The price of being bold? Relationship between personality and endoparasitic infection in a tree squirrel

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Abstract

Individual variation in behaviour can contribute to the heterogeneous distribution of parasites among hosts for example by affecting the probability of encountering infective stages (larvae). Here, we investigated the relationship between host boldness/exploration tendency and gastro-intestinal helminth infection in invasive Eastern grey squirrels (*Sciurus carolinensis*). We used direct helminth counts, data rarely available in host-parasite studies that often used less reliable indirect estimates of infection. We predicted that bolder animals with a strong exploration tendency will have higher parasite load than shy, less explorative hosts. We examined two parameters of parasite infection: infection status and intensity of infection. Individual personality of 207 grey squirrels was assessed by capture-mark-recapture (CMR), calculating the trappability and trap diversity indices as estimates of boldness and exploration, respectively. Since both indices were strongly correlated, we used PCA to derive a single score (first component) which had a high value for bold, exploring animals. At the end of the study, 77 individuals were euthanized and gastro-intestinal helminths were identified and counted. Overall 73% of grey squirrels were infected by *Strongyloides robustus* with the intensity of infection varying from 1 to 86 worms (mean ± SE = 10.7 ± 2.1 helminths per host). We found that bolder, more explorative animals were more likely to be infected by *S. robustus*. However, host personality was not related to parasite intensity, which was instead positively associated with host body mass. Our results confirm that differences in personality-related host behaviour can influence the distribution of infections within host populations and stimulate further questions on whether such personality-parasite relationships may affect the invasion process.

Keywords: personality; *Strongyloides robustus*; invasive species; parasite infection; *Sciurus carolinensis*.

Introduction

Among vertebrates, individual variation in personality has been demonstrated to affect variation in fitness among animals within a population (Biro and Stamps 2008; Smith and Blumstein 2008; Dingemanse and Réale 2013). Personality is defined as consistent between-individual differences in behaviour, maintained over time and/or across ecological contexts (Réale et al. 2007). Differences in individual personality are often measured through traits such as risk-taking (boldness), exploring under new situations, aggressiveness and sociability (Réale et al. 2007). Discovering important resources (Wolf et al. 2007), acquiring intraspecific dominance and increasing survival (Smith and Blumstein 2008) are benefits that have been associated with animal boldness and/or exploration tendency. However, other studies described contrasting patterns with high costs related to boldness/exploration, such as increased chance to encounter predators (Sih et al. 2004; Boon et al. 2008), or reduced survival or reproductive success (Réale et al. 2009; Réale and Festa-Bianchet 2003; Sih et al. 2004).

Another potential ecological effect of personality is its influence on the probability of acquiring parasite infections (Hawley et al. 2011; Ezenwa et al. 2016). Risk-taking behaviour, exploration tendency, activity and sociality are all traits that may affect hosts' exposure to parasites by modulating contact rates among individuals and/or increasing chances of encountering parasite infective stages in the environment (Altizer et al. 2003; Kortet et al. 2010; Hawley et al. 2011; Ezenwa et al. 2016). For example, an indirect effect of activity-exploration on tick load was demonstrated in an introduced population of Siberian chipmunks (*Tamias sibiricus*) in France: tick load increased with space use, which in turn was positively affected by chipmunk's activity-exploration tendency (Boyer et al. 2010). On the other hand, several parasite species are known to manipulate behaviour as a strategy to enhance transmission, and indirect alterations of behaviour may also arise from physiological or immunological changes induced by infection (Kortet et al. 2010; Poulin 2013; Ezenwa et al. 2016). Establishing the causality in personality-infection relationships may therefore not be straightforward, especially when dealing with wild animals who are naturally infected prior to the study. In any case, independently from the directionality of the relationship, differences in individual personality can be associated with differences in exposure to parasite infective stages, and consequently with the distribution of parasites within host populations (Hawley et al. 2011; Ezenwa et al. 2016).

Here, we use the Eastern grey squirrel (*Sciurus carolinensis*), an invasive alien species in Northern Italy, as a study system to test the hypothesis that individual differences in personality will be associated with (endo)parasite infection. Eastern grey squirrels have been introduced in the British Isles and in parts of Italy (O'Teangana et al. 2000; Bertolino et al. 2014; Gurnell et al. 2015), where they have caused the decline of native Eurasian red squirrels (*Sciurus vulgaris*) by competing for food resources and increasing physiological stress in the native species (Wauters et al. 2002a, b; Gurnell et al. 2004, Santicchia et al. 2018a), and, exclusively on the British Isles, through disease-mediated competition (Tompkins et al. 2002; Romeo et al. 2018). Both male and female grey squirrels have strongly overlapping home ranges and neighbouring females can be close kin (Gurnell et al. 2001). The mating system is promiscuous with most matings done by older, dominant males (Koprowski 1994). By using an invasive species which is currently subject to a control program as a model system, we obtained direct counts of adult gastro-intestinal parasites, and did not have to rely on indirect measures of endoparasite loads (e.g. faecal egg counts) which offer less reliable estimates of actual parasite intensity (Tompkins and Hudson 1999;Villanúa et al. 2006; Romeo et al. 2014a). In Italy, invasive grey squirrels have fewer macroparasites (both ecto- and endoparasites) than in their native range and only one dominant gastro-intestinal helminth, *S. robustus*, which they carried along from N. America (prevalence 57% Romeo et al. 2014b). *S. robustus* is an obligate gastro-intestinal parasitic nematode common in many species of nearctic tree squirrels (e.g. Chandler 1942; Bartlett 1995). In Italy *S. robustus* was found to spill over to Eurasian red squirrels (Romeo et al. 2015). Nematodes within the genus *Strongyloides* have a direct (i.e. with no intermediate hosts) and peculiar life-cycle, which in most species includes parasitic generations consisting only of parthenogenic females and free-living dioecious generations with sexual reproduction (Viney and Lok 2015). However, Bartlett (1995) hypothesised that *S. robustus* may completely lack a free-living generation and sexual reproduction. In this species, parasitic, parthenogenetic females inhabit the proximal part of the small intestine, reproduce by parthenogenesis and release their eggs in the lumen where they are shed with host faeces. Eggs hatch in the environment where L1 larvae undergo two moults before reaching the infective stage. Infective larvae (L3) infect their host by skin-penetration, undergo a final moult into adult females and migrate through host tissues until they reach the intestine. The life-span of *Strongyloides* spp. is generally short compared to other helminth species: for instance, longevity of parasitic females *S. ratti* is on average 1-3 months, but it is believed that a small proportion of individuals can persist up to 1 year (Gems 2000; Wilkes et al. 2004). To our knowledge, there are no specific data about the longevity of *S. robustus* parasitic females.

