Animal Behaviour 157 (2019) 219–226

Contents lists available at ScienceDirect

Animal Behaviour

journal homepage: www.elsevier.com/locate/anbehav

Behavioural repeatability is affected by early developmental conditions in a butterfly

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ARTICLE INFO

Article history:
Received 7 February 2019
Initial acceptance 12 March 2019
Final acceptance 8 July 2019
Available online 16 October 2019
MS number 19-00108R

Keywords:
animal personality
anthropogenic landscapes
early environment
Lepidoptera
Pararge aegeria
urbanization

For developing organisms, early environmental conditions are critical as they provide cues about their environment and are thus helpful to make decisions for the short and long term. As such, the early environment is known to affect several phenotypic traits, and these can persist after developmental growth. However, the role of these early environmental conditions in shaping personality traits remains largely unknown. Here, we used a reciprocal transplant experiment to explore the effect of landscape of origin versus landscape of development on boldness and activity in a butterfly, *Pararge aegeria*. Larvae of woodland, agricultural and urban population origins were reared in situ in their landscape of origin or under the two alternative environmental conditions. We then repeatedly quantified boldness and activity in the F1 adults under laboratory conditions. While the landscape of development appeared to have no effect on mean trait values, it affected trait repeatability through changes in among-individual variation. Additionally, males of agricultural origin had higher mean boldness scores than woodland and urban origin males. Also, average boldness declined with testing sequence in individuals of woodland origin, but not in agricultural and urban origin individuals. Overall, our results suggest that (1) conspecifics originating from distinct habitat types differ in some aspects of boldness, and (2) early developmental conditions can affect behavioural consistency without changing mean behavioural phenotypes.

In most species, environmental conditions interact with development and lead to the production of distinct phenotypes from the same genotype (i.e. phenotypic plasticity; West-Eberhard, 2003). As such, early environmental conditions are of crucial importance as they can have long-lasting effects on an individual's phenotype and performance. For example, developing *Daphnia cucullata* waterfleas exposed to predator kairomones grow long helmets that help reduce predation rates (Agrawal, Laforsch, & Tollrian, 1999). Thus, both biotic and abiotic environmental cues picked up by developing organisms are important to understand the phenotype later in life.

Animal personality refers to individual behavioural differences that are consistent across time and/or contexts (Réale, Reader, Sol, McDougall, & Dingemanse, 2007). Both genetic and environmental factors contribute to personality variation, each of which accounts for about 50% (Dochtermann, Schwab, & Sih, 2015). Yet, the role of early environmental conditions in shaping personality traits later in life remains poorly understood. Recent studies have explored some aspects of it, highlighting long-lasting effects of environmental factors such as conspecific density (Han & Brooks, 2015; Müller, Küll, & Müller, 2016), food quality (e.g. Han & Dingemanse, 2017; Krause, Honarmand, Wetzel, & Naguib, 2009; Tremmel & Müller, 2013), incubation temperature (Bertin et al., 2018; Siviter et al., 2017) and physical complexity of the rearing environment (Liedtke, Redekop, Schneider, & Schuett, 2015) on adult animal personality.

Most invertebrate species lack morphological or behavioural strategies that may partly protect their offspring from external influences (e.g. vivipary and parental care), leaving embryos much more exposed to external conditions than in some other taxa. Invertebrates may hence be expected to show increased sensitivity to early environmental cues. Here, we studied developmental plasticity in personality traits in response to landscape-related cues in the speckled wood butterfly, *Pararge aegeria*. During the last few decades this species has expanded its ecological niche in Western Europe; while it was once primarily a woodland species, it has now colonized agricultural areas (Dover & Sparks, 2000; Merckx, Van

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https://doi.org/10.1016/j.anbehav.2019.08.006
0003-3472/

Dyck, Karlsson, & Leimar, 2003) and occurs in wooded parks in urban settings too (Kaiser, Merckx, & Van Dyck, 2018). The colonization of these anthropogenic, fragmented landscapes exposes developing individuals to environmental cues that differ substantially from those encountered in the ancestral habitat. For instance, agricultural and urban areas are typically warmer and/or thermally more variable than wooded areas (e.g. Kaiser, Merckx, & Van Dyck, 2016; Merckx, Serruys, & Van Dyck, 2015; Merckx, Van Dongen, Matthysen, & Van Dyck, 2008).

