Urbanisation and sex affect the consistency of butterfly personality across metamorphosis

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Abstract

Animal behaviour may change with age, when young individuals experience different environments from adults. However, the role of ontogeny in personality traits is still open to debate and it is not clear whether individual differences in behaviour are maintained across important ontogenetic changes such as metamorphosis. Here, we repeatedly quantified personality in rural and urban populations of the speckled wood butterfly (*Pararge aegeria*) at the larval, pupal and adult stages. We detected significant repeatability at all life stages, showing that personality is already present at the immature stages. We found no evidence for landscape-related differences in personality traits, but adult males were bolder and more active than adult females. Adults also became bolder with trial sequence, suggesting habitation to the experimental procedure. Urbanization, together with sex, affected relationships among larval and adult personality traits. More active larvae with short latencies resulted in more explorative adults. However, this was the case in males of urban origin only, and we detected no such correlation in females or in rural males. We suggest that harsh conditions prevailing in cities may lead to stronger trait integration across metamorphosis, but also that these urbanization-related selective pressures may act differently on males and females.

Significance statement

In many taxa, metamorphosis marks the transition between the juvenile and the adult stages. During this crucial developmental step, morphology and physiology are deeply remodelled, which may have important consequences for the behaviour of individuals. Although personality has emerged as a hot topic in behavioural ecology, little is known about the consequences of metamorphosis for personality stability. We studied rural and urban speckled wood butterflies at the immature and adult stages to examine whether insect personality is retained over metamorphosis. Sexes differed regarding boldness and activity, but rural and urban butterflies behaved similarly. Nevertheless, urbanization affected relationships among larval and adult

personality traits. Some larval and adult traits correlated in urban males, whereas this was not the case in females or in rural males. This suggests that urbanization may alter trait combinations across metamorphosis, but this in a sex-specific manner.

Keywords:

Behavioural syndrome – global change – Lepidoptera – ontogeny – sexual differences

Introduction

Animal personality refers to individual behavioural differences that are consistent over time and/or across situations (Réale et al. 2007). Importantly, personality trait values are not necessarily fixed during an individual's lifespan. Instead, differences between individuals are expected to be largely maintained (Réale et al. 2007; Stamps and Groothuis 2010). Such personality traits have been documented in a wide range of organisms, including both vertebrate and invertebrate taxa (Kralj-Fišer and Schuett 2014; Vonk et al. 2017). Additionally, personality is known to be present already in the early life stages (e.g. Wuerz and Krüger 2015; Carter et al. 2016) and its consistency over a large amount of time has been demonstrated in several species (e.g. Gyuris et al. 2012; Debeffe et al. 2015). This suggests that personality traits may be robust and resilient to important ontogenetic processes such as sexual maturation.

In some taxa, internal changes occurring during ontogeny are coupled with abrupt morphological changes. This is especially the case for most amphibians and holometabolous insects, as their complete metamorphosis involves a deep remodelling of the neural and motoric system (Consoulas et al. 2000). In these groups, weak correlations between larval and adult personality traits are expected because personality is hypothesised to be linked to internal factors which undergo strong changes during metamorphosis (Amat et al. 2018). Additionally, some species experience a drastic habitat shift during ontogeny. For example, dragonfly larvae are aquatic and bee larvae live inside closed cells while adults are terrestrial in both cases. For such species, adults and larvae occupy distinct niches, use different habitat resources and face different predators. Consequently, selection pressures are likely to be very different for juveniles and adults and personality is expected to be uncoupled between these life stages (Sih et al. 2004). However, these general predictions are only partly supported by the few empirical studies available (Amat et al. 2018). For example, activity and exploration were correlated across metamorphosis in the lake frog (*Rana ridibunda*) (Wilson and Krause 2012) and larval

behavioural type relates to adult activity and boldness in the damselfly *Lestes congener* (Brodin 2009), which are both species with habitat-associated niche shifts. In species with both terrestrial larvae and adults, Rodrigues et al. (2016) detected correlations between larval and adult behavioural traits in the lady beetle *Eriopis connexa*. Contrastingly, personality variation was not consistent across metamorphosis in several beetle species (Müller and Müller 2015; Wexler et al. 2016; Monceau et al. 2017), despite larvae and adults using similar habitats and feeding on identical resources, nor in *Drosophila* fruit flies (Anderson et al. 2016). This suggests that there may not be a single pattern applying to all species undergoing complete metamorphosis, but instead that personality retention could be influenced by ecological factors other than the juvenile to adult niche shift only.