In this system, we predict that bolder, more explorative grey squirrels are more likely to encounter free-living infective stages (larvae) of *Strongyloides robustus*, and consequently are more likely to become infected by the endoparasite. However, once the host is infected, we expect that other factors, related to host susceptibility and immune response (Perkins et al. 2003; Paterson and Viney 2002), may become predominant in determining individual intensity of infection (number of helminths per infected host). Hence, we expect only a weak or no relationship between a host's personality and intensity of endoparasite infection.

Materials and Methods

Study areas, trapping and handling squirrels

We trapped grey squirrels in 5 study areas (PIO, RS, BER, COM, MOR) located in Piedmont, Northern Italy (Table S1). All areas are woodlands or parks with a similar composition of mature deciduous trees and ornamental conifers surrounded by agricultural landscapes. Food availability was high in all study areas with seeds of oak (*Quercus petraea, Q. robur*), hornbeam (*Carpinus betulus*), black walnut (*Juglans nigra*) and lime (*Tilia cordata*) as major food resources used by grey squirrels (Wauters et al. 2001). We used areas with comparable habitat type and food availability to avoid potential confounding effects of habitat type on the personality-parasite relationship. Moreover, we included study area as a random factor in the models related to parasite infection and personality to account for other potential sources of variability between sites (see *Relationship between parasite infection and personality*). Trapping areas corresponded with total woodland/park areas surrounded by unsuitable habitat (open, cultivated fields). Grey squirrels had strongly overlapping home ranges (e.g. Gurnell et al. 2001) and all squirrels had access to both edge and central traps, avoiding the issue of edge-trap effects (Boyer et al. 2010).

In each site, we first carried out at least 2 capture-mark-recapture (CMR) sessions (one every two months) lasting 4 to 5 days to collect data for the estimation of indirect personality indices (details in Table

S1). In 4 areas (PIO, RS, BER and COM) CMR sessions were then followed by a final removal session in which individuals were culled (Table 1). Number of traps used varied slightly between sessions and/or study areas. A trapping session involved the use of 16 (PIO), 16 (RS), 17 (BER), 30 (COM), 48 (MOR) ground-placed Tomahawk live-traps (model 202, Tomahawk Live Trap Co, Wisconsin, USA) evenly spaced throughout the areas. We baited traps with hazelnuts and walnuts and checked them three times/day (details in SM1: ethical note). We flushed the trapped animal in a zipper-tube handling cloth and marked it at first capture with a Monel 1005-1L1 ear-tag (National Band & Tag Co. Newport, KY, USA). We measured length of the right hind foot with a thin ruler (precision 0.5mm) and weighed the squirrel with a Pesola spring-balance (precision 5g). We determined sex and age as described in detail elsewhere (Gurnell et al. 2001). During removal sessions, we euthanized grey squirrels by CO_2 inhalation following EC and AVMA guidelines (Close et al. 1996; Leary et al. 2013), and stored the carcasses at -20°C until examination.

Personality indices

We calculated two indirect indices of personality: a trappability and trap diversity index. An individual's willingness to enter a baited trap is often used as a measure of its tendency for risk-taking (boldness, Réale et al. 2007; Boon et al. 2008; Le Coeur et al. 2015). The number of different traps visited is considered a measure of the animal’s propensity to explore the environment (Réale et al. 2007; Boyer et al. 2010). We estimated trappability using the ratio of number of captures on the length of capture period (i.e. occasions: the number of trap checks from the first to the last trapping session for a given animal), and trap diversity by the ratio of number of different traps in which an animal was caught on the number of available traps. Subsequently, we standardised both ratios ((x-mean)/SD) within each study area to account for differences between study areas in trap numbers, squirrel density or capture history (Santicchia et al. 2018b). Recent studies have shown that personality traits to some degree can change over time and with age (flexible component of personality, Dingemanse and Réale 2013), therefore we excluded juvenile squirrels from the analysis. In addition, to avoid any potential bias due to squirrel dispersal during late summer-autumn (Koprowski 1994), and any possible effect of removal, we calculated trappability and trap diversity considering only capture sessions from late October to May, hence without using the final removal session.

