

This item is the archived peer-reviewed author-version of:

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

Reference:

Santicchia Francesca, Romeo Claudia, Ferrari Nicola, Matthysen Erik, Vanlauw e Laura, Wauters Luc, Martinoli Adriano.- The price of being bold? Relationship between personality and endoparasitic infection in a tree squirrel
Zeitschrift für Säugetierkunde - ISSN 0044-3468 - 97(2019), p. 1-8
Full text (Publisher's DOI): <https://doi.org/10.1016/J.MAMBIO.2019.04.007>
To cite this reference: <https://hdl.handle.net/10067/1601300151162165141>

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

3

4 Francesca Santicchia^{a†}, Claudia Romeo^{b†}, Nicola Ferrari^{b,c}, Erik Matthysen^d, Laure Vanlauwe^d, Lucas A
5 Wauters^{a,d*}, Adriano Martinoli^a

6

7 († contributed equally to this manuscript)

8

9 ^aEnvironment Analysis and Management Unit - Guido Tosi Research Group - Department of Theoretical
10 and Applied Sciences, Università degli Studi dell'Insubria, Varese, Italy

11 ^bDepartment of Veterinary Medicine, Università degli Studi di Milano, Via Celoria 10, 20133 Milan, Italy

12 ^cCentro di Ricerca Coordinata Epidemiologia e Sorveglianza Molecolare delle Infezioni, Università degli
13 Studi di Milano, Milano, Italy

14 ^dDepartment of Biology, Evolutionary Ecology Group, University of Antwerp, Universiteitsplein 1, 2610
15 Wilrijk, Belgium

16

17 *Corresponding author (Wauters L.A.): Department of Theoretical and Applied Sciences, Università degli
18 Studi dell'Insubria, via J.H. Dunant, 3 – 21100 – Varese. Italy. Telephone: (+39) 0332 421538. email:

19 l.wauters@uninsubria.it

20

21

22 **Abstract**

23

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

42

43

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

45

46

47

48

49

50 **Introduction**

51

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

63 Another potential ecological effect of personality is its influence on the probability of acquiring
64 parasite infections (Hawley et al. 2011; Ezenwa et al. 2016). Risk-taking behaviour, exploration tendency,
65 activity and sociality are all traits that may affect hosts' exposure to parasites by modulating contact rates
66 among individuals and/or increasing chances of encountering parasite infective stages in the environment
67 (Altizer et al. 2003; Kortet et al. 2010; Hawley et al. 2011; Ezenwa et al. 2016). For example, an indirect
68 effect of activity-exploration on tick load was demonstrated in an introduced population of Siberian
69 chipmunks (*Tamias sibiricus*) in France: tick load increased with space use, which in turn was positively
70 affected by chipmunk's activity-exploration tendency (Boyer et al. 2010). On the other hand, several parasite
71 species are known to manipulate behaviour as a strategy to enhance transmission, and indirect alterations of
72 behaviour may also arise from physiological or immunological changes induced by infection (Kortet et al.
73 2010; Poulin 2013; Ezenwa et al. 2016). Establishing the causality in personality-infection relationships may
74 therefore not be straightforward, especially when dealing with wild animals who are naturally infected prior
75 to the study. In any case, independently from the directionality of the relationship, differences in individual
76 personality can be associated with differences in exposure to parasite infective stages, and consequently with
77 the distribution of parasites within host populations (Hawley et al. 2011; Ezenwa et al. 2016).

