

Personality, immune response and reproductive success: an appraisal of the pace-of-life syndrome hypothesis

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Summary

1. The pace-of-life syndrome (POLS) hypothesis is an extended concept of the life-history theory that includes behavioural traits. The studies challenging the POLS hypothesis often focus on the relationships between a single personality trait and a physiological and/or life-history trait. While pathogens represent a major selective pressure, few studies have been interested in testing relationships between behavioural syndrome, and several fitness components including immunity.

2. The aim of this study was to address this question in the mealworm beetle, *Tenebrio molitor*, a model species in immunity studies. The personality score was estimated from a multidimensional syndrome based of four repeatable behavioural traits.

3. In a first experiment, we investigated its relationship with two measures of fitness (reproduction and survival) and three components of the innate immunity (haemocyte concentration, and levels of activity of the phenoloxidase including the total proenzyme and the naturally activated one) to challenge the POLS hypothesis in *T. molitor*. Overall, we found a relationship between behavioural syndrome and reproductive success in this species, thus supporting the POLS hypothesis. We also showed a sex-specific relationship between behavioural syndrome and basal immune parameters.

4. In a second experiment, we tested whether this observed relationship with innate immunity could be confirmed in term of differential survival after challenging by entomopathogenic bacteria, *Bacillus thuringiensis*. In this case, no significant relationship was evidenced.

5. We recommend that future researchers on the POLS should control for differences in evolutionary trajectory between sexes and to pay attention to the choice of the proxy used, especially when looking at immune traits.

Key-words: *Bacillus thuringiensis*, behavioural syndrome, fitness, immunity, longevity, survival

Introduction

Variation in behaviour among individuals of a given species was disregarded for a long time because it was considered a simple variance around an adaptive mean. Within the last decade, this opinion has been progressively replaced by an individual-based view of behavioural

variability (see Wilson 1998 as a first base and Carere & Maestripieri 2013 for a recent synthesis). Several studies have shown that individuals consistently behave differently from each other across a wide range of contexts (i.e. predation and/or competition) for several behavioural traits (such as activity, boldness, exploration and/or neophobia, see Carere & Maestripieri 2013). These traits, called personality traits, and their correlates, namely behavioural syndromes, have been then studied in a large variety of species within the animal kingdom (Bell, Hankinson & Laskowski 2009; Kralj-Fišer & Schuett 2014).

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How these consistent behavioural differences are maintained and evolve within populations is a central issue in behavioural ecology because of their ecological and evolutionary implications, as for example in the context of biological invasions (Chapple, Simmonds & Wong 2012; Carere & Gherardi 2013) or disease transmission (Dizney & Dearing 2013; Ezenwa *et al.* 2016; see also Wolf & Weissing 2012 for an overview). Interestingly, before the rise of personality studies, behavioural traits were rarely integrated into the classical life-history theory framework (Stearns 1976). Between-individual differences in behaviour can, however, be associated to different life-history strategies, and thus to fitness payoff. Therefore, personality traits can be integrated into an extended concept that already includes life-history and physiological traits, called the pace-of-life syndrome hypothesis (POLS, Réale *et al.* 2010). The POLS hypothesis can be summarized as 'live fast, die young'. This assumes that individuals, who are growing the fastest, are also those that reproduce the earliest and exhibit the shortest life span. In order to fuel highly demanding metabolic activity, these individuals (namely proactive) are expected to show high-risk behaviours such as high level of activity and exploration (Réale *et al.* 2007, 2010). In line with this, boldness and growth rate were found positively correlated in the crayfish *Cherax destructor*, whatever the context (Biro, Adriaenssens & Sampson 2014). The POLS as a mechanism explaining the maintenance of personality and behavioural syndromes is still debated. Indeed, some studies found evidence supporting the POLS hypothesis (e.g. Niemelä *et al.* 2013), while others failed to evidence any relationship between behaviour and life-history and/or physiological traits (e.g. Klueen, Siitari & Brommer 2014; Royauté *et al.* 2015). Studies testing the POLS hypothesis often focus on the relationship between a single personality trait and a single life-history and/or physiological trait (Bijleveld *et al.* 2014; Biro, Adriaenssens & Sampson 2014; Cole & Quinn 2014; Krams, Kivleniece, Kuusik, Krama, Freeberg, Mänd, Vrublevska *et al.* 2013; Krams, Kivleniece, Kuusik, Krama, Freeberg, Mänd, Rantala *et al.* 2013; Krams *et al.* 2014; Montiglio *et al.* 2014 but see Klueen, Siitari & Brommer 2014). Assessing the correlation between only one personality trait and only one life-history trait is highly questionable. Indeed, personality or life-history traits are fundamentally multidimensional (Réale *et al.* 2010). Reliable assessment of the relationship between these traits should thus be based on multidimensional measures.

