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

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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

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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 (Φ) 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 (Φ) 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 Φ 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 β_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 (β or β_i) of the
287 regression between relative fitness (ω) and the standardized (per sex) value of a trait (x or x_i), α
288 represents the intercept and ε 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 (γ) and selection gradients (γ_i for each trait x_i) as well as correlational selection
303 gradients (γ_{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), α represents the intercept, β and β_i represent respectively the linear selection differential and the linear selection gradient for each trait x_i , and ε 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 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.

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 ± 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×HA² interaction; however, the second best and
401 equivalent model (Table 4, $\Delta AICc < 2$) did not include this interaction while it retained a quadratic HA²
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).

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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)

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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	7.2×10^{-10}
Breath rate index	874	146	0.440	0.055	0.34-0.547	2.06×10^{-14}
Exploration score	850	139	0.523	0.050	0.428-0.627	2.98×10^{-13}

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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)		

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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	Δ AICc
	Handling Aggression				
	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
	Breath Rate				
	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
	Exploration Score				
	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

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

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858 **Table 5:** Estimates of survival (Φ) 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
ϕ	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

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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	0.132 ± 0.046	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	0.230 ± 0.100
	city	247		0.011 ± 0.058	-0.034 ± 0.117

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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 ²	BRI ²	ES ²	HA*BRI	HA*ES	BRI*ES
females	forest	103	0.030 ± 0.055	-0.051 ± 0.038	0.015 ± 0.067	-0.098 ± 0.046	0.081 ± 0.045	-0.044 ± 0.046
	city	276		-0.056 ± 0.031	-0.015 ± 0.069			
males	forest	93	0.018 ± 0.064	0.070 ± 0.031	-0.088 ± 0.060	-0.018 ± 0.048	0.033 ± 0.051	-0.006 ± 0.052
	city	247						

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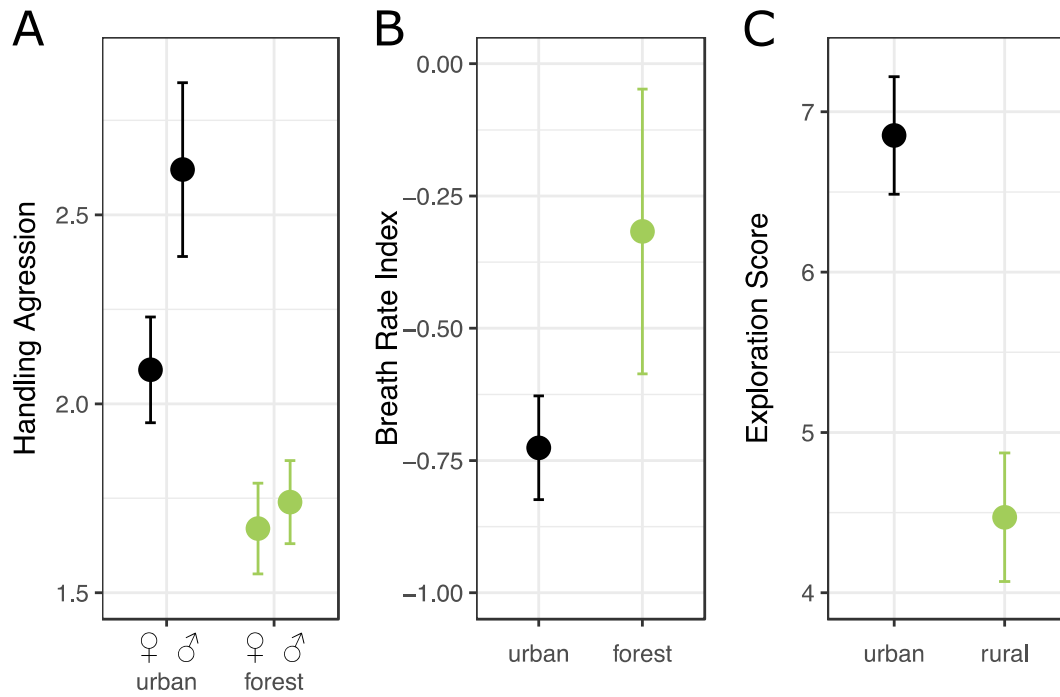
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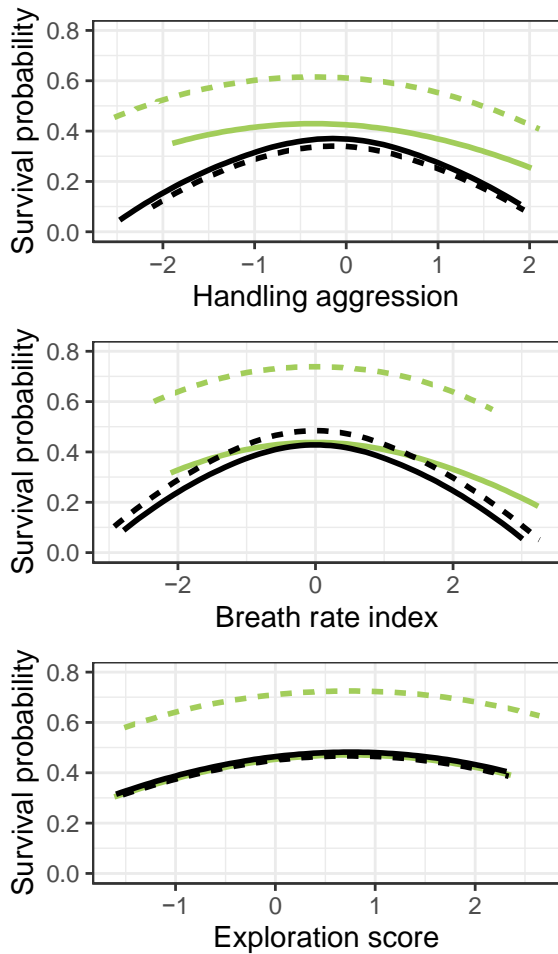
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FIGURES



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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.



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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 β estimates provided by the CMR models
 894 containing the trait effect and the lower AICc (**Table 4**, β_1 = linear selection differential, and
 895 β_2 = quadratic selection differential).

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