

1 An experimental test of changed personality in butterflies from  
2 anthropogenic landscapes

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15 **Abstract**

16 During the last century, the human footprint on natural ecosystems has increased strongly and  
17 human-altered habitats such as urban and agricultural areas have extended globally. Despite  
18 their negative impacts on biodiversity, these habitats offer unique opportunities to study how  
19 native species respond to novel environmental conditions. Here, we studied phenotypic  
20 divergence associated with colonization of human-altered habitats in the Speckled wood  
21 (*Pararge aegeria*). We reared butterflies of woodland, urban and agricultural origins under  
22 common garden conditions and we measured boldness and activity at the adult stage. Both  
23 behavioural traits were repeatable at the individual level (i.e. personality traits), but we found  
24 weak evidence for ecotype-related differences in mean boldness and activity. In line with urban  
25 areas being stressful habitats, we found that boldness and activity traits correlate in urban  
26 butterflies, while we found no such syndrome pattern in woodland and agricultural butterflies.  
27 Our results show that urbanization can alter some aspects of personality in an insect species,  
28 but they do not support the assumption that anthropogenic habitats favour boldness.

29

30 **Significance statement**

31 Human activities such as urbanization and intensive agriculture strongly alter terrestrial  
32 ecosystems and they are among the most significant threats to biodiversity. To tolerate human-  
33 dominated landscapes, many vertebrate species show behavioural shifts towards bold  
34 personalities, but similar responses remain rather overlooked in invertebrate taxa. Here, we  
35 studied the progeny of woodland, agricultural and urban Speckled woods reared under common  
36 garden conditions and we assessed their personality. We found little evidence for differences in  
37 personality traits among landscape types, but the behavioural syndrome linking boldness and

38 activity was detected only in urban butterflies. This shows that urbanization can indeed shape  
39 some aspects of personality in invertebrates.

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41 **Keywords:**

42 Animal personality – Behavioural syndrome – Ecotypic differentiation – Human-induced rapid  
43 environmental change – Urbanization

44

45 **Declarations**

46 *Funding*

47 AK is a research fellow with the Belgian Fund of Scientific Research F.S.R.-FNRS. The  
48 research was supported by PDR grant FNRS PDR T.0188.14 and an ARC research grant of  
49 Fédération Wallonie-Bruxelles and UCLouvain (ARC-grant 17/22-086) to HVD.

50 *Conflict of interest*

51 The authors declare that they have no conflict of interest.

52 *Ethical approval*

53 Our experimental protocol complies with all institutional guidelines at UCLouvain and the  
54 F.R.S.-FNRS on invertebrate research. No permit was necessary to perform the experiments  
55 described in the study.

56 *Data availability*

57 The data set generated and analysed during the current study is available from the corresponding  
58 author on reasonable request.

## 59 **Introduction**

60 Human activities have become a major evolutionary driver for other species. Through  
61 behaviours such as selective hunting, large-scale harvesting, species translocation and  
62 landscape modifications, *Homo sapiens* has proven to be an effective selective pressure  
63 affecting the phenotype of a wide range of wild species (Allendorf & Hard 2009; Sullivan *et al.*  
64 2017). The process of urbanization, i.e. the replacement of (semi)-natural vegetation (including  
65 agricultural land uses) by buildings and other impermeable surfaces and infrastructure, involves  
66 some of the most extreme forms of human-caused landscape changes. It results in increased  
67 fragmentation of natural habitats and in altered biophysical processes (Parris 2016).  
68 Consequently, urban landscapes typically have reduced species richness compared to  
69 surrounding natural habitats (McKinney 2008; Concepción *et al.* 2015; Ramírez-Restrepo &  
70 MacGregor-Fors 2017; Merckx *et al.* 2018a, b; Piano *et al.* 2020). Yet, in addition to human  
71 commensals (e.g. feral pigeons, rats), cities typically harbour native species that persisted  
72 locally despite severe landscape modifications and species that have colonized urban areas more  
73 recently from adjacent (semi-) natural habitats. In Europe, one of the most iconic examples of  
74 such colonization is the European blackbird (*Turdus merula*), which was originally a forest  
75 specialist, but started breeding in urban gardens and parks about 200 years ago (Luniak *et al.*  
76 1990). The urban ecotype then spread over most of the species range in Western and Central  
77 Europe following independent colonization events (Evans *et al.* 2009).

