



Personality and ectoparasitic mites (*Hemipteroseius adleri*) in firebugs (*Pyrrhocoris apterus*)



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ABSTRACT

Individuals of the same species often consistently differ in their behaviour across time and context. These stable differences are usually termed ‘animal personality’. Parasitism is known to significantly influence the evolution of animal personality at least in part because more explorative individuals may meet parasites more frequently than less explorative ones. Previously, we have demonstrated that consistent individual differences (i.e. boldness, activity, exploration) can be measured in firebugs. As continuation, we examined here the relationship between firebug personality traits and their ectoparasitic mite loads in a wild population. We showed that bugs that behaved in a more explorative way have more mites and we also found a marginally significant interaction between sex, boldness and activity: bolder and more active female firebugs were more infected. In addition, we experimentally tested whether an artificial infection causes any alteration in the bug’s behaviour and whether there is any relationship between firebug personality and immune response. This treatment did not induce any alteration in bugs’ personality. We found that bugs become more explorative but less active when repeating the experiment, but at the same time all personality traits (boldness, activity and exploration) were repeatable. Furthermore, firebugs with a stronger immune response behaved more boldly but also less actively.

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1. Introduction

An increasing amount of evidence shows that animals have personalities, i.e. differences in behaviour between individuals that are maintained over time and across different contexts (e.g. Gosling, 2001; Bell, 2007; Dingemanse and Wolf, 2010). For instance, if an individual is shy in a feeding context, it will also be shy in a mating context. In other words, it will take fewer risks than a bold individual would do in these situations. This consistency does not mean that behaviour cannot be altered by age or environmental conditions but that the behavioural differences between individuals should be maintained. The adaptive significance of risk taking may depend on how the cost of being bold changes from situation to situation and over time. For example, brave individuals can have higher fitness benefits if predation risk is low, whereas shy

individuals can benefit more if predation risk is high (Sih et al., 2004).

Besides predation, parasitism can also be a crucial, although rarely considered, ecological factor that will have significant influence on the evolution of personality (Kortet et al., 2010; Barber and Dingemanse, 2010). According to a common definition of parasitism, the health status of a parasitised individual is deteriorated through a wide variety of mechanisms compared to an unparasitised one (Bush et al., 2001; Poulin, 2007). Parasitism can have essential effects on the hosts’ fitness, similar to predation (e.g. Raffel et al., 2008; Schmid-Hempel, 2009). However, major differences exist between predation and parasitism. Predation has immediate fitness effects if the prey is killed or injured, in contrast to parasitism, which has a gradual, but long-lasting fitness consequence. It is possible to find examples of parasites causing low fecundity (Newey and Thirgood, 2004), or decreasing the host’s mating success, due to the other sex’s avoidance of parasitised individuals as mating partners (Hamilton and Zuk, 1982). Parasites can also have an effect on the hosts’ feeding rate, sociability and migration (Lozano, 1991; Hart, 1997; Jog and Watve, 2005). Furthermore, parasites can manipulate the hosts’ behaviour in order to facilitate their

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own transmission by making the host behave in a more risk-prone way thus reducing their longevity (Libersat et al., 2009). Many of these effects might arise because parasites can modify the personality of their hosts, although the causes and consequences of this are not always obvious.

