Larval personality does not predict adult personality in a holometabolous insect

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Although personality (consistent inter-individual differences in behavioural traits across time and/or contexts) and behavioural syndromes (suites of correlated personality traits) have been widely studied in the last decades, the origin and development of these traits during ontogeny are still underexplored. In this context, species undergoing metamorphosis are of special interest. To date, however, the persistence of personality traits has been only little investigated in organisms undergoing complete metamorphosis such as in holometabolous insects, although this kind of studies may provide important insights from a functional point of view. Here, we tested whether the personality and the behavioural syndrome are maintained through metamorphosis in *Tenebrio molitor*, a holometabolous insect species. We found that personality and behavioural syndrome were present in both larval and adult stages. However, larval personality and behavioural syndrome did not predict adult behaviour. We suggest that the complete reorganization during metamorphosis may have profound effect on the behaviour of the beetles. These results challenge the established common thought that personality should persist along an individual lifespan.

ADDITIONAL KEYWORDS: behavioural syndrome – consistency – metamorphosis – pace of life syndrome – *Tenebrio molitor*.

INTRODUCTION

In the last decades, animal personality has received considerable interest due to its multiple implications in ecological and evolutionary processes (Chapple, Simmonds & Wong, 2012; Wolf & Weissing, 2012; Carere & Gherardi, 2013; David & Dall, 2016; Modlmeier *et al.*, 2015). Many studies have shown the existence of personality traits (i.e. consistent inter-individual differences in behavioural traits across time and/or contexts, Réale *et al.*, 2007) and behavioural syndromes (suites of personality traits correlated to each other, Réale *et al.* 2007), both in vertebrates and invertebrates (Bell, Hankison & Laskowski, 2009; Kralj-Fišer & Schuett, 2014). Although the major part of the literature aims at understanding how these consistent inter-individuals differences across contexts can be maintained and evolve in

natural populations (see Carere & Maestripieri, 2013 for a review), the genesis of personality traits and how they progress during ontogeny are still underexplored (Stamps & Groothuis, 2010; Wilson & Krause, 2012a; Herde & Eccard, 2013; Sweeney *et al.*, 2013).

Ontogeny is characterized by successive transitions from immature to mature stages until the death of the individual. In species undergoing metamorphosis, the individuals endure an extensive reorganization of neural tissues as well as major physiological, morphological, and/or behavioural modifications (Wigglesworth, 1954; Truman & Riddiford, 2002). These physical modifications are frequently linked to ecological niche shift and are thus associated with changes in the selective pressures exerted on individuals (Stoks & Córdoba-Aguilar, 2012). Despite these fundamental changes, memory acquired during immature stages can be maintained over lifetime and influence adult behaviour. For example, the chemical recognition acquired during larval stage is maintained in adults for nest mate recognition, host and/or host plant preference

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as demonstrated in Hymenoptera (*Hyssopus pallidus*: Gandolfi, Mattiacci & Dorn, 2003 and *Aphaenogaster senilis*: Signorotti, Jaisson & d'Ettorre, 2014) and in Lepidoptera (*Manduca sexta*: Blackiston, Silva Casey & Weiss, 2008; *Spodoptera littoralis*: Proffit *et al.*, 2015). To date, different studies have evidenced that rank order for personality traits as well as behavioural syndrome could be maintained during ontogeny (Herde & Eccard, 2013; Petelle *et al.*, 2013; Sweeney *et al.*, 2013; McCowan & Griffith, 2014) and across metamorphosis in anurans (Wilson & Krause, 2012b) and heterometabolous insects (both in hemimetabolous metamorphosis: Brodin, 2009 and paurometabolous metamorphosis: Gyuris, Feró & Barta, 2012; Hedrick & Kortet, 2012; Niemelä *et al.*, 2012). Some level of plasticity could be adaptive during ontogeny (Fischer *et al.*, 2014; Nettle & Bateson, 2015). However, the range of expression of such a plasticity is genetically determined and should exhibit inter-individual differences, which should be conserved along ontogeny (Sih *et al.*, 2004; Wilson & Krause, 2012b). These results are in line with the work by Sokolowski (2001) on the genetic determinism of the behavioural type rover and sitter in *Drosophila melanogaster*. However, unlike anurans and heterometabolous insects, holometabolous insects (e.g. Diptera, Coleoptera, Lepidoptera) undergo extreme transformations during a complete metamorphosis, including a profound remodelling of the nervous system (Kalogianni, Consoulas & Theophilidis, 1989; Consoulas *et al.*, 2000; Tissot & Stocker, 2000). To our knowledge, although it may represent a major constraint for the evolution of personality in these insect species, the persistence of personality and behavioural syndrome in insects with complete metamorphosis has only been tested twice so far, in *Phaedon cochleariae* (Müller & Müller, 2015) and in *Tribolium castaneum* (Wexler *et al.*, 2016). In both cases, personality traits were present in larvae or adults but were not consistent across metamorphosis.

In the present study, we tested whether personality and behavioural syndrome are maintained across the ontogeny of the holometabolous insect, *Tenebrio molitor* (Coleoptera: Tenebrionidae). This species represents a common pest that feeds on food resources stored for human purposes, and its use for laboratory studies presents two major advantages. First, it easily breeds under laboratory conditions closed to field conditions. Second, the neurophysiological reorganization occurring during metamorphosis has been widely studied in this species (see for example Breidbach, 1990a, b, c). Four behavioural traits, activity, exploration, food neophobia (that is a measure of boldness), and gregariousness, all commonly used to describe the different dimensions of personality (Réale *et al.*, 2007) were tested here. Different trajectories (i.e. evolution of the traits) over the ontogeny have been found in

holometabolous (Müller & Müller, 2015) and heterometabolous insect species (Brodin, 2009; Gyuris *et al.*, 2012; Hedrick & Kortet, 2012; Niemelä *et al.*, 2012). In *T. molitor*, there is neither a habitat nor dietary ontogenic shift: larvae and adults live in the same environment with overlapping generations and low dispersal, exploit the same resources, and are thus exposed to similar environmental constraints. We expected that, if existing at the larval stage, personality traits and behavioural syndrome should persist across metamorphosis in this insect species, unless the functional reorganization that occurs during metamorphosis influences the maintenance of these traits (Mather & Logue, 2013). The ontogenetic trajectories may also differ between males and females due to sexual selection. Indeed, conspicuous sex-specific behaviours such as male calling in *Gryllus integer* may select for lower boldness in males (Hedrick & Kortet, 2012). Since no such behaviours are known in *T. molitor*, we did not expect a difference between females and males *T. molitor* for their ontogenetic trajectories.

