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ORIGINAL ARTICLE

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

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 and 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

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reduced species richness compared to surrounding natural habitats (McKinney 2008; Concepción et al. 2015; Ramírez-Restrepo and 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; Merckx et al. 2018a; Kern and Langerhans 2018) and life-history or physiological traits (Sprau et al. 2017; Tüzün et al. 2017b; Sepp et al. 2018; Diamond 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 and 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 and 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. Talloen et al. 2004; Oliver et al. 2015), we therefore expect tightened behavioural syndromes in butterflies from anthropogenic landscapes.

Methods

Study species and sampling sites

The Speckled wood (*P. aegeria* L.) is a common multivoltine butterfly species that occurs over most of Europe (Settele et al. 2008). Larvae feed on the leaves of various grass species

(Shreeve, 1986). In June and July 2015, we collected gravid females from ten regions in central Belgium. In each region, we selected one woodland, one urban and one agricultural site (see Fig. S1). Woodland sites consisted of continuous forests typically larger than 100 ha; agricultural sites of systems of small woodlots and hedgerows surrounded by arable fields and pastures; urban sites of wooded parks and woodlots surrounded by buildings and roads (Kaiser et al. 2019a). We captured one or two females at each site, which in total resulted in 14 woodland, 12 urban and 15 agricultural families (see Table S1 for capture details). Collected females were transported to the laboratory for oviposition in individual cages on the grass *Poa pratensis*, while they could feed ad libitum on honey-soaked (10% solution) cotton pads.

Butterfly rearing

The offspring (i.e. F1-generation) of the wild-caught females were reared on potted *P. pratensis* plants (grown on a standardized soil mixture under identical light and temperature conditions) in a climate room (photoperiod: L:D 16 h:8 h; day temperature: 25 °C; night temperature: 16 °C). Each host plant contained four full-sib caterpillars and was enclosed in nylon netting. Pots were checked on a regular basis for pupating larvae. Pupae were removed from their plant and placed individually in labelled plastic cups. We checked all cups twice a day for emergence. We used 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: $12 \times 4 \times 2$ m; installed in a larger glass greenhouse) whose floor was taped to delineate two rows of eight rectangles (each 1.5×2 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 and 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 and 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 and 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 and 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 (Fig. 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) (Fig. 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; Lapiedra et al. 2017; Charmantier 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. 2017a). Anthropogenic landscapes typically have altered plant and animal

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 ($r_{\text{Individual}}$, with 95% credible intervals)

	$\sigma^2_{\text{Individual}}$	σ^2_{Family}	σ^2_{Region}	$\sigma^2_{\text{Residual}}$	$r_{\text{Individual}}$
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]

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.

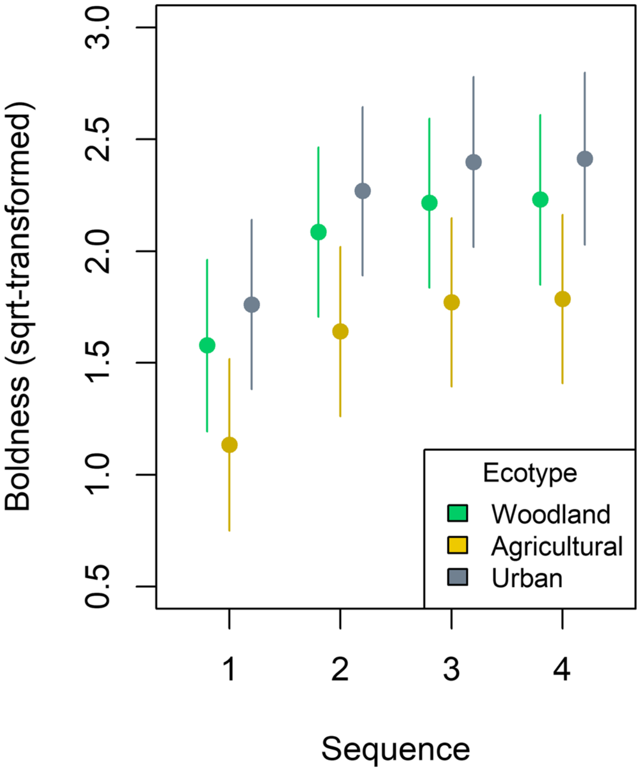

Fig. 1 Effects of sequence and ecotype on boldness. Points show the expected mean ± 95% confidence intervals based on the model output

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

Within-individual correlation between the two behavioural traits was low in all studied populations. Contrastingly, we detected a significant among-individual correlation between boldness and activity, but only for butterflies of urban origin. Urban butterflies that were on average bolder were less active in the greenhouse test. Although the direction of this correlation is unexpected given that boldness and activity are

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]

expected to be positively related (Réale et al. 2010), our results suggest that cities favour different behavioural syndromes compared to rural areas. Interestingly, studies on birds showed that behavioural syndromes tend to break down with urbanization (Scales et al. 2011; Bókony et al. 2012; Carrete and Tella 2017; but see Hardman and Dalesman 2018), while we found that urban butterflies actually have tighter behavioural syndromes. We believe this difference among taxa probably relates to distinct effects of urbanization on birds versus small-sized animals such as butterflies. Cities are highly productive habitats for city-dwelling birds (Shochat et al. 2006) and bird predation rates are lower than in surrounding rural areas (Eötvös et al. 2018). Therefore, at least some of the selective pressures in cities can be assumed to be relaxed for birds. By contrast, urban-heat-island effects (Merckx et al. 2018b; Brans et al. 2018), mechanical disturbances due to green infrastructure management, and high turnover of semi-natural areas (Parris 2016) are important 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. 2017a), 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.

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

Compliance with ethical standards

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.

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