Using a reciprocal transplant approach, we reared speckled wood larvae originating from these three distinct landscape types both in their landscape of origin and in the two alternative landscape types. This approach allowed us to test whether environmental cues available from the landscape of development during the larval stage affect repeatability and mean levels of two well-studied personality traits (i.e. boldness and activity) at the adult stage. While effects of land conversion to agricultural area on animal personality have attracted little attention overall (but see Cornelius, Awade, Cândia-Gallardo, Sieving, & Metzger, 2017), urban/rural differences in personality traits are being increasingly documented. For example, flight-initiation distance is shorter in urban birds and lizards (e.g. Cavalli, Baladrón, Isacch, Biondi, & Bó, 2018; Lapiedra, Chejanovski, & Kolbe, 2017; Sol et al., 2018) and urban great tits, *Parus major*, and beetles are more exploratory than rural conspecifics (Charmantier, Demeyrier, Lambrechts, Perret, & Grégoire, 2017; Schuett et al., 2018). Overall, these traits point towards a more proactive/fast lifestyle (Réale et al., 2010) in urbanized areas. Whether the urban/rural phenotypic divergence in behaviour reflects genetic differentiation or arises from phenotypic plasticity (and possibly from early experience; Miranda, Schielzeth, Sonntag, & Partecke, 2013) remains unresolved in most cases (see Charmantier et al., 2017). If early environmental conditions indeed play a role in shaping these differences, we expected butterflies that developed in anthropogenic landscapes (i.e. urban and agricultural sites) to display higher levels of boldness and activity. We also investigated whether the three ecotypes differed in the level of developmental plasticity for these personality traits. While large woodlands provide buffered and relatively stable conditions, hedgerows and small woodlots in agricultural and urban areas can experience high levels of disturbance as a consequence of human activities (e.g. Parris, 2016). As such, we predicted higher levels of developmental plasticity in agricultural and urban populations compared to populations of woodland origin.

METHODS

Study Species and Sampling Sites

The speckled wood is a multivoltine butterfly species with a wide distribution throughout Europe (Settele et al., 2008). Eggs are laid singly on grass leaves and larvae feed on various grass species (Shreeve, 1986). In May 2016, we collected gravid females from four regions (ca. 100 km^2 each) in central Belgium. In each region, we selected one woodland, one urban and one agricultural site. Woodland sites consisted of continuous forests dominated by deciduous tree species and were typically larger than 100 ha (except one site that was about 30 ha); agricultural sites were systems of small woodlots and hedgerows surrounded by crop fields and pastures; urban sites consisted of wooded parks and cemeteries and small woodlots surrounded by a matrix of buildings and motorways. We captured one or two females at each site, which in total resulted in four woodland, seven agricultural and eight urban families (see Table A1 for capture details and Fig. A1 for a map of the study region). Collected females were brought to the laboratory (photoperiod: 16:8 h light:dark; day temperature: 25 °C; night temperature: 16 °C) 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 and Split-brood Field Experiment

For each of the 19 females, we randomly selected 18 first- or second-instar larvae which we spread over six *P. pratensis* plants per female. Thus, each potted plant (enclosed in a nylon netting) contained three full-sib larvae. Plants were grown from seeds on a standardized soil mixture and under standard conditions in a climate room (16:8 h light:dark, 25 °C:16 °C). They were watered every other day during growth. The offspring of each female was then spread equally between the three landscape types within the region of origin of the mother. The fine-mesh netting (height: 30 cm; diameter: 15 cm) surrounding the host plant was sealed at the bottom and top to prevent the larvae from escaping (see Fig. A2). The netting also prevented potential predators from preying on the larvae. Potted plants were placed on site between 21 May and 10 June. At each site, one of the pots contained a terrestrial probe (iButton, Thermochron DS1923, Maxim Integrated, San Jose, CA, U.S.A.; resolution: 0.06 °C) that logged air temperature within the pot approximately 10 cm above the ground for 26 days. These probes showed that woodland sites are about 0.5 °C cooler than agricultural and urban sites (woodland sites: 15.5 ± 0.2 °C; agricultural sites: 16.0 ± 0.2 °C; urban sites: 16.0 ± 0.2 °C; mean ± SE). Every 5 days we checked all enclosures for pupae, which we removed from the plant and individually stored in labelled plastic cups in a climate room (16:8 h light:dark, 25 °C:16 °C) until emergence.

Behavioural Tests

We submitted adult offspring to two behavioural assessments: (1) a boldness test and (2) an activity test. We tested a total of 146 (67 females and 79 males) virgin butterflies (see Table A2 for the precise number of tested butterflies for each combination of landscape of origin, landscape of development and sex). Assessments started on the day following emergence, i.e. on day 1, and we retested most individuals on days 2, 3 and 4. At the end of each day, butterflies could feed ad libitum on a cotton pad soaked with a 10% honey solution. A single observer (A.K.) conducted all behavioural observations. Prior to behavioural assessments, each butterfly received a unique, noninformative identifier that prevented the observer knowing which treatment the butterfly had received during its larval development.