MATERIAL AND METHODS

ETHICAL NOTE

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. *Tenebrio molitor* larvae of different ages were obtained from a local producer (Insectes Production Vente, Dannemarie-sur-Crête, France) where they were fed on bran flour and supplemented with carrots and potatoes. They were obtained as larvae and then reared in the laboratory in large tanks (60 × 40 × 20 cm) containing a mix of bran and piglet flour (2:1 ratio), water ad libitum to limit cannibalism (Johnston & Lee, 1990), and large pieces of paper towel as refuge. No sign of parasitism or fungal/bacterial infection were observed. Insects were maintained at 28 ± 1 °C and 60 ± 5% relative humidity in the absence of light as they naturally avoid light (Cloudsley-Thompson, 1953). Experiments were performed under red light in order to create a darkness condition compatible with an observation by experimenters. Handling was restricted to the minimum required by the experiment to limit stress. The minimum number of individuals was used in this study to provide a sufficient sample size for the analyses.

GENERAL DESIGN

The experiment consisted of four behavioural tests realized sequentially in the same order for all individuals: (1) activity, (2) exploration, (3) food neophobia (i.e. reaction to a novel food source), and (4) gregariousness. All individuals were tested in the afternoon for standardization purpose. The exact lifetime of *T. molitor* is

highly variable according to the rearing conditions. In these experimental conditions, the adults typically live for 1–2 months. The larval developmental period is *c.* 160 days (see Kim *et al.*, 2015), but the number of instars is also highly variable (from 14 to 20 instars; Park *et al.*, 2014). Similar to the works by Wilson & Krause (2012b) and Wexler *et al.* (2016), we chose to repeat the sequences of the four tests on consecutive days (i.e. with a 24-h interval) for each larva or adult. Such an interval between two consecutive tests allowed assessing repeatability of the measurements within developmental stage and not across stages.

Twenty-four hours before the first test, larvae were individually isolated in Petri dishes (Ø 9 cm) containing ad libitum bran and piglet flour (2:1 ratio), water, and a piece of paper towel as refuge. Larvae significantly reduced their activity and feeding behaviour 24 h before metamorphosis as previously observed by Howard (1955), and there was no unambiguous a priori cue for assessing the time to metamorphosis. We therefore had to rely on a conservative a posteriori criterion to select our experimental individuals. Additionally, the manipulation of the larvae seemed to shorten the delay until the beginning of metamorphosis (Karine Monceau, personal observation). We thus limited our manipulation to two replicates for each behavioural test per individual at the larval and the adult stages. Only larvae for which the metamorphosis began at least 3 days after the second session of behavioural tests were considered in the analysis (*n* = 96, 44 females, 52 males). We also verified that no variation in activity was observed below this limit. The mean delay between the second session of larval tests and the onset of metamorphosis was 13 ± 5 days (mean ± SD, median = 12 days). After the behavioural assessments at the larval stage, larvae were kept individually in their Petri dish until adulthood in the same conditions as previously described. Therefore, they could be followed individually from the larval to the adult stages. Behavioural assessments of the adults were performed at 11 ± 1 days after emergence. All individuals were sexed after the last behavioural experiment to limit handling stress. All tests were video monitored (Sony HDR-CX405 HD Handycam), and video records were subsequently analyzed by the same person (JR) to avoid inter-observer bias in scoring (Burghardt *et al.*, 2012).

Behavioural tests

The four tests were designed to quantify inter-individual variance in behaviour (see Supporting Information 1). Each behavioural test was performed for 5 min as preliminary tests proved that such a duration was sufficient to assess between-individual variability. A 1-min acclimatization period occurred prior to each test: the focal individual was kept in a small plastic cup (Ø 3 cm × 3 cm height) in the middle of the arena for exploration and gregariousness tests and at the opposite of the food source for the neophobia test. During the acclimatization period prior to the activity test, the focal individual was free to move in the arena without being isolated under a plastic cup. The recording of the behaviour began when the acclimatization period ended. Except for the food neophobia test, no resource (water or food) was provided within the apparatus.

Activity test

The activity arena was made of a 9-cm-diameter Petri dish fixed upside down in the centre of a 14-cm Petri dish, forming a 2.5-cm-wide circular pathway. The bottom of the arena was lined with a paper sheet on which eight radial sectors were drawn. The paper sheet was changed after each test to prevent bias caused by odour released by the insect. The number of transitions between sectors was recorded as the individual score of activity: the higher the score, the more the individual was active.

Exploration test

The exploration arena was made of a 9-cm-diameter Petri dish regularly divided in three radial sectors, each containing substrates of different grain size: sand (0.1 mm), small (*c.* 2–3 mm), and large gravels (*c.* 5–6 mm). These three different substrates differed in term of sensitive environment and burrowing potential, which is an important behaviour in *T. molitor* (Cloudsley-Thompson, 1953). The substrates were changed after each individual test to remove potential pheromonal marking. A circular area (Ø 3 cm) in the centre of the Petri dish where the individual was acclimatized was free of substrate. The number of transitions between sectors was recorded as the exploration score: the higher the score, the more the individual was explorative.

