Behavioral Ecology and Sociobiology

An experimental test of changed personality in butterflies from anthropogenic landscapes

--Manuscript Draft--

Manuscript Number:	BEAS-D-20-00066R2	
Full Title:	An experimental test of changed personality in butterflies from anthropogenic landscapes	
Article Type:	Original Article	
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Funding Information:	Fonds De La Recherche Scientifique - FNRS (Aspirant Grant)	Mr. Aurélien Kaiser
	Fonds De La Recherche Scientifique - FNRS (PDR grant FNRS PDR T.0188.14)	Prof. Hans Van Dyck
	Fédération Wallonie-Bruxelles (ARC-grant 17/22-086)	Prof. Hans Van Dyck

An experimental test of changed personality in butterflies from anthropogenic landscapes

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Abstract

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

Significance statement

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

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

Keywords:

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

Declarations

Funding

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

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

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

Data availability

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

Introduction

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

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

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

Animal personality refers to consistent behavioural differences among individuals (Réale *et al.* 2007). Within a species, individuals may consistently differ in behavioural traits such as boldness, activity, exploration, aggressiveness or sociability, which are the major personality axes (Réale *et al.* 2007). Moreover, personality traits tend to be grouped into behavioural syndromes, whereby the average phenotype of individuals in one context is correlated with the average phenotype of the same individuals in another context (i.e. between-individual correlation – Dingemanse *et al.* 2012). For instance, Great tits (*Parus major*) that explore a new environment more rapidly are also more aggressive towards conspecifics (Verbeek *et al.* 1996). Personality traits and behavioural syndromes are relevant in the context of human-induced rapid environmental changes, as they have wide-reaching implications for ecological and evolutionary processes (Sih *et al.* 2004; Sol *et al.* 2013). Several studies also reported shifts in personality traits linked to urbanization. For instance, a study across multiple European cities showed that wild urban Great tits displayed more distress calling and higher pecking rates (i.e. an index of handling aggression) than their rural conspecifics (Senar *et al.* 2017). Overall, urban vertebrates show personality traits associated with a more proactive life-style (Koolhaas *et al.* 1999): urban animals tend to be more explorative, bolder and more active than their rural conspecifics. Such a shift towards a more proactive style is supposed to be adaptive as it would reduce fear reactions towards novel objects and it would facilitate the acquisition of new food resources (e.g. Sol *et al.* 2013; Senar *et al.* 2017). Also, urbanization was found to uncouple personality traits, with behavioural syndrome breakdowns observed in some bird species (e.g. Evans *et al.* 2010; Bókony *et al.* 2012).

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

133 Talloen *et al.* 2004; Oliver *et al.* 2015), we therefore expect tightened behavioural syndromes
134 in butterflies from anthropogenic landscapes.

135 **Methods**

136 *Study species and sampling sites*

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

148 *Butterfly rearing*

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

to the ecotype and it ensured that the observer was blind to the origin of the butterfly during the behavioural tests.

Behavioural tests

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

Boldness

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

We maintained constant light conditions and a room temperature of 25°C during the test. All butterflies but one were submitted four times to this test, with one day elapsing between two successive trials. Butterflies were then weighed using a microbalance (Ohaus Explorer;

accuracy: ± 0.1 mg). After weighing, butterflies returned to their individual plastic cup until the activity test.

Activity

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

Statistical analysis

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

Effects of fixed variables on mean behaviour

We used linear mixed models (package *lme4*) to test for differences in behaviour among ecotypes and sexes, and to unravel dynamics of these traits relative to the testing sequence. Prior to the analyses, we applied a square-root transformation to boldness and activity to achieve normality of the residuals. Boldness and activity were the response variables, while ecotype (i.e. woodland, urban or agricultural), sex (i.e. male or female), sequence (as a

categorical variable) were included as explanatory variables. We initially included the ecotype × sex and ecotype × sequence interactions, but they were never significant ($P \geq 0.1$) and we thus removed them from the final models. Region of origin, family ID and individual ID were included as random factors (i.e. random intercepts) in all models. Body mass was added as a covariate for both behavioural traits. For activity, we added temperature in the greenhouse tunnel (in °C) and solar irradiance (in W/m^2) during the test as additional covariates. Continuous variables (i.e. covariates) were scaled prior to the analyses.