78 Species confronted with novel habitats face numerous challenges in the form of changes to the  
79 physical and/or biotic environment (Hobbs *et al.* 2009). Consequently, successful colonization  
80 of anthropogenic landscapes is likely to require adjustments at multiple levels and urban  
81 animals often differ from their rural conspecifics in terms of behaviour (reviewed in Sol *et al.*  
82 2013), morphology (Winchell *et al.* 2016; Janssens *et al.* 2017; Kern & Langerhans 2018;  
83 Merckx *et al.* 2018a) and life-history or physiological traits (Sprau *et al.* 2017; Tüzün *et al.*

84 2017a; Diamond *et al.* 2018; Sepp *et al.* 2018). Both genetic differentiation and phenotypic  
85 plasticity may contribute to such landscape-related differences, although the relative  
86 contribution of each mechanism is often difficult to assess. Nonetheless, we now know that  
87 some of these differences indeed have at least partly a genetic basis (Alberti *et al.* 2017).

88 Animal personality refers to consistent behavioural differences among individuals (Réale *et al.*  
89 2007). Within a species, individuals may consistently differ in behavioural traits such as  
90 boldness, activity, exploration, aggressiveness or sociability, which are the major personality  
91 axes (Réale *et al.* 2007). Moreover, personality traits tend to be grouped into behavioural  
92 syndromes, whereby the average phenotype of individuals in one context is correlated with the  
93 average phenotype of the same individuals in another context (i.e. between-individual  
94 correlation – Dingemanse *et al.* 2012). For instance, Great tits (*Parus major*) that explore a new  
95 environment more rapidly are also more aggressive towards conspecifics (Verbeek *et al.* 1996).

96 Personality traits and behavioural syndromes are also relevant in the context of human-induced  
97 rapid environmental changes, as they have wide-reaching implications for ecological and  
98 evolutionary processes (Sih *et al.* 2004; Sol *et al.* 2013). Several studies also reported shifts in  
99 personality traits linked to urbanization. For instance, a study across multiple European cities  
100 showed that wild urban Great tits displayed more distress calling and higher pecking rates (i.e.  
101 an index of handling aggression) than their rural conspecifics (Senar *et al.* 2017). Overall, urban  
102 vertebrates show personality traits associated with a more proactive life-style (Koolhaas *et al.*  
103 1999): urban animals tend to be more explorative, bolder and more active than their rural  
104 conspecifics. Such a shift towards a more proactive style is supposed to be adaptive as it would  
105 reduce fear reactions towards novel objects and it would facilitate the acquisition of new food  
106 resources (e.g. Sol *et al.* 2013; Senar *et al.* 2017). Also, urbanization was found to uncouple  
107 personality traits, with behavioural syndrome breakdowns observed in some bird species (e.g.  
108 Evans *et al.* 2010; Bókony *et al.* 2012).

109 Comparatively few studies investigated whether similar shifts also occur in invertebrate taxa  
110 (Schuett *et al.* 2018). In this regard, the Speckled wood (*Pararge aegeria* L.) is an interesting  
111 study system for exploring changes in personality traits linked to anthropogenic activities. In  
112 NW-Europe, the species inhabits large forest patches, but also small woodlots and hedgerows  
113 in agricultural areas (e.g. Merckx & Van Dyck 2005, 2007) and forested gardens and parks in  
114 cities (e.g. Bergerot *et al.* 2012). Together, the three landscape types represent a gradient from  
115 relatively natural landscapes (i.e. woodlands) to moderately and strongly human-altered  
116 landscapes (i.e. agricultural and urban areas, respectively). Moreover, several studies confirmed  
117 that the Speckled wood exhibits consistent behavioural differences (Ducatez *et al.* 2014; Kaiser  
118 *et al.* 2018, 2019a, b). In a previous study, we used a field reciprocal transplant approach  
119 including all three ecotypes of the Speckled wood and we detected higher boldness levels in  
120 agricultural males (Kaiser *et al.* 2019a). Here, we tested for evolved personality differences (i.e.  
121 boldness and activity) between the three ecotypes using butterflies reared under common garden  
122 conditions using families collected from 10 regions in central and northern Belgium. This  
123 experimental design aims to provide one of the most comprehensive studies so far of human-  
124 induced shifts in personality traits and behavioural syndromes in an invertebrate species. We  
125 expect butterflies from anthropogenic (i.e. agricultural and urban) landscapes to be bolder and  
126 more active, like what is observed in vertebrate taxa. Additionally, there is evidence that  
127 adverse conditions, including high predation pressure (Bell 2005; Dingemanse *et al.* 2007;  
128 Urszán *et al.* 2015) or high pesticide levels (Tüzün *et al.* 2017a, but see Royauté *et al.* 2015),  
129 can impact the structure of behavioural syndromes. Because warmer and dryer climatic  
130 conditions in agricultural and urban landscapes (e.g. Serruys & Van Dyck 2014; Kaiser *et al.*  
131 2016; Merckx *et al.* 2018b) are expected to provide stressful conditions for a drought-sensitive  
132 species such as the Speckled wood (e.g. Talloen *et al.* 2004; Oliver *et al.* 2015), we therefore  
133 expect tightened behavioural syndromes in butterflies from anthropogenic landscapes.