To date, the effect of the interaction between personality and pathogens on fitness has been neglected (see for a recent review Ezenwa *et al.* 2016), while it is considered as a major selective force shaping life-history strategies (Barber & Dingemanse 2010; Kortet, Hedrick & Vainikka 2010). On the one hand, pathogens can manipulate the behaviour of their intermediate host by increasing risk-taking behaviours, which ultimately favours parasite transmission to its final host (see for review Adamo 2013; Poulin 2013). Therefore, infection is susceptible to modify

host personality. On the other hand, host behaviour could also affect risk of parasitism thus impacting infectious disease epidemiology (Combes 2001; Barber & Dingemanse 2010; Hawley *et al.* 2011; Wolf & Weissing 2012; Dizney & Dearing 2013). For instance, the probability of being infected by Sin Nombre virus is three times lower in shy than in bold deer mice because the former engage less often in social context than the latter (Dizney & Dearing 2013). The immune system is the last line of defence against pathogens. It is divided into two major lines: one generic, i.e. the constitutive immune response and one specific, i.e. the induced immune response that supposes several expositions to the pathogens (Siva-Jothy, Moret & Rolff 2005). Fast-living/high risk-taking individuals that are more exposed to novel environments and potential pathogens (Barber & Dingemanse 2010) are thus supposed to be selected for efficient immune defence and more specifically, for generalist (i.e. constitutive) immunity. Therefore, under POLS hypothesis, fast-living/high risk-taking individuals should invest more in constitutive immune response than slow-living/low risk-taking individuals, while the reverse should be observed for induced immune defence (Ricklefs & Wikelski 2002; Lee 2006). For example, Lee *et al.* (2008) showed that the duration of incubation in birds was positively correlated with the antibody level suggesting that birds with longer incubation period can invest more in specific immune defence. In the house finch, *Haemorhous mexicanus*, the individuals that are more exploratory (risk-taking) have also higher level of antibodies (Zylberberg, Klasing & Hahn 2014).

Potential association between personality, life-history and immune function has been recently investigated in field crickets (Niemelä *et al.* 2013). However, this study was restricted to (i) a single dimension of the immune function (namely encapsulation), and (ii) two behavioural measures (defreezing and overall boldness) recorded in a single test, and therefore corresponding to a single personality dimension, the boldness. The authors used an inert nylon filament to stimulate the encapsulation of a macroparasite. Although this technique is widely used, it does not actually provide an integrative view of the activation of the whole immune system contrary to the injection of live bacteria for instance (Charles & Killian 2015). Indeed, caution should be taken in the way immunological proxies are interpreted because immunity is also a multidimensional function (Biard *et al.* 2015) and might not be sufficient to actually predict immune efficacy (Pham & Schneider 2008). In the present study, we assessed the relationship between (i) personality and fitness and (ii) personality and immunity. In order to account for multidimensionality of personality, fitness and immunity, our tests were based on composite indices taking into account several measures for each component. These experiments were performed on the mealworm beetle, *Tenebrio molitor*, which presents two major advantages. First, it easily breeds in laboratory in conditions closed to the field. Second, this is a model species for immuno-ecology studies (see Moret & Siva-Jothy 2003;

Moret 2006; Zanchi *et al.* 2011, 2012; Moreau *et al.* 2012). Four behavioural traits, activity, exploration, food neophobia (that is a measure of boldness) and gregariousness, all describing the proactive (i.e. fast-living/high risk-taking)–reactive (i.e. slow-living/low risk-taking) continuum (Sih, Bell & Johnson 2004; Sih *et al.* 2004; Laubu *et al.* 2016), were examined similar to Monceau *et al.* (2017a). Then, in a first experiment, we tested for potential relationships between personality traits and (i) two measures of fitness, the number of viable offspring and adults survival duration, and (ii) measures of the constitutive immune response, such as haemocyte concentration, and levels of activity of the phenoloxidase enzymes. Indeed, haemocyte concentration and enzymes of the phenoloxidase cascade are major component of innate immunity in invertebrates, which are often good predictors of insect investment into immunocompetence (Eslin & Prévost 1998; Fellowes, Kraaijeveld & Godfray 1998; Kraaijeveld, Limentani & Godfray 2001; Siva-Jothy, Moret & Rolff 2005). In a subsequent experiment, we exposed mealworm beetles to more stringent conditions of rearing by increasing the ambient temperature to enhance possible trade-offs between personality traits and an integrative measure of immunity that is the resistance to infection. Once tested for their behaviour, individuals were challenged with the entomopathogenic bacterium, *Bacillus thuringiensis* (Bt), to evaluate their ability to survive the bacterial infection. According to POLS hypothesis, proactive mealworm beetles were expected to exhibit (i) shorter life span and higher number of offspring, (ii) higher level of constitutive immune response but (iii) to be less affected by bacteria infection than the reactive individuals.

Materials and methods

ETHICAL STATEMENT

All applicable international, national and/or institutional guidelines for the care and use of animals were followed.

INSECT REARING AND ISOLATION

The mealworm beetles originated 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 kept in large tanks (60 × 40 × 20 cm) containing a mix of bran and piglet flour (3 : 1 ratio) and water *ad libitum* in standard laboratory conditions (24 ± 1 °C, 60 ± 5% RH, permanent darkness). In experiment 1, adult beetles were additionally supplemented with carrots once a week. Carrots are a source of carotenoids that are antioxidants involved in immune function (Cornet, Biard & Moret 2007; Babin, Biard & Moret 2010; Babin *et al.* 2015). In experiment 2, as we constrained the conditions to exacerbate the trade-offs involving immunity, the beetles were not supplemented with carrots and were maintained at 28 ± 1 °C and 60 ± 5% RH in the absence of light as they naturally avoid light (Cloudsley-Thompson 1953).