Next, we verified the consistency of individual trappability and trap diversity across sessions. On a restricted sample of 127 squirrels, that were caught in at least two capture sessions, we calculated the repeatability in the number of different captures per session and in trap diversity per session. We estimated the repeatability and its confidence intervals from linear mixed-effects models through the function *rpt*, using bootstrap and permutation, as implemented in the R package *rptR* (Stoffel et al. 2017). We included study area, sex, session, number of occasions, number of active traps as fixed effects and squirrel identity as random intercept. The likelihood ratio test (LRT) was used to test for significance of the random intercept of each model (Martin and Réale 2008).

Before the analysis of parasite infection parameters, we checked for independence between the two standardised personality indices (Santicchia et al. 2018b). Since trappability and trap diversity were strongly and positively correlated ($r = 0.85$; $n = 207$; $p < 0.001$), we used a principal component analysis (PCA) to derive new variables which maximize the variance among individual squirrels. The first component (PC1) explained 92% of total variance (loadings: 0.707 trappability index + 0.707 trap diversity index), thus we only retained this variable as a single measure of an individual's personality. PC1 had a high score for those animals that are captured often and in many different traps, so it can be considered as a parameter that includes both boldness and exploration. Finally, since in several species personality traits can be related to body size or body mass (Martin and Réale 2008), we also checked for correlations between squirrel's body size (foot length) and mass and PC1 score.

Parasitological examination

On a total of 95 euthanized adult and subadult grey squirrels (31 males, 65 females), 77 also had trappability and trap-diversity estimates and were examined for gastro-intestinal helminths following standard parasitological procedures (SM2, see also Romeo et al. 2013, 2014b). *S. robustus* individuals were identified morphologically (Chandler 1942; Sato et al. 2007) and counted as described in SM2.

Relationship between parasite infection and personality

We explored the effect of PC1 score on three different descriptors/parameters of parasite infection, each one used as response variable in a specific Generalized Linear Mixed Model (GLMM). We first explored variation in *S. robustus* abundance (number of helminths per host, including uninfected hosts), which can be considered as a combined measure of both infection status (a host is infected or not) and parasite intensity (number of helminths per infected host). Then we ran two specific models on *S. robustus* infection status (infected/not infected) and intensity to disclose whether PC1 score is equally related to different aspects of parasite infection. Variation in infection status (n = 77) was explored through logistic regression, whilst variation in *S. robustus* abundance (n = 77) and intensity (n = 56) was analysed through GLMMs with negative binomial error distribution, in order to account for the aggregate distribution of parasites within the host population (Shaw et al. 1998). In all models, we examined the effect of PC1 score, body mass and sex on infection parameters, including study area as a random intercept to account for extrinsic heterogeneities among sites (such as food abundance, host density, see Table 1). Since helminth infections can differ between the sexes and with host body mass (e.g. Poulin 1996; Martin and Réale 2008), we also included the interactions between sex and PC1 score and sex and body mass in full models, but these interactions were eliminated when they did not contribute significantly to the model fit. Seasonal variation in infection parameters was not considered because all parasitological data were obtained from squirrels culled from the end of October to early January. All covariates were examined for collinearity using correlation coefficients (all $r < 0.4$) and pairwise scatterplots (Zuur et al. 2010), which indicated there were no collinearity problems in our data. Unless otherwise specified, all values and parameter estimates are reported as mean (± SE). All the statistical analyses were carried out using the software R 3.3.3 (R Development Core Team 2018).

Results

Personality indices and phenotypic variables

We captured a total of 207 individuals. Number of captures per individual grey squirrel varied between 1 and 10 (median 2, mean ± SE = 2.61 ± 0.12 captures), and number of different traps from 1 to 7 (median 2, mean ± SE = 1.98 ± 0.08 traps). There was no difference between the sexes in either mean number of captures

($F_{1, 201}$= 1.24, p = 0.27) or mean number of different traps ($F_{1, 201}$= 1.79, p = 0.18). Further details are given in SM3.

We observed significant and consistent within-individual variation, across CMR sessions, in number of captures (n = 127; repeatability = 0.25, 95% CI = 0.14 – 0.41, Likelihood ratio test LRT = 13.9; df = 1; p < 0.0001) and in trap diversity (n = 127; repeatability = 0.29, 95% CI = 0.18 – 0.45, Likelihood ratio test LRT = 18.9; df = 1; p < 0.0001).

Body mass was positively correlated with both foot length and PC1 score. When analysed per sex, this pattern was consistent in females, but not in males where body mass was not correlated with PC1 score (Table 2).

Parasitological examination

Overall 56 out of 77 grey squirrels were infected by *S. robustus* [prevalence = (the number of infected hosts/total number of examined squirrels)*100 = 73% ± 5.1%]. Parasite abundance varied from 0 to 86 helminths per squirrel (mean ± SE = 7.8 ± 1.6) and mean intensity (mI) of *S. robustus* was 10.7 ± 2.1 helminths per infected host. Only 12 grey squirrels hosted also other helminth species: *Trichostrongylus spp*. was found in 10 squirrels (13%, mean Intensity ± SE: 2.9 ± 0.6) and in two hosts we found 6 oxyurid nematodes and 1 specimen of cestode, respectively, but these parasites were not included in the analyses. Results of parasitological analysis by study area are given in Table 1.