## 134 **Methods**

### 135 *Study species and sampling sites*

136 The Speckled wood (*P. aegeria* L.) is a common multivoltine butterfly species that occurs over  
137 most of Europe (Settele *et al.* 2008). Larvae feed on the leaves of various grass species (Shreeve,  
138 1986). In June and July 2015, we collected gravid females from ten regions in central Belgium.  
139 In each region, we selected one woodland, one urban and one agricultural site (see Figure S1).  
140 Woodland sites consisted of continuous forests typically larger than 100 ha; agricultural sites  
141 of systems of small woodlots and hedgerows surrounded by arable fields and pastures; urban  
142 sites of wooded parks and woodlots surrounded by buildings and roads (Kaiser *et al.* 2019a).  
143 We captured one or two females at each site, which in total resulted in 14 woodland, 12 urban  
144 and 15 agricultural families (see Table S1 for capture details). Collected females were  
145 transported to the laboratory for oviposition in individual cages on the grass *Poa pratensis*,  
146 while they could feed *ad libitum* on honey-soaked (10% solution) cotton pads.

### 147 *Butterfly rearing*

148 The offspring (i.e. F1-generation) of the wild-caught females were reared on potted *P. pratensis*  
149 plants (grown on a standardized soil mixture under identical light and temperature conditions)  
150 in a climate room (photoperiod: L:D 16h:8h; day temperature: 25°C; night temperature: 16°C).  
151 Each host plant contained four full-sib caterpillars and was enclosed in nylon netting. Pots were  
152 checked on a regular basis for pupating larvae. Pupae were removed from their plant and placed  
153 individually in labelled plastic cups. We checked all cups twice a day for emergence. We used  
154 a unique alphanumeric code to identify all individuals. The code was non-informative regarding  
155 to the ecotype and it ensured that the observer was blind to the origin of the butterfly during the  
156 behavioural tests.

### 157 *Behavioural tests*

158 We subjected adult offspring to two behavioural assessments: (i) a boldness test, and (ii) an  
159 activity test conducted in a novel environment. We tested 50 woodland (20 males; 30 females),  
160 53 urban (22 males; 31 females) and 47 agricultural (20 males; 27 females) butterflies. The  
161 number of tested individuals per family is presented in Table S2. Assessments started on the  
162 day following emergence, i.e. on day 1, and we retested some individuals on day 2, 3 and 4 (see  
163 below). Butterflies had no access to food, but they all had access to a water-soaked cotton pad  
164 at the end of each day. A single observer (AK) conducted all behavioural observations. The  
165 exact procedure for assessing boldness and activity in the study system has been described *in*  
166 *extenso* elsewhere (Kaiser *et al.* 2019a, b), so we only briefly describe the methodology  
167 hereafter.

#### 168 Boldness

169 We placed the butterfly (with closed wings) in the centre of a semi-transparent glassine  
170 envelope and we counted the number of struggles for one minute. Struggles are defined as series  
171 of leg, head and/or wing movements, interrupted from other such series by pauses of inactivity.  
172 Pauses typically lasted at least a couple of seconds, which allowed a clear distinction between  
173 successive struggle bouts. Note that the focus was on the number of bouts and we did not  
174 consider their intensity (e.g. duration or number of leg movements within a bout).