We know that parasites can influence the evolution of animal personality in multiple ways, as suggested by Kortet et al. (2010) and Barber and Dingemanse (2010). First, parasites can exert a selective pressure on personality and behavioural traits, as the risk of acquiring parasites can be altered by an individual's personality. For instance, Boyer et al. (2010) found a positive correlation between tick load and boldness in Siberian chipmunks (*Tamias sibiricus*), suggesting that proactive individuals have the opportunity to encounter parasites more often than reactive ones and therefore possess a higher risk of infection. Secondly, parasites can promote individual differences in a host's behavioural type in a population in three different ways (Poulin, 1994; Thomas et al., 2005): (1) behavioural change as a result of the unavoidable "sickness effect", an evolutionarily neutral side effect which has no benefit for either the host or the parasite; (2) behavioural alteration can be adaptive for the host in order to reduce the fitness cost of infection (e.g. terminal investment, Minchella and Loverde, 1981); and, (3) parasites alter the host's behaviour in a way beneficial to the former because it increases their transmission rate. Two well-known examples of the latter are the loss of aversion to cats in rodents infected with *Toxoplasma gondii* (Berdoy et al., 2000) and the water-seeking behaviour caused by the Gordian worm in its grasshopper host (Thomas et al., 2002). Although these associations between parasitism and behaviour can also be sex dependent, the actual mechanisms are not always clear because predictions usually depend on the mode of transmission of the parasite (Dunn et al., 2011). For instance, Møller et al., (2004) found that infection by malaria parasites delayed arrival dates in male swallows but not females. Immune defence is one key factor in the fight against parasites and pathogens and also correlates with numerous life-history variables (e.g. Rantala and Roff, 2005). For instance, developmental time and mature body size can be associated with level of immune defence and recent papers have also demonstrated that the efficiency of the immune system should also be associated with personality (Kortet et al., 2010; Butler et al., 2012; Niemelä et al., 2012). Establishing and maintaining an efficient immune system is costly (e.g. Owens and Wilson, 1999; McKean et al., 2008), however, and personalities can adjust whether an individual pay that cost, or not. One would assume that certain personalities can afford this extra cost: Individuals with a strong immune system should behave in a bolder and more active way (to gain the energy needed for an efficient immune system) since, the cost of encountering parasites is lower than in the case of individuals with a weak immune system (Kortet et al., 2010). This was also observed in male eastern chipmunks (*Tamias striatus*), where bolder individuals have a higher reproductive success and also have a higher parasite load explained by either a depleted energy reserve or the immunosuppressive effects of testosterone (Patterson and Schulte-Hostedde, 2011). Consequently, we expect differences between sexes because they also differ in their behaviour during reproduction that might also lead to differences in exposure to parasites (Dunn et al., 2011).

To study the effects of parasites on their hosts' personality, firebugs (*Pyrrhocoris apterus*) and their ectoparasitic mite (*Hemipteroseius adleri*) offer an ideal model system. We have shown that firebug individuals differ consistently in behaviour, phenomena that we interpret as personality (Gyuris et al., 2011, 2012). There are several studies (e.g. Socha and Zemek, 2004, 2007) that have investigated the behavioural differences between the two morphological forms of firebugs (i.e. long-winged and short winged) that have shown that differences are usually more expressed in females but not in males. In addition, firebugs harbour

ectoparasitic mites which are found under their wings where they suck haemolymph (Zhang, 1995). These mites appear to have a negative effect on firebugs as suggested by the findings of Lewandowski and Szafranek (2005); these workers observed that firebugs with more than three mites suffer higher winter mortality presumably because they enter hibernation in a bad condition due to their mite infection. This mite also appears to be widely distributed over the geographical range of its host species (Fend'a, 2011) and has recently been detected in our study population of firebugs in Hungary (Kontschán and Gyuris, 2010). Despite their common co-occurrence, however, very little is known about the mites' effect on their hosts' behaviour.

Here we investigate the following related questions: First, is there any correlation between the personality of firebugs (*P. apterus*) and the intensity of infection by the ectoparasitic mite (*H. adleri*)?; Secondly, is there any difference in the correlation structure of behavioural traits between infected and non-infected bugs?; Thirdly, is firebug personality altered by an experimentally induced artificial immune challenge?; and fourth, to what extent is the immune reaction activated by the experimental challenge associated with personality traits? We expect that bugs with higher exploratory behaviour would be more infected and that these differences are more expressed in female firebugs. Finally, we predict that the level of immune defence is associated with behaviour.

2. Materials and methods

2.1. Studied species and experimental design

The firebug (*Pyrrhocoris apterus*) is a common and widely distributed Palearctic species of the heteropteran family Pyrrhocoridae. It can be found mostly around linden trees, the seed of which provides its main food. The parasitic mite, *Hemipteroseius adleri* (Costa, 1967), is the only species of the *Hemipteroseius* genus described from Europe (Poland and Lithuania: Lewandowski and Szafranek, 2005; Chmielewski, 2006 Hungary: Kontschán and Gyuris, 2010) and the Middle East (Israel: Costa, 1967) and which is also associated with firebugs. This mite also uses the red bug, *Scanthius aegyptius*, as an alternative host (Costa, 1967). *H. adleri* was described from our studied population (Kontschán and Gyuris, 2010), so we used two groups of firebugs for this study: In one of them we investigated correlations between host behaviour and mite infection, while in the other we performed an experimental immune challenge to test its effect on behaviour.