78 Here, we use the Eastern grey squirrel (*Sciurus carolinensis*), an invasive alien species in Northern Italy, as a
79 study system to test the hypothesis that individual differences in personality will be associated with
80 (endo)parasite infection. Eastern grey squirrels have been introduced in the British Isles and in parts of Italy
81 (O'Teangana et al. 2000; Bertolino et al. 2014; Gurnell et al. 2015), where they have caused the decline of
82 native Eurasian red squirrels (*Sciurus vulgaris*) by competing for food resources and increasing physiological
83 stress in the native species (Wauters et al. 2002a, b; Gurnell et al. 2004, Santicchia et al. 2018a), and,
84 exclusively on the British Isles, through disease-mediated competition (Tompkins et al. 2002; Romeo et al.
85 2018). Both male and female grey squirrels have strongly overlapping home ranges and neighbouring
86 females can be close kin (Gurnell et al. 2001). The mating system is promiscuous with most matings done by
87 older, dominant males (Koprowski 1994). By using an invasive species which is currently subject to a
88 control program as a model system, we obtained direct counts of adult gastro-intestinal parasites, and did not
89 have to rely on indirect measures of endoparasite loads (e.g. faecal egg counts) which offer less reliable
90 estimates of actual parasite intensity (Tompkins and Hudson 1999; Villanúa et al. 2006; Romeo et al. 2014a).
91 In Italy, invasive grey squirrels have fewer macroparasites (both ecto- and endoparasites) than in their native
92 range and only one dominant gastro-intestinal helminth, *S. robustus*, which they carried along from N.
93 America (prevalence 57% Romeo et al. 2014b). *S. robustus* is an obligate gastro-intestinal parasitic
94 nematode common in many species of nearctic tree squirrels (e.g. Chandler 1942; Bartlett 1995). In Italy *S.*
95 *robustus* was found to spill over to Eurasian red squirrels (Romeo et al. 2015). Nematodes within the genus
96 *Strongyloides* have a direct (i.e. with no intermediate hosts) and peculiar life-cycle, which in most species
97 includes parasitic generations consisting only of parthenogenic females and free-living dioecious generations
98 with sexual reproduction (Viney and Lok 2015). However, Bartlett (1995) hypothesised that *S. robustus* may
99 completely lack a free-living generation and sexual reproduction. In this species, parasitic, parthenogenic
100 females inhabit the proximal part of the small intestine, reproduce by parthenogenesis and release their eggs
101 in the lumen where they are shed with host faeces. Eggs hatch in the environment where L1 larvae undergo
102 two moults before reaching the infective stage. Infective larvae (L3) infect their host by skin-penetration,
103 undergo a final moult into adult females and migrate through host tissues until they reach the intestine. The
104 life-span of *Strongyloides* spp. is generally short compared to other helminth species: for instance, longevity
105 of parasitic females *S. ratti* is on average 1-3 months, but it is believed that a small proportion of individuals

106 can persist up to 1 year (Gems 2000; Wilkes et al. 2004). To our knowledge, there are no specific data about
107 the longevity of *S. robustus* parasitic females.

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

115

116 **Materials and Methods**

117 *Study areas, trapping and handling squirrels*

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

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

132 S1). In 4 areas (PIO, RS, BER and COM) CMR sessions were then followed by a final removal session in
133 which individuals were culled (Table 1). Number of traps used varied slightly between sessions and/or study
134 areas. A trapping session involved the use of 16 (PIO), 16 (RS), 17 (BER), 30 (COM), 48 (MOR) ground-
135 placed Tomahawk live-traps (model 202, Tomahawk Live Trap Co, Wisconsin, USA) evenly spaced
136 throughout the areas. We baited traps with hazelnuts and walnuts and checked them three times/day (details
137 in SM1: ethical note). We flushed the trapped animal in a zipper-tube handling cloth and marked it at first
138 capture with a Monel 1005-1L1 ear-tag (National Band & Tag Co. Newport, KY, USA). We measured
139 length of the right hind foot with a thin ruler (precision 0.5mm) and weighed the squirrel with a Pesola
140 spring-balance (precision 5g). We determined sex and age as described in detail elsewhere (Gurnell et al.
141 2001). During removal sessions, we euthanized grey squirrels by CO₂ inhalation following EC and AVMA
142 guidelines (Close et al. 1996; Leary et al. 2013), and stored the carcasses at -20°C until examination.

143

144 *Personality indices*

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

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

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

177

178 *Parasitological examination*

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

183

184 *Relationship between parasite infection and personality*

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

205

206 **Results**

207 *Personality indices and phenotypic variables*

208 We captured a total of 207 individuals. Number of captures per individual grey squirrel varied between
209 1 and 10 (median 2, mean \pm SE = 2.61 ± 0.12 captures), and number of different traps from 1 to 7 (median 2,
210 mean \pm SE = 1.98 ± 0.08 traps). There was no difference between the sexes in either mean number of captures

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

213 We observed significant and consistent within-individual variation, across CMR sessions, in number
214 of captures ($n = 127$; repeatability = 0.25, 95% CI = 0.14 – 0.41, Likelihood ratio test LRT = 13.9; $df = 1$; p
215 < 0.0001) and in trap diversity ($n = 127$; repeatability = 0.29, 95% CI = 0.18 – 0.45, Likelihood ratio test
216 LRT = 18.9; $df = 1$; $p < 0.0001$).