Boldness

We placed butterflies individually in a semitransparent glassine envelope (63 x 97 mm), which allowed the observer to see the butterfly's movements. We positioned the butterfly (with closed wings) in the centre of the envelope and maintained it in this position by gently pressing two opposite corners of the envelope. We counted struggles for 1 min. Here, we defined a struggle as a series of leg, head and/or wing movements, interrupted from other such series by pauses of inactivity (Kaiser et al., 2018). As the butterfly was unable to move freely in the envelope, we assumed the test exposed the butterfly to stressful conditions that mimic a predator's attack (e.g. butterfly being stuck in a spider's web or held in a bird's beak). Tests took place in a 25 °C room under constant light conditions. All butterflies were tested four times on 4 consecutive days. They were then weighed using a microbalance (Ohaus Explorer; accuracy: ± 0.1 mg) and put back in their labelled plastic cup for at least 1 h before the activity test.

Table 1
Results from model selection to identify predictors of adult boldness and activity

	k	LogLik	AICc	ΔAICc	Model weight
Boldness					
Saturated model: Body mass + (Development + Origin + Sequence + Sex)^2					
Origin + Sequence + Sex + Origin*Sequence + Origin*Sex	13	-940.650	1908.0	0.00	0.454
Origin + Sequence + Sex + Origin*Sequence + Origin*Sex + Sequence*Sex	14	-940.125	1909.0	1.05	0.269
Origin + Sequence + Sex + Origin*Sequence + Origin*Sex + Body mass	14	-940.598	1909.9	2.00	0.167
Development + Origin + Sequence + Sex + Development*Sex + Origin*Sequence + Origin*Sex	17	-937.842	1910.8	2.83	0.110
Activity					
Saturated model: Body mass + Temperature + Time of the day + (Time of the day)^2 + (Development + Origin + Sequence + Sex)^2					
Sequence + (Time of the day)^2 + Body mass + Temperature + Sex + Sequence*Sex	11	-1395.592	2813.7	0.00	0.184
Sequence + Time of the day + (Time of the day)^2 + Body mass + Temperature + Sex + Sequence*Sex	12	-1394.738	2814.1	0.38	0.152
Sequence + (Time of the day)^2 + Body mass + Sex + Sequence*Sex	10	-1397.272	2815.0	1.28	0.097
Sequence + (Time of the day)^2 + Temperature + Sex + Sequence*Sex	10	-1397.281	2815.0	1.30	0.096
Sequence + Time of the day + (Time of the day)^2 + Body mass + Sex + Sequence*Sex	11	-1396.244	2815.0	1.30	0.096
Sequence + Time of the day + (Time of the day)^2 + Temperature + Sex + Sequence*Sex	11	-1396.325	2815.1	1.47	0.089
Sequence + Time of the day + (Time of the day)^2 + Sex + Sequence*Sex	10	-1397.640	2815.7	2.01	0.067
Origin + Sequence + (Time of the day)^2 + Body mass + Temperature + Sex + Sequence*Sex	13	-1394.568	2815.8	2.14	0.063
Sequence + (Time of the day)^2 + Sex + Sequence*Sex	9	-1398.758	2815.8	2.17	0.062
Origin + Sequence + Time of the day + (Time of the day)^2 + Body mass + Temperature + Sex + Sequence*Sex	14	-1393.737	2816.3	2.59	0.051
Sequence + Body mass + Sex + Sequence*Sex	9	-1399.142	2816.6	2.94	0.042

We report the degrees of freedom (*k*), log-likelihood (LogLik), corrected Akaike information criterion value (AICc) and model weight of each model. ΔAICc refers to the difference between a model's AICc and the best-ranked model. For the saturated model, we only show the fixed effects (see the Statistical analysis section for a detailed description of the random structure). Models with ΔAICc > 3 are not shown and models with ΔAICc < 2 are indicated in bold.

Activity

After the boldness test, butterflies were submitted to an activity test. We released butterflies individually at one extremity of an empty plastic greenhouse tunnel (12 x 4 m and 2 m high) whose concrete floor was taped to delineate 16 rectangles of 1.5 x 2 m. The flight tunnel was installed in a much larger permanent glass greenhouse maintained at a minimum temperature of 21 °C. Each butterfly was allowed to move freely in the tunnel for 4 min while the observer recorded the number of transitions between squares (used as a proxy for activity). We additionally recorded ambient temperature during the test (Aria temperature probe). All tests were conducted between 1130 and 2040 hours (UTC+1). Because of time constraints, and since a few individuals ($N = 6$) died before the end of the test period, there is some variation in the number of times individuals were tested for activity. Ten butterflies were tested twice, 15 were tested three times and 121 were tested four times. As for the boldness assessments, 1 day elapsed between two successive trials. After the last behavioural test, butterflies were killed and stored by freezing (-21 °C).