Apart from ontogeny-related changes, personality traits *per se* and behavioural syndromes (i.e. suites of correlated behavioural traits - Sih et al. 2004) are likely to be influenced by intrinsic and extrinsic factors. Males and females, for example, differ in repeatability (Bell et al. 2009) and often show behavioural differences because both sexes have distinct roles and demands. In invertebrates, males typically spend a significant proportion of their time looking for mating opportunities, whereas females do so looking for suitable oviposition sites. As such, intersexual differences in personality traits (e.g. Wexler et al. 2016; Stanley et al. 2017) and in their repeatability (e.g. Hedrick and Kortet 2012; Wexler et al. 2016) are known to occur in this group. Landscape context is another potential source of variation in personality. For example, Australian desert gobies (*Chlamydogobius eremius*) inhabiting groundwater spring rivers were less active and bolder than conspecifics from riverine waterholes (Moran et al. 2016), and populations from the two habitat types also differed in behavioural correlations (Moran et al. 2017). Similarly, colonization of a novel habitat by an aquatic isopod leads to changes in its personality and behavioural syndrome structure compared to isopods from the ancestral habitat (Karlsson Green et al. 2016). In both cases, the shifts are supposed to be caused by altered

predation risk (see also Bell and Sih 2007; Dingemanse et al. 2007) and/or by changing intensity of intraspecific competition. These examples show that different landscapes, as a result of diverging selective pressures, not only select for specific behavioural types, but also reshape relationships among traits. This is of particular interest for urban ecosystems that often show altered biotic and abiotic selective pressures compared to more natural, rural settings (Alberti 2015; Alberti et al. 2017) and thus require behavioural adjustments for a successful city life (Sol et al. 2013). This includes changes in mean trait value (Lapiedra et al. 2017; Charmantier et al. 2017; Senar et al. 2017) as well as behavioural consistency (Miranda et al. 2013; Hardman and Dalesman 2018) and trait integration (Scales et al. 2011; Carrete and Tella 2017). However, studies investigating urban-rural differences in behaviour are still largely biased toward vertebrates (Miranda et al. 2013), and only a handful of studies have looked at such differences in invertebrates (but see Tüzün et al. 2015; Kralj-Fišer et al. 2017; Schuett et al. 2018).

Here, we investigated whether personality is conserved across the larval and adult stages in the speckled wood butterfly (*Pararge aegeria*). Despite important differences in food resources among stages, both are terrestrial and there is some evidence that butterfly larvae and adults share at least part of their predators – notably birds – (Feltwell 1982). As such, we predict that this species does not experience a strong metamorphosis-associated habitat shift. Additionally, Blackiston et al. (2008) showed that learned behaviours can be retained through metamorphosis, suggesting that at least some regions of the brain are conserved during this important ontogenetic step. Hence, we expect behavioural trait correlation across metamorphosis (i.e. a behavioural syndrome across metamorphosis) in butterflies. Additionally, we expect adult urban butterflies to be bolder, more explorative and more active compared to non-urban butterflies, due to lower predation pressure from invertebrate predators and parasitoids in urbanised landscapes (e.g. Denys and Schmidt 1998; Shochat et al. 2004; Lagucki et al. 2017). We also expect intersexual differences, but only at the adult stage. Speckled wood males are

116 known to use active mate-locating strategies, whereas females make short flight bouts close to
117 the vegetation, stopping frequently to lay eggs and bask (Shreeve 1986). We therefore predict
118 adult males to be bolder and more active than adult females.

Methods

Study species and sampling sites

The Speckled wood (*Pararge aegeria* L.) is a satyrine butterfly common throughout Europe. This multivoltine species primarily occurs in woodlands but also in wooded parks in densely built-up areas (Bergerot et al. 2012). Females lay eggs singly on the leaves of various grass species upon which the larvae feed (Shreeve 1986). For the cohort following the direct developmental pathway, estimated timespans of the larval, pupal and adult stages are 24, 15 and 18 days, respectively (Bink 1992), while Davies (1978) calculated a mean life expectancy of about 7 days for adult males based on capture-recapture data and mean residence times. In September 2014, we captured gravid females at four sites in central Belgium. Rural sites (i.e. Bois de Lauzelle and Meerdaalwoud – Fig. S1) consist of large deciduous woodlands located in landscapes dominated by woodlands; urban sites (i.e. ULB Campus 'La Plaine' and Parc Schuman – Fig. S2) are parks (46 and 13 ha, respectively) of the Brussels agglomeration surrounded by a dense urban matrix (i.e. buildings and motorways) with a high prevalence of impervious surfaces. Overall, urban sites appear to be located in more fragmented landscapes. In line with recent studies on urbanization in the same region (Kaiser et al. 2016; Merckx et al. 2018), weather probes placed at two sites show warmer temperature (+1.5°C in night-time temperature) and lower relative humidity (-3% in night-time relative humidity) in urban areas. Landscape characteristics of each site and climatic data are available at Table S1 of the Supplementary material. At each site we collected three female butterflies and brought them to the laboratory for oviposition on the grass *Poa trivialis* in individual cages, which hence resulted in having 12 families in total.

Host plant and butterfly rearing

Host plants (*P. trivialis*) were grown from seeds on a standardized soil mixture and under standard conditions in a climate room (16L:8D; 25°C:16°C). We randomly selected seven eggs of each female and transferred them attached to their grass leave to individually labelled Petri dishes. We provided a piece of wet cotton to increase ambient humidity and checked every day for hatching. Larvae were fed in their Petri dish for two weeks. Leaves were changed every day and we removed excrement pellets to avoid bacterial contamination. After this period, they were transferred individually to a labelled potted plant, enclosed in nylon netting. Next, pupae were placed individually in labelled plastic cups and checked twice a day for emergence.