Food neophobia

Food neophobia was measured as the feeding response to new food items that our focal individuals never encountered either at the larval or adult stage. A new piece of novel food item (*c.* 125 mm³) known to be accepted by both larvae and adults of *T. molitor* was used for each test. Larvae were transferred in a new 14-cm-diameter Petri dish and were tested first with cucumber and second with orange. Similarly, adults were tested first with apple and second with banana. These food sources were used for two reasons. First, they are known to be used by some raisers to feed their beetles. Second, these fruits and vegetables contain more than 70% moisture that is susceptible to attract

T. molitor (Baek *et al.*, 2015). The latency to reach the food source was recorded as the individual score of neophobia: the higher the score, the more the individual is neophobic. In order to avoid the confounding effect of individual differences in the degree of starvation, all the individuals have been fed ad libitum prior experiments. Thus, the latency to reach the new food item should reflect more the boldness of the individual than its motivation for feeding.

Gregariousness

Gregariousness was tested in a rectangular plastic box ($16 \times 10 \times 10$ cm^3) divided in three sectors: two small compartments (1.5-cm large) at each side of the box separated from a large central compartment (13-cm large) by opaque plastic separators, perforated with regularly spaced holes (Ø 2 mm), allowing chemical communication and direct physical contact (see Weaver & McFarlane, 1989; Weaver, McFarlane & Alli, 1989; Carazo *et al.*, 2004, 2009). The focal individual was placed in the central compartment. Four unrelated individuals were placed in one of the two small compartments (hereafter referred as 'social compartment') at random, whereas the other small compartment at the opposite side was kept empty. These individuals were randomly chosen from the stock culture: four larvae were used when assessing a focal larva, while two adult males and two adult females were used when focal adults were assessed (to prevent from sexual attraction bias). The individual score of gregariousness was recorded as the time required for the insect to reach the social zone which was defined as the 1.5-cm-wide area (i.e. one body length) next to the social compartment: the higher the score, the more the individual is solitary compared to the other individuals from the experimental group. While *T. molitor* is described as a gregarious species, the aim of this test was to quantify consistent individual differences in this behaviour. The individual score of gregariousness has to be understood as a relative measure compared to the average response at the group level.

Paper sheets, substrate, Petri dishes, plastic boxes, and cups were used for a single individual test. They were either discarded and replaced after each test or carefully washed and dried before reuse to prevent interaction with odours along successive tests (see Weaver & McFarlane, 1989; Weaver *et al.*, 1989; Carazo *et al.*, 2004, 2009).

STATISTICAL ANALYSES

Statistical analyses were realized with R 3.1.2 (R Core Team, 2014). Repeatability between the first and the second replicates of each behavioural test was estimated for larvae and adults separately. The scores of activity and exploration followed a negative binomial

distribution. Following Nakagawa & Schielzeth (2010), repeatability for these measurements was assessed using concordance correlation coefficients (Lin, 1989, 2000; Carrasco, 2010; Schweitzer, Motreuil & Dechaume-Moncharmont, 2015) using the package epiR (Stevenson *et al.*, 2016). The scores of food neophobia and gregariousness (or their transformation) did not follow any classical distribution. For these measurements, we therefore assessed the repeatability based on ranks using Spearman's correlation coefficients. In each case, repeatability coefficients were reported with their bootstrapped 95% confidence interval (95% CI). To assess the potential impact of the distance to the metamorphosis, we divided our sample of larvae based on the delay to metamorphosis (below and above the median of our sample, i.e. 12 days) and then computed de novo the repeatability coefficients that allow direct comparisons of the 95% CI for the two groups. Differences between sexes for each replicate were tested using the Cliff's δ assorted with its 95% CI using effsize package (Torchiano, 2014). This effect size estimator was preferred to the classical Cohen's *d* because the data did not fit with classical probability distribution (Cliff, 1996; Nakagawa & Cuthill, 2007; Macbeth, Razumiejczyk & Ledesma, 2011; Ivarsson *et al.*, 2013). The Cliff's δ was also used to compare the score at the larval and adult stages. The interpretation of the magnitude of the differences between sexes or between larval and adult stages was based on thresholds provided by Romano *et al.* (2006): δ < 0.147: negligible differences, δ < 0.33: small differences, δ < 0.474: medium differences, and δ ≥ 0.474: large differences.

For repeatable measurements, we computed the average of individual scores measured during the first and the second behavioural tests and used it as data point thereafter. In order to characterize behavioural syndromes, the correlations between repeatable personality traits were tested using Spearman's rank correlation with Benjamini–Hochberg correction for multiple tests in order to minimize inflation of Type 1 error (Benjamini & Hochberg, 1995). The first axis of the principal componentanalysis (PCA) taking into account all the repeatable personalitytraits correlated in syndrome was then used as asynthetic personality score for the larvae and theadults separately. The link between the synthetic score and each personality trait of the individuals at the larval and adult stages was assessed using Spearman's correlation coefficient.