Relationship between personality and parasite infection

There were no significant differences between males and females in either of the infection parameters and interactions of host sex with either PC1 score or body mass were not significant and thus were excluded from all the final models (all p > 0.25). The first, general model on *S. robustus* infection showed that endoparasite abundance in grey squirrels increased with both the host's PC1 score and body mass (Table 3). However, further models showed that infection status is only related to PC1 score (p = 0.03; Figure 1; Table 3), with bolder,more explorative individuals having a higher probability of being infected by *S. robustus*. Conversely, intensity of infection did not vary with PC1 score, but was positively related with host body mass (p = 0.02; Figure 2; Table 3).

Discussion

We studied whether Eastern grey squirrel personality differences were associated with individual variation in endoparasite infection and confirmed our prediction that bolder-more explorative animals had a higher abundance of *S. robustus* than shy-less explorative grey squirrels. However, we also showed that host personality was mainly related to the probability of acquiring *S. robustus*, whereas we found no relationship between boldness/exploration and parasite intensity, which was instead related to host body mass.

Indirect indices of personality and statistical inference

In several studies on free-ranging rodents in natural environments, animal personality has been estimated through capture-mark-recapture data (Boyer et al. 2010; Patterson and Shulte-Hostedde 2011; Le Coeur et al. 2015). These studies are based on the fact that consistent behavioural (personality) traits affect trappability, independent of other phenotypic factors such as size and sex (Biro 2013). Nevertheless, care must be taken when using an individual's captures record as a measure of a personality trait such as boldness and/or exploration when access to traps may vary among animals in the population (Boon et al. 2008; Boyer et al. 2010; Biro 2013). We are convinced that in this study, this bias risk is small for the following reasons: first, we observed a large inter-individual variability in number of captures and number of different traps used by squirrels, suggesting that we sampled both individuals with a high and a low trappability. Second, trapping probabilities estimated from long-term capture-mark-recapture studies on tree squirrels indicate high probabilities (>90%) for an animal that is present in the population of being captured at least once per year (Wauters et al. 2008; Santicchia et al. 2018b). Third, even if our sample was biased (e.g. shyest and/or most strongly infected animals are missed), this would lead to lack of power, but not to spurious results, making our tests and interpretations more conservative.

Recently, there is much debate about which are the most appropriate methods to study animal personality in the wild (e.g. Carter et al. 2013; Brehm and Mortelliti 2018). One point on which most studies agree is that a method can be considered reliable only as far as it has high individual repeatability, as personality traits must be maintained through time/space. Another point that strongly emerges from studies dealing with personality, is that methods are difficult to standardize as their efficiency depends on species-specific traits such as social and mating system, foraging habits, space use patterns: what works best to measure a chosen trait in one species might fail for another taxa, even if closely related (Rèale et al. 2007; Carter et al. 2013). In many studies on free-ranging sciurid rodents in natural environments, animal personality has been successfully measured through capture-mark-recapture data, using individual trappability (i.e. number of captures per animal during a trapping session) as an index of boldness/tendency to take risks, and trap diversity (i.e. number of different traps in which the same animal is caught) as a measure of exploration tendency (Boyer et al 2010; Patterson and Shulte-Hostedde 2011; Montiglio et al 2012; Le Coeur et al. 2015; Santicchia et al. 2018b; Mazzamuto et al. 2018a), These studies reported high and significant repeatabilities, and also in this study, trappability and trap diversity indices were statistically significant and consistent between trapping sessions, even when calculated only over two short trapping periods. Moreover, on a subset of the squirrels used in this study, PC1 score correlated significantly with personality indices derived from arena tests (Mazzamuto et al. 2018b; Santicchia et al. unpubl. data).

Personality and body mass

Relationships between body mass and personality traits such as boldness or exploration are highly variable among mammal species (Bighorn sheep, *Ovis canadensis*: Réale et al. 2009; Eastern chipmunk, *Tamias striatus*: Martin and Réale 2008; Muroid rodents: Careau et al. 2009; laboratory rat: Rödel and Meyer 2011). Among invasive grey squirrels, we found that body mass was correlated with boldness-exploration in females but not in males. In some non-territorial tree squirrels, heavier adult females seem to engage more frequently in aggressive interactions than animals of lower body mass (Wauters and Dhondt 1989; Koprowski 1994). Hence, a body mass, boldness-exploration correlation could suggests a behavioural syndrome (Sih et al. 2004) with bolder female squirrels being also the more aggressive ones, which could be investigated using specific tests, such as mirror image stimulation to measure aggressiveness (Réale et al. 2007). Alternatively, bold-explorative individuals could acquire access to more or higher quality resources and thus become heavier than shy-less explorative squirrels. This hypothesis could be further examined by observing foraging behaviour and space use of individually marked squirrels with known personality.

Personality-parasite relationship

Our findings revealed a general positive relationship between boldness-exploration and *S. robustus* abundance. However, when the two components that determine parasite abundance (infection status and intensity of infection) were analysed separately, we found that personality was related to the probability of becoming infected by *S. robustus*, but not to the amount of nematodes harboured by infected animals. Conversely, we found that host body mass was not associated with infection status, but related positively with intensity of infection, thereby confirming earlier results from a larger scale study on the same species (Romeo et al. 2014b). In other words, bolder-more explorative animals are more likely to acquire the parasite, reasonably as a consequence of a higher encounter probability with infective stages, but once infected, heavier grey squirrels tend to host more *S. robustus* worms than animals of lower body mass.