Repeatability of behavioural traits

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

Correlations among behavioural traits

To test for correlations among behavioural traits, we fitted ecotype-specific bivariate mixed models with the *MCMCglmm* package (Hadfield 2010). Models contained boldness and activity (both square-root transformed) as response variables, sex, sequence and body mass as fixed effects, and region of origin, family ID and individual ID as random effects. We used a Gaussian distribution and a non-informative inverse-Wishart prior. We used 300,000 iterations, from which we discarded the first 30,000 (burn-in), while using a thinning interval of 100. This resulted in low autocorrelation. We performed three runs to confirm robustness of the model

outputs. We report within- and between-individual correlations as they both contribute to phenotypic correlations. The former represents the correlation between an individual's change in one trait between *t* and *t+1* and the change in another trait over the same period (Dingemanse & Dochtermann 2013). The latter represents the correlation between individual mean values of two traits and it is thus a measure of behavioural syndromes *sensu stricto* (Dingemanse *et al.* 2012). Within- and between-individual correlations were estimated following equations in Dingemanse and Dochtermann (2013). Correlations whose 95% credible intervals do not overlap with zero were considered significant.

Results

Boldness and activity

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

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

Correlations among traits

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

Discussion

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

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

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

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

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

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

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

Conclusion

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

Acknowledgements

We thank two anonymous reviewers for their constructive comments, and Camille Turlure for help with catching and rearing butterflies. This is publication number BRC 363 of the Biodiversity Research Centre (ELI/ELIB, UCLouvain).

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Tables

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

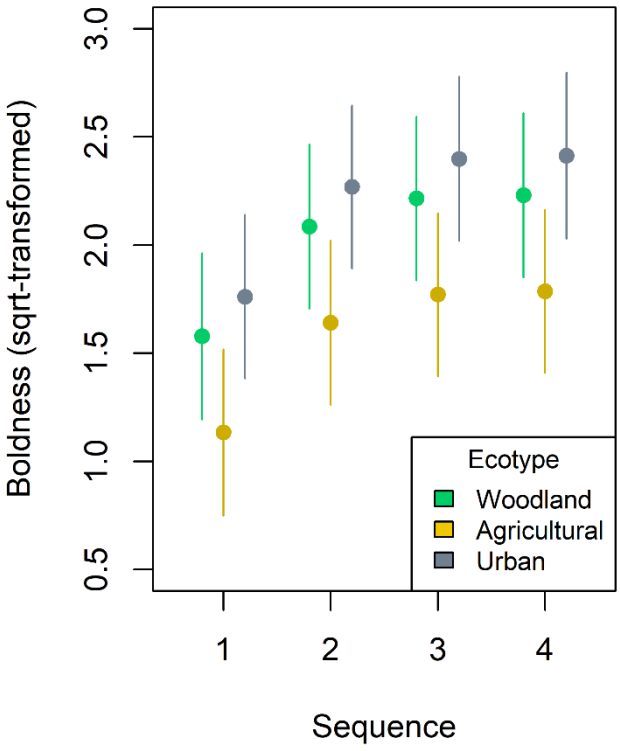
	$\sigma^2_{Individual}$	σ^2_{Family}	σ^2_{Region}	$\sigma^2_{Residual}$	$r_{Individual}$
Boldness	0.846 [0.692 ; 1.027]	0.105 [0.059 ; 0.164]	0.000 [0.000 ; 0.000]	0.896 [0.816 ; 1.024]	0.442 [0.400 ; 0.503]
Activity	1.368 [1.024 ; 0.970]	0.609 [0.372 ; 0.970]	0.010 [0.003 ; 0.030]	8.769 [7.449 ; 9.806]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