RESULTS

The scores for all tests are presented in Figure 1. For all replicates, no differences between sexes were detected either in larvae or in adults (Table 1). Adults were always more active and explorative
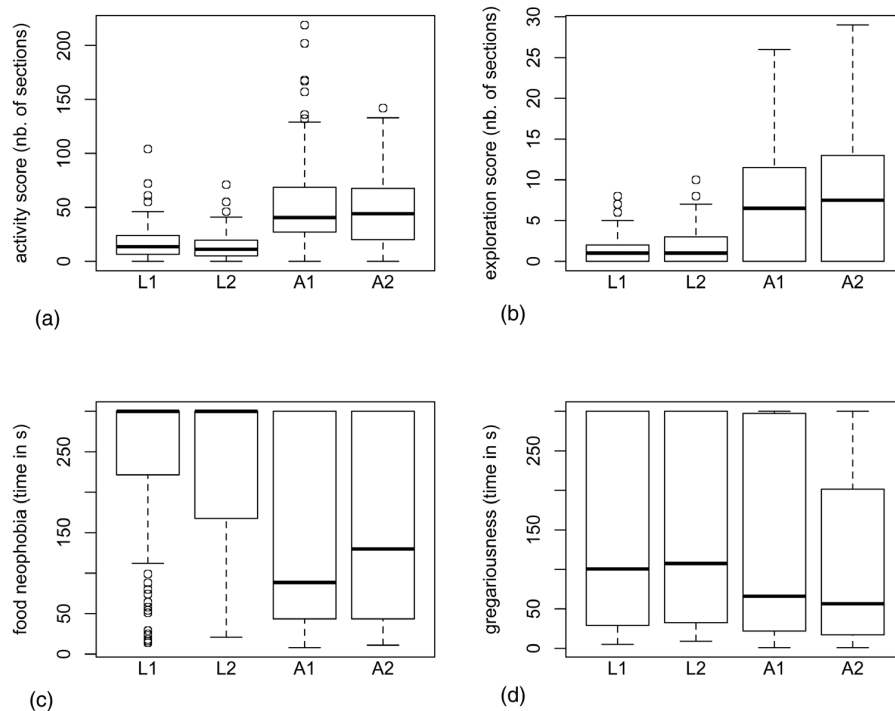
Figure 1. Behavioural scores of the *Tenebrio molitor* (n = 96) tested twice at the larval (L1 and L2) stage and twice at the adult (A1 and A2) stage for each behavioural test: (a) activity, (b) exploration, (c) food neophobia, and (d) gregariousness. Bold lines, boxes, dashed lines, and open circles stand for medians, middle two quartiles, 1.5× interquartile ranges, and extreme values, respectively.

Table 1. Sexual dimorphism in the four behavioural traits for each replicate (R1 and R2) at the larval and the adult stages measured with Cliff's δ and its 95% confidence interval on 44 females and 52 males

Behavioural trait	Replicate	Larvae	Adults
Activity	R1	0.09 (−0.14; 0.32)	0.12 (−0.11; 0.35)
	R2	0.13 (−0.10; 0.35)	0.12 (−0.12; 0.34)
Exploration	R1	0.01 (−0.21; 0.24)	0.22 (−0.008; 0.43)
	R2	0.11 (−0.12; 0.32)	0.07 (−0.17; 0.29)
Food neophobia	R1	0.01 (−0.18; 0.20)	0.03 (−0.21; 0.26)
	R2	−0.12 (−0.30; 0.07)	0.14 (−0.09; 0.36)
Gregariousness	R1	0.07 (−0.16; 0.30)	−0.01 (−0.24; 0.22)
	R2	−0.004 (−0.24; 0.23)	0.11 (−0.13; 0.34)

Traits in which intervals cross 0 are not dimorphic. R1, replicate 1; R2, replicate 2.

[activity: Cliff's δ = 0.73 with 95% CI = (0.61; 0.82) and exploration: δ = 0.65 with 95% CI = (0.50; 0.76)], less food neophobic and gregarious than larvae [food neophobia: δ = −0.56 with 95% CI = (−0.68; −0.42) and gregariousness: δ = −0.17 with 95% CI = (−0.33; −0.01)]. For all traits, the two groups of larvae (less than 12 days and more than 12 days to metamorphosis) did not differ. Therefore, we pooled these two stages and considered the overall sample of larvae

in the subsequent analysis (Supporting Information 2). We found short-term repeatability for the activity and exploration scores both at the larval and adult stages with similar level of repeatability (Table 2). Measurements of food neophobia were also found repeatable but at the adult stage only, while gregariousness was repeatable neither at the larval nor at the adult stage. Activity and exploration were positively correlated in both larvae and adults (Table

3). In adults, exploration was also negatively correlated to food neophobia, but there was no correlation between activity and food neophobia (Table 3). In order to allow comparisons between larval and adult personality scores, the PCA for both stages were computed using the same behaviours, exploration and activity scores. For larvae, the first axis of the PCA explained 73.41% of the total variance (factor loadings: activity and exploration, −0.71 both, Fig. 2a). Low synthetic behavioural scores (i.e. low PC1 score) corresponded to highly active and explorative larvae and high scores to the less active and exploratory larvae (negative factor loadings). For adults, the first axis of the PCA explained 66.75% of the total variance (factor loadings: activity and exploration, 0.71 both, Fig. 2b). Low synthetic behavioural scorescorresponded to the less activeand exploratory adults and, high scores to highly active and explorative adults. Finally, the behaviour of the individuals at the larval and the adult stages was not correlated [Spearman's rank correlation: activity: $r_s = 0.02$, $N = 96$, $P = 0.84$, 95% CI = $(−0.18; 0.22)$; exploration: $r_s = −0.01$, $N = 96$, $P = 0.89$, 95% CI = $(−0.22; 0.19)$; synthetic behavioural score: $r_s = −0.02$, $N = 96$, $P = 0.82$, 95% CI = $(−0.19; 0.23)$, Fig. 3]. This result was consistent in males and females [synthetic behavioural score, males: $r_s = 0.10$, $N = 52$, $P = 0.44$, 95% CI = $(−0.17; 0.36)$ and females: $r_s = −0.07$, $N = 44$, $P = 0.68$, 95% CI = $(−0.38; 0.22)$].

Table 2. Repeatability assorted with their 95% confidence interval for each behavioural trait for individuals at the larval and adult stages ($n = 96$)

Behavioural trait	Larvae	Adults
Activity*	**0.41 (0.23; 0.55)**	**0.50 (0.34; 0.63)**
Exploration*	**0.26 (0.06; 0.43)**	**0.29 (0.09; 0.46)**
Food neophobia†	0.05 (−0.17; 0.26)	**0.33 (0.15; 0.51)**
Gregariousness†	0.18 (−0.008; 0.36)	0.12 (−0.08; 0.32)

Traits in which intervals do not cross 0 are significantly repeatable (bold).
*Repeatability tested with Lin's concordance correlation coefficient.
†Repeatability tested with Spearman's rank correlation.