Ethical Note

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

Statistical Analysis

All analyses were carried out with R 3.5.1 (R Core Team, 2018).

Prior to the analyses, we applied a square-root transformation to boldness and activity scores to achieve normality of the residuals. To identify predictors of boldness and activity, we used a model selection procedure (Burnham, Anderson, & Huyvaert, 2011; Symonds & Moussalli, 2011) based on the corrected Akaike information criterion, AICc, using the MuMIn package. For each of the two response variables, we first built a saturated model. For boldness, explanatory variables included landscape of origin and of development (i.e. woodland, urban or agricultural), sex (i.e. male or female), sequence (from 1 to 4; a continuous variable) and their two-way interactions. We also included body mass as a covariate. Region of origin, family ID and individual ID were included as random factors (i.e. random intercept). Individual ID was nested within family ID which itself was nested within region of origin. The saturated model for activity included the same predictors, but we also added temperature in the greenhouse tunnel, time of day (in minutes from 1100 onwards) and the quadratic effect of the latter as additional covariates. The random structure was identical to the random structure of the saturated model built for adult boldness. For each trait, we retained models with ΔAICc within 2 of the model with the lowest AICc. These models were considered as the set of candidate models from which we calculated the variable weight of each predictor. Because the best-ranked models (i.e. those with the lowest AICc) were rather poorly supported (i.e. model weight < 0.9; see Table 1 for the sets of candidate models), we then used a full-model averaging procedure based on the set of candidate models to get estimates and standard errors of each predictor (Symonds & Moussalli, 2011).

The repeatability of each behavioural trait was calculated based on the best-ranked models. 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 as the between-individual variance divided by the sum of the between-individual and residual variances (Nakagawa & Schielzeth, 2010). We first calculated repeatabilities for the entire data set. Because we were also interested in investigating the effects of developmental conditions (i.e. landscape of development) on the consistency of behavioural traits, repeatability was estimated separately for butterflies that developed under woodland, agricultural and urban conditions. Repeatability was considered significant when 95% credible intervals did not overlap zero. Similarly, repeatability estimates were considered as different when 95% credible intervals did not overlap.

RESULTS

Predictors of the Mean Behaviour

The model selection procedure suggests that adult boldness (mean level differences) was predicted by the Origin*Sequence

interaction (Table 1). More specifically, boldness declined with sequence in butterflies of woodland origin, while there was no sequence-related change in boldness in butterflies of the agricultural and urban origins (Fig. 1, Table 2). There was also support for a significant two-way interaction involving sex and the landscape of origin to affect boldness. In males, individuals of an agricultural origin had on average higher boldness scores than their woodland and urban counterparts, while there was no such landscape-related difference in females (Fig. 2, Table 2). Note that none of the models from the set of candidate models included landscape of development as a predictor, either as a main effect or in an interaction (see Table 1).

Mean levels of adult activity were associated with a two-way interaction involving sequence and sex (Table 1): activity increased with sequence in females, but not in males (Fig. 3, Table 2). Additionally, there was support for a relationship between activity and the quadratic effect of time of day, which indicates that activity peaked during mid-afternoon. Again, there was no support for an influence of the landscape of development on the mean level of activity (Table 1).

Repeatability of Behavioural Traits

Boldness and activity both showed moderate repeatability when calculated on the entire data set (Table 3). When analysed separately by landscape of development, repeatability estimates differed between groups. Boldness repeatability of butterflies that developed under urban conditions was higher than in conspecifics that developed under woodland or agricultural conditions. However, this pattern was reversed for activity: repeatability was lower for individuals that developed in an urban landscape compared to the woodland and agricultural groups. In both cases, these differences in repeatability were caused by changes in among-individual variation (Table 3).

Table 2
Model-averaged estimates (± SE) for factors predicting boldness and activity (both were square-root transformed) in speckled wood butterflies

Fixed effects	Estimate ± SE
Boldness	
Intercept[a]	1.861 ± 0.222
Origin [Urban]	0.263 ± 0.273
Origin [Woodland]	0.285 ± 0.302
Sequence	-0.056 ± 0.083
Sex [Male]	0.767 ± 0.273
Sequence*Origin [Urban]	0.047 ± 0.106
Sequence*Origin [Woodland]	-0.260 ± 0.117
Sex [Male]*Origin [Urban]	-0.995 ± 0.360
Sex [Male]*Origin [Woodland]	-1.065 ± 0.340
Sequence*Sex [Male]	-0.028 ± 0.066
Body mass	0.007 ± 0.049
Activity	
Intercept[b]	5.742 ± 0.437
Sequence	0.633 ± 0.191
Time of day	-0.087 ± 0.131
(Time of day)^2	-0.239 ± 0.111
Body mass	-0.362 ± 0.319
Temperature	-0.189 ± 0.171
Sex [Male]	-0.363 ± 0.608
Sequence*Sex [Male]	-0.762 ± 0.256

[a] Reference group: female of agricultural origin.
[b] Reference group: female.