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

	Among-individual correlation	Within-individual correlation
Woodland	-0.333 [-0.825 ; 0.398]	-0.200 [-0391 ; 0.025]
Agricultural	0.181 [-0.597 ; 0.813]	-0.046 [-0.174 ; 0.217]
Urban	**-0.699 [-0.902 ; -0.137]**	0.029 [-0.145 ; 0.179]

Figure captions

Fig. 1 Effects of sequence and ecotype on boldness. Points show the expected mean ± 95% confidence intervals based on the model output



Electronic supplementary material

Behavioral Ecology and Sociobiology

An experimental test of changed personality in butterflies from anthropogenic landscapes

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Figure S1: Capture sites within the Sint-Niklaas region (See Table S1). Red, blue and yellow points represent the urban, woodland and agricultural site, respectively. Black circles represent 1-kilometre radius buffers around the sites. The upper panel (A) shows the aerial view of the capture sites (aerial views were accessed online from the Flemish geographic portal – https://www.geopunt.be/). The lower panel (B) shows the landscape composition within the 1-km buffers. For (B), red areas within buffers represent imperviousness and blue areas represent the tree cover density. The intensity of the colour is proportional to the cover (both on a 0-100% scale). White areas are other types of surfaces, in this region mainly consisting of grasslands and croplands (i.e. agricultural land-use). Imperviousness (High Resolution Layer: Imperviousness Density (IMD) 2015) and tree cover density (High Resolution Layer: Tree Cover Density (TCD) 2015) were accessed from the Copernicus Programme (https://land.copernicus.eu/pan-european/high-resolution-layers/).