Pupae were randomly collected in stock populations and newly emerged adults were maintained individually in Petri dishes (Ø 9 cm) containing bran and piglet flour (3 : 1 ratio), water *ad libitum* and a piece of paper towel as refuge. When 10 days old (± 1 day), the beetles were included into the experiments. Experiment 1 used a total of 82 individuals (41 males and 41 females) and experiment 2 used a total of 236 individuals (109 males and 127 females).

EXPERIMENTAL DESIGN

Overview (Fig. 1)

Prior to both experiments, beetle personality was evaluated on four behavioural traits. In experiment 1, the individuals were successively tested for (i) their constitutive immune system, and (ii) their fitness (reproduction and survival). In experiment 2, they were only challenged with *Bt* and their survival to the infection was monitored.

Personality tests

Personality tests were realized in the same conditions of temperature and humidity as those used for rearing, and under red light

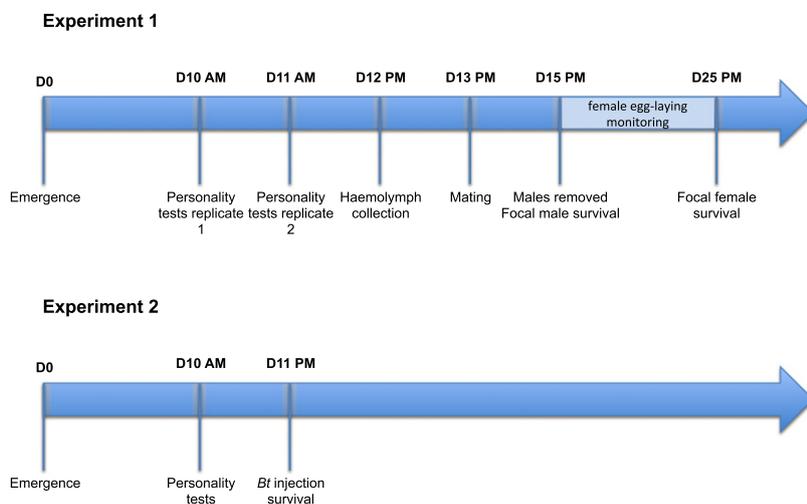


Fig. 1. Experimental design overview. The chronology of the experiment is given based on the age of the beetle from their emergence at day 0 (D0). [Colour figure can be viewed at wileyonlinelibrary.com]

in order to create darkness condition compatible with observation by experimenters. All individuals were tested in the morning. The four behavioural traits were tested sequentially in the same order for all individuals: (i) activity, (ii) exploration, (iii) food neophobia (i.e. reaction to novel food source) and (iv) gregariousness following Monceau *et al.* (2017a, see a complete description of the tests in Appendix S1, Supporting Information). Briefly, activity score was assessed as the number of transitions between sections in a round arena divided into eight sectors of equal size. Measure of individual exploration was obtained in a circular arena from the number of transitions between three radial sectors of equal size, each being recovered with substrates of different granulometry (either sand, fine gravel or large gravel) that stimulated burrowing, which is an ecologically relevant behaviour in *T. molitor* (Cloudsley-Thompson 1953). Food neophobia was estimated by the latency to reach a food item that beetles have never eaten (the higher the score, the more neophobic the individual was). Finally, gregariousness was assessed by the latency to reach the social zone constituted of four beetles (two males and two females) (the higher the score, the more solitary the individual was). After testing for behavioural traits, the insects were kept in their Petri dish until the next experiment was performed in the same conditions as those used for rearing.

Petri dishes and plastic boxes used for the tests were used for only one individual and one test, and then carefully washed and dried before reuse. All tests were video monitored, and the records were subsequently analysed by the same person (RC for experiment 1 and CL for experiment 2) using JWatcher software (v 1.0, Blumstein & Daniel 2007). Repeatability of the behavioural measurements was assessed using individuals from experiment 1 ($n = 82$), which were tested twice (two replicates for each behavioural test) at 24 h of interval. This time interval is within the range of intervals used in previous studies using biological models of similar longevity than *T. molitor* (see for few examples Pruitt, Riechert & Jones 2008; Pruitt, Grinsted & Settepani 2013; Wexler *et al.* 2016; Monceau *et al.* 2017a). A summary of the behavioural scores for each experiment is given in Table S1. As the behavioural traits were repeatable in experiment 1, individuals from experiment 2 were only assessed just once in order to limit manipulation that would induce stress as reported in previous studies on *T. molitor* larvae (Monceau *et al.* 2017a). Additionally, in invertebrates, the effects of a stress differ as a function of the immune parameter considered (Adamo 2012). Increasing the number of repeated tests may thus impact the outcome of the challenge by *Bt*.