175 We maintained constant light conditions and a room temperature of 25°C during the test. All  
176 butterflies but one were submitted four times to this test, with one day elapsing between two  
177 successive trials. Butterflies were then weighed using a microbalance (Ohaus Explorer;  
178 accuracy:  $\pm 0.1$  mg). After weighing, butterflies returned to their individual plastic cup until the  
179 activity test.

#### 180 Activity



181 The activity test took place in an empty plastic greenhouse tunnel (length × width × height: 12  
182 x 4 x 2 m; installed in a larger glass greenhouse) whose floor was taped to delineate two rows  
183 of eight rectangles (each 1.5 x 2 m). Each butterfly was released individually at one extremity  
184 of the tunnel and it could move freely for four minutes while the observer recorded the number  
185 of transitions between rectangles (used as a proxy for activity). Here, we did not consider  
186 vertical movements as these generally occurred in a gradual way during transitioning between  
187 rectangles. Ambient temperature was on average  $30.0 \pm 5.8^{\circ}\text{C}$  (mean  $\pm$  SD). Hence, this  
188 provided optimal thermal conditions for flight (Shreeve 1984; Van Dyck & Matthysen 1998).  
189 Due to time constraints, individuals vary in the number of times they were tested for activity.  
190 72 butterflies were tested four times, 33 were tested three times, 8 were tested twice, 8 were  
191 tested once and 29 butterflies could not be tested at all. The number of tested individuals per  
192 ecotype for the activity test is presented in Table S3. Again, one day elapsed between two  
193 successive trials.

#### 194 *Statistical analysis*

195 All statistical analyses were performed with R 3.5.1 (R Core Team 2020).

#### 196 Effects of fixed variables on mean behaviour

197 We used linear mixed models (package *lme4*) to test for differences in behaviour among  
198 ecotypes and sexes, and to unravel dynamics of these traits relative to the testing sequence.  
199 Prior to the analyses, we applied a square-root transformation to boldness and activity to  
200 achieve normality of the residuals. Boldness and activity were the response variables, while  
201 ecotype (i.e. woodland, urban or agricultural), sex (i.e. male or female), sequence (as a  
202 categorical variable) were included as explanatory variables. We initially included the ecotype  
203 × sex and ecotype × sequence interactions, but they were never significant ( $P \geq 0.1$ ) and we  
204 thus removed them from the final models. Region of origin, family ID and individual ID were

205 included as random factors (i.e. random intercepts) in all models. Body mass was added as a  
206 covariate for both behavioural traits. For activity, we added temperature in the greenhouse  
207 tunnel (in °C) and solar irradiance (in W/m<sup>2</sup>) during the test as additional covariates. Continuous  
208 variables (i.e. covariates) were scaled prior to the analyses.

#### 209 Repeatability of behavioural traits

210 We calculated individual repeatability to validate that consistent individual differences (i.e.  
211 personality) in boldness and activity occurred in our study system. Repeatability is the fraction  
212 of the total phenotypic variance that can be attributed to between-individual differences  
213 (Nakagawa & Schielzeth 2010). From the mixed models presented above, we simulated the  
214 posterior distribution of the random terms based on 2000 simulations using the *sim* function  
215 from the *arm* package. We calculated the adjusted repeatability of boldness and activity based  
216 on Nakagawa & Schielzeth (2010). Repeatability was considered significant when 95% credible  
217 intervals did not overlap zero.

#### 218 Correlations among behavioural traits

219 To test for correlations among behavioural traits, we fitted ecotype-specific bivariate mixed  
220 models with the *MCMCglmm* package (Hadfield 2010). Models contained boldness and activity  
221 (both square-root transformed) as response variables, sex, sequence and body mass as fixed  
222 effects, and region of origin, family ID and individual ID as random effects. We used a Gaussian  
223 distribution and a non-informative inverse-Wishart prior. We used 300,000 iterations, from  
224 which we discarded the first 30,000 (burn-in), while using a thinning interval of 100. This  
225 resulted in low autocorrelation. We performed three runs to confirm robustness of the model  
226 outputs. We report within- and between-individual correlations as they both contribute to  
227 phenotypic correlations. The former represents the correlation between an individual's change  
228 in one trait between  $t$  and  $t+1$  and the change in another trait over the same period (Dingemanse

229 & Dochtermann 2013). The latter represents the correlation between individual mean values of  
230 two traits and it is thus a measure of behavioural syndromes *sensu stricto* (Dingemanse *et al.*  
231 2012). Within- and between-individual correlations were estimated following equations in  
232 Dingemanse and Dochtermann (2013). Correlations whose 95% credible intervals do not  
233 overlap with zero were considered significant.