The relationship between animal personality and parasitism has been investigated in different mammals, where boldness, exploration or activity were found to influence infestation by ectoparasites (Siberian chipmunk: Boyer et al. 2010; Barbary ground squirrels: Piquet et al. 2018) or infection by virus (domestic cat: Natoli et al. 2005) or endoparasites (mainly gastro-intestinal helminths, Eastern chipmunk: Patterson and Shulte-Hostedde 2011). However, the relationship between different personality traits and the risk of acquiring parasites will vary depending on the specific infection dynamics and transmission strategies of the parasite (VanderWaal and Ezenwa 2016; Piquet et al. 2018). Concerning the *S. robustus* parasitic cycle (see study system), infective stages will likely be aggregated on the ground beneath tree canopies, and in general in those areas most used by arboreal squirrels. Additionally, squirrels' nests (dens and dreys) have been suggested as a microenvironment that might favour infective larval development and survival (Wetzel and Weigl 1994; Bartlett 1995; Romeo et al. 2015). Hence, other than on the ground, infection by free-living, skin-penetrating larvae could occur inside nests, and transmission might be facilitated by nest-sharing which occurs regularly (Koprowski 1994; Romeo et al. 2015). As a consequence, if bolder-more explorative squirrels forage more widely, interact more frequently with other squirrels and/or use more different nest sites (red squirrels, Wauters and Dhondt 1990; grey squirrels, Koprowski 1994), they will be more likely to encounter free-living infective stages and become infected by *S. robustus.*

Concerning the directionality of the personality-infection relationship observed in our study, we are aware that, as in most previous studies on personality – parasite associations, our research was performed on individuals that were naturally infected prior to the personality measures. It is therefore impossible to discriminate whether certain personality traits cause greater infection risk, or whether infection causes changes in the expression of personality traits (Ezenwa et al. 2016). Parasitic manipulation of host behaviour, and parasites affecting a host's personality have been demonstrated in various taxa (Barber and Dingemanse 2010; Poulin 2013; Ezenwa et al. 2016) and we cannot rule out this possibility. However, based on the biology and transmission cycle of *S. robustus*, we believe that in this specific study-system, it is more likely that personality affects macroparasite infection and not the opposite. Firstly, *S. robustus* has a direct life cycle (i.e. with no intermediate hosts) and in the case of helminths, direct manipulation of host behaviour, including personality, is typically observed in intermediate hosts as a strategy to enhance transmission to the definitive hosts through predation (Poulin 2013). Another possibility would be that the parasite affects animal personality through indirect pathways, such as weakening host condition or affecting trade-offs between investment in self-maintenance and certain costly personality traits (Poulin 2013; Ezenwa et al. 2016), and such indirect effects of infection on host behaviour can arise also in infections by nematodes with direct cycles. For example, Kavaliers and Colwell (1995) found that mice infected by *Heligmosomoides poligyrus* showed a decrease in spatial learning abilities likely induced by immuno-mediated mechanisms. However, this does not seem to be the case in infections by *Strongyloides* spp., as Braithwaite et al. (1998) found no evidence of impaired learning and memory in rats experimentally infected by the nematode *Strongyloides ratti*.

Finally, despite personality being associated to the probability of becoming infected by *S. robustus*, the number of helminths harboured by the host does not seem to be influenced by re-exposure to infective stages, as it was not related to bold-explorative behaviours, but rather to host body mass. Nematodes within the genus *Strongyloides* are known to elicit complex, density-dependent immune responses (Paterson and Viney 2002; Romeo et al. 2014a), and it is thus likely that re-exposure, and, in turn, personality will have a negligible impact on their numbers, which are instead mainly regulated by immune-mediated processes.

Hence, a tentative explanation for the positive relationship between *S. robustus* intensity and hosts' body mass may be that heavier, often older and more dominant squirrels invest more energy in fat reserves, spacing behaviour (i.e. defending larger home ranges, Don 1983), survival and reproduction (Wauters and Dhondt 1989; Lane et al. 2010) than in the immune system (Sheldon and Verhulst 1996). Such energy allocation trade-offs could lead in turn to a less effective immune response against parasite infection (Shoenle et al. 2018 and references therein). Complex relationships between parasites, personality and body mass (or body condition index) have also been documented in other sciurid species such as least chipmunks (*Tamias minimus*), where ectoparasite abundance was positively influenced by both exploration tendency and body condition index (Bohn et al. 2017).

In conclusion, the observed personality – parasite infection relationship in invasive grey squirrels and their co-introduced helminth parasite could potentially affect the invasion process and the interactions with native species. Compared to shy-less explorative individuals, bold-more explorative animals are more likely to be the first to invade new areas (Cote et al. 2010), and, based on our findings, they are also more likely to become infected and to carry along helminths. In our study, we focused on a single, dominant parasite, but if our results could be extended to other parasite species, this would imply that the observed relationship could favour both the spread of alien pathogens and the acquisition of local ones. As a consequence, a positive relationship between boldness-exploration and infection by parasites could increase the chances for spillover and spill-back processes towards native species (Kelly et al. 2009; Romeo et al. 2014b) or even slow down the invasion, due to the costs associated with infection. Whether this is actually a general pattern in invasive host-parasite systems (Chapple et al. 2012; Piquet et al. 2018) could be further investigated by studying personality–parasite relationships along an invasion wave or by comparing the degree of boldness-exploration tendency between native and introduction ranges. Our findings of personality – parasitism interactions in invasive mammals allow a better prediction of their potential role in spreading infectious agents, supporting the assessment of their impact on biological conservation.