DISCUSSION

This study supports the existence of personality both in larvae and adults of *T. molitor*. Overall, individuals at the adult stage were more active and explorative but less neophobic and gregarious than at the larval stage. Activity and exploration were found to be repeatable and correlated, thus forming a so-called behavioural syndrome. The existence of such a syndrome is consistent with previous studies on animal (vertebrates and invertebrates) personality because these behaviours are closely related to general locomotion abilities, although they were tested differently (Réale *et al.*, 2007). However, in our study, the personality traits and the behavioural syndrome of the larvae did not predict those of the adult. This result contrasts with previous studies (Sokolowski, 2001; Brodin, 2009; Gyuris *et al.*, 2012; Niemelä *et al.*, 2012; Wilson & Krause, 2012b) but is in agreement with the results obtained in holometabolous species (Müller & Müller, 2015; Wexler *et al.*, 2016).

Personality and behavioural syndrome are assumed to be maintained within population due to their association with life-history and physiological traits, thus resulting in different fitness payoff strategies that form a continuum of slow to fast living individuals. This theoretical framework is an extension of the life-history theory, the so-called pace-of-life syndrome (POLS) hypothesis (Réale *et al.*, 2010). Several studies suggested for instance a functional relationship between personality and energy metabolism (see Careau *et al.*, 2008, 2015). In *T. molitor*, Krams *et al.* (2013) reported a link between metabolic rate and boldness that is also linked to higher mortality risk under predation. However, the POLS hypothesis implicitly assumes that an individual born as slow or fast living type belongs to this category for its entire life, except when the individuals experienced contrasting pressures during ontogeny (Fischer *et al.*, 2014; Nettle & Bateson, 2015). Yet, in *T. molitor*, larvae and adults share the same resources and live in high promiscuity (Howard, 1955). To our knowledge, sexual behaviour has been poorly investigated in this species, and no obvious sex differences are reported to date. Anyway, we might have found sex differences similar to *T. castaneum* (Wexler *et al.*, 2016)
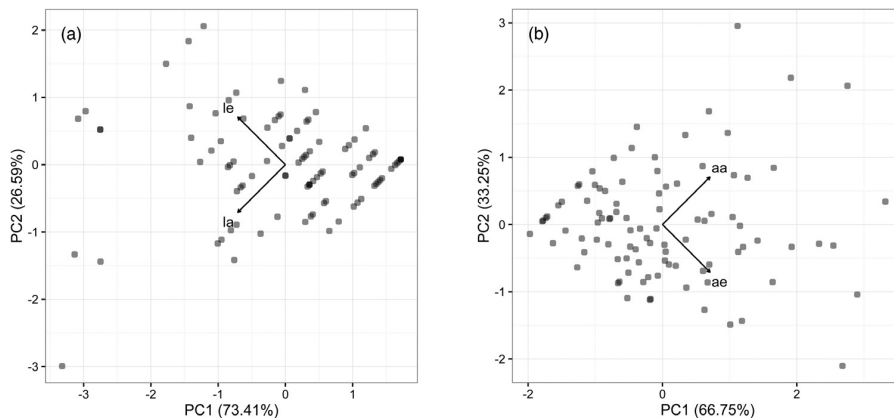
Table 3. Matrix of correlations between repeatable personality traits in larvae (above diagonal) and adults (below diagonal)

	Activity	Exploration	Food neophobia
Activity	–	**0.52 (0.35; 0.66)**	NT
Exploration	**0.35 (0.17; 0.53)**	–	NT
Food neophobia	−0.10 (−0.31; 0.11)	**−0.37 (−0.55; −0.16)**	–

Spearman's rank coefficients are provided with their 95% confidence interval. Correlations in which intervals do not cross 0 are significant (bold). NT, not tested.

Figure 2. Principal component analysis (PCA) graphical representation for (a) larvae and (b) adults. Arrows represent the personality traits included in the PCA. aa, adult activity; ae, adult exploration; la, larval activity; le, larval exploration.
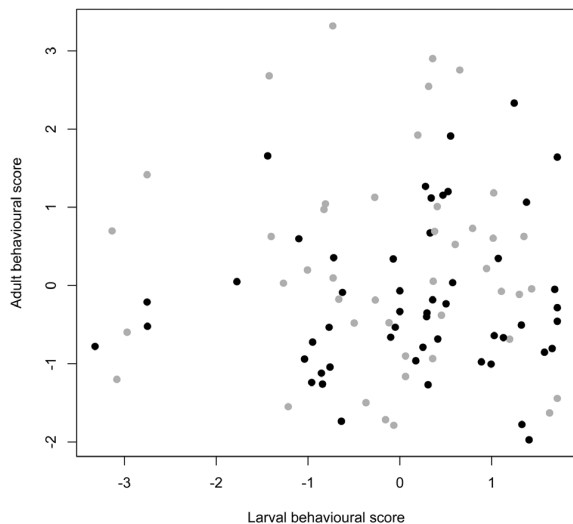

Figure 3. Relationship between synthetic behavioural scores (first axis of the principal component analysis) at the larval and adult stages (females in grey dots and males in black dots). In adults, positive synthetic behavioural score corresponds to highly active and explorative individuals (positive factor loadings) while in larvae, positive synthetic behavioural score corresponds to the less active and explorative individuals (negative factor loadings).

due to the phylogenetical proximity between these two species (Zhang *et al.*, 2016). However, no such difference was detected in *T. molitor* either in larvae or in adults. Therefore, no fundamental shift in behaviour was expected here, except those that would arise from the metamorphosis process itself.