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

220

221 *Parasitological examination*

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

229

230 *Relationship between personality and parasite infection*

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

237 intensity of infection did not vary with PC1 score, but was positively related with host body mass ($p = 0.02$;
238 Figure 2; Table 3).

239

240 **Discussion**

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

246

247 *Indirect indices of personality and statistical inference*

248 In several studies on free-ranging rodents in natural environments, animal personality has been estimated
249 through capture-mark-recapture data (Boyer et al. 2010; Patterson and Shulte-Hostedde 2011; Le Coeur et al.
250 2015). These studies are based on the fact that consistent behavioural (personality) traits affect trappability,
251 independent of other phenotypic factors such as size and sex (Biro 2013). Nevertheless, care must be taken
252 when using an individual's captures record as a measure of a personality trait such as boldness and/or
253 exploration when access to traps may vary among animals in the population (Boon et al. 2008; Boyer et al.
254 2010; Biro 2013). We are convinced that in this study, this bias risk is small for the following reasons: first,
255 we observed a large inter-individual variability in number of captures and number of different traps used by
256 squirrels, suggesting that we sampled both individuals with a high and a low trappability. Second, trapping
257 probabilities estimated from long-term capture-mark-recapture studies on tree squirrels indicate high
258 probabilities (>90%) for an animal that is present in the population of being captured at least once per year
259 (Wauters et al. 2008; Santicchia et al. 2018b). Third, even if our sample was biased (e.g. shyest and/or most
260 strongly infected animals are missed), this would lead to lack of power, but not to spurious results, making
261 our tests and interpretations more conservative.

262 Recently, there is much debate about which are the most appropriate methods to study animal
263 personality in the wild (e.g. Carter et al. 2013; Brehm and Mortelliti 2018). One point on which most studies

264 agree is that a method can be considered reliable only as far as it has high individual repeatability, as
265 personality traits must be maintained through time/space. Another point that strongly emerges from studies
266 dealing with personality, is that methods are difficult to standardize as their efficiency depends on species-
267 specific traits such as social and mating system, foraging habits, space use patterns: what works best to
268 measure a chosen trait in one species might fail for another taxa, even if closely related (Réale et al. 2007;
269 Carter et al. 2013). In many studies on free-ranging sciurid rodents in natural environments, animal
270 personality has been successfully measured through capture-mark-recapture data, using individual
271 trappability (i.e. number of captures per animal during a trapping session) as an index of boldness/tendency
272 to take risks, and trap diversity (i.e. number of different traps in which the same animal is caught) as a
273 measure of exploration tendency (Boyer et al 2010; Patterson and Shulte-Hostedde 2011; Montiglio et al
274 2012; Le Coeur et al. 2015; Santicchia et al. 2018b; Mazzamuto et al. 2018a), These studies reported high
275 and significant repeatabilities, and also in this study, trappability and trap diversity indices were statistically
276 significant and consistent between trapping sessions, even when calculated only over two short trapping
277 periods. Moreover, on a subset of the squirrels used in this study, PC1 score correlated significantly with
278 personality indices derived from arena tests (Mazzamuto et al. 2018b; Santicchia et al. unpubl. data).

279

280 *Personality and body mass*

281 Relationships between body mass and personality traits such as boldness or exploration are highly variable
282 among mammal species (Bighorn sheep, *Ovis canadensis*: Réale et al. 2009; Eastern chipmunk, *Tamias*
283 *striatus*: Martin and Réale 2008; Muroid rodents: Careau et al. 2009; laboratory rat: Rödel and Meyer 2011).
284 Among invasive grey squirrels, we found that body mass was correlated with boldness-exploration in
285 females but not in males. In some non-territorial tree squirrels, heavier adult females seem to engage more
286 frequently in aggressive interactions than animals of lower body mass (Wauters and Dhondt 1989;
287 Koprowski 1994). Hence, a body mass, boldness-exploration correlation could suggests a behavioural
288 syndrome (Sih et al. 2004) with bolder female squirrels being also the more aggressive ones, which could be
289 investigated using specific tests, such as mirror image stimulation to measure aggressiveness (Réale et al.
290 2007). Alternatively, bold-explorative individuals could acquire access to more or higher quality resources

291 and thus become heavier than shy-less explorative squirrels. This hypothesis could be further examined by
292 observing foraging behaviour and space use of individually marked squirrels with known personality.