Our first study group was established using 86 experimental animals collected from a wild population between September–October 2011, in Debrecen, northeast Hungary (47.52° N, 21.62° E; 130 m a.s.l.; subcontinental climate). After collection, the bugs were put individually into jars and kept under temperate laboratory conditions until observations were conducted (for further details on our laboratory conditions, see Gyuris et al., 2011). Sunflower seeds as food and water were provided ad libitum. After 1–2 days of accommodation we carried out personality tests (see below) with each bug individually. After the personality tests the bugs were put into 97% propanol in Eppendorf vials to allow us to measure body lengths and to enable us to count mites with the help of a microscope at two-fold magnification. Since we found only one macropter (long winged) specimen (out of 87) we omitted this individual from further analyses (Gyuris et al., 2011).

For our second study group, firebugs (all short-winged) were taken from laboratory stock cultures which were established from a wild population in Debrecen and had been kept in the laboratory since May 2012. We used 90 individuals (30 per treatment group), but because two bugs died before the treatment we conducted experiments with the following sample sizes: control group = 30,

♂:15, ♀:15; puncture group=30, ♂:15, ♀:15; implant group=28, ♂:13, ♀:15. We treated groups in three different ways (see below) and conducted personality test series (henceforth referred as ‘run’) twice: before and after the treatment. We did the second test the day after the first test and applied the treatments outlined below.

2.1.1. ‘Implant group’

To measure immune response intensity we punctured the third abdominal segment of the bugs on the right side and inserted a sterile 2 mm long nylon monofilament (diameter 0.18 mm). This is a widely used technique in ecological immunity studies to generate an acute immune response by melanin encapsulation in insects (Gillespie et al., 1997; Rantala and Roff, 2007). This method is also useful for avoiding complications caused by the uncontrolled parasite–host actions by using a standardised antigen (nylon monofilament) (Bouletreau, 1986). We put the implant into the bugs after the first behavioural test and left it inserted till the second tests. The average time the firebugs had the implant was 15.7 ± 1.57 (mean \pm SD) hours. The implant was removed before the second test and photographed from two different angles under a microscope. The pictures were then analysed using the ImageJ program (Rasband, 1997–2015) and the degree of encapsulation was estimated as the cumulative grey values of implants (Rantala and Roff, 2007).

2.1.2. ‘Puncture group’

We punctured an approximately 2 mm-deep hole in the same way as in the case of the previous treatment but did not insert an implant.

2.1.3. ‘Control group’

Firebugs in this group did not receive any treatment (neither puncture nor implant).

2.2. Personality tests

We used the same behavioural tests as Gyuris et al. (2011), briefly summarised below, in an arena 55 cm in diameter. The floor of the arena was covered with filter paper and four plugs (made of gum, 0.75 cm high, and 1.83 cm in diameter) were placed on the floor as novel objects. The filter paper and plugs were replaced before every run to avoid uncontrolled chemical cues from previous runs. Our personality tests comprised two parts: (i) a “boldness test” and (ii) an “open field test.” In the first test we put a bug into a vial, covered it with paper and waited for one minute to let it accommodate to its new environment. After one minute, we flicked the vial (to be sure that the bug is on the bottom of the vial), removed the paper, laid the vial on its side and waited (for a maximum of ten minutes) until the bug’s antenna appeared in the vial opening, registered as the “emergence”. After the appearance of the antenna or ten minutes, whichever occurred first, we moved to the second stage of the test.

The second stage (open field test) also began with a one minute habituation in the vial. After the habituation we shook the bug into the centre of the arena, registered when it started to move (hereafter “walking latency”) and recorded its movement pattern with a webcam. In this way we were able to measure how soon the bug responded to a startling stimulus when it entered a new environment, how thoroughly it explored its new environment (quantified by counting the “number of novel objects” visited), and the time it took to reach the arena wall (hereafter “wall time”). Bug movement in the open field test was also characterised by calculating the following variables: (i) mean; and, (ii) variance of step size (distance between two consecutive positions, one second apart); (iii) mean; and, (iv) variance of turning (the turning angle for a given point of the path is the angle between the line determined by the point and

the previous point and the line given by the point and the subsequent point). This is a previously applied method for measuring the boldness, explorativeness and activity personality axes in firebugs (Gyuris et al., 2011, 2012).