DISCUSSION

During development, organisms rely on multiple environmental cues that are integrated to produce the individual phenotype. However, human activities may alter these signals or provide

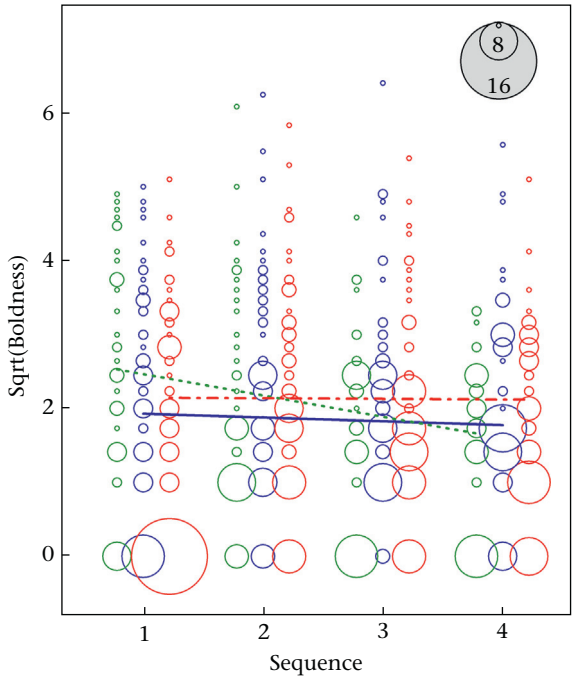

Figure 1. Boldness (square-root transformed) in relation to test sequence. Lines represent predicted values based on model-averaged estimates. Green points and the dotted line represent butterflies of woodland origin; blue points and the solid line represent butterflies of agricultural origin; red points and the dash-dotted line represent butterflies of urban origin. Points are jittered horizontally to avoid overlap and improve clarity. Point size is proportional to sample size, as exemplified by the inset, with the smallest points representing $N = 1$.

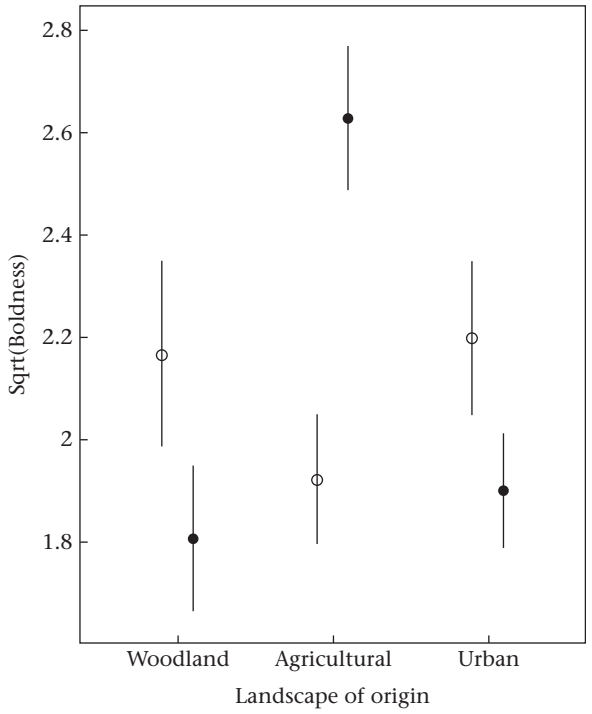

Figure 2. Boldness (square-root transformed; raw data) in relation to sex and landscape of origin. Open and filled circles refer to females and males, respectively. For each group, we show the mean ± SE.