293

294 *Personality-parasite relationship*

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

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

318 sites (red squirrels, Wauters and Dhondt 1990; grey squirrels, Koprowski 1994), they will be more likely to
319 encounter free-living infective stages and become infected by *S. robustus*.

320 Concerning the directionality of the personality-infection relationship observed in our study, we are
321 aware that, as in most previous studies on personality – parasite associations, our research was performed on
322 individuals that were naturally infected prior to the personality measures. It is therefore impossible to
323 discriminate whether certain personality traits cause greater infection risk, or whether infection causes
324 changes in the expression of personality traits (Ezenwa et al. 2016). Parasitic manipulation of host behaviour,
325 and parasites affecting a host’s personality have been demonstrated in various taxa (Barber and Dingemanse
326 2010; Poulin 2013; Ezenwa et al. 2016) and we cannot rule out this possibility. However, based on the
327 biology and transmission cycle of *S. robustus*, we believe that in this specific study-system, it is more likely
328 that personality affects macroparasite infection and not the opposite. Firstly, *S. robustus* has a direct life
329 cycle (i.e. with no intermediate hosts) and in the case of helminths, direct manipulation of host behaviour,
330 including personality, is typically observed in intermediate hosts as a strategy to enhance transmission to the
331 definitive hosts through predation (Poulin 2013). Another possibility would be that the parasite affects
332 animal personality through indirect pathways, such as weakening host condition or affecting trade-offs
333 between investment in self-maintenance and certain costly personality traits (Poulin 2013; Ezenwa et al.
334 2016), and such indirect effects of infection on host behaviour can arise also in infections by nematodes with
335 direct cycles. For example, Kavaliers and Colwell (1995) found that mice infected by *Heligmosomoides*
336 *poligyryus* showed a decrease in spatial learning abilities likely induced by immuno-mediated mechanisms.
337 However, this does not seem to be the case in infections by *Strongyloides* spp., as Braithwaite et al. (1998)
338 found no evidence of impaired learning and memory in rats experimentally infected by the nematode
339 *Strongyloides ratti*.

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

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

355 In conclusion, the observed personality – parasite infection relationship in invasive grey squirrels
356 and their co-introduced helminth parasite could potentially affect the invasion process and the interactions
357 with native species. Compared to shy-less explorative individuals, bold-more explorative animals are more
358 likely to be the first to invade new areas (Cote et al. 2010), and, based on our findings, they are also more
359 likely to become infected and to carry along helminths. In our study, we focused on a single, dominant
360 parasite, but if our results could be extended to other parasite species, this would imply that the observed
361 relationship could favour both the spread of alien pathogens and the acquisition of local ones. As a
362 consequence, a positive relationship between boldness-exploration and infection by parasites could increase
363 the chances for spillover and spill-back processes towards native species (Kelly et al. 2009; Romeo et al.
364 2014b) or even slow down the invasion, due to the costs associated with infection. Whether this is actually a
365 general pattern in invasive host-parasite systems (Chapple et al. 2012; Piquet et al. 2018) could be further
366 investigated by studying personality–parasite relationships along an invasion wave or by comparing the
367 degree of boldness-exploration tendency between native and introduction ranges. Our findings of personality
368 – parasitism interactions in invasive mammals allow a better prediction of their potential role in spreading
369 infectious agents, supporting the assessment of their impact on biological conservation.

370

371 **Acknowledgements**

372 We thank all woodland owners for giving us access to their estates. We are also grateful to Mattia Panzeri
373 and Lara Bernasconi for help with the fieldwork. Comments by two anonymous referees helped to improve

374 the paper. Grey squirrel control was carried out as part of post-LIFE activities (LIFE09 NAT/IT/000095 EC-
375 SQUARE). This research did not receive any specific grant from funding agencies in the public, commercial,
376 or not-for-profit sectors.

377

378 **Data references**

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

380

381 **References**

382

383 Altizer, S., Nunn, C.L., Thrall, P.H., Gittleman, J.L., Antonovics, J., Cunningham, A.A., Dobson, A.P.,

384 Ezenwa, V., Jones, K.E., Pederson, A.B., Poss, M., Pulliam, J.R.C., 2003. Social organization and parasite

385 risk in mammals: integrating theory and empirical studies. *Annual Rev. Ecol. Evol. Syst.* 34, 517-547.