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Data references

The datafile will be made available upon acceptance of the paper.

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Table 1. Grey squirrel population size and density per study area with data on *Strongyloides robustus* infection. N = number of examined squirrels; n = number of infected hosts; p = prevalence; mA = mean abundance (n. parasites/examined hosts); mI = mean intensity (n. parasites/infected hosts).

Study area	**Squirrel population size***	**Squirrel density(ind/ha**)**	***Strongyloides robustus* infection**		
			n (p)	**mA ± SE**	**mI ± SE**
BER (N = 8)	30	6.1	1 (12.5%)	0.25 ± 0.25	2.0
COM (N = 27)	49	15.2	18 (67%)	2.26 ± 0.61	3.39 ± 0.79
PIO (N = 18)	29	11.3	15 (83%)	19.0 ± 5.9	22.8 ± 6.62
RS (N = 24)	46	7.8	22 (92%)	8.17 ± 1.57	8.91 ± 1.62

* Population size was estimated using mark recapture model with Schumacher Eschmeyer estimator as implemented in the R (R Development Core Team 2017) package *fishmethods* (Nelson 2017).

** BER (4.9 ha); COM (3.2 ha); PIO (2.6 ha); RS (5.9 ha)

Table 2. Pearson's correlation between foot length, body mass and PC1 in grey squirrels. Sample size between brackets.

	Foot length	**PC1 (Boldness and exploration)**
Both sexes (n = 77)		
Body mass	r = 0.41, p = 0.001	r = 0.28, p = 0.01
Foot length		r = 0.07, p = 0.58
Males (n = 29)		
Body mass	r = 0.42, p = 0.03	r = 0.25, p = 0.20
Foot length		r = 0.21, p = 0.30
Females (n= 48)		
Body mass	r = 0.43, p = 0.008	r = 0.32, p = 0.03
Foot length		r = -0.05, p = 0.77

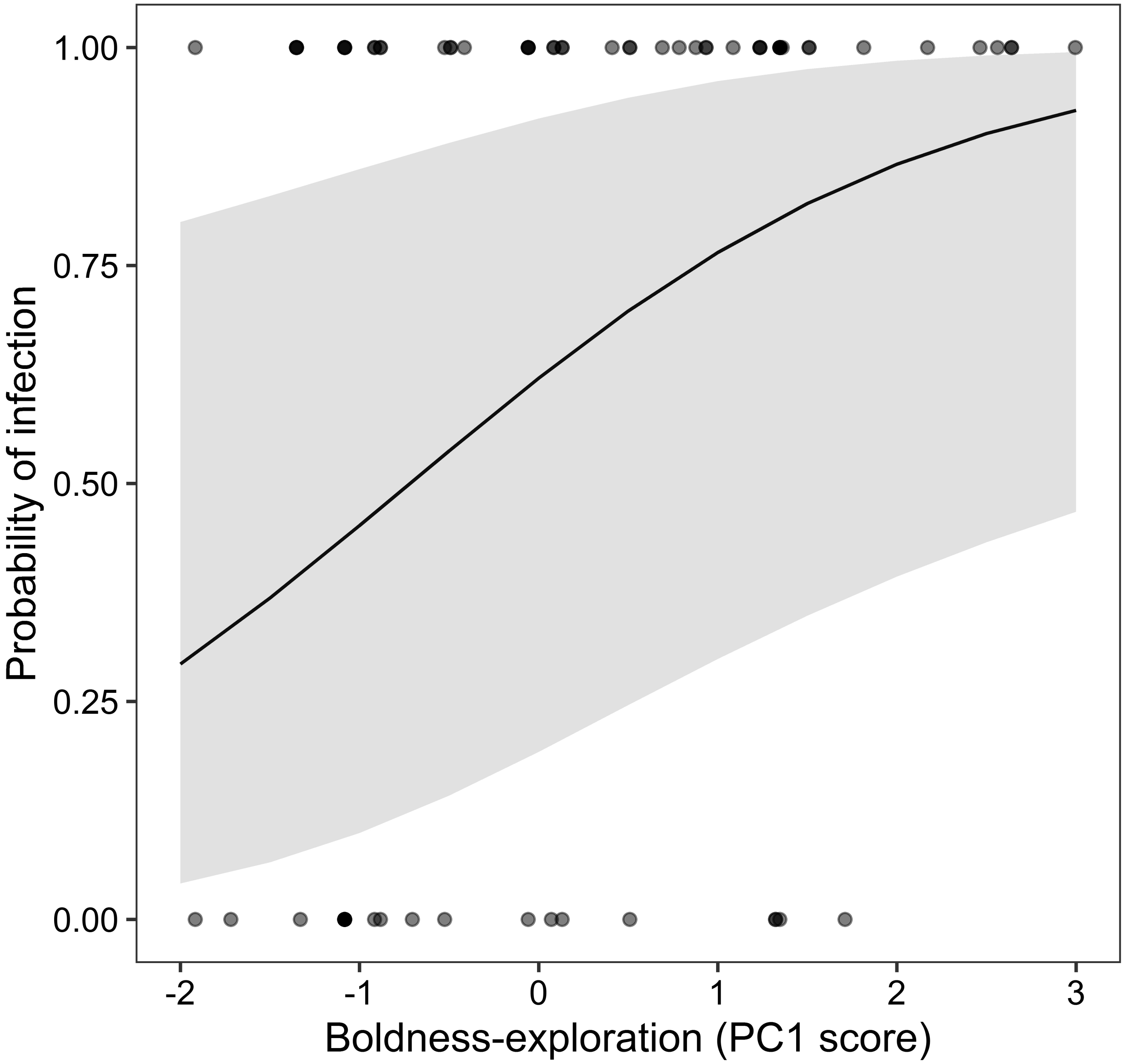
Table 3. Selected models explaining observed variation in infection by *Strongyloides robustus* in grey squirrel hosts.