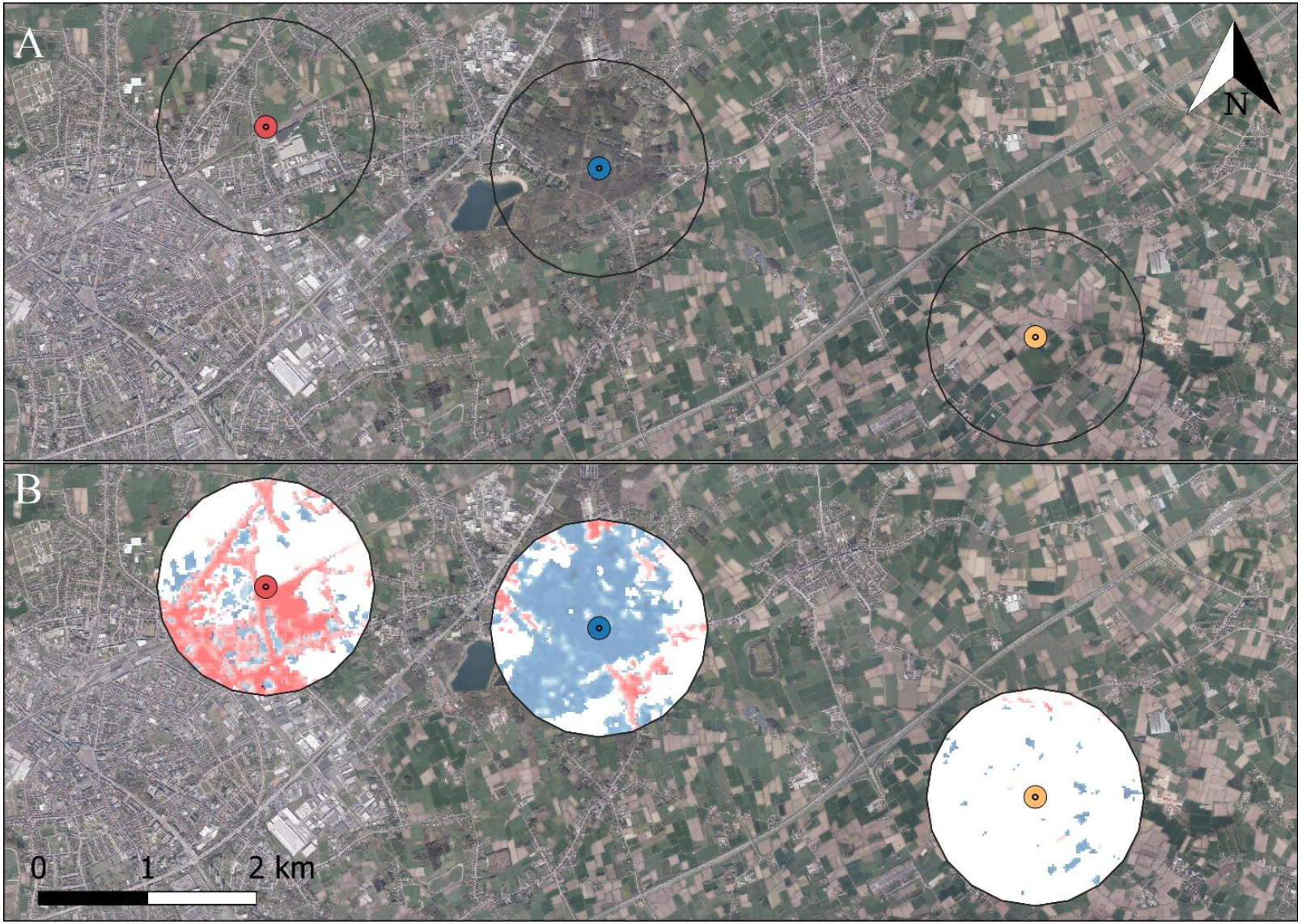

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Table S1: Geographical coordinates of the studied populations (i.e. capture sites). For each population, we provide the region of origin, the landscape type (i.e. ecotype) as well as the number of tested families (i.e. number of females captured at each site).

Population	Region	Type	Latitude (N) & longitude (E)	Families
Aalst	Aalst	Urban	50.939192, 4.057537	1
Moorsel	Aalst	Agricultural	50.942857, 4.105700	2
Dorpveld	Aalst	Woodland	50.931644, 4.156294	1
Antwerpen	Antwerpen	Urban	51.195447, 4.430365	1
Morkhoven	Antwerpen	Agricultural	51.102210, 4.832670	1
Herentals	Antwerpen	Woodland	51.203081, 4.860671	1
Bruxelles	Bruxelles	Urban	50.818266, 4.400313	2
Kortenberg	Bruxelles	Agricultural	50.926738, 4.648412	2
Halle	Bruxelles	Woodland	50.705179, 4.291698	1
Geraardsbergen	Geraardsbergen	Urban	50.767818, 3.864286	1
Tollembeek	Geraardsbergen	Agricultural	50.745164, 4.005191	2
Noir-Jambon	Geraardsbergen	Woodland	50.637608, 3.968643	1
Leuven	Leuven	Urban	50.888521, 4.698123	2
Rillaar	Leuven	Agricultural	50.972280, 4.867586	2
Walenbos	Leuven	Woodland	50.929560, 4.874904	2
Mechelen	Mechelen	Urban	51.011605, 4.464534	1
Ekspoel	Mechelen	Agricultural	51.039280, 4.388389	1
Arkenbos	Mechelen	Woodland	51.059365, 4.393013	2
Namur	Namur	Urban	50.444624, 4.889939	1
Petit-Waret	Namur	Agricultural	50.520923, 5.040287	1

Seilles	Namur	Woodland	50.510625, 5.117124	2
Oudenaarde	Oudenaarde	Urban	50.846709, 3.607512	1
Lede	Oudenaarde	Agricultural	50.880937, 3.554040	1
Ename	Oudenaarde	Woodland	50.858297, 3.647626	2
Sint-Niklaas	Sint-Niklaas	Urban	51.178730, 4.163672	1
Beestenhoek	Sint-Niklaas	Agricultural	51.161232, 4.264872	2
Vossekot	Sint-Niklaas	Woodland	51.175141, 4.207527	1
Tienen	Tienen	Urban	50.810404, 4.921161	1
Hoegaarden	Tienen	Agricultural	50.768442, 4.874433	1
Meerdaal	Tienen	Woodland	50.801001, 4.699343	1

Table S2: Number of individuals tested per family. Number into brackets indicate the number of tested males (M) and females (F).