386 doi: 10.1146/annurev.ecolsys.34.030102.151725.

387 Barber, I., Dingemanse, N.J., 2010. Parasitism and the evolutionary ecology of animal personality. *Phil.*

388 *Trans. R. Soc. B.* 365, 4077–4088. doi: 10.1098/rstb.2010.0182.

389 Bartlett, C., 1995. Morphology, homogonic development, and lack of a free-living generation in

390 *Strongyloides robustus* (Nematoda, Rhabditoidea), a parasite of North American sciurids. *Folia Parasitol.*

391 42, 102-114.

392 Bell, A.M., Hankisonm S.J., Laskowski, K.L., 2009. The repeatability of behaviour: a meta analysis. *Anim.*

393 *Behav.* 77, 771-783. doi: 10.1016/j.anbehav.2008.12.022.

394 Bertolino, S., Montezemolo, N.C., Preatoni, D.G., Wauters, L.A., Martinoli, A., 2014. A grey future for

395 Europe: *Sciurus carolinensis* is replacing native red squirrels in Italy. *Biol. Inv.* 16, 53-62.

396 doi: 10.1007/s10530-013-0502-3.

397 Biro, P.A., Stamps, J.A., 2008. Are animal personality traits linked to life-history productivity? *Trends Ecol.*

398 *Evol.* 23, 361-368. doi: 10.1016/j.tree.2008.04.003.

399 Biro, P.A., 2013. Are most samples of animals systematically biased? Consistent individual trait differences
400 bias samples despite random sampling. *Oecologia* 171, 339-345. doi: 10.1007/s00442-012-2426-5.

401 Bohn, S.J., Webber, Q.M.R., Florko, K.R.N., Paslawski, K.R., Peterson, A.M., Piche, J.E., Menzies, A.K.,
402 Willis, C.K.R., 2017. Personality predicts ectoparasite abundance in an asocial sciurid. *Ethology*
403 doi: 10.1111/eth.12651.

404 Boon, A.K., Réale, D., Boutin, S., 2008. Personality, habitat use, and their consequences for survival in
405 North American red squirrels *Tamiasciurus hudsonicus*. *Oikos* 117, 1321-1328. doi: 10.1111/j.0030-
406 1299.2008.16567.x.

407 Boyer, N., Réale, D., Marmet, J., Pisanu, B., Chapuis, J-L., 2010. Personality, space use and tick load in an
408 introduced population of Siberian chipmunks *Tamias sibiricus*. *J. Anim. Ecol.* 79, 538-547.
409 doi: 10.1111/j.1365-2656.2010.01659.x.

410 Braithwaite, V.A., Salkeld, D.J., McAdam, H.M., Hockings, C.G., Ludlow, A.M., Read, A.F., 1998. Spatial
411 and discrimination learning in rodents infected with the nematode *Strongyloides ratti*. *Parasitology* 117, 145-
412 154.

413 Brehm, A.M., Mortelliti, A. 2018. Mind the trap: large-scale field experiment shows that trappability is not a
414 proxy for personality. *Anim. Behav.* 142, 101-112.

415 Careau, V., Bininda-Emonds, O.R.P., Thomas, D.W., Réale D., Humphries M.M., 2009. Exploration
416 strategies map along fast–slow metabolic and life-history continua in muroid rodents. *Funct. Ecol.* 23, 150-
417 156. doi: 10.1111/j.1365-2435.2008.01468.x.

418 Carter, A.J., Feeney, W.E., Marshall, H.H., Cowlshaw, G., Heinsohnm R., 2013. Animal personality: what
419 are behavioural ecologists measuring? *Biol. Rev.* 88, 465-475. doi: 10.1111/brv.12007.

420 Chandler, A.C., 1942. Helminths of tree squirrels in Southeast Texas. *J. Parasitol.* 28, 135-140.
421 doi: 10.2307/3272725.

422 Chapple, D.G., Simmonds, S.M., Wong, B.B.M., 2012. Can behavioral and personality traits influence the
423 success of unintentional species introductions? *Trends Ecol. Evol.* 27, 57-64.
424 doi: 10.1016/j.tree.2011.09.010.