2.3. Statistical analyses

2.3.1. Observational data

First we used observational data (see above Section 2.1.1.) to analyse the relationship between measured behavioural traits and degree of infection to determine the prevalence value of mites in our studied population (Rózsa, 2005). Since, it was 23% and the distribution in the number of mites was highly skewed we categorised the collected bugs based on whether they had any mites or not (i.e. infected vs. non-infected bugs). We also calculated the relationship between bugs’ body length and number of mites to investigate whether longer bugs have more mites using Spearman rank correlations. Since, we did not find any correlation between body length and number of mites ($r_s = 0.094$, $p = 0.393$), body length was not included in subsequent analysis.

To evaluate differences in the correlation structure of behavioural variables (*structural consistency*; Stamps and Groothuis, 2010; Gyuris et al., 2012) in the two groups (infected vs. non-infected) we computed a Spearman rank correlation matrix and then compared them using a Mantel randomisation test (Manly, 1997). We calculated a Spearman rank-correlation matrix for the eight behavioural variables and performed agglomerative clustering using one minus the absolute value of correlation coefficients as dissimilarity measures (Gyuris et al., 2012). We then calculated clustering of the behavioural variables using the “agnes” function in the cluster (Maechler et al., 2014) package (Ward’s clustering method). On this basis we identified groups that characterise the firebugs’ personality traits (boldness, activity, explorativeness) and evaluated a composite rank variable for each group of behaviours by summing the individuals’ ranks for all variables that correspond to a given group of behavioural traits. We checked the correlation (Spearman rank correlation) between composite variable (boldness, activity and exploration) and each behavioural traits (emergence, walking latency, wall time, mean step size, variance of step size, mean turning angle, variance of turning angle and number of novel objects visited) and found strong positive correlations in all cases ($R > 0.277$, $p < 0.01$).

Next, we investigated the relationship between personality and the degree of infection by fitting separate generalised linear models (GLMs) for each trait (boldness, activity and explorativeness). We entered the given composite rank variable (boldness, activity, explorativeness), sex (male, female) and their interaction as explanatory variables while the degree of infection (infected vs. non-infected) was the response variable. We used binomial error distribution in GLMs, the likelihood ratio test and eliminated the non-significant interactions and main effects with backward elimination to derive a minimal model (Crawley, 2007).

2.3.2. Artificial infection

To identify personality traits in the second database (see above Section 2.1.2.) we carried out a cluster analysis using the same procedure as discussed above. We found the same grouping of behavioural variables and therefore calculated composite rank variables (in the same way as discussed in Section 2.3.1.). We analysed the effect of treatment on each personality trait with mixed-effects models (LmerTest, Kuznetsova et al., 2014) entering the individuals’ ID as the random factor and ‘run’ (first or second), treatment (control, puncture, and implant), sex (male, female) and all of their interactions as explanatory variables. We fitted separate models for each personality trait (boldness, activity and explorativeness) as the response variable and omitted non-significant