234

## 235 **Results**

### 236 *Boldness and activity*

237 Boldness showed a moderate repeatability (Table 1) and this behavioural trait was influenced  
238 by testing sequence ( $F_{3,467.6} = 12.71$ ;  $P < 0.0001$ ): boldness increased from the first to the second  
239 day and then remained stable until the last test on day 4 (Figure 1). The overall effect of ecotype  
240 was not statistically significant ( $F_{2,25.5} = 3.16$ ;  $P = 0.059$ ), but post-hoc comparisons revealed  
241 that agricultural butterflies had lower boldness scores on average than urban butterflies ( $P =$   
242  $0.042$  after Bonferroni adjustment for multiple comparisons) (Figure 1). Other post-hoc  
243 comparisons were non-significant (Post-hoc test:  $P > 0.20$  after Bonferroni adjustment). We did  
244 not detect any effect of sex ( $F_{1,362.2} = 1.33$ ;  $P = 0.249$ ), nor of body mass ( $F_{1,508.9} = 0.58$ ;  $P =$   
245  $0.448$ ) on this trait.

246 Activity was also repeatable, although the repeatability was lower than for boldness (Table 1).  
247 Contrary to boldness, none of the fixed effects considered had a significant effect on activity  
248 (all  $P$ -values  $\geq 0.09$ ).

### 249 *Correlations among traits*

250 A significant among-individual correlation between boldness and activity was present in the  
251 urban ecotype only (Table 2). For all ecotypes, within-individual correlation between boldness  
252 and activity was low and non-significant.

253

## 254 **Discussion**

255 As the human pressure on natural systems increases, many species are confronted with novel  
256 environmental conditions. Using Speckled woods of woodland, agricultural and urban  
257 population origins reared under common garden conditions, we show weak differentiation in  
258 mean boldness (but not in mean activity) among populations from the three landscape types.  
259 Yet, the behavioural syndrome (i.e. among-individual correlation) linking these two traits was  
260 only detected in urban-origin butterflies. This provides an example of a phenotypic change  
261 associated with human-altered habitats whose footprint is forecasted to increase globally over  
262 the next decades.

263 Evidence for personality differences among rural and urban populations is accumulating in  
264 animals (e.g. Miranda *et al.* 2013; Charmantier *et al.* 2017; Lapiedra *et al.* 2017; Senar *et al.*  
265 2017; Schuett *et al.* 2018; Baxter-Gilbert *et al.* 2019). However, most studies on urban-rural  
266 behavioural differences focused on wild individuals. It is therefore unclear whether these  
267 differences reflect a genetic basis or arise due to phenotypic plasticity (but see Tüzün *et al.*  
268 2017b). Anthropogenic landscapes typically have altered plant and animal communities and  
269 often show increased disturbance due to human activities (e.g. recreational activities or  
270 agricultural management practices). Just as observed in vertebrate species, traits associated with  
271 a proactive style may facilitate the exploitation of new types of resources (e.g. new nectar  
272 sources or prey items) and increase the resilience to disturbance in invertebrate taxa too. We  
273 may thus expect that high boldness and activity levels would be adaptive in urban (and

274 agricultural) environments and that this would translate into evolved (i.e. genetically based)  
275 differences, even though responses to urbanization are likely to depend ultimately on species-  
276 specific attributes (e.g. sensory ecology, ecological niche). Contrary to our expectations, we  
277 found little evidence for ecotype-related differences in mean boldness and not at all for activity  
278 in our Speckled wood samples reared under a common garden setting. For example, woodland  
279 and urban butterflies show no difference in mean levels of personality traits even though they  
280 originate from the most contrasting landscape types in our system. This result echoes our  
281 previous findings (Kaiser *et al.* 2018). While it remains to be tested whether wild Speckled  
282 wood individuals show differences in mean levels of personality traits among ecotypes, this  
283 result implies that such differences would be mainly caused by behavioural plasticity. We may  
284 even speculate on the developmental stage at which behavioural differences among urban and  
285 woodland butterflies would arise. Indeed, a reciprocal experiment conducted under field  
286 conditions showed that conditions (i.e. different landscape types) experienced during the larval  
287 and early pupal stages had no effect on mean levels of personality traits in the Speckled wood  
288 (Kaiser *et al.* 2019a). Thus, conditions during the late pupal or imago stage (e.g. predator  
289 abundance, conspecific density, adult food resources) would likely cause ecotype-related  
290 differences in mean personality.