425 Close, B., Banister, K., Baumans, V., Bernoth, E.M., Bromage, N., Bunyan, J., Erhardt, W., Flecknell, P.,
426 Gregory, N., Hackbarth, H., Morton, D., Warwick, C., 1996. Recommendations for euthanasia of
427 experimental animals: Part 1. *Lab. Anim.* 30, 293-316. doi: 10.1258/002367796780739871.

428 Cote, J., Clobert, J., Brodin, T., Fogarty, S., Sih, A., 2010. Personality-dependent dispersal: characterization,
429 ontogeny and consequences for spatially structured populations. *Philos. Trans. R. Soc. B* 365, 4065-4076.
430 doi: 10.1098/rstb.2010.0176.

431 Dingemanse, N.J., Réale, D., 2013. What is the evidence for natural selection maintaining animal personality
432 variation? In: Carere, C., Maestripieri, D. (Eds.) *Animal personalities: behavior, physiology, and evolution.*
433 1st ed, Chicago University Press, Chicago (IL), pp. 201-220.

434 Don, B.A.C., 1983. Home range characteristics and correlates in tree squirrels. *Mammal Rev.* 13, 123-132.
435 doi: 10.1111/j.1365-2907.1983.tb00273.x.

436 Ezenwa, V.O., Archie, E.A., Craft, M.E., Hawley, D.M., Martin, L.B., Moore, J., White, L., 2016. Host
437 behaviour–parasite feedback: an essential link between animal behaviour and disease ecology. *Proc. R. Soc.*
438 *B* 283. doi: 10.1098/rspb.2015.3078.

439 Gems, D., 2000. Longevity and ageing in parasitic and free-living nematodes. *Biogerontology* 1,
440 289–307. doi: [10.1023/A:1026546719091](https://doi.org/10.1023/A:1026546719091)

441 Gurnell, J., Wauters, L.A., Preatoni, D., Tosi, G., 2001. Spacing behaviour, kinship and dynamics of the grey
442 squirrel in a newly colonised deciduous woodland in north Italy. *Can. J. Zool.* 79, 1533-1543.
443 doi: 10.1139/z01-109.

444 Gurnell, J., Wauters, L.A., Lurz, P.W.W., Tosi, G., 2004. Alien species and interspecific competition: effects
445 of introduced eastern grey squirrels on red squirrel population dynamics. *J. Anim. Ecol.* 73, 26-35.
446 doi: 10.1111/j.1365-2656.2004.00791.x.

447 Gurnell, J., Lurz, P.W.W., Wauters, L.A., 2015. Years of interactions and conflict in Europe: competition
448 between Eurasian red squirrels and North American grey squirrel. In: Shuttleworth, C.M., Lurz, P.W.W.,
449 Hayward, M.W. (Eds) Red squirrels: Ecology, Conservation & Management in Europe. England, pp. 19–37.

450 Hawley, D.M., Etienne, R.S., Ezenwa, V.O., Jolles, A.E., 2011. Does animal behavior underlie covariation
451 between hosts' exposure to infectious agents and susceptibility to infection? Implications for disease
452 dynamics. *Integr. Comp. Biol.* 51, 528-539. doi: 10.1093/icb/acr062.

453 Kavaliers, M., Colwell, D.D., 1995. Reduced spatial learning in mice infected with the nematode,
454 *Heligmosomoides polygyrus*. *Parasitology* 110, 591-597. doi: 10.1017/S0031182000065318.

455 Kelly, D.W., Paterson, R.A., Townsend, C.R., Poulin, R., Tompkins, D.M., 2009. Parasite spillback: A
456 neglected concept in invasion ecology? *Ecology* 90, 2047-2056. doi: 10.1890/08-1085.1.

457 Koprowski, J.L., 1994. *Sciurus carolinensis*. *Mammalian Species* 480, 1-9. doi: 10.2307/3504224.

458 Kortet, R., Hedrick, A.V., Vainikka, A., 2010. Parasitism, predation and the evolution of animal
459 personalities. *Ecology Letters* 13, 1449-1458. doi: 10.1111/j.1461-0248.2010.01536.x

460 Lane, J.E., Boutin, S., Speakman, J.R., Humphries, M.M., 2010. Energetic costs of male reproduction in a
461 scramble competition mating system. *J. Anim. Ecol.* 79, 27-34. doi: 10.1111/j.1365-2656.2009.01592.x.