Response variable	**Predictor**	**Parameter estimate**	**df**	**z-value**	**p**
Abundance	Sex[†]	0.30 ± 0.29	1	1.04	0.30
	Body mass	0.008 ± 0.003	1	2.41	0.016
	PC1 score	0.24 ± 0.12	1	2.02	0.043
Infection status	Sex[†]	0.76 ± 0.68	1	1.11	0.27
	Body mass	0.011 ± 0.009	1	1.29	0.20
	PC1 score	0.69 ± 0.31	1	2.02	0.028
Intensity	Sex[†]	0.12 ± 0.24	1	0.49	0.63
	Body mass	0.007 ± 0.003	1	2.43	0.015
	PC1 score	0.101 ± 0.103	1	0.99	0.32

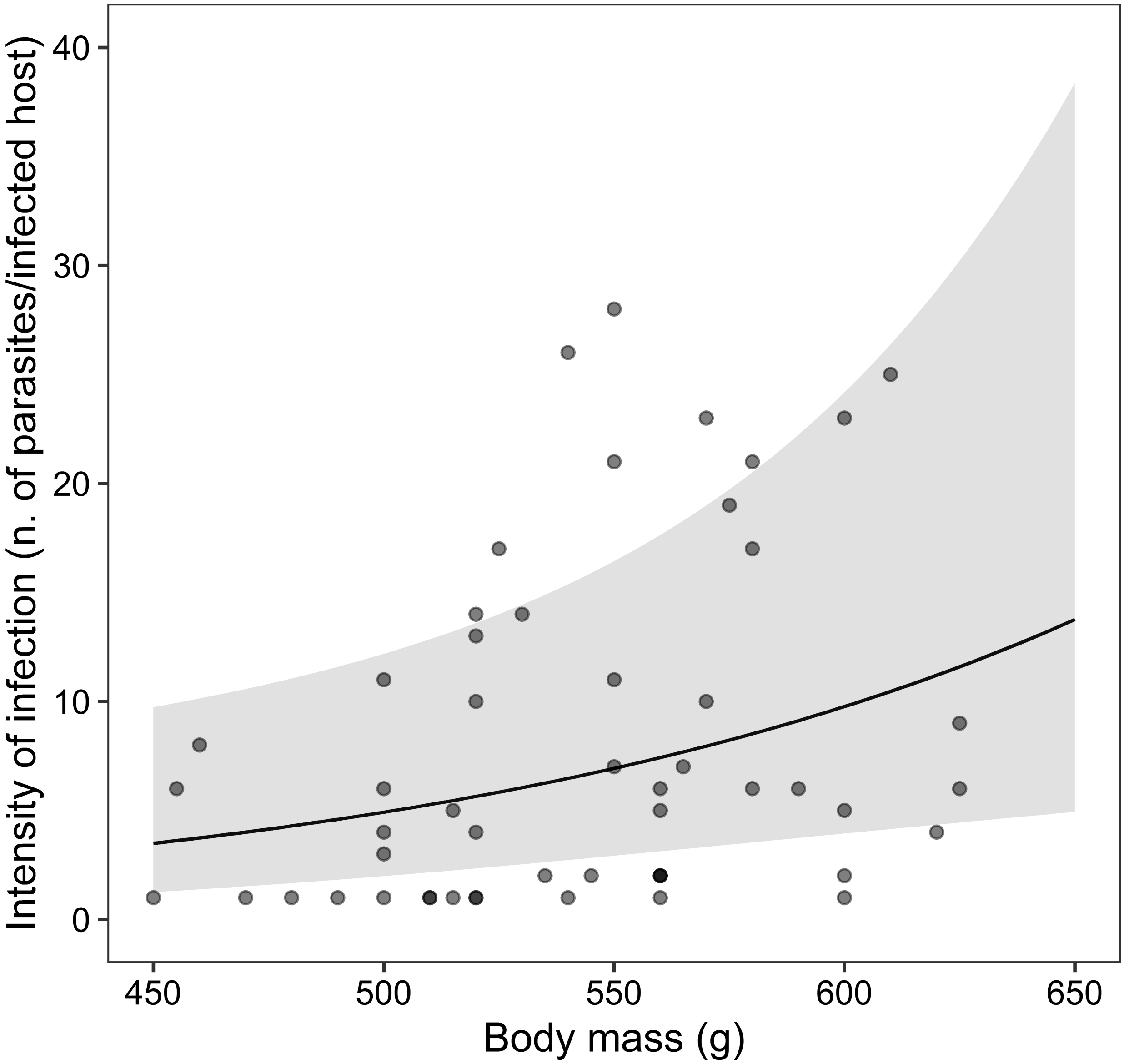
[†] females held as reference level

Figure 1. Relationship between *Strongyloides robustus* infection status and host boldness-exploration (PC1 score) in grey squirrels: observed values (full circles) and probability of infection predicted by the model (line). Grey band indicates 95% CI. Colour of circles is darker when different points overlap.