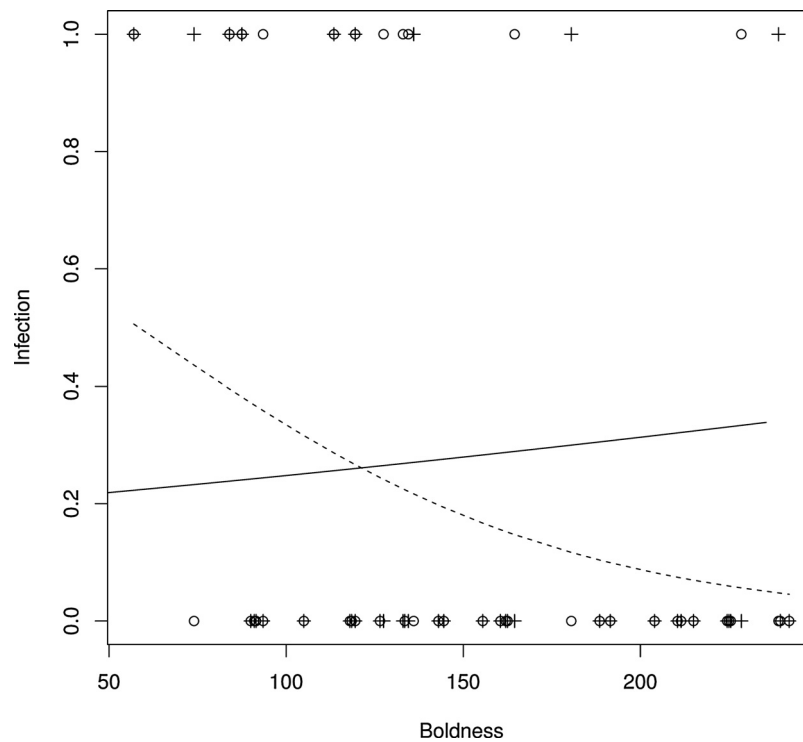


Fig. 1. The relationship between infection and boldness in male (solid line) and female (dashed line) firebugs. The lower value of boldness means the bugs are bolder (i.e. emerge from refuge sooner, response to an alarm faster and reach the wall sooner). Infection variable was dichotomized: infected (1) vs. non-infected (0). Points and solid line refers to male, while crosses and dashed line to female firebugs. Regression lines were predicted by generalised linear models (GLMs, for further details see the main text).

interactions and the main effects using a likelihood ratio test and backward elimination (Crawley, 2007). We tested for the significance level of repeatability of each personality trait by comparing the variance explained by the random term to the error variance in the minimal model with the rand function of the lmerTest R package (Kuznetsova et al., 2014).

Finally, we tested the effect of immune response (level of encapsulation) on personality traits with mixed effects' linear models. We used only those bugs in these analyses that were treated with a monofilament implant. The longer time the implant was inside the body the higher the encapsulation rate so we fitted a linear model to encapsulation rate as a function of duration time and then used the residuals from this for further analyses. Each composite rank variable (boldness, activity and explorativeness) was entered as a response variable into a separate model where the explanatory variables were sex, run and residuals of encapsulation. We fitted the individuals' ID as the random factor and the effect of the explanatory variables was computed using likelihood ratio tests (Crawley, 2007).

All statistical analyses were carried out in the R interactive statistical environment (R Development Core Team, 2014), using specifically the cluster (Maechler et al., 2014), lme4 (Bates et al., 2014), lmerTest (Kuznetsova et al., 2014) and ade4 (Dray and Dufour, 2007) packages.

3. Results

3.1. The relationship between parasite infection and personality

We found that 20 (σ : 11, φ : 9) out of 86 (σ : 43, φ : 43) individuals (23%) were infected by *H. adleri*. Based on the cluster analysis, our behavioural variables can be divided into the following groups: (1) emergence, walking latency and wall time can be considered on the boldness personality axis; (2) mean step size, variance of step size

can be identified as *activities*; and, (3) mean turning angle, variance of turning angle and number of novel objects describe the *explorativeness* personality axis. The result of the Mantel test showed that the structure of behavioural variables was similar in groups of infected and non-infected bugs ($r_s = 0.750$, $p < 0.001$). Therefore we calculated composite rank variables for the whole dataset.

Although we found that the interaction of sex and boldness is marginally significant ($\beta = -0.019 \pm 0.010$, $p = 0.067$), individually neither sex ($\beta = 2.400 \pm 1.444$, $p = 0.096$) nor boldness ($\beta = 0.003 \pm 0.005$, $p = 0.577$) had any impact on degree of infection (residual deviance: 88.627 on 82 degrees of freedom). We showed that boldness correlated with degree of infection in a different way in the different sexes as presented in Fig. 1: there is a positive correlation in females where bolder individuals tend to be more infected. The GLMs also indicated that explorativeness has a strong influence on the probability of infection (residual deviance: 86.118 on 83 degree of freedom); more explorative bugs had more mites ($\beta = 0.014 \pm 0.005$, $p = 0.012$; Fig. 2). The interaction between sex and explorativeness was non-significant ($\beta = 0.005 \pm 0.011$, $p = 0.641$) and sex alone had no effect ($\beta = -0.230 \pm 0.534$, $p = 0.667$). We found a marginally significant interaction between activity and sex ($\beta = 0.021 \pm 0.012$, $p = 0.071$) showing that the bugs' activity explains the degree of infection (residual deviance: 89.255 on 82 degree of freedom) differently in sexes. As Fig. 3 shows, more active females were more infected. We also identified a weak relationship between sex and the degree of infection, i.e. females were marginally less infected than males ($\beta = -2.216 \pm 1.243$, $p = 0.074$). Activity alone had no effect on infection ($\beta = -0.006 \pm 0.007$, $p = 0.412$).