Behavioural tests

A single observer (AK) conducted all behavioural observations. We initially aimed to test all individuals twice in each of the tests, but this was not always feasible because some individuals did not survive till adulthood and some larvae were missed due to their small size and camouflage colours during the time spent looking for them in their potted plant. Individuals that died before adult emergence (i.e. for which sex was unknown) and adult butterflies with wing deformation were discarded from the analyses.

Larval latency and activity

Three-weeks old larvae (N=67) were submitted to an open-field test which took place in a 25°C climate room under constant light conditions. Larvae were removed from their host plant and placed on a sheet of white paper, in the centre of a 25 mm diameter circle, covered with a glass Petri dish (diam. 15 cm). At that moment, all larvae were in a defensive posture (i.e. curled), typically adopted when disturbed. We video-recorded activity during 10 minutes after an initial 30 seconds acclimation period. From the videos, we retrieved: (1) latency (in s) to resume activity (i.e. leaving the defensive posture); and (2) net displacement (in cm) (i.e. distance to the centre of the arena at the end of the test). We assume the latter behaviour reflects activity.

166 Individuals that did not resume activity within the observation period were given the maximal
167 value (i.e. 600 s – occurring in 27.6% (37/134) of the trials) and an activity score of zero. For
168 each individual, we repeated the test after 64 ± 25 minutes (mean ± SD) on the same day.

169 Pupal anti-predator behaviour

170 We conducted an anti-predator test on pupae (N=73) four days after pupation. Although
171 butterfly pupae show no mobility once attached to the pupation substrate, they are nonetheless
172 able to move abdominal segments in the form of quick movements of short duration ("jerks")
173 to scare predators and parasitoids (Cole 1959; Brakefield et al. 1992). In order to simulate an
174 encounter with a predator, individual pupae were repeatedly touched with a wooden stick. Some
175 pupae reacted to this stimulation within a few seconds. We then recorded the amount of
176 individual jerks. When individuals did not respond during the 30 s of the trial, we stopped and
177 gave a value of zero to these individuals. Each pupa was tested twice; tests were conducted in
178 early morning and late afternoon of the same day.

179 Adult boldness, exploration and activity

180 Behavioural tests on adult butterflies (N=66) started on the day following emergence, i.e. at day
181 1 (but eleven butterflies – 2 rural and 2 urban females, 3 rural and 4 urban males – were tested
182 at day 2 and two butterflies – 1 rural and 1 urban male – at day 3). After the last test, butterflies
183 were killed and stored by freezing (-21°C).

184 We designed a boldness test (i.e. activity in stressful conditions) inspired by docility tests
185 conducted on vertebrates (e.g. Martin and Réale 2008; Brommer and Kluen 2012). A butterfly
186 was placed in a semi-transparent glassine bag (63 x 97 mm envelope), which allowed to see the
187 butterfly's movements. We positioned the butterfly (with closed wings) in the centre of the bag
188 and maintained it in this position by gently pressing two of the corners of the envelope. We
189 started a stopwatch and counted the number of struggles during one minute. Here, we define

struggles as series of leg, head or wing movements interrupted by pauses of inactivity. The test took place in a 25°C room under constant light conditions. The elapsed time between the first and the second trial was around two hours.

After boldness tests, we quantified exploration and activity for each individual. Prior to each test, butterflies were placed at 30°C in an incubator for five minutes in order to raise body temperature close to the optimum to initiate voluntary flight (30-34°C; Van Dyck and Matthysen 1998). Each butterfly was then released at the extremity of a greenhouse tunnel (9 m x 4 m x 2 m; installed in a larger greenhouse maintained at a temperature of 21°C) whose floor was taped to delineate twelve squares of 1.5 m x 2 m. During the test, the observer stood still at the entrance of the tunnel and moved as little as possible. Each butterfly was allowed to move freely in the tunnel during four minutes and the observer recorded: (1) the number of squares visited at least once (hereafter adult exploration); (2) time elapsed before the first landing (hereafter adult activity). For each individual, time between the two tests was 154 ± 58 minutes (mean ± SD).

Statistical analysis

Effects of fixed variables on behavioural traits

In order to test for differences among landscapes, sexes and trials, we ran several univariate (generalized) linear mixed-effects models (*lme4* and *MASS* packages). Each of the measured behaviours was treated as the response variable. Landscape of origin (rural *versus* urban), sex, test number (sequence; first *versus* second trial) and their interactions were included as fixed effects in all models. We additionally added larval body surface (in cm^2 - retrieved from the video when larvae were in the defensive posture) and adult forewing area (in cm^2 - standardized within each sex) as proxies for larval and adult body size, respectively. For adult exploration and activity (i.e. traits measured in the greenhouse), ambient temperature was included as an

additional covariate. All models included individual identity (each individual was given a single identifier), family and sampling site of the mother as random effects.

Activity at the larval and adult stages were log-transformed, then analysed with a linear mixed model. Pupal anti-predator strategy was modelled with a Poisson distribution error, whereas adult boldness and exploration were modelled with a quasi-Poisson distribution error with the square-root link function, since equivalent generalized linear models with Poisson distribution errors showed signs of overdispersion. Larval latency time was right-censored, as larvae still immobile at the end of the test were treated as censored data. Therefore, it was modelled with a Cox proportional hazards model allowing to include random effects (*coxme* package).