Experiment 1

Basal immune parameters. In the afternoon following the second series of behavioural tests, the baseline level of constitutive immune defences including haemocyte concentration, and activity levels of the phenoloxidase enzymes were assessed. Prior to haemolymph collection, insects were weighted (Sartorius ED124S, precision ± 1 mg Sartorius AG, Goettingen, Germany) and anaesthetized on ice for 5 min. Then, a 3- μ L sample of haemolymph was collected from a wound in the membrane between the thorax and the abdomen using a chilled glass capillary (Ringcaps[®], Hirschmann[®]) and flushed into 17 μ L of ice cold phosphate-buffered saline (PBS 10 mM, pH 7.4). Once sampled for their haemolymph, insects were kept individually in their Petri dish in the same conditions as previously exposed until the next

day, waiting to be assessed for reproduction and longevity (see section below). Haemocyte concentration was immediately estimated using 10 μ L of the homogenized solution in a Neubauer haemocytometer under a phase contrast microscope (magnification $\times 400$, Eclipse e200; Nikon, Tokyo, Japan). The remaining 10 μ L was snap-frozen in liquid nitrogen and stored in -20 °C freezer until use for later measurement of the phenoloxidase activity. For each individual haemolymph sample, the activity of naturally activated phenoloxidase (PO) enzymes only (hereafter PO activity) and the activity of the proenzymes (proPO) in addition to that of the PO (hereafter total-PO activity) were measured using a spectrophotometer (Zanchi *et al.* 2011). PO activity was quantified without further activation, while total-PO activity required the activation of the proPO into PO with chymotrypsin. Both measures provide complementary information about the level of investment and use of this immune defence system. The relative level of use of the phenoloxidase system might have important implication in the context of the study of the POLS hypothesis because its activity is often associated to self-damage (Sadd & Siva-Jothy 2006). To this purpose, frozen haemolymph samples were thawed on ice and centrifuged (2300 g, 5 min, 4 °C). Five microlitres of the supernatant was added to a microplate well (Sero-Well; 96 wells, Sterilin, UK) containing 20 μ L of PBS and either 140 μ L of distilled water for PO activity or 140 μ L of 0.07 mg mL⁻¹ chymotrypsin solution (Sigma, St. Louis, MO, USA) for total-PO activity. Then, 20 μ L of 4 mg mL⁻¹ L-dopa solution (L-3, 4-dihydroxyphenylalanine, Sigma-Aldrich) was added to each well. Enzymatic reactions were performed at 30 °C during 40 min and optic densities were monitored in spectrophotometer (Versamax; Molecular Devices, Sunnyval, CA, USA) every 15 s at 490 nm. Enzymatic activities were therefore calculated as the slope in the initial rate period using software SOFT-Max Pro (v.4.0; Molecular Devices).

Fitness measurements (reproduction and survival). The day following haemolymph collection, focal insects (males and females) were randomly paired in Petri dishes (\varnothing 9 cm) containing a shelter (piece of paper towel), bleach flour and water *ad libitum* with a virgin mate of the opposite sex and of the same age (collected and reared in the same conditions as the focal insect but not tested for personality nor their immunity). The fitness of an individual depends not only of its own quality but also of the quality of its partner (Muller, Arenas *et al.* 2016; Muller, Thiéry *et al.* 2016). The randomized pair formation over a large number of replicates was a conservative procedure limiting this partner effect and allowing the interpretation of a significant correlation between the fitness and the immune or behavioural parameters, everything else being equal. Bleach flour was preferred to bran and piglet flour because it is easier to sieve for egg detection. Couples were allowed copulating freely for 48 h. Males were then returned to their individual Petri dish, while females were moved into a new Petri dish containing bleach flour every second day for 10 days in order to count the number of larvae emerging from eggs. At the end of the 10th day, females were moved back to their initial Petri dish. After assessing their reproduction, all focal males and females were maintained individually and followed daily for their longevity. Here, fitness was assessed in focal females by counting the number of offspring they produced and in focal males by counting the number of offspring their female mate produced.

Experiment 2

Resistance to infection. After being assessed for their behaviour, 11-day-old (± 2 days) beetles were infected with the Gram-positive bacterium, *Bt* (Institut Pasteur, CIP 53.1), commonly used as biological insecticide, and which is considered as a major pathogen of *T. molitor* (Dubuffet *et al.* 2015). This method has recently proven to be useful in the mealworm beetle, *T. molitor*, to estimate change in immunity according previous experience of the insects with bacterial pathogens (Dhinaut, Chogne & Moret 2017). *Bt* was grown overnight in 20 mL of broth medium (LB: 10 g of tryptone, 5 g of yeast extract, 10 g of NaCl in pure water, pH 7) at 28 °C. The *Bt* solution was then centrifuged at 3500 g at 4 °C for 30 min. The supernatant was discarded and the bacteria pellet was used for infection. Beetles were inoculated by dipping a sterilized 0.03-mm diameter needle (Fine Science Tools® no. 26000-25) into the bacteria pellet and pricking the animal through the pleural membrane between the second and third abdominal tergites. Once treated, insects were weighted (precision ± 1 mg) and then kept in their Petri dish in rearing conditions, supplied with water and food *ad libitum*, and monitor daily until death. A control experiment was also performed to make sure that *Bt* does indeed reduce survival compare to control beetles (see Appendix S2).