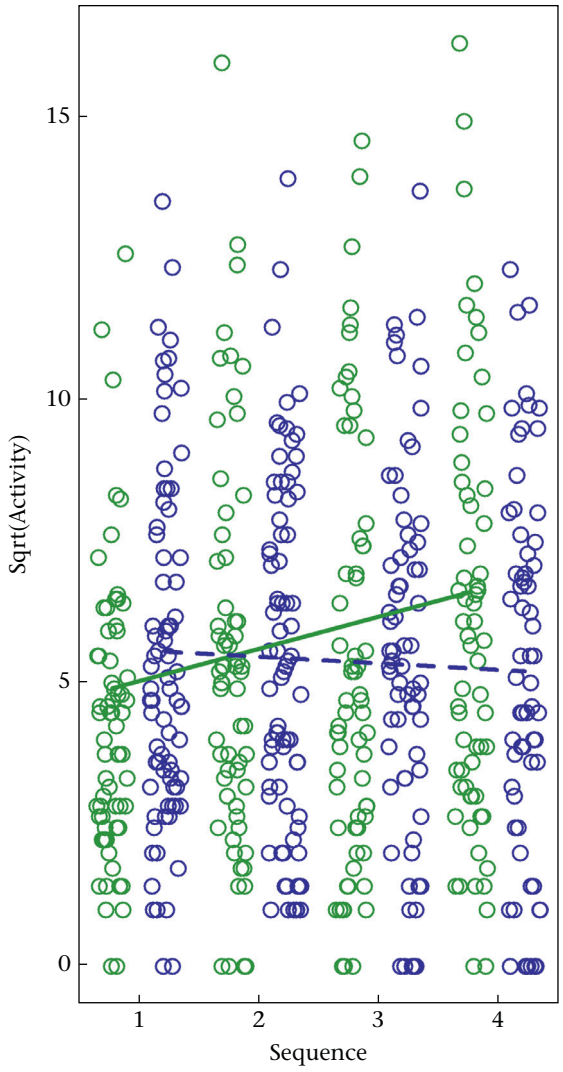

Figure 3. Activity (square-root transformed) in relation to test sequence. Lines represent predicted values based on model-averaged estimates and points are the observed values (raw data). Green points and the solid line represent females; blue points and the dashed line represent males. Points are jittered horizontally to avoid overlap and improve clarity.

developing organisms with new ones. Here, we tested for differences in adult personality traits related to the landscape of development (and the landscape of origin) in the speckled wood butterfly. While we found no difference in mean boldness and activity scores related to the landscape of development, urban conditions affected behavioural consistency (i.e. repeatability) by altering among-individual variation. We also found that mean boldness was influenced by the landscape of origin (in interaction with sequence and sex). The latter result suggests that some ecotype-related differences in personality traits can be robust to development in conditions that differ substantially from the landscape of origin conditions.

Differences between the three developmental conditions (i.e. landscapes of development) are obviously not restricted to a unique environmental component, but instead involve a cocktail of (a)biotic conditions. Microclimatic differences are the most commonly reported differences between woodland, agricultural and urban areas (e.g. Kaiser et al., 2016; Merckx et al., 2018; Serruys & Van Dyck, 2014). Here, agricultural and urban sites were on average 0.5 °C warmer than woodland sites. However, other environmental features too are likely to differ between landscape types, and such differences may influence the development of personality traits. For example, anthropogenic habitats often harbour altered communities of invertebrate predators (e.g. Dahirel, Dierick, De Cock, & Bonte, 2017; Lagucki, Burdine, & McCluney, 2017; Rocha & Fellowes, 2018), which can drive between-population differences in boldness (e.g. Harris, Ramnarine, Smith, & Pettersson, 2010; Moran, Mossop, Thompson, & Wong, 2016; but see ; Sommer-Trembo et al., 2017). Despite these multicomponent differences between landscape types, we did not detect any influence of the landscape of development on the mean expression of personality traits at the adult stage. This suggests a certain robustness of boldness and activity to contrasting developmental conditions, at least under the range of environmental conditions tested here. Similar results were found for house crickets, *Acheta domesticus*, in which early life diets did not impact personality trait averages (Royauté & Dochtermann, 2017; Royauté, Garrison, Dalos, Berdal, & Dochtermann, 2019). Similarly, Sweeney et al. (2013) found little differences in average foraging aggressiveness and boldness between field- and laboratory-reared spiders. Likewise, our results do not support our initial hypothesis that both agricultural and urban butterflies would show increased levels of developmental plasticity for behavioural traits compared to woodland origin butterflies, which typically experience more buffered developmental conditions. Hence, this may differ from the role of plasticity relative to landscape of development for other phenotypic traits like adult morphology (Merckx & Van Dyck, 2006).

Nevertheless, landscape of origin did affect boldness, although this effect occurred in two-way interactions with sequence and with sex. At the population level, boldness decreased with sequence in butterflies of woodland origin, while there was no such decrease in butterflies of agricultural or urban origin. Because the speckled wood butterfly is a short-lived species (Davies, 1978), it is important to stress that sequence (in days) integrates not only processes of learning (e.g. habituation) to the experimental

Table 3
Among-individual and residual variance (Var_{ID} and Var_R), adjusted repeatabilities (R adj.) and their 95% confidence intervals (95% CI)