Repeatability of behavioural traits

Based on the final model retained in the previous step, we used the *rptR* package (Stoffel et al. 2017) to calculate an adjusted repeatability (i.e. accounting for fixed effects) for all behavioural traits. In the case of larval latency, data were log-transformed then treated with a Gaussian error distribution. Note that we report repeatability on the link-scale for non-Gaussian data as advised by Nakagawa and Schielzeth (2010).

Within- and among-stage syndromes

In individuals for which all behavioural traits were known (N = 12 for rural females; 15 for rural males; 11 for urban females; and 19 for urban males) we tested for within- and between-stage behavioural associations. We first averaged each behavioural trait over the two successive measurements. Then we conducted a series of linear mixed models with the mean score at one test as response variable and the mean score at another test (together with landscape of origin, sex and their interactions with the behavioural score) as explanatory variables. Family and sampling site of the mother were included as random effects. When the behavioural score

included as an explanatory variable was involved in a significant interaction with sex and/or landscape of origin, we further tested the slope for significance of each group separately.

Because we expect larval traits to predict behaviour at a subsequent developmental stage, larval behavioural traits were treated as explanatory variables when adult behavioural traits were included as the response variable. When considered as the response variable, larval activity and adult boldness were log(x+1) transformed and adult activity was log-transformed to improve residual distribution, but note that this did not alter the shape of the relationship.

Since only 14% (8/57) of individuals showed a mean pupal anti-predator score greater than zero, we did not test for relationships between this trait and other larval or adult behaviour traits.

We used type II Wald tests to obtain P-values for the fixed effects. Non-significant interactions (P-value > 0.10) were removed from the model, starting with the interaction with the highest P-value.

All statistical analyses were performed with R 3.4.2 (R Core Team 2018).

Data availability

The datasets analysed during the current study are available from the corresponding author on reasonable request.

Results

Repeatability of behavioural traits

We found significant (adjusted) repeatability for all behavioural traits (Table 1). Repeatability estimates range from 0.23 for the two larval traits to 0.98 for the pupal anti-predator behaviour.

Effects of fixed variables on behavioural traits

For adult boldness, sex ($\chi^2_1 = 9.630$; $P = 0.002$) and the sequence ($\chi^2_1 = 13.226$; $P < 0.001$) sorted effects: males behaved bolder than females (males: 5.80 ± 0.54; females: 3.55 ± 0.51; mean ± SE), and adults scored higher during the second trial (first trial: 4.57 ± 0.59; second trial: 5.19 ± 0.52; mean ± SE). We also found an effect of sex on adult activity ($F_{1,55.5} = 8.799$; $P = 0.004$): the duration of the first flight bout was on average 20 seconds longer in males than in females (males: 47.18 ± 5.99 s; females: 27.69 ± 5.25 s; mean ± SE). For adult exploration, larger individuals tended to explore more (forewing area: $\chi^2_1 = 3.53$; $P = 0.060$), but there was no such effect of body size for the other adult traits (boldness: $\chi^2_1 = 0.712$; $P = 0.398$; activity: $F_{1,53.2} = 1.065$; $P = 0.306$). Neither landscape of origin as a main effect, nor its interaction with sex or sequence, had an effect on adult personality traits (all P-values > 0.10). Contrastingly, none of the tested fixed effects (sex, landscape of origin, sequence – and their interactions – and larval body size) had an influence on larval and pupal traits (all P-values > 0.10). Mean values (± SE) for all measured behaviours and associated sample sizes are available in the Supplementary material (Tables S2-7).

Within-stage correlations

We found significant within-stage correlations among behavioural traits (Table 2). Larval latency and activity were related, but the relationship between these two traits was affected by the interaction between sex and the landscape of origin. Larval latency and activity were negatively related in all groups, but the relationship was weaker in males of urban origin. Post-

hoc comparisons revealed that rural and urban females had similar slopes (rural females *versus* urban females: P = 0.860) while slopes differ between rural and urban males (rural males *versus* urban males: P = 0.004). At the adult stage, exploration and boldness were positively related as well as activity and exploration.

Among-stage associations

We detected significant associations between larval and adult traits (Table 3). Adult boldness and larval activity were negatively related (Fig. 1). The relationships between adult exploration and larval latency and between adult exploration and larval activity both depended on the interaction between sex and the landscape of origin. In urban males, larval latency and activity tend to be related to adult exploration, whereas this was not the case in females nor in males of rural origin (Fig. 2 and 3). Post-hoc comparisons showed that in both cases, slopes of the relationship were similar in rural and urban females (adult exploration and larval latency: rural females *versus* urban females: P = 0.357; adult exploration and larval activity: rural females *versus* urban females: P = 0.661), but differed between rural and urban males (adult exploration and larval latency: rural males *versus* urban males: P = 0.027; adult exploration and larval activity: rural males *versus* urban males: P = 0.012).

Discussion

We studied the stability of individual behavioural variation in the speckled wood butterfly (*Pararge aegeria*), an insect species that undergoes a complete metamorphosis. All behavioural traits were repeatable, suggesting that personality is not restricted to the adult stage in this species. While males were more active and bolder than females, mean values of the measured personality traits did not differ among individuals from rural and urban populations. Nevertheless, the consistency of personality across metamorphosis tended to depend on complex sex by landscape of origin interactions in some cases, with only urban males displaying correlations between larval and adult personality traits.