STATISTICAL ANALYSES

Experiment 1

All statistical analyses were performed with R software (v.3.1.2., R Core Team 2014). For each behavioural trait and replicates, the range and the median of the scores assorted with their 95% confidence interval (95% CI) calculated using the R package *boot* (Canty & Ripley 2016) were given. The repeatability between the first and the second replicate for each behavioural trait was estimated first for the whole sample, and then separately for males and females. As activity and exploration scores followed a negative binomial distribution, repeatability was assessed using concordance correlation coefficient (Lin 1989, 2000; Carrasco 2010; Schweitzer, Motreuil & Dechaume-Moncharmont 2015; Monceau *et al.* 2017a) using the package *EPIR* (Stevenson *et al.* 2016) following Nakagawa & Schielzeth (2010). Scores for food neophobia and gregariousness (or their transformation) did not follow any classical distribution. We thus assessed rank repeatability using Spearman's correlation coefficient for these scores (Monceau *et al.* 2017a). In each case, repeatability coefficients were reported with their bootstrapped 95% CI. As all behavioural measurements were significantly repeatable (see results), scores in the first and the second replicate were averaged for each behavioural trait. Subsequent analyses were based on these average scores. Sexual differences in behaviour were also estimated, and the associated effect size was reported using a nonparametric estimator, the Cliff's δ assorted with its 95% CI using *EFFSIZE* package (Torchiano 2014). This estimator was preferred to more classical Cohen's d because the data were not normally distributed (Cliff 1996; Nakagawa & Cuthill 2007; Macbeth, Razumiejczyk & Ledesma 2011; Ivarsson *et al.* 2013). We also tested for differences between sexes using classical Wilcoxon rank sum tests. In order to determine behavioural syndromes, correlations between all personality traits were tested using Spearman's rank correlation. P -values

were adjusted for multiple comparisons using Benjamini–Hochberg's correction to control for false discovery rate (Benjamini & Hochberg 1995) from the R package *MUTOSS* (MuToss Coding Team *et al.* 2014). A principal component analysis (PCA) taking into account all correlated personality traits was then performed to estimate a synthetic personality score. This synthetic score was then used to test the relationship between personality and immune parameters or fitness traits. A PCA was also performed to obtain a synthetic variable for innate immunity estimated by haemocyte concentration, PO and total-PO activities. The relationship between behavioural syndrome and immune parameters was tested using a linear regression after checking for normality and homoscedasticity. The linear regression also included sex as explanatory variable in order to control for potential differences between males and females. Three individuals (two females and one male) were excluded from this analysis due to missing values for haemocyte concentration. The number of larvae (and its transformation) did not follow any known distribution. Therefore, we could not rely on parametric models to assess the effect of personality on fecundity. As mother's body mass was expected to affects the number of offspring, we tested the correlation between number of larvae, behavioural syndrome and mother's body mass using partial Spearman's correlations (Schielzeth 2010) with the *PPCOR* package (Kim 2015). The effect of behavioural syndrome on longevity was tested using a Cox proportional hazards regression model from *SURVIVAL* package (Therneau 2014). The significance of the overall model (including a sex effect) was tested using log-likelihood ratio test. Some individuals ($n = 31$, aged from 85 to 111 days post-emergence) were still alive at the end of the experiment and were thus included as censored data (but they did not exhibit differences in their behaviour: Wilcoxon test, $W = 893$, $P = 0.28$, $N_{\text{censored}} = 31$ and $N_{\text{uncensored}} = 51$).

Experiment 2

Similar to experiment 1, a PCA including the four personality traits was used to test the effect of behaviour on resistance to *Bt* through a survival analysis using a Cox proportional hazards regression model. The significance of the overall model (including a sex effect in interaction with the behavioural syndrome and a body mass effect) was tested using log-likelihood ratio test. Some individuals ($n = 82$, aged from 31 to 45 days post-emergence) were still alive at the end of the experiment and were thus included as censored data (but they did not exhibit differences in their behaviour Wilcoxon test, $W = 6698$, $P = 0.44$, $N_{\text{censored}} = 82$ and $N_{\text{uncensored}} = 154$).

Results

PERSONALITY AND BEHAVIOURAL SYNDROME (EXPERIMENT 1)

No difference between sexes was detected for the four behavioural traits (see 95% CI and Wilcoxon rank sum test: activity: $W = 931$, $P = 0.40$; exploration: $W = 840$, $P = 1.00$; food neophobia: $W = 665$, $P = 0.10$; gregariousness: $W = 806$, $P = 0.75$). Considering all the data pooled together regardless of the gender, all traits were found repeatable (Table 1). Female food neophobia and

Table 1. Repeatability (see text for details about the procedure used) for each behavioural trait for the whole sample and for each sex, assorted with their 95% confidence interval (between square brackets). Sexual dimorphism for each trait is also presented with Cliff's δ and its 95% confidence interval. For each category, the sample size is given between brackets

	Overall ($N = 82$)	Male ($N = 41$)	Female ($N = 41$)	Dimorphism ($N = 82$)
Activity	0.46 [0.28; 0.61]	0.63 [0.40; 0.78]	0.29 [-0.001; 0.54]	0.11 [-0.15; 0.34]
Exploration	0.37 [0.18; 0.54]	0.26 [-0.04; 0.52]	0.50 [0.26; 0.68]	-0.0006 [-0.25; 0.25]
Food neophobia	0.23 [0.005; 0.44]	0.39 [0.09; 0.66]	0.09 [-0.23; 0.42]	-0.21 [-0.41; 0.04]
Gregariousness	0.37 [0.17; 0.55]	0.52 [0.24; 0.74]	0.21 [-0.11; 0.48]	-0.04 [-0.29; 0.21]