Previous works that investigated the effects of metamorphosis on the behaviour of *T. molitor* were however in accordance with the prediction of behavioural type persistence of the POLS hypothesis. These experiments aimed at testing the retention of learning across metamorphosis; interestingly, they found that memory was preserved in this species suggesting that at least some parts of the brain remain stable over this radical process (Borsellino, Pierantoni & Schieti-Cavazza, 1970; Alloway, 1972). Here, we show that larval personality does not predict the adult personality although the behavioural measurements were repeatable within each stage. These results suggest that the existence of such a within-stage consistency is constitutive, that is genetically determined while the intensity of the behaviour (i.e. the score per se) would be functionally determined, that is related to the metabolism, physiological, and/or neurological processes or under genetic regulation that could be modified during metamorphosis (Krams *et al.*, 2013). In *Schistocerca americana* (Orthoptera), changes in the metabolism of locomotory behaviour were observed between young and adult individuals although this species is heterometabolous (Kirkton & Harrison, 2006; Kirkton, Nyberg & Fox, 2011). Some proteins such as heat shock proteins (Hsp) whose expression is related to the resistance to stress are known to be weakly related between life stages (Sørensen, Kristensen & Loeschcke, 2003). In a recent study on *T. molitor*, Lardies *et al.* (2014) suggested that heat-inducible genes might differ between larvae and adults. This kind of regulation (i.e. age-dependent genetic regulation) may thus provide a potential mechanism that could explain why some behaviours may differ between the larval and the adult stages. We would like to emphasize here that testing behavioural traits closer to the metamorphosis was almost impossible due to the major change in activity level

we observed in the late instar larvae and in the newly emerged non-melanized adults.

CONCLUSION AND PERSPECTIVES

Although our results challenge the POLS hypothesis as a way to explain the consistency of personality traits and behavioural syndrome, they also question the adaptive value of this shift in behaviour observed across metamorphosis. This study also opens new perspectives in a similar way of those highlighted by Class & Brommer (2015), who stressed the importance of considering behavioural syndromes at different stages of the development of the individuals. Indeed, although the emphasis has been already put on the need of understanding the evolution of personality during development, the effects of ontogeny on personality and behavioural syndrome are still rarely considered (Stamps & Groothuis, 2010; Groothuis & Trillmich, 2011; Wilson & Krause, 2012a; Biro & Stamps, 2015; Class & Brommer, 2015). To date, we still lack evidence for a functional basis of personality, but *T. molitor* might represent an ideal model species to understand the role of metamorphosis.

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REFERENCES

Alloway TM. 1972. Retention of learning through metamorphosis in the grain beetle (*Tenebrio molitor*). *American Zoologist* **12:** 471–477.

Baek S, Perez AE, Turcotte RM, White JB, Adedipe F, Park Y-L. 2015. Response of *Tenebrio molitor* (Coleoptera: Tenebrionidae) adults to potato: implications for monitoring and sampling. *Journal of Stored Products Research* **60:** 5–10.

Bell AM, Hankison SJ, Laskowski KL. 2009. The repeatability of behaviour: a meta-analysis. *Animal Behaviour* **77:** 771–783.

Benjamini Y, Hochberg Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society. Series B (Methodological)* **57:** 289–300.

Biro PA, Stamps JA. 2015. Using repeatability to study physiological and behavioural traits: ignore time-related change at your peril. *Animal Behaviour* **105:** 223–230.

Blackiston DJ, Silva Casey E, Weiss MR. 2008. Retention of memory through metamorphosis: can a moth remember what it learned as a caterpillar? *PLoS ONE* **3:** e1736.

Borsellino A, Pierantoni R, Schieti-Cavazza B. 1970. Survival in adult mealworm beetles (*Tenebrio molitor*) of learning acquired at the larval stage. *Nature* **225:** 963–964.

Breidbach O. 1990a. Reorganization of persistent motoneurons in a metamorphosing insect (*Tenebrio molitor* L., Coleoptera). *The Journal of Comparative Neurology* **302:** 173–196.

Breidbach O. 1990b. Serotonin-immunoreactive brain interneurons persist during metamorphosis of an insect: a developmental study of the brain of *Tenebrio molitor* L. (Coleoptera). *Cell & Tissue Research* **259:** 345–360.

Breidbach O. 1990c. Constant topological organization of the coleopteran metamorphosing nervous system: analysis of persistent elements in the nervous system of *Tenebrio molitor*. *Journal of Neurobiology* **21:** 990–1001.

Brodin T. 2009. Behavioural syndrome over the boundaries of life—carryovers from larvae to adult damselfly. *Behavioral Ecology* **20:** 30–37.

Burghardt GM, Bartmess-LeVasseur JN, Browning SA, Morrison KE, Stec CL, Zachau CE, Freeberg TM. 2012. Perspectives—minimizing observer bias in behavioral studies: a review and recommendations. *Ethology* **118:** 511–517.

Carazo P, Font E, Forteza-Behrendt E, Desfilis E. 2009. Quantity discrimination in *Tenebrio molitor*: evidence of numerosity discrimination in an invertebrate? *Animal Cognition* **12:** 463–470.

Carazo P, Sanchez E, Font E, Desfilis E. 2004. Chemosensory cues allow male *Tenebrio molitor* beetles to assess the reproductive status of potential mates. *Animal Behaviour* **68:** 123–129.

Careau V, Montiglio P-G, Garant D, Pelletier F, Speakman JR, Humphries MM, Réale D. 2015. Energy expenditure and personality in wild chipmunks. *Behavioral Ecology and Sociobiology* **69:** 653–661.

Careau V, Thomas D, Humphries MM, Réale D. 2008. Energy metabolism and animal personality. *Oikos* **117:** 641–653.

Carere C, Gherardi F. 2013. Animal personalities matter for biological invasions. *Trends in Ecology and Evolution* **28:** 5–6.

Carere C, Maestripieri D. 2013. *Animal personalities: behavior, physiology, and evolution*. Chicago: University of Chicago Press.

Carrasco JL. 2010. A generalized concordance correlation coefficient based on the variance components generalized linear mixed models for overdispersed count data. *Biometrics* **66:** 897–904.

Chapple DG, Simmonds SM, Wong BBM. 2012. Can behavioral and personality traits influence the success of unintentional species introductions? *Trends in Ecology and Evolution* **27:** 57–64.

Class B, Brommer JE. 2015. A strong genetic correlation underlying a behavioural syndrome disappears during development because of genotype–age interactions. *Proceedings of the Royal Society B: Biological Sciences* **282:** 20142777.

Cliff N. 1996. Answering ordinal questions with ordinal data using ordinal statistics. *Multivariate Behavioral Research* **31:** 331–350.