Repeatability of behavioural traits in P. aegeria

Personality traits have been documented in several butterfly species, including our study species (Ducatez et al. 2012, 2014; Ducatez and Baguette 2016). However, these studies have focused on adults only, without considering the immature stages. Here, we detected significant repeatability for all behavioural trait, spread across the three life stages of *P. aegeria*. This shows that not only adult butterflies behave consistently between the two successive trials, but that larvae too exhibited consistent individual differences in behaviour. This adds to a growing body of literature describing the existence of personality traits in the larval stage of several insect species (e.g. Alcalay et al. 2014; Wexler et al. 2016; Monceau et al. 2017; Stanley et al. 2017). Additionally, we show that pupal anti-predator behaviour was repeatable, which is, to our knowledge, the first evidence of a personality trait at this life stage. Although the pupa may be viewed as a largely immobile stage, we propose that it should not be ignored in behavioural studies. For example, pupal movements are known to deter parasitoids (Cole 1959) in some Lepidoptera, and they may thus be linked to survival. In some butterfly species, pupae are able to produce sounds and vibrations used not only in defensive contexts (Downey and Allyn 1973; Álvarez et al. 2014), but also to elicit mutualistic interactions with ants (Travassos and Pierce

2000). This behaviour has not been studied in the context of personality, but based on our results, it would be of interest to investigate inter-individual variation and consistency in pupal sound production and potential effects on ant guarding.

As a consequence of our experimental design, these estimates should be seen as short-term repeatability. Indeed, estimates were obtained from tests conducted on the same day, usually only a few hours apart. Repeatability is expected to decrease with increasing intervals between consecutive behavioural tests (Bell et al. 2009). This may at least partly explain why our repeatability estimates are relatively high (ranging from 0.23 to 0.98). Nevertheless, we expect this relatively short time interval between tests to be ecologically relevant for our study species. As already mentioned by Ducatez et al. (2012), most butterfly species are short-lived animals –the expected adult lifespan is less than two weeks for temperate-zone species without adult diapause (Scott 1974)– for which a couple of hours hence represent a significant part of their lifespan.

Sex and test sequence, but not landscape of origin, influence personality

While we did not detect any effect of the landscape of origin on the mean value of personality traits, we found strong effects of sex in two out of three adult behavioural traits. Flight bouts before the first landing were longer in males, and they also had higher boldness scores compared to females. We argue that these intersexual differences reflect the respective strategies of each sex, i.e. the type of activities they devote their time to. Under natural conditions, males either defend a small territory against conspecifics where they await females ("perching" strategy – involving frequent fast flights to intercept and chase intruders) or engage in extensive mate-location flights ("patrolling" strategy) (Shreeve 1984; Merckx and Van Dyck 2005). In contrast, females usually only make relatively short flight bouts, frequently interrupted to oviposit or hide in the vegetation (Shreeve 1986). We believe higher levels of activity and boldness are advantageous for males in order to locate mates or to gain access to high-quality territories by

deterring rivals (see for example Rudin and Briffa 2012; Lane and Briffa 2017), whereas such investment is not required for females. Interestingly, sex-related differences were not observed in larvae or pupae of the speckled wood, suggesting that intersexual differences in personality traits arise late in development in this species (Chippindale et al. 2001). Although differences among sexes in feeding and anti-predator behaviours, for example, are known to occur in the larval stage of several insects (e.g. Baker et al. 1992; Karl and Fischer 2008; Wormington and Juliano 2014), Monceau et al. (2017) found no sex-related differences in personality traits in the flour beetle *Tenebrio molitor*, neither at the larval nor at the adult stage. Thus, the developmental stage at which sex-related difference in behaviour arises is likely to be trait-specific, depending on sex- and stage-specific needs and constraints.

In addition to the sexual dimorphism in adult behavioural traits, we also observed a sequence effect on adult boldness with both males and females having higher scores during the second trial. This increase in boldness is likely a result of individual butterflies becoming more familiar with the test. Organisms usually show stress responses (i.e. 'freezing' or trying to escape) when confronted with unfamiliar conditions, but progressively overcome initial fear when repeatedly exposed to these conditions. This may take the form of an increase or decrease in the focal trait with test order, depending on the system and the trait of interest (Dingemanse et al. 2012), indicating habituation *sensu lato*. For example, activity decreased with trial order in eastern chipmunks (Martin and Réale 2008), whereas exploration increased in great tits when birds are tested repeatedly (Dingemanse et al. 2012). The here observed increase in boldness suggests that similar processes are at work in invertebrates too (see also Kralj-Fišer et al. 2017; Müller and Müller 2017).