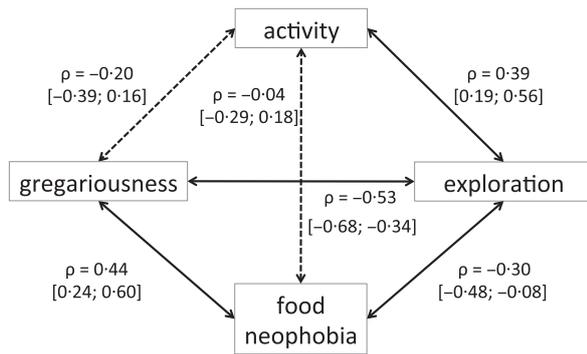


Fig. 2. Correlations between the four personality traits in the mealworm beetles. Spearman's ρ correlation coefficients are given with their 95% confidence interval (95% CI) in brackets. Correlations are significant when their 95% CI does not cross 0. Significant and non-significant correlations are represented by plain and dashed double arrows respectively.

gregariousness were not repeatable, although with values consistent with those of males (the 95% confidence intervals overlap), which may indicate a lack of statistical power due to limited sample size. These four personality traits were correlated with at least one other trait thus revealing a behavioural syndrome (Fig. 2). We therefore calculated a synthetic personality score based on the first axis of the PCA ($PC1_{beh}$) that represented 48.56% of the total variance (factor loadings, activity: 0.37, exploration: 0.54, food neophobia: -0.48, gregariousness: -0.58). Highly positive values of $PC1_{beh}$ correspond to proactive (i.e. fast-living/high risk-taking) individuals exhibiting high level of activity, exploration, food neophily and gregariousness, while highly negative value corresponded to reactive individuals.

RELATIONSHIP BETWEEN BEHAVIOURAL SYNDROME AND FITNESS (EXPERIMENT 1)

A summary of the fitness parameters is presented in Appendix S3. After controlling for mother's body mass, there was a significant positive partial correlation between the number of larvae and behavioural syndrome in females (partial Spearman's correlation $\rho = 0.36$, 95% CI = [0.06; 0.60], Fig. 3), whereas there was no correlation in males ($\rho = -0.14$, 95% CI = [-0.46; 0.22]), and these partial correlation coefficients were significantly

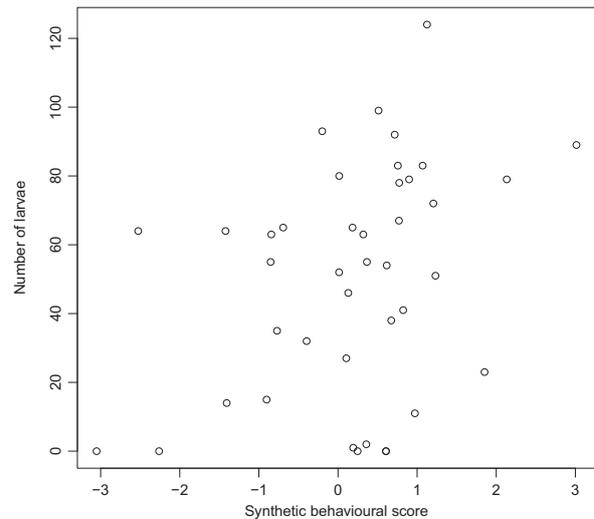


Fig. 3. Correlation between the number of larvae and the female's personality score (min: reactive individual, max: proactive individual).

different between sexes ($P = 0.025$). Longevity was sex-dependent; in average, females died earlier than males (Cox proportional hazard model: standardized estimate = -0.64, odds ratio = 0.53, $\chi^2 = 5.00$, d.f. = 1, $P = 0.02$) but neither behavioural syndrome (standardized estimate = -0.31, odds ratio = 0.73, $\chi^2 = 1.37$, d.f. = 1, $P = 0.24$) nor their interaction (standardized estimate = 0.14, odds ratio = 1.15, $\chi^2 = 0.21$, d.f. = 1, $P = 0.65$) was correlated with longevity.

RELATIONSHIP BETWEEN BEHAVIOURAL SYNDROME AND BASAL IMMUNE PARAMETERS (EXPERIMENT 1)

A summary of the immune parameters is presented in Appendix S4. In order to avoid inflation of type I error arising from multiple comparisons, we assessed the relationship between personality and basal immune parameters by first estimating a synthetic immune score using PCA. The first axis of this PCA ($PC1_{imm}$) represented 54.76% of the total variance. PO and total-PO activities mostly contributed to $PC1_{imm}$ (factor loadings for PO: 0.67, total-PO: 0.69, haemocytes: -0.25). This immune score was not explained by body mass ($F = 3.55$, 1 and 74 d.f., $P = 0.13$), while it was significantly explained by the

