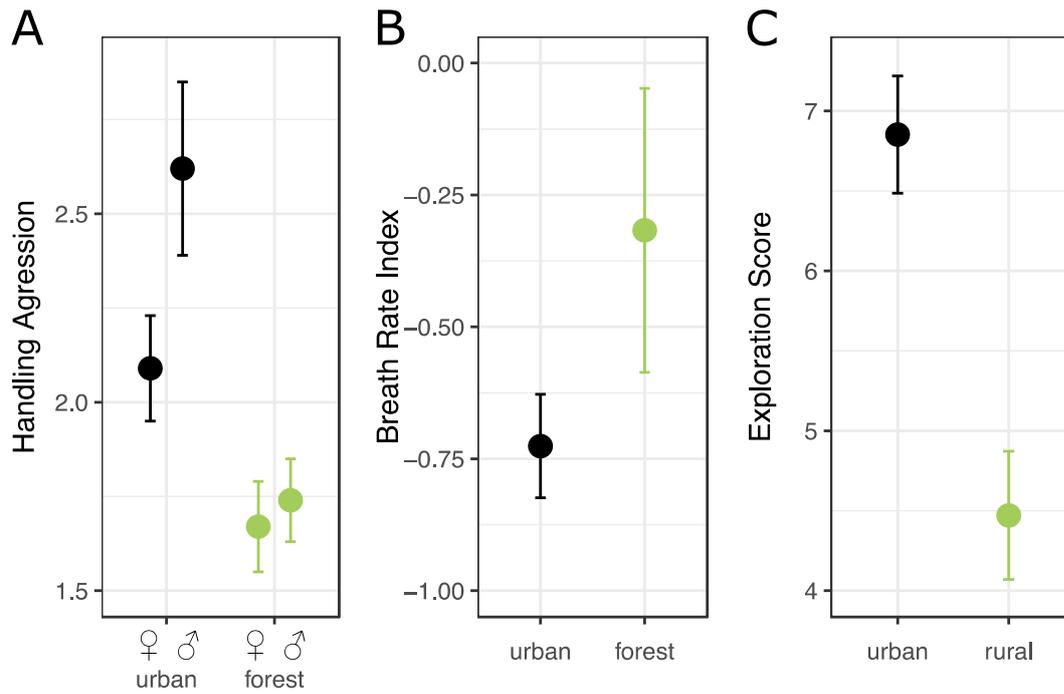
877

878

879

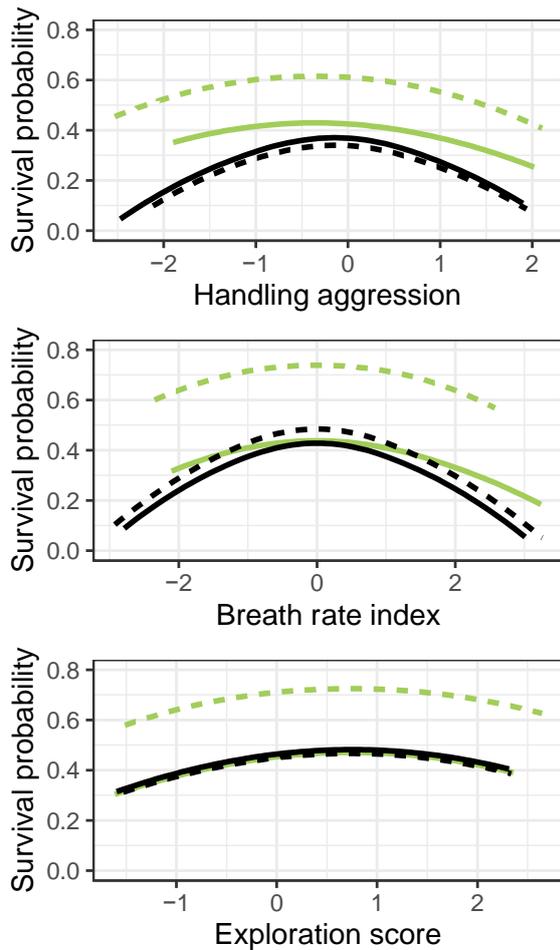
880

## FIGURES



881

882 **Figure 1:** Divergence between urban and forest great tits on 3 behavioural and stress-related  
 883 traits (A) handling aggression (presented for each sex), (B) breath rate index and (C)  
 884 exploration score (mean±SE estimated from models accounting for effects of year, sex, age,  
 885 rank of capture, temperature, habitat×year, habitat×sex, (and body mass and hour for BRI) see  
 886 Table S6). Note that the breath rate measured corresponds to the time for 30 breaths hence  
 887 lower values of breath rate index imply a higher breath rate.



888

889 **Figure 2:** Survival rate of forest (green) and urban (black) great tits in relation to Handling  
 890 Aggression (A), Breath Rate Index (B) and Exploration Score (C). Dashed lines represent  
 891 yearling individuals and solid lines represent adult birds of 2 years or more. Individual values  
 892 of survival are calculated using the following equation  $\phi_{ind} = (1 / (1 + \exp(-\beta_0 + \beta_1 * x_{ind} + \beta_2 * x_{ind}^2)))$   
 893 with  $x_{ind}$  the individual value of a trait and  $\beta$  estimates provided by the CMR models  
 894 containing the trait effect and the lower AICc (**Table 4**,  $\beta_1$ = linear selection differential, and  
 895  $\beta_2$ = quadratic selection differential).

896

897