Larval and adult personalities are linked by an urbanization × sex interaction

The existence of ontogenetic consistency of behavioural traits in insects is still a matter of debate, and evidence of such consistency remains scarce. Amat et al. (2018) found only six

368 studies investigating the link between larval and adult personality traits in holometabolous
369 insects. Interestingly, five out of these six studies showed personality uncoupling across
370 metamorphosis, with all five conducted on Coleopterans, suggesting that our current knowledge
371 on the relationship between personality consistency and metamorphosis may be taxonomically
372 biased. Here, we observed that butterfly larvae that were more active developed into shy adults.
373 In the small white butterfly (*Pieris rapae*), larval and adult life-history traits are genetically
374 associated (Tanaka 1989). Our results suggest that behavioural traits at different life-stages also
375 share a genetic basis or are linked to intrinsic variables (i.e. hormone levels and metabolic rates
376 – see Niemelä and Dingemanse 2018) in *P. aegeria*. These relationships appear robust to
377 metamorphosis despite the profound internal changes occurring during this ontogenetic step.

378 However, in some cases, relationships among larval and adult behavioural traits also depended
379 on both the landscape of origin and sex. Larval latency and activity were related to adult
380 exploration in males of urban origin, but not in females nor in rural males. Behavioural
381 syndromes may differ among populations, and those differences can sometimes be linked to
382 diverging ecological conditions such as differences in predation risk (Bell 2005; Dingemanse
383 et al. 2007) or in pesticide exposure (Royauté et al. 2014). Our results suggest that the same
384 could apply to behavioural syndromes across metamorphosis too, and that larval-adult trait
385 relationships can be shaped by both environmental conditions and intrinsic factors. In our study,
386 two of the three detected across-stage correlations were only present in urban males. Although
387 care must be taken regarding the relatively small number of tested families, these results offer
388 novel perspectives on rural-urban differences in behavioural syndromes. Urbanization has
389 repeatedly been shown to alter behavioural syndrome structures in birds (Evans et al. 2010;
390 Bókony et al. 2012). Similarly, Tüzün et al. (2017) detected an activity-boldness syndrome in
391 urban but not in rural damselfly larvae in a pesticide-free behavioural trial. The authors suggest
392 that cities provide relatively harsher conditions compared to more natural settings, and that they

hence favour tighter behavioural correlations as a result of stronger selection pressures. We believe this may be the case in our system too. *P. aegeria* is primarily a drought-sensitive (Oliver et al. 2015), woodland species whose juvenile survival tends to be negatively affected by warm and dry conditions (Schweiger et al. 2006). Elevated temperature and low humidity in urban areas (Kaiser et al. 2016; Merckx et al. 2018) are expected to impose strong selective pressures on this species through direct (i.e. egg and/or larval mortality) and indirect (i.e. changes in host-plant quality) effects (see Talloen et al. 2004). Such unfavourable developmental conditions would favour stronger trait integration and promote personality stability across metamorphosis in urban areas. Although this provide an interesting framework for explaining urban-rural differences in male behavioural syndromes in our study, a detailed evaluation of this hypothesis would be needed, especially because urbanization typically involves a cocktail of co-occurring environmental changes and its effects are likely to depend on the species' ecology. The absence of equivalent behavioural correlations in urban females probably reflects sex-specific selective pressures of urbanization (Bonier et al. 2007) due to intersexual differences in resource acquisition for growth and daily activities (i.e. mainly mate-location for males *versus* oviposition and feeding for females).

Summary and perspectives

Our results show that personality traits are not adult-specific but are also present in juvenile stages of a species with a complex life-cycle. Additionally, our study suggests that urbanization, together with sex, plays a role in shaping relationships among behavioural traits. Detailed knowledge about the fitness consequences of personality is currently lacking in our study system. Such information remains scarce for most invertebrates (but see Goulet et al. 2016). As such, we believe unravelling this link should be the next step to fully understand the ecological relevance of personality traits.

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644

Tables

Table 1: Variance of the random terms and adjusted repeatability of behavioural traits, with their associated P-value (tested by likelihood ratio test) and sample size (N). Note that some of the variances associated with the *Population* and *Family* terms are reported as zero as they are smaller than 0.001.

	Larval latency	Larval activity	Pupal anti-predator strategy	Adult boldness	Adult exploration	Adult activity
Individual	0.530	0.108	77.630	0.580	0.238	0.336
Family	0.596	0.125	0.000	0.324	0.000	0.178
Population	0.000	0.000	0.000	0.000	0.000	0.000
Residual	1.182	0.235	1.566	0.255	0.668	0.651
Adjusted Repeatability	0.23	0.23	0.98	0.50	0.26	0.28
P-value	0.006	0.005	< 0.0001	< 0.0001	0.021	0.003
N	67	67	73	66	66	66

650 Table 2: Within-stage behavioural correlations. Slopes (and their associated individual P-value)
651 are given for each group separately when interactions are significant.

652

Response variable	Explanatory variables	F-statistics	Slope ± SE
Larval activity log (x+1)	Larval latency × landscape × sex	$F_{1,43.0} = 4.494$ P = 0.039	Rural females: -0.0027 ± 0.0003 P < 0.001
			Urban females: -0.0028 ± 0.0003 P < 0.001
			Rural males: -0.0030 ± 0.0003 P < 0.001
			Urban males: -0.0017 ± 0.0003 P < 0.001
Adult exploration	Adult activity	$F_{1,50.2} = 15.593$ P < 0.001	0.0437 ± 0.0103
Adult exploration	Adult boldness	$F_{1,40.8} = 8.687$ P = 0.005	0.2793 ± 0.0865

653

654 Table 3: Among-stage behavioural correlations. Slopes (and their associated individual P-
655 value) are given for each group separately when interactions are significant.