Figure 2. Relationship between *Strongyloides robustus* intensity of infection and host body mass in grey squirrels: observed values (full circles) and values predicted by the model (line). Grey band indicates 95% CI. Colour of circles is darker when different points overlap.



1.00
0.75
0.50
0.25
0.00
Probability of infection
-2
-1
0
1
2
3
Boldness-exploration (PC1 score)


Intensity of infection (n. of parasites/infected host)
0
10
20
30
40
450
500
550
600
650
Body mass (g)

Declaration of interest form

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. Authors have no competing interests. As corresponding author, I declare that the work described has not been published previously, that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder.

Santicchia et al. supplementary material

Table S1. Study area coordinates, dates of CMR sessions and the grey squirrel removal session.

Study area (size, ha)	Coordinates	CMR sessions	Removal session
PIO (2.6)	44°56'13" N; 7°35'30" E	January 2015 March 2015 May 2015	October 2015
RS (5.9)	44°57’01" N; 7°35'41" E	January 2015 March 2015 May 2015	October 2015
BER (4.9)	44°47'25"N; 7°38'59"E	December 2014 February 2015 April 2015	November 2015
COM (3.2)	44°50'20"N; 7°46'05"E	November 2016 December 2016	January 2017
MOR (25.0)	44°55'42"N; 7°44'43" E	December 2015 February 2016 April 2016	No removal

SM1. Ethical note on trapping and handling procedures

Grey squirrels were trapped using Tomahawk Live Traps (Model 202, Tomahawk Live Trap Co., Hazelhurst, WI, USA), partly covered with dark plastic sheets to provide animals with shelter. Traps were baited with hazelnuts and walnuts, and were checked three times/day to minimize time in trap. Before handling the trap was completely covered with a cloth to reduce stress. Trapped animals were flushed in a zipper-tube handling cloth to reduce direct contact with the operator and marked at first capture with a Monel 1005-1L1 ear-tag (National Band & Tag Co. Newport, KY, USA). Handling time was kept as short as possible (less than 5 minutes). Trapping and handling methods complied with the Italian national law on wildlife (L.N. 157 of 1992) and were based on the scientific guidelines and opinion from the Italian Institute for Environmental Protection and Research (ISPRA). At the end of the study, animals had to be killed as part of a control program on Invasive Alien Species (sample size 95 grey squirrels, 31 males and 64 females, all adults or subadults; LIFE09 NAT/IT/000095 EC-SQUARE Project) and followed national and European guidelines (ITA Decree No. 230/2017; EU Regulation No. 1143/2014). Specific permits were granted by the competent authorities for wildlife research and management of Turin and Cuneo Provinces (permit nr. D.D. 294-34626 of 2014 and Prot. n. 0002624 of 13/01/2014, respectively).Trapped squirrels were shifted from the zipper-tube to a plastic container where they were euthanized by CO_2 overdose, following EC and

AVMA guidelines (Close et al. 1996; Leary et al. 2013). All the operators were trained by veterinary staff specialised in animal welfare. Each carcass was placed in a sealed plastic bag and stored at -20°C for later examination. Tissue and blood samples collected from carcasses and not used in this study are either stored for future studies or have already been utilized within other research projects, in line with the principles set out in the 3Rs.

SM2: Details on helminth counts

The whole gastrointestinal tract from the oesophagus to the rectum was removed during post-mortem examination. Each tract (stomach, small intestine, caecum and colon to rectum) was dissected separately and washed with tap water, and its content was filtered through two sieves (lumen 0.40 and 0.038 mm, respectively). The content of each tract was then examined under a stereomicroscope (×10 magnification), and adult *S. robustus* were counted.

SM3: Differences in trappability and trap diversity between study areas

The average number of captures ($F_{4,\ 201}= 4.58$, $p = 0.001$) and number of different traps in which a squirrel was caught differed significantly between study areas ($F_{4,\ 201}= 5.41$, $p = 0.0004$). These differences between areas did not influence our results since trapping indices were standardised within study areas.

Trappability was higher in PIO and BER than in the other three study areas (DLSM, all $p < 0.05$; BER n = 33, 3.19 ± 0.32 captures; PIO n = 32, 3.44 ± 0.30 captures; COM n = 40, 2.00 ± 0.20 captures; RS n = 47, 2.43 ± 0.22 captures; MOR n = 55, 2.4 ± 0.26 captures). Also trap diversity differed between study areas. It was significantly higher in PIO than in COM, MOR and RS (DLSM, all $p < 0.05$; PIO n = 32, 2.53 ± 0.22 traps; COM n = 40, 1.8 ± 0.17 traps; MOR n = 55, 1.67 ± 0.12 traps; RS n = 47, 1.83 ± 0.13 traps), and also higher in BER than in MOR (DLSM, $p < 0.05$; BER n = 33, 2.39 ± 0.20 traps).