3.2. Effect of immune treatment on behaviour

We tested the effect of treatment on each personality trait separately. Based on the results of the mixed-effects models, immune

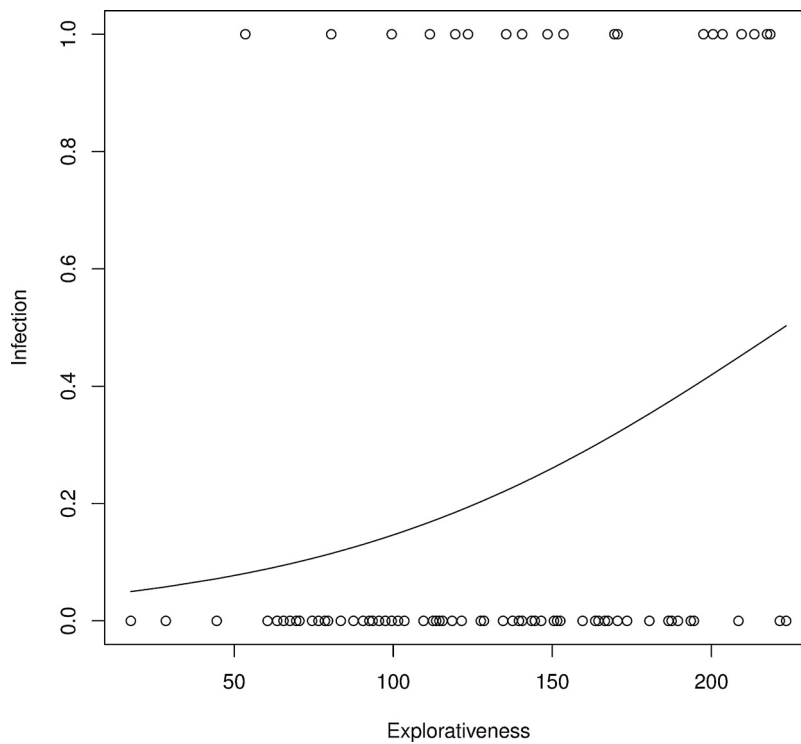


Fig. 2. The relationship between infection and explorativeness. The higher value of exploration describes the bugs explore their environment more thoroughly. Since we did not find differences between sexes we did not present the sexes separately. Regression lines was predicted by generalised linear models (GLMs). Infection variable was dichotomized: infected (1) vs. non-infected (0).

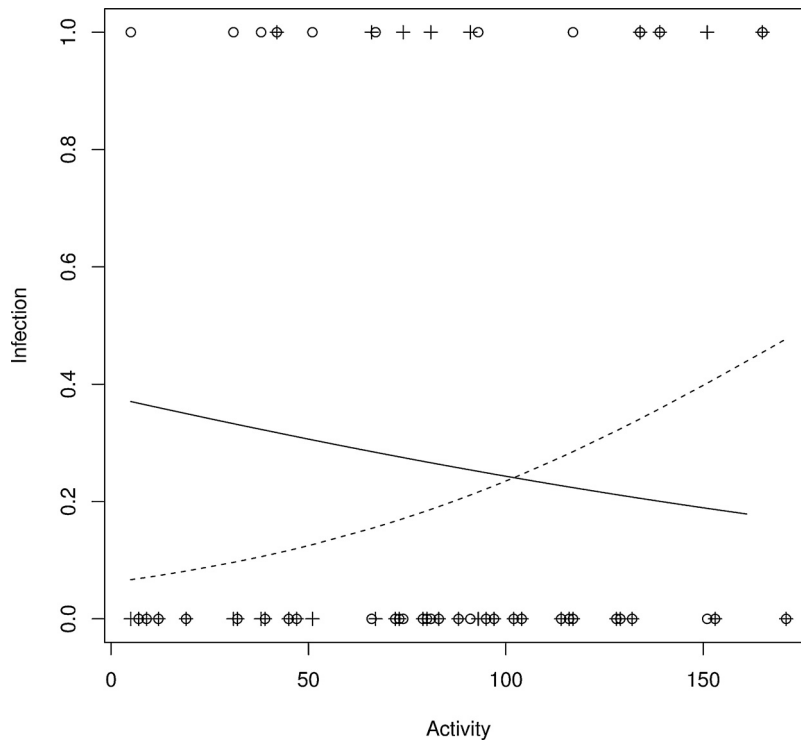


Fig. 3. The relationship between infection and activity in male (points and solid line) and female (crosses and dashed line) firebugs. Higher value of activity represents more active bugs. Infection variable was dichotomized: infected (1) vs. non-infected (0).