656

Response variable	Explanatory variables	F-statistics	Slope ± SE
Adult boldness log (x+1)	Larval activity	$F_{1,25.7} = 5.483$ P = 0.027	-0.15884 ± 0.05927
Adult exploration	Larval latency × landscape × sex	$F_{1,44.9} = 4.460$ P = 0.040	Rural females: 0.0016 ± 0.0037 P = 0.655
			Urban females: 0.0066 ± 0.0038 P = 0.084
			Rural males: 0.0051 ± 0.0039 P = 0.194
			Urban males: -0.0059 ± 0.0030 P = 0.054
Adult exploration	Larval activity × landscape × sex	$F_{1,47.7} = 4.239$ P = 0.044	Rural females: -0.4724 ± 0.4292 P = 0.271
			Urban females: -0.7474 ± 0.4589 P = 0.103
			Rural males: -0.5224 ± 0.3498 P = 0.135
			Urban males: 1.2375 ± 0.6092 P = 0.042

657

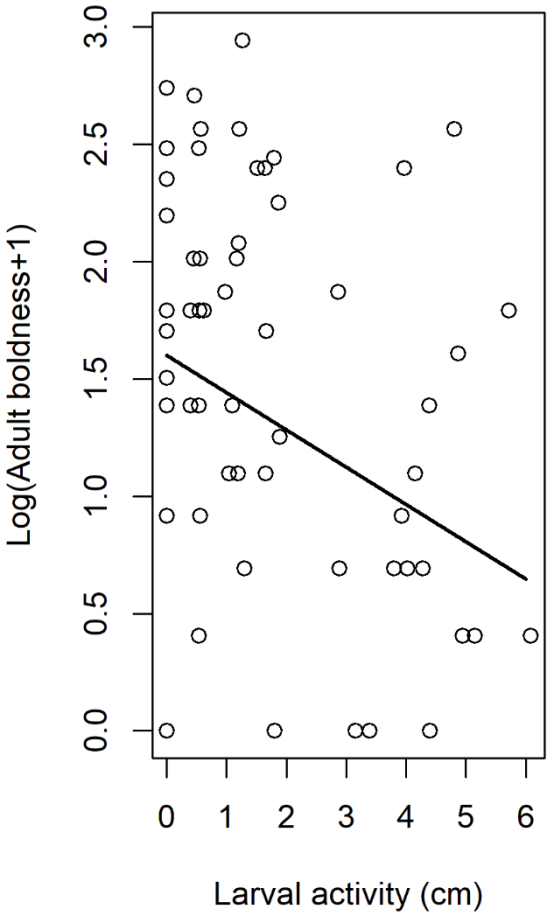
Figure captions

Fig. 1: Relationship between larval activity (in cm) and adult boldness (number of individual movements in an envelope).

Fig. 2: Relationship between larval latency (in s) and adult exploration (number of visited squares in the greenhouse tunnel) for females (left panel) and males (right panel). Open circles and dashed lines refer to rural butterflies; filled circles and plain lines refer to urban butterflies.

Fig. 3: Relationship between larval activity (in cm) and adult exploration (number of visited squares in the greenhouse tunnel) for females (left panel) and males (right panel). Open circles and dashed lines refer to rural butterflies; filled circles and plain lines refer to urban butterflies.