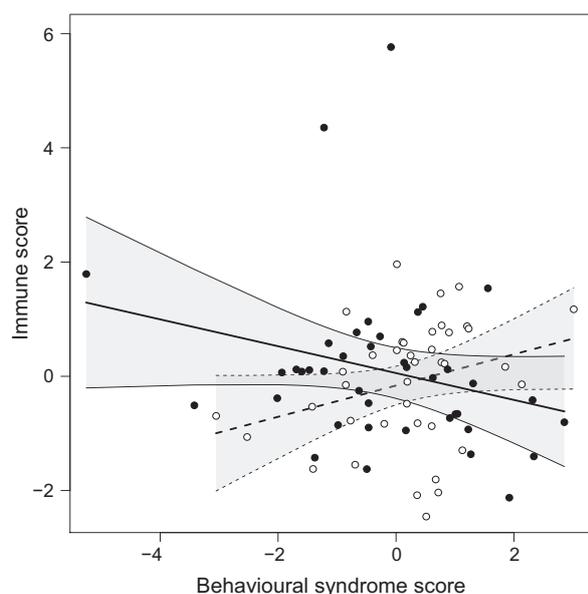


Fig. 4. Correlation between the behavioural syndrome score (min: reactive individual, max: proactive individual) and the immune score (min: low PO-PPO and high haemocyte concentration, max: high PO-PPO and low haemocyte concentration) by sexes. Females are in white dots/dashed line and 95% confidence interval and males are in black dots/plain line and 95% confidence interval.

interaction between sex and behavioural syndrome ($F = 8.80$, 1 and 74 d.f., $P = 0.02$, Fig. 4). The direction of the correlation was sex-specific (Pearson's correlation coefficient: $r = 0.29$, 95% CI = $[-0.03; 0.55]$ in females and $r = -0.26$, 95% CI = $[-0.52; 0.06]$ in males) and these levels of correlation were significantly different between sexes ($P = 0.017$). Both 95% CI slightly overlapped with 0 possibly due to a lack of statistical power as the analyses were based on 39 females and 40 males. Power analyses revealed that two and half times larger sample sizes would be sufficient to obtain significant correlations.

RELATIONSHIP BETWEEN BEHAVIOURAL SYNDROME AND RESISTANCE TO INFECTION (EXPERIMENT 2)

The synthetic personality score was estimated using PCA based on the four behavioural traits. The first axis represented 32.46% of the total variance, and according to factor loadings, this personality score was consistent with experiment 1 in describing a proactive–reactive continuum (activity: 0.66, exploration: 0.56, food neophobia: 0.009, gregariousness: -0.49). The probability to survive to the *Bt* infection was not linked to sex (Cox proportional hazard model: standardized estimate = -0.23 , odds ratio = 0.80, $\chi^2 = 1.88$, d.f. = 1, $P = 0.17$), behavioural syndrome (standardized estimate = -0.04 , odds ratio = 0.96, $\chi^2 = 0.55$, d.f. = 1, $P = 0.46$) or their interaction (standardized estimate = -0.05 , odds ratio = 0.95, $\chi^2 = 0.10$, d.f. = 1, $P = 0.77$) and only depended on body

mass (standardized estimate = -0.23 , odds ratio = 0.80, $\chi^2 = 7.73$, d.f. = 1, $P < 0.01$).

Discussion

The aim of this study was to assess the relationship between personality, fitness and immunity of adult mealworm beetles. A first crucial step was to check the existence of correlated personality traits in a behavioural syndrome in this species. The four behavioural traits (activity, exploration, food neophobia and gregariousness) measured herein were found to be repeatable, and their repeatability scores (ranging from 0.23 to 0.46) were congruent with those previously reported in the meta-analysis by Bell, Hankison & Laskowski (2009). These traits were also correlated in a syndrome. Activity and exploration have been associated with dispersal abilities (Dingemans *et al.* 2003; Cote, Clobert *et al.* 2010; Hoset *et al.* 2011). Gregariousness (i.e. sociability) was negatively correlated with exploration (Cote, Clobert *et al.* 2010; Cote, Fogarty *et al.* 2010). Contrary to high level of activity and exploration that are estimates of a risky behaviour, food neophobia is a mechanism to avoid dangerous food sources (Rozin & Vollmecke 1986) and therefore reflects low risk-taking behaviour. Food neophobia was also reported to be affected by social context (Oostindjer *et al.* 2011; Costa *et al.* 2014). In our study, food neophobia and gregariousness were positively correlated meaning that sociable individuals were more reluctant at feeding on new food sources. Overall, *T. molitor* behavioural syndrome can be defined as a continuum of low risk-taking individuals (gregarious, neophobic, low activity and explorative skills) to high risk-taking individuals (solitary, neophilic, high activity and explorative skills) that resumes the reactive–proactive continuum (Réale *et al.* 2007, 2010).

The POLS hypothesis suggests that the behavioural syndrome is associated to different fitness payoff strategies (Réale *et al.* 2010). According to POLS hypothesis, high risk-taking individuals were expected to exhibit shorter life span, rapid egg laying and higher number of offspring. Here, we only found a relationship between female behavioural syndrome and their number of offspring produced, but not with other components of fitness. High risk-taking females produced more offspring than low risk-taking females, as expected from the POLS hypothesis. Such a relationship was often observed between a single personality trait and reproductive success (Biro & Stamps 2008; Smith & Blumstein 2008) but has rarely been reported in insects to date (see however Logue *et al.* 2009). Reproduction being classically traded off against life span (Arnqvist & Nilsson 2000), proactive females are expected to show higher investment into reproduction at the expense of longevity. In the present case, females suffered reduced longevity compared to males (as expected due to the larger reproductive costs) but independently of their behavioural syndrome thus suggesting that increased offspring production is not

associated with reduced longevity in proactive females. However, the experiments allowed solely one reproduction event that was perhaps not sufficient (i.e. not costly enough for females) to contrast strategies. Additionally, the experiments did permit to assess a potential trade-off between number and quality in the offspring. Indeed, females may strategically invest in smaller clutch but of higher quality (Arnqvist & Nilsson 2000). Further investigations should thus consider the life-history traits of the offspring. Interestingly, in their meta-analysis, Smith & Blumstein (2008) found that only boldness but not exploration was found associated to reproductive success. Such an effect was stronger in males than in females. In contrast to these synthetic results, we found that personality scores were not correlated with reproductive success in *T. molitor* males, even though these traits were found correlated in females.