Cloudsley-Thompson JL. 1953. Studies in diurnal rhythms. IV. Photoperiodism and geotaxis in *Tenebrio*

molitor L. (Coleoptera: Tenebrionidae). *Proceedings of the Royal Entomological Society of London. Series A, General Entomology* **28:** 10–12.

Consoulas C, Duch C, Bayline RJ, Levine RB. 2000. Behavioral transformations during metamorphosis: remodeling of neural and motor systems. *Brain Research Bulletin* **53:** 571–583.

David M, Dall SRX. 2016. Unravelling the philosophies underlying 'animal personality' studies: a brief re-appraisal of the field. Ethology 122:1–9.

Fischer B, van Doorn GS, Dieckmann U, Taborsky B. 2014. The evolution of age-dependent plasticity. *American Naturalist* **183:** 108–125.

Gandolfi M, Mattiacci L, Dorn S. 2003. Preimaginal learning determines adult response to chemical stimuli in a parasitic wasp. *Proceedings of the Royal Society B: Biological Sciences* **270:** 2623–2629.

Groothuis TGG, Trillmich F. 2011. Unfolding personalities: the importance of studying ontogeny. *Developmental Psychobiology* **53:** 641–655.

Gyuris E, Feró O, Barta Z. 2012. Personality traits across ontogeny in firebugs, *Pyrrhocoris apterus*. *Animal Behaviour* **84:** 103–109.

Hedrick AV, Kortet R. 2012. Sex differences in the repeatability of boldness over metamorphosis. *Behavioral Ecology and Sociobiology* **66:** 407–412.

Herde A, Eccard JA. 2013. Consistency in boldness, activity and exploration at different stages of life. *BMC Ecology* **13:** 49.

Howard RS. 1955. The biology of the grain beetle *Tenebrio molitor* with particular reference to its behavior. *Ecology* **36:** 262–269.

Ivarsson A, Andersen MB, Johnson U, Lindwall M. 2013. To adjust or not adjust: nonparametric effect sizes, confidence intervals, and real-world meaning. *Psychology of Sport and Exercise* **14:** 97–102.

Johnston SL, Lee RE Jr. 1990. Regulation of supercooling and nucleation in a freeze intolerant beetle (*Tenebrio molitor*). *Cryobiology* **27:** 562–568.

Kalogianni E, Consoulas C, Theophilidis G. 1989. Anatomy and innervation of the abdominal segment muscles in larval and adult *Tenebrio molitor* (Coleoptera). *Journal of Morphology* **202:** 271–279.

Kim SY, Park JB, Lee YB, Yoon HJ, Lee KY, Kim NJ. 2015. Growth characteristics of mealworm Tenebrio molitor. *Journal of Sericultural and Entomological Science* **53:** 1–5.

Kirkton SD, Harrison JF. 2006. Ontogeny of locomotory behaviour in the American locust, *Schistocerca americana*: from marathoner to broad jumper. *Animal Behaviour* **71:** 925–931.

Kirkton SD, Nyberg ET, Fox KM. 2011. Ontogenetic changes in citrate synthase and lactate dehydrogenase activity in the jumping muscle of the American locust (*Schistocerca americana*). *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* **160:** 110–116.

Kralj-Fišer S, Schuett W. 2014. Studying personality variation in invertebrates: why bother? *Animal Behaviour* **91:** 41–52.

Krams I, Kivleniece I, Kuusik A, Krama T, Freeberg TM, Mänd R, Vrublevska J, Rantala MJ, Mänd M. 2013. Predation selects for low resting metabolic rate and consistent individual differences in anti-predator behavior in a beetle. *Acta Ethologica* **16:** 163–172.

Lardies MA, Arias MB, Poupin MJ, Bacigalupe LD. 2014. Heritability of hsp70 expression in the beetle *Tenebrio molitor*: ontogenetic and environmental effects. *Journal of Insect Physiology* **67:** 70–75.

Lin LI. 1989. A concordance correlation coefficient to evaluate reproducibility. *Biometrics* **45:** 255–268.

Lin LI. 2000. A note on the concordance correlation coefficient. *Biometrics* **56:** 324–325.

Macbeth G, Razumiejczyk E, Ledesma RD. 2011. Cliff's delta calculator: a non-parametric effect size program for two groups of observations. *Universitas Psychologica* **10:** 545–555.

Mather JA, Logue DM. 2013. The bold and the spineless: invertebrate personalities. In: Carere C, Maestripieri D, eds. *Animal personalities: behavior, physiology, and evolution*. Chicago: University of Chicago Press, 13–35.

McCowan LSC, Griffith SC. 2014. Nestling activity levels during begging behaviour predicts activity level and body mass in adulthood. *PeerJ* **2:** e566.

Modlmeier AP, Keiser CN, Wright CM, Lichtenstein JLL, Pruitt JN. 2015. Integrating animal personality into insect population and community ecology. *Current Opinion in Insect Science* **9:** 77–85.

Müller T, Müller C. 2015. Behavioural phenotypes over the lifetime of a holometabolous insect. *Frontiers in Zoology* **12:** S8.

Nakagawa S, Cuthill IC. 2007. Effect size, confidence interval and statistical significance: a practical guide for biologists. *Biological Reviews* **82:** 591–605.

Nakagawa S, Schielzeth H. 2010. Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. *Biological Reviews* **85:** 935–956.

Nettle D, Bateson M. 2015. Adaptive developmental plasticity: what is it, how can we recognize it and when can it evolve? *Proceedings of the Royal Society B: Biological Sciences* **282:** 20151005.

Niemelä PT, Vainikka A, Hedrick A, Kortet R. 2012. Integrating behaviour with life history: boldness of the field cricket, *Gryllus integer*, during ontogeny. *Functional Ecology* **26:** 450–456.

Park JB, Choi WH, Kim SH, Jin HJ, Han YS, Lee YS, Kim NJ. 2014. Developmental characteristics of *Tenebrio molitor* larvae (Coleoptera: Tenebrionidae) in different instars. *International Journal of Industrial Entomology* **28:** 5–9.