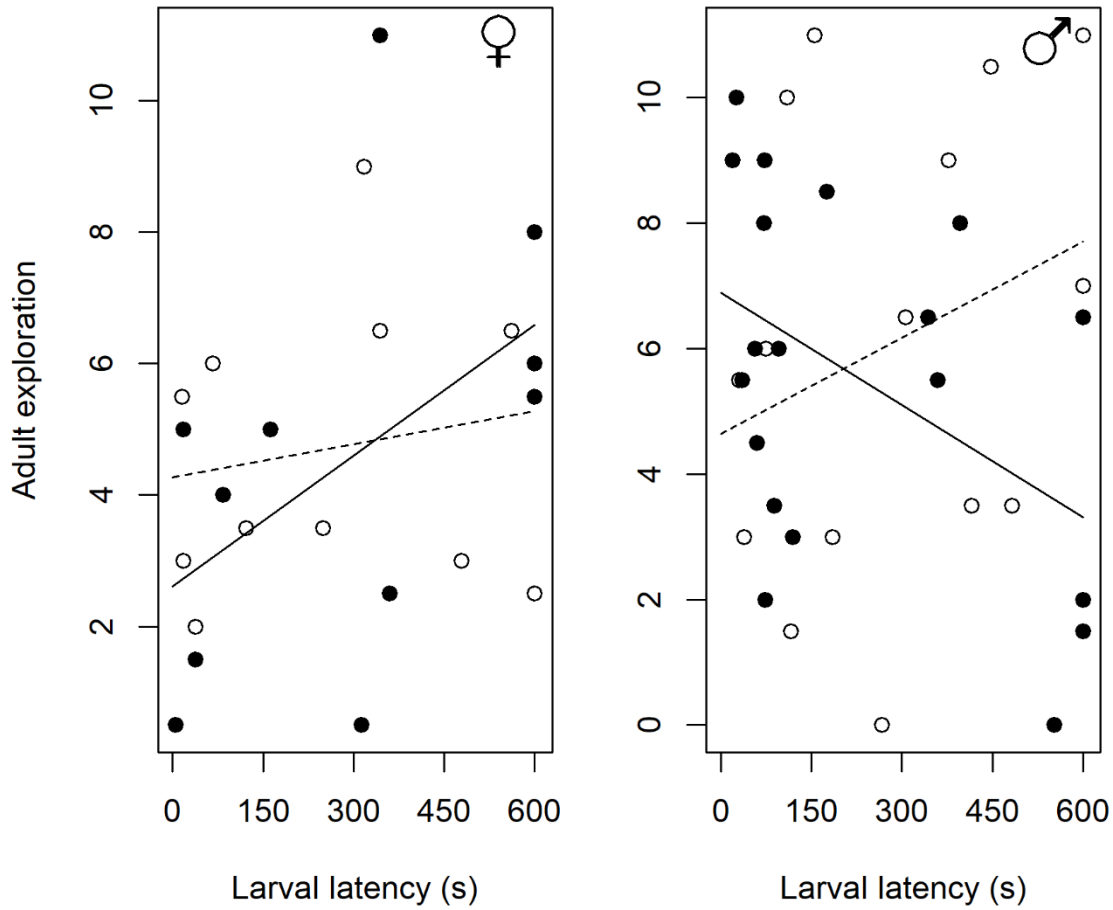
671 **Fig. 1**



672

673

674 **Fig. 2**


Adult exploration
0
2
4
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Larval latency (s)
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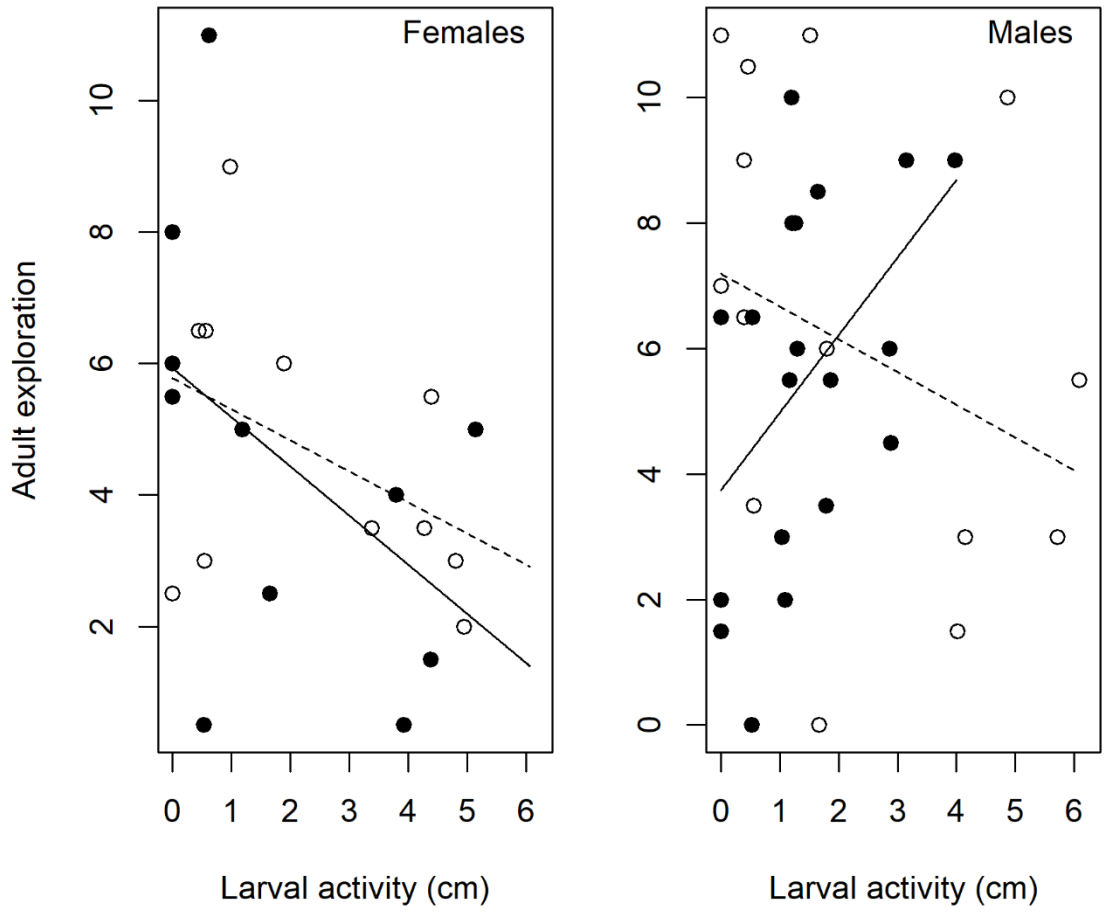
Larval latency (s)
♂

675

676 **Fig. 3**


Females
Males
Adult exploration
Larval activity (cm)
Larval activity (cm)
0
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677