Basically, males and females differ in the way they can maximize fitness according to Bateman's principle. As females maximize fitness through their longevity, they should invest in immunity, whereas males that maximize fitness through multiple mating, should allocate preferentially their resource to the expression of costly traits associated to inter-sexual selection or intra-sexual selection (Schulenburg *et al.* 2009). Sex differences in investment in immunity derive from Bateman's principle and are common in both vertebrates and invertebrates (Rolff 2002; Zuk *et al.* 2004; Nunn *et al.* 2009; Schulenburg *et al.* 2009). Here, we found contrasted patterns of correlation between immunity score and behavioural syndrome in males (a negative relationship) and in females (a positive relationship). Such a difference in immune defences between males and females observed in experiment 1 may reflect different strategies of resource allocation to the immune function as a consequence of differences of constraints between sexes (Schwenke, Lazzaro & Wolfner 2016). However, whatever their behavioural profile, males and females did not differ in their ability to survive a bacterial infection. While the results for this direct assessment of the immune response by testing resistance to a bacterial infection in experiment 2 appear to contrast with those from immune parameters measured in experiment 1, they may not be inconsistent. Indeed, if males and females gain fitness through different life-history strategies (see above), then they may also adopt different immune strategies to increase fitness when challenged with a pathogen: resistance and tolerance (Schneider & Ayres 2008). Resistance corresponds to the ability to clear pathogens, whereas tolerance corresponds to the ability to endure pathogens. Each immune strategy may lead to similar outcomes in terms of survival to a pathogenic infection and fitness, but they are expected to have different temporal fitness payoff after infection (Kutzer & Armitage 2016). Resistance to the infection is expected to immediately decrease short-term reproduction, which might be then restored later on. When relying on this strategy, females mitigate the immediate infection risks by the mean of larger immune defence in order to ensure long-term

reproductive success. By contrast tolerance is expected to maintain the reproductive function, but increasing pathogen load may then finally kill the host. Contrary to reactive males, proactive males might use this strategy: they save the costs of immune defence in favour of multiple mating events before dying from the infection. Further studies are requested to assess this hypothesis. Taking together, our results question the evolutionary significance of the apparent link between basal immune parameters and behaviour, which is not confirmed by differential survival to infection. Indeed, the role of immune system is to be an efficient gatekeeper against pathogens and thus to increase individual survival. Our data thus question the validity of studies that link personality and immune parameters only in non-challenging conditions without any assessment of the overall capacity of the individual to survive infections (see also Krams *et al.* 2016).

Conclusions

The studies challenging the POLS hypothesis often focus on either a single personality, physiology and/or life-history trait correlated with each other (Bijleveld *et al.* 2014; Biro, Adriaenssens & Sampson 2014; Cole & Quinn 2014; Krams *et al.* 2014; Montiglio *et al.* 2014). Here, four personality traits, two measures of fitness and several components of the immune function were used to challenge the POLS hypothesis in *T. molitor*. This study reveals a relationship between behavioural syndrome and reproductive success in this species, thus deserving the POLS hypothesis. Additionally, it shows for the first time in an invertebrate species, a sex-specific relation between behavioural syndrome and basal immune parameters, which, however, is not confirmed in a functional perspective. We recommend that future research on the link between immunity and behavioural syndrome control for sex differences to take into account the differences between sexes for evolutionary trajectories and we urge researchers to pay attention to the choice of the proxy used to test POLS hypothesis to avoid misleading results (Biard *et al.* 2015).

Authors' contributions

K.M., F.X.D.M., J.M. and Y.M. conceived the ideas and designed methodology; K.M., C.L., R.C. and S.M. collected the data; K.M., C.L., R.C. and F.X.D.M. analysed the data; K.M., F.X.D.M., J.M. and Y.M. wrote the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Acknowledgements

We thank Jenny Sauvage for her technical help during the experiments. We also thank Indrikis Krams and two anonymous reviewers whose constructive comments helped in improving the manuscript.

Data accessibility

Data available from the Dryad Digital Repository <https://doi.org/10.5061/dryad.c67k3> (Monceau *et al.* 2017b).

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Received 28 November 2016; accepted 7 April 2017

Handling Editor: Daniel Ardia

Supporting Information

Details of electronic Supporting Information are provided below.

Table S1. Summary of the scores for each behavioural test and their replicates.

Appendix S1. Behavioural tests realized on *Tenebrio molitor*.

Appendix S2. Control experiment for *Bt* effect on *Tenebrio molitor*.

Appendix S3. Summary of the fitness parameters.

Appendix S4. Summary of the immune parameters.