Petelle MB, McCoy DE, Alejandro V, Martin JGA, Blumstein MT. 2013. Development of boldness and docility in yellow-bellied marmots. *Animal Behaviour* **86:** 1147–1154.

Proffit M, Khallaf MA, Carrasco D, Larsson MC, Anderson P. 2015. 'Do you remember the first time?' Host plant preference in a moth is modulated by experiences during larval feeding and adult mating. *Ecology Letters* **18:** 365–374.

R Core Team. 2014. *R: a language and environment for statistical computing, version 3.1.2*. Vienna: R Foundation for Statistical Computing.

Réale D, Garant D, Humphries MM, Bergeron P, Careau V, Montiglio P. 2010. Personality and the emergence of the pace-of-life syndrome concept at the population level. *Philosophical Transactions of the Royal Society B: Biological Sciences* **365:** 4051–4063.

Réale D, Reader SM, Sol D, McDougall PT, Dingemanse NJ. 2007. Integrating animal temperament within ecology and evolution. *Biological Reviews* **82:** 291–318.

Romano J, Kromrey JD, Coraggio J, Skowronek J. 2006. Appropriate statistics for ordinal level data: should we really be using t-test and Cohen's d for evaluating group differences on the NSSE and other surveys? Annual Meeting of the Florida Association of Institutional Research, Cocoa Beach, 1–3 February.

Schweitzer C, Motreuil S, Dechaume-Moncharmont F-X. 2015. Coloration reflects behavioural types in the convict cichlid, Amatitlania siquia. *Animal Behaviour* **105:** 201–209.

Signorotti L, Jaisson P, d'Ettorre P. 2014. Larval memory affects adult nest-mate recognition in the ant *Aphaenogaster senilis*. *Proceedings of the Royal Society B: Biological Sciences* **281:** 20132579.

Sih A, Bell AM, Johnson JC, Ziemba RE. 2004. Behavioural syndromes: an integrative overview. *Quarterly Review of Biology* **79:** 241–277.

Sokolowski MB. 2001. *Drosophila*: genetics meets behaviour. *Nature Reviews Genetics* **2:** 879–890.

Sørensen JG, Kristensen TN, Loeschcke V. 2003. The evolutionary and ecological role of heat shock proteins. *Ecology Letters* **6:** 1025–1037.

Stamps J, Groothuis TGG. 2010. The development of animal personality: relevance, concepts and perspectives. *Biological Reviews* **85:** 301–325.

Stevenson M, Nunes T, Heuer C, Marshall J, Sanchez J, Thornton R, Reiczigel J, Robison-Cox J, Sebastiani P, Solymos P, Yoshida K, Jones G, Pirikahu S, Firestone S, Kyle R. 2016. epiR: tools for the analysis of epidemiological data, R package version 0.9-74. Available at: http://CRAN.R-project.org/package=epiR. Accessed 6 September 2016.

Stoks R, Córdoba-Aguilar A. 2012. Evolutionary ecology of Odonata: a complex life cycle perspective. *Annual Review of Entomology* **57:** 240–265.

Sweeney K, Gadd RDH, Hess ZL, McDermott DR, MacDonald L, Cotter P, Armagost F, Chen JZ, Berning AW, DiRienzo N, Pruitt JN. 2013. Assessing the effects of rearing environment, natural selection, and developmental stage on the emergence of a behavioral syndrome. *Ethology* **119:** 436–447.

Tissot M, Stocker RF. 2000. Metamorphosis in drosophila and other insects: the fate of neurons throughout the stages. *Progress in Neurobiology* **62:** 89–111.

Torchiano M. 2014. effsize: efficient effect size computation, R package version 0.5.2. Available at: https://cran.r-project.org/web/packages/effsize/index.html. Accessed 15 May 2016.

Truman JW, Riddiford LM. 2002. Endocrine insights into the evolution of metamorphosis in insects. *Annual Review of Entomology* **47:** 467–500.

Weaver DK, McFarlane JE. 1989. Aggregation in yellow mealworms, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) larvae: II. Observations and analyses of behavioral parameters in aggregation. *Journal of Chemical Ecology* **15:** 1617–1627.

Weaver DK, McFarlane JE, Alli I. 1989. Aggregation in yellow mealworms, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) larvae: I. Individual and group attraction to frass and isolation of an aggregant. *Journal of Chemical Ecology* **15:** 1605–1615.

Wexler Y, Subach A, Pruitt, JN, Scharf I. 2016. Behavioral repeatability of flour beetles before and after metamorphosis and throughout aging. *Behavioral Ecology and Sociobiology* **70:** 745–753.

Wigglesworth VB. 1954. *The physiology of insect metamorphosis*. London: Cambridge University Press.

Wilson ADM, Krause J. 2012a. Metamorphosis and animal personality: a neglected opportunity. *Trends in Ecology and Evolution* **27:** 529–531.

Wilson ADM, Krause J. 2012b. Personality and metamorphosis: is behavioural variation consistent across ontogenetic niche shifts? *Behavioral Ecology* **23:** 1316–1323.

Wolf M, Weissing FJ. 2012. Animal personalities: consequences for ecology and evolution. *Trends in Ecology and Evolution* **27:** 452–461.

Zhang H-L, Liu B-B, Wang X-Y, Han Z-P, Zhang D-X, Su C-N. 2016. Comparative mitogenomic analysis of species representing six subfamilies in the family Tenebrionidae. *International Journal of Molecular Sciences* **17:** 841.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Supporting Information 1. Experimental apparatus for the four behavioural tests.
Supporting Information 2. Lin's concordance correlation coefficient assorted with their 95%CI. (Lin's coefficient for activity and exploration, Spearman's coefficient for food neophobia and gregariousness). The sample was divided into two groups: the larvae tested at more than 12 days (23 females and 24 males) and the larvae tested at less than 12 days (21 females and 28 males) prior to metamorphosis.