		Var_{ID}	Var_{ID} 95% CI	Var_R	Var_R 95% CI	R adj	R adj 95% CI
Boldness							
	Full data set	0.593	0.479–0.731	1.177	1.070–1.343	0.337	0.286–0.379
Landscape of development	Woodland	0.223	0.114–0.381	1.237	0.956–1.649	0.150	0.086–0.224
	Agricultural	0.265	0.192–0.406	1.378	1.141–1.636	0.170	0.126–0.225
	Urban	0.855	0.610–1.211	0.955	0.813–1.176	0.502	0.393–0.564
Activity							
	Full data set	1.610	1.268–2.001	7.881	7.091–8.899	0.169	0.139–0.199
Landscape of development	Woodland	1.826	1.018–2.788	8.398	6.365–10.711	0.176	0.114–0.250
	Agricultural	2.302	1.476–3.068	7.560	6.270–9.179	0.238	0.169–0.292
	Urban	0.223	0.129–0.314	8.596	6.881–10.217	0.026	0.016–0.035

For each behavioural trait, variance components and repeatabilities are calculated for the full data set and separately for individuals from the three landscapes of development.

procedure, but also age. As such, this interaction between sequence and landscape of origin could indicate that woodland butterflies differ in their rates of habituation compared to conspecifics from human-altered habitats (e.g. Cavalli et al., 2018), or alternatively that age-related changes in boldness (i.e. rates of senescence; see Class & Brommer, 2016) differ between populations. This requires further testing. We also found that agricultural males had higher boldness scores than woodland and urban males, while such landscape-related differences were absent in females. Because large sunlit patches, allowing efficient mate detection (Bergman & Wiklund, 2009), are likely to be rarer, and speckled wood densities higher, in agricultural than in woodland areas (Merckx & Van Dyck, 2005), we assume that competition among males for favourable territories is higher in agricultural landscapes. In this context, high levels of boldness may be beneficial as bold individuals are expected to have an advantage when competing for resources (Briffa, Sneddon, & Wilson, 2015). However, the precise ecological relevance of boldness in our study system remains to be determined.

We also note that the average activity increased with sequence in females. Again, we should stress that sequence refers to concomitant effects of age and habituation. However, we believe that this increase in activity largely results from ageing virgin females that become more inclined to actively seek males to reduce costs linked to delayed mating (Bergman, Gotthard, & Wiklund, 2011). Here, females remained unmated until 4 days after emergence, which would increase fitness costs (see Wickman & Jansson, 1997) and the motivation of females to engage in active mate location. The observed increased activity for virgin females of advanced age, allowed for by the ad libitum access to honey solution, may thus increase the chances of encountering a male (Bergman et al., 2011).

While we did not detect any effect of the landscape of development on mean levels of boldness and activity, developmental conditions affected repeatability of these behavioural traits. Surprisingly, the direction of this effect differed between the two traits. Butterflies that developed under urban conditions were more repeatable for boldness than individuals reared in woodland or agricultural landscapes. However, this pattern was reversed for activity. We found that changes in repeatability mainly arose from changes in among-individual variation. As such, our results agree with recent studies showing that early developmental conditions can shape personality traits by altering variation components (i.e. within- and among-individual variation) (Han & Dingemanse, 2017), even if they had little or no effect on the mean phenotype (e.g. DiRienzo, Niemelä, Hedrick, & Kortet, 2016; Royauté & Dochtermann, 2017). Here we have shown that developmental conditions can increase among-individual variation in some, but not all, traits. In a spider species, the largest effect of social rearing on among-individual variation was observed on attack behaviour, while other behaviours were less affected or unaffected by the treatment (DiRienzo, Johnson, & Dornhaus, 2019). DiRienzo et al. (2019) suggested that this might be due to the high relevance of attack behaviour for conspecific interactions. In our case, the reason why urban conditions would trigger an increase or a decrease in among-individual variation is less clear as the ecological relevance of personality traits in our system is not fully understood (although activity might be related to territorial fighting; Kaiser, Merckx, & Van Dyck, 2019). This aspect would therefore require further investigation.

The role of developmental plasticity in shaping animal personality remains understudied. Here, we have shown limited effects of the landscape of development and associated environmental cues on mean behavioural trait values. However, we have shown that developmental conditions can affect behavioural consistency. While our reciprocal transplant experiment provides a sound approach to investigate questions related to developmental plasticity of animal personality, further mechanistic experimental approaches are needed to assess whether specific early environmental cues affect personality traits later in life.

Acknowledgments

A.K. is a research fellow with the Belgian National Fund for Scientific Research F.S.R.-FNRS, Fédération Wallonie-Bruxelles, Belgium. The research was supported by PDR-grant T.0188.14 of the F.R.S. – FNRS Fund and ARC-research grant 17/22-086 of UCLouvain and the Fédération Wallonie-Bruxelles. We thank Camille Turlure for help with rearing butterflies. This is publication number BRC 352 of the Biodiversity Research Centre (ELI/ELIB, UCL).