challenge treatment had no effect on behaviour. However, we found a marginally significant interaction between sex and run in the case of boldness (Table 1). It showed that the difference between the two runs of boldness were higher in females than in males. In addition,

female bugs behaved in a less active way and in general firebugs were less active along the second run. We also found an effect of run on explorativeness, i.e. bugs behaved in a more explorative way in the second run (Table 1). Analysing the effect of the random fac-

Table 1
Minimal models presenting the effect of run and sex on each composite rank behavioural variable separately in the immune treatment experiment (see Section 2.3.2.). *P* values in bold are significant.

	Estimate (β)	Std. Error	<i>T</i> value	<i>P</i> value
Boldness ~				
Run(2)	−6.721	18.708	−0.359	0.720
Sex(female)	−32.308	23.569	−1.371	0.172
Run(2):sex(female)	49.699	26.162	1.900	0.060
Activity ~				
Run(2)	−30.818	8.256	−3.733	<0.001
Sex(female)	−50.299	18.911	−2.660	<0.001
Explorativeness ~				
Run (2)	32.432	11.255	2.881	<0.001

tor (individual's ID) showed that all personality traits (boldness, activity, exploration) were repeatable (all *p* value ≤ 0.001).

3.3. The relationship between immune response and personality

To investigate degree of encapsulation (later referred to as 'Resid_encaps'), we used only bugs that were implanted and added this variable as an explanatory variable into mixed-effects models. We found a marginally significant effect on boldness, i.e. the bolder the bugs were, the higher encapsulation they exhibited ($\beta = -0.5270 \pm 0.3059$, $t = -1.723$, $p = 0.098$). In addition, we also found a relationship between activity and encapsulation rate: less active individuals had a higher immune response ($\beta = -0.6817 \pm 0.3393$, $t = -2.009$, $p = 0.056$). No significant relationship was found between explorativeness and immune response.

4. Discussion

One of the key questions in behavioural ecology is how evolution shapes behaviour in response to ecological conditions (Davies et al., 2012). Even though the existence of animal personalities is widely recognised, only a few studies have examined the importance of the different ecological factors that underlie their expression (Kekäläinen et al., 2014). It has, for example, been proposed that parasites may play a crucial role in shaping animal personalities because behavioural differentiation can have a large impact on the prevalence and dynamics of different diseases (Wolf and Weissing, 2012). Indeed, in many cases parasites can exert remarkable effects on their hosts' state by affecting their development, physiology, morphology and behaviour (Price, 1980; Beckage, 1997; Lefevre et al., 2009; Thomas et al., 2010).

In this paper, on the one hand, we examined the relationship between a range of behavioural traits and infection caused by an ectoparasitic mite (*H. adleri*) in a wild population of firebugs. On the other hand, we simulated an artificial infection under laboratory circumstances: (1) to see if we can promote alteration in bugs' behaviour, and; (2) to measure how the immune response of the bug is associated with their personality traits. In the first case, even though the correlational structures of behavioural variables were similar in infected and non-infected bugs, we found differences in the composite rank variables (i.e. in personality traits). We observed that firebugs who explored their environment more thoroughly are more infected by mites. Variation in exploratory behaviour can be especially important in determining individual variation in exposure to parasites (e.g. Dingemans et al., 2002; Drent and Marchetti, 1999). It is suggested that more exploratory individuals are more likely to encounter novel objects or environments, and also come into contact with novel parasites or pathogens more often (Barber and Dingemans, 2010). These explorative individuals usually spend more time moving over larger geographical areas (e.g. van Overveld and Matthysen,

2010) which also supports the findings that they have an increased exposure to parasites or pathogens. For instance, more explorative pumpkinseed sunfish (*Lepomis gibbosus*) have significantly higher level of trematode infections (Wilson et al., 1993). Another study (Boyer et al., 2010) also found that exploratory behaviour was an important explanatory factor in predicting tick load in Siberian chipmunks (*Tamias sibiricus*).