Family	Population	Region	Type	Nbr of tested individuals
1	Aalst	Aalst	Urban	5 ind. (3M + 2F)
2	Moorsel	Aalst	Agricultural	2 ind. (1M + 1F)
3	Moorsel	Aalst	Agricultural	3 ind. (1M + 2F)
4	Dorpveld	Aalst	Woodland	5 ind. (2M + 3F)
5	Antwerpen	Antwerpen	Urban	5 ind. (2M + 3F)
6	Morkhoven	Antwerpen	Agricultural	4 ind. (2M + 2F)
7	Herentals	Antwerpen	Woodland	3 ind. (3F)
8	Bruxelles	Bruxelles	Urban	4 ind. (2M + 2F)
9	Bruxelles	Bruxelles	Urban	2 ind. (1M + 1F)

10	Kortenberg	Bruxelles	Agricultural	2 ind. (1M + 1F)
11	Kortenberg	Bruxelles	Agricultural	3 ind. (2M + 1F)
12	Halle	Bruxelles	Woodland	7 ind. (2M + 5F)
13	Geraardsbergen	Geraardsbergen	Urban	6 ind. (2M + 4F)
14	Tollembeek	Geraardsbergen	Agricultural	2 ind. (1M + 1F)
15	Tollembeek	Geraardsbergen	Agricultural	3 ind. (1M + 2F)
16	Noir-Jambon	Geraardsbergen	Woodland	5 ind. (3M + 2F)
17	Leuven	Leuven	Urban	2 ind. (2F)
18	Leuven	Leuven	Urban	2 ind. (1M + 1F)
19	Rillaar	Leuven	Agricultural	3 ind. (1M + 2F)
20	Rillaar	Leuven	Agricultural	2 ind. (1M + 1F)
21	Walenbos	Leuven	Woodland	4 ind. (2M + 2F)
22	Walenbos	Leuven	Woodland	1 ind. (1F)
23	Mechelen	Mechelen	Urban	6 ind. (2M + 4F)
24	Ekspoel	Mechelen	Agricultural	5 ind. (2M + 3F)
25	Arkenbos	Mechelen	Woodland	4 ind. (2M + 2F)
26	Arkenbos	Mechelen	Woodland	2 ind. (1M + 1F)
27	Namur	Namur	Urban	4 ind. (2M + 2F)
28	Petit-Waret	Namur	Agricultural	5 ind. (2M + 3F)
29	Seilles	Namur	Woodland	3 ind. (1M + 2F)
30	Seilles	Namur	Woodland	2 ind. (1M + 1F)
31	Oudenaarde	Oudenaarde	Urban	6 ind. (2M + 4F)
32	Lede	Oudenaarde	Agricultural	2 ind. (2F)
33	Ename	Oudenaarde	Woodland	1 ind. (1M)

34	Ename	Oudenaarde	Woodland	2 ind. (1M + 1F)
35	Sint-Niklaas	Sint-Niklaas	Urban	5 ind. (2M + 3F)
36	Beestenhoek	Sint-Niklaas	Agricultural	3 ind. (1M + 2F)
37	Beestenhoek	Sint-Niklaas	Agricultural	2 ind. (1M + 1F)
38	Vossekot	Sint-Niklaas	Woodland	5 ind. (2M + 3F)
39	Tienen	Tienen	Urban	6 ind. (3M + 3F)
40	Hoegaarden	Tienen	Agricultural	6 ind. (3M + 3F)
41	Meerdaal	Tienen	Woodland	6 ind. (2M + 4F)

Table S3: Number of individuals tested per ecotype for the activity test.

	Number of activity tests				
	0	**1**	**2**	**3**	**4**
Woodland	10	4	3	13	20
Agricultural	14	2	2	11	18
Urban	5	2	3	9	34