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Appendix

Table A1
Geographical coordinates of the studied populations (i.e. capture sites)

Site	Region	Type	Coordinates	No. of families
Zoniënwoud	Brussels	Woodland	50.817169 N, 4.451454 E	1
Nossegem	Brussels	Agricultural	50.885436 N, 4.501197 E	1
Evere	Brussels	Urban	50.868429 N, 4.411816 E	2
Heverleebos	Leuven	Woodland	50.851864 N, 4.680525 E	1
Winksele	Leuven	Agricultural	50.895566 N, 4.654337 E	2
Abdij Keizersberg	Leuven	Urban	50.887761 N, 4.698649 E	2
Schiplakenbos	Mechelen	Woodland	50.978020 N, 4.513523 E	1
Bos van Aa	Mechelen	Agricultural	51.002477 N, 4.396765 E	2
Industriepark Mechelen-Zuid	Mechelen	Urban	51.011586 N, 4.464538 E	2
Wissebos	Tienen	Woodland	50.809795 N, 5.002819 E	1
Hoegaarden	Tienen	Agricultural	50.768475 N, 4.874297 E	2
Station Tienen	Tienen	Urban	50.811842 N, 4.919830 E	2

For each population, we provide the region of origin, the landscape type and the number of tested families (i.e. number of females captured at each site).

Table A2
Number of tested butterflies for each combination of landscape of origin, landscape of development and sex

Origin development	Woodland	Agricultural	Urban
Woodland	F: 7 M: 2	F: 5 M: 6	F: 5 M: 7
Agricultural	F: 4 M: 10	F: 11 M: 10	F: 12 M: 12
Urban	F: 6 M: 8	F: 10 M: 9	F: 7 M: 15

F: females; M: males.

Figure A2. Picture of on-site host plants showing the experimental set-up .

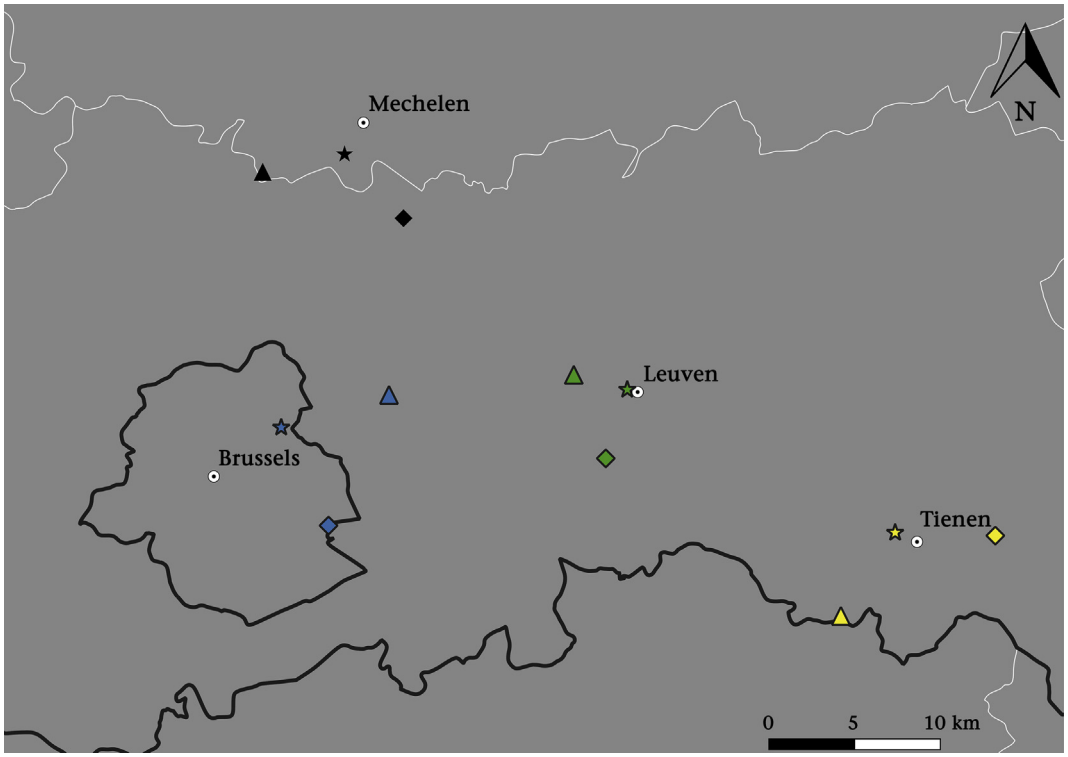

Figure A1. Location of the studied populations in Belgium. Stars, triangles and diamonds stand for urban, agricultural and woodland populations, respectively. Colours refer to the region of origin (see Table A1). Black and white lines refer to regional and provincial borders, respectively .