In the context of boldness and activity, we found marginally significant interactions between sex and boldness and also sex and activity; these two behavioural traits variously explain degree of infection in the different sexes. We also demonstrate that bolder and more active females are more infected by mites (Figs. 1 and 3), in concert with earlier results from deer mice, where bolder individuals were shown to be more likely infected by a virus and exhibited specific behaviours connected with virus transmission (Disney and Dearing, 2013). Similarly, another study also found evidences for the relationship between parasitism and behaviour: Giles, (1983) reported that infected sticklebacks (*Schistocephalus solidus*) approach the model predator more readily than non-infected ones, while Barber et al. (2004) found reduced escape responses after infection in the same species. Webster (1994) demonstrated an increased activity in rats infected by *T. gondii* while reduced activity was found in a copepod parasitised by *Diphyllobothrium* (Pasternak et al., 1995). These workers, however, did not find differences between the sexes. Previously we found behavioural differences between the two morphological forms (winged and un-winged) in female, but not in male, firebugs—macropter females behave more boldly and more exploratively than brachypter ones (Gyuris et al., 2011). This finding also supports expected differences between sexes in behaviour, since females usually invest more into their reproduction, hence life-history strategies could have a stronger effect on females. Because of the reproductive interest of males and females this difference can conduct to adaptations and even counter-adaptations where one sex will act for selfish gain (Modlmeier et al., 2015). For instance, more aggressive female fishing spiders (*Dolomedes fimbriatus*) are more likely to kill their mates before copulation (Kralj-Fišer et al., 2013). This behaviour is costly for mating, but female fishing spiders increase efficiency during foraging and territory maintenance. Besides, another study found that the mating system can be altered as a result of group composition and the presence of a given behavioural type: male water striders dominate their mating pool and exclude the females for a while, then within the pools hyper aggressive males appear (Sih et al., 2014; Wey et al., 2015).

Barber and Dingemans (2010) suggest that high levels of activity and exploratory behaviour could increase an individual's chance of being infected because they can be exposed to a wider array of parasites. An alternative explanation may be that infected individuals become more active, bold and exploratory because parasites change their behaviour to increase the chance of their transmission into their next hosts (Liberat et al., 2009). At the moment we cannot say which of these explanations might account for the observed correlations between behaviour and infection status in firebugs in the studied wild population. Nevertheless, the fact that we cannot find structural differences of observed behavioural traits between infected and non-infected bugs (*structural consistency*, Stamps and Groothuis, 2010) might suggest that the first scenario is more plausible (more active and explorative bugs face more parasites). This is also supported by our finding that artificial infection does not modify the behaviour of firebugs.

Insects have internal immune defences based on humoral and cellular responses to pathogens that are able to cross the cuticle (Gillespie et al., 1997). The encapsulation of standardised antigen (nylon monofilament) is commonly used to measure immune competence and it was shown that the strength of the encapsulation response correlates with parasite and disease resistance (e.g.

Rantala and Roff, 2007; Gorman et al., 1996). Here we measured firebugs immune response and found that its degree was related to activity and boldness. Firebugs with a stronger immune response behaved less actively, but at the same time more boldly. However, we noticed that the latter was only marginally significant. It partially supports our previous expectation namely individuals with a stronger immune system should be bolder – to gain energy for an efficient immune system (Kortet et al., 2010); at the same time we found an opposite picture in the case of activity. Since these individuals originated from a laboratory stock culture we did not have information about the magnitude of parasitism or predation risk in the original population in the wild. Besides, we assume that the stronger immune response associated with bolder behaviour can be adaptive to compensate the potential risk of being parasitised as also found in field crickets (Kortet et al., 2007). Nevertheless if an individual invests in one trait (here the strong immune system) it might not be able to afford to invest in another energy-demanding function such as activity as we found in this study.

In order to truly understand why animals show different personalities we need to consider ecological variables like population density, predation or even parasite infection. This paper is an initial explorative study, which examines the relationship between firebugs' personality traits and their ectoparasitic mites. Further empirical studies are needed to gain an accurate view about behavioural consequences of parasitism in animal personality context, because individuals whose personality promotes a high parasite infection may be responsible for the high parasite transmission in a population, therefore play a crucial role in parasite and disease persistence (Natoli et al., 2005; Perkins et al., 2003). This suggests that the personality profile of populations can have important evolutionary consequences.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.beproc.2015.11.011>.

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