291 Additionally, there was a trend towards lower boldness scores in agricultural butterflies  
292 compared to urban conspecifics. This is unexpected because we found in a previous experiment  
293 that agricultural males were bolder than woodland and urban conspecifics, independently from  
294 their landscape of development (Kaiser *et al.* 2019a). The two experiments notably differ by  
295 their design. As mentioned above, Kaiser *et al.* (2019a) used a reciprocal transplant design  
296 conducted under field conditions, while here we used a common garden approach. Depending  
297 on developmental conditions (i.e. laboratory *versus* outdoor), the ranking of the agricultural  
298 ecotype – relative to the woodland and urban ecotypes – appears to change, which may indicate

299 that boldness in the Speckled wood is shaped by gene  $\times$  environment interactions (Stamps &  
300 Groothuis 2010; Niemelä & Dingemanse 2014). Similarly, sequence affected boldness only as  
301 a main effect here (see also Kaiser *et al.* 2018), while it interacted with the ecotype in the  
302 reciprocal transplant experiment (Kaiser *et al.* 2019a). In both experiments, the sequence effect  
303 most likely reflects a habituation process, but outdoor developmental conditions seem to have  
304 promoted ecotype-related differences in habituation. Nevertheless, we should remain cautious  
305 when interpreting results from these two experiments together at this stage and this hypothesis  
306 warrants further study.

307 Within-individual correlation between the two behavioural traits was low in all studied  
308 populations. Contrastingly, we detected a significant among-individual correlation between  
309 boldness and activity, but only for butterflies of urban origin. Urban butterflies that were on  
310 average bolder were less active in the greenhouse test. Although the direction of this correlation  
311 is unexpected given that boldness and activity are expected to be positively related (Réale *et al.*  
312 2010), our results suggest that cities favour different behavioural syndromes compared to rural  
313 areas. Interestingly, studies on birds showed that behavioural syndromes tend to break down  
314 with urbanization (Scales *et al.* 2011; Bókony *et al.* 2012; Carrete & Tella 2017; but see  
315 Hardman & Dalesman 2018), while we found that urban butterflies actually have tighter  
316 behavioural syndromes. We believe this difference among taxa probably relates to distinct  
317 effects of urbanization on birds versus small-sized animals such as butterflies. Cities are highly  
318 productive habitats for city-dwelling birds (Shochat *et al.* 2006) and bird predation rates are  
319 lower than in surrounding rural areas (Eötvös *et al.* 2018). Therefore, at least some of the  
320 selective pressures in cities can be assumed to be relaxed for birds. By contrast, urban-heat-  
321 island effects (Brans *et al.* 2018; Merckx *et al.* 2018b), mechanical disturbances due to green  
322 infrastructure management, and high turnover of semi-natural areas (Parris 2016) are important  
323 stressors for city invertebrates. In particular, (micro-) climatic alterations and the increased

324 likelihood of drought events, as well as their indirect impacts on food quantity and quality, are  
325 expected to be important for ectothermic invertebrates such as *P. aegeria*, which was originally  
326 a woodland species and is hence drought-sensitive (Oliver *et al.* 2015). In line with recent work  
327 on rural-urban differentiation in damselflies (Tüzün *et al.* 2017b), urban areas may thus  
328 represent generally more stressful low quality habitats, favouring the emergence of particular  
329 behavioural syndromes in city invertebrates. Our sampling design, including families from ten  
330 regions, reduces the probability that the observed difference is primarily driven by genetic drift,  
331 which may be an important non-adaptive process shaping urban-rural differences (Rivkin *et al.*  
332 2019). Consequently, future laboratory experiments simulating drought stress (see Talloen *et*  
333 *al.* 2004), may provide useful insights on the adaptive value of trait integration under stressful  
334 conditions.

### 335 **Conclusion**

336 Using a butterfly species that has recently expanded its ecological niche, we aimed to unravel  
337 the effect of human-altered landscapes on personality traits. While mean levels of boldness (but  
338 not activity) were weakly related to the ecotype, covariation between personality traits differed  
339 according to the landscape of origin. Although we cannot fully rule out maternal effects, our  
340 common garden approach suggests that this difference has at least partly a genetic basis and  
341 results from micro-evolutionary changes (Alberti *et al.* 2017). Currently, about half of the  
342 Earth's land surface is covered by anthropogenic habitats (Ellis *et al.* 2010), some of which –  
343 like urban areas – are expected to experience a global expansion on the relatively short term  
344 (Seto *et al.* 2012). Therefore, understanding how novel environmental conditions shape the  
345 evolution of successful species is crucial to implement effective mitigation measures to reduce  
346 the negative impact of human-altered habitats on biodiversity in general.

347

348 **Acknowledgements**

349 We thank two anonymous reviewers for their constructive comments, and Camille Turlure for  
350 help with catching and rearing butterflies. This is publication number BRC XXX of the  
351 Biodiversity Research Centre (ELI/ELIB, UCLouvain).

352

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523

524 **Tables**

525 Table 1: Variance components associated with each of the random effects included in the analyses of boldness and activity, with 95% confidence  
 526 intervals. We also show the individual repeatability for each trait ( $r_{\text{Individual}}$ , with 95% credible intervals)

	$\sigma^2$ Individual	$\sigma^2$ Family	$\sigma^2$ Region	$\sigma^2$ Residual	$r$ Individual
Boldness	0.846 [0.692 ; 1.027]	0.105 [0.059 ; 0.164]	0.000 [0.000 ; 0.000]	0.896 [0.816 ; 1.024]	0.442 [0.400 ; 0.503]
Activity	1.368 [1.024 ; 0.970]	0.609 [0.372 ; 0.970]	0.010 [0.003 ; 0.030]	8.769 [7.449 ; 9.806]	0.133 [0.102 ; 0.159]

527

528 Table 2: Among- and within-individual correlation coefficients between boldness and activity (with 95% credible intervals), for each ecotype  
 529 separately. Significant correlation estimates are highlighted in bold

	Among-individual correlation	Within-individual correlation
Woodland	-0.333 [-0.825 ; 0.398]	-0.200 [-0.391 ; 0.025]
Agricultural	0.181 [-0.597 ; 0.813]	-0.046 [-0.174 ; 0.217]
Urban	<b>-0.699 [-0.902 ; -0.137]</b>	0.029 [-0.145 ; 0.179]

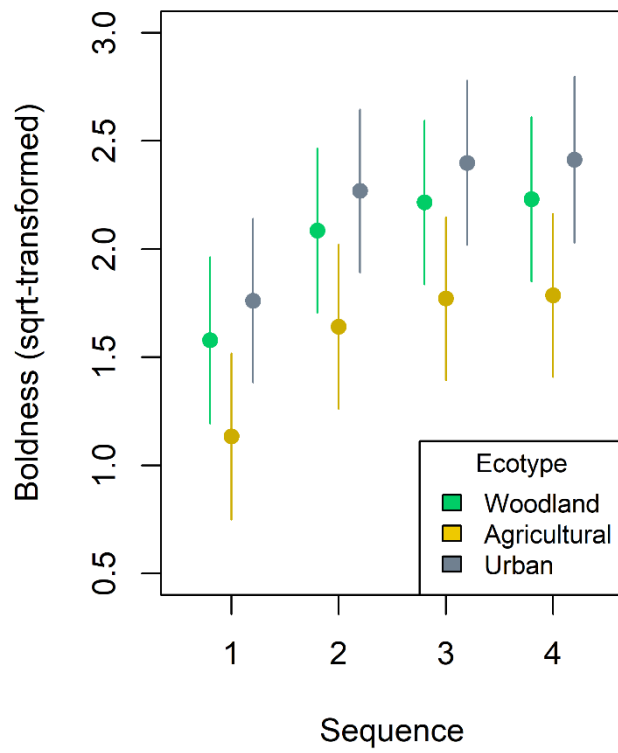
530



531 **Figure captions**

532 **Fig. 1** Effects of sequence and ecotype on boldness. Points show the expected mean  $\pm$  95%

533 confidence intervals based on the model output



534