462 Le Coeur, C., Thibault, M., Pisanu, B., Thibault, S., Chapuis, J-L., Baudry, E., 2015. Temporally fluctuating
463 selection on a personality trait in a wild rodent population. *Behav. Ecol.* 26, 1285-1291.
464 doi: 10.1093/beheco/arv074.

465 Leary, S., Underwood, W., Anthony, R., Cartner, S., Corey, D., Grandin, T., Greenacre, C.B., Gwaltney-
466 Bran, S., McCrackin, M.A., Meyer, R., Miller, D., Shearer, J., Yanong, R., 2013. AVMA Guidelines for the
467 Euthanasia of Animals: 2013 Edition.

468 Martin, J.G.A., Réale, D., 2008. Temperament, risk assessment and habituation to novelty in eastern
469 chipmunks, *Tamias striatus*. *Anim. Behav.* 75, 309-318. doi: 10.1016/j.anbehav.2007.05.026.

470 Mazzamuto, M.V., Wauters, L.A., Preatoni, D.G., & Martinoli, A., 2018b. Behavioural and population
471 responses of ground-dwelling rodents to forest edges. *Hystrix* 29, 211–215. doi:10.4404/hystrix-00119-2018

472 Mazzamuto, M.V., Cremonesi, G., Santicchia, F., Preatoni, D.G., Martinoli, A., Wauters, L.A., 2018b.
473 Rodents in the arena: a critical evaluation of methods measuring personality traits. *Eth. Ecol & Evol.* Doi
474 10.1080/03949370.2018.1488768.

475 Montiglio, P-O., Garant, D., Pelletier, F., R eale, D., 2012. Personality differences are related to long-term
476 stress reactivity in a population of wild eastern chipmunks, *Tamias striatus*. *Anim. Behav.* 84, 1071-1079.

477 Natoli, E., Say, L., Cafazzo, S., Bonanni, R., Schmid, M., Pontier, D., 2005. Bold attitude makes male urban
478 feral domestic cats more vulnerable to Feline Immunodeficiency Virus. *Neurosci. Biobehav. Rev.* 29, 151-
479 157. doi: 10.1016/j.neubiorev.2004.06.011.

480 Nelson, G.A., 2017. fishmethods: Fisheries Methods and Models in R.
481 <http://CRAN.Rproject.org/package=fishmethods>.

482 O'Teangana, D., Reilly, S., Montgomery, W.I., Rotchford, J., 2000. The distribution and status of the Red
483 squirrel (*Sciurus vulgaris*) and Grey squirrel (*Sciurus carolinensis*) in Ireland. *Mammal Rev.* 30, 45-56.
484 doi: 10.1046/j.1365-2907.2000.00054.x.

485 Paterson, S., Viney, M.E., 2002. Host immune responses are necessary for density dependence in nematode
486 infections. *Parasitology* 125, 283-292. doi: 10.1017/S0031182002002056.

487 Patterson, L.D., Schulte-Hostedde, A.I., 2011. Behavioural correlates of parasitism and reproductive success
488 in male eastern chipmunks, *Tamias striatus*. *Anim. Behav.* 81, 1129-1137.
489 doi: 10.1016/j.anbehav.2011.02.016.

490 Pelletier, F., Page, K.A., Ostiguy, T., Festa-Bianchet, M., 2005. Fecal counts of lungworm larvae and
491 reproductive effort in bighorn sheep, *Ovis canadensis*. *Oikos* 110, 473-480. doi: 10.1111/j.0030-
492 1299.2005.14120.x.

493 Perkins, S.E., Cattadori, I.M., Tagliapietra, V., Rizzoli, A., Hudson, P.J., 2003. Empirical evidence for key
494 hosts in persistence of a tick-borne disease. *Int. J. Parasitol.* 33, 909–917. doi:10.1016/S0020-7519(03)
495 00128-0.

496 Piquet, J.C, López-Darias, M., van der Marel, A., Nogales, M., Waterman, J., 2018. Unraveling behavioral
497 and pace-of-life syndromes in a reduced parasite and predation pressure context: personality and survival of
498 the Barbary ground squirrel. *Behav. Ecol. Sociobiol.* 72, 147. doi: 10.1007/s00265-018-2549-8

499 Poulin, R., 1996. Sexual inequalities in helminth infections: a cost of being a male? *Am. Nat.* 147, 287-295.

500 Poulin, R., 2013. Parasite manipulation of host personality and behavioural syndromes. *J. Exp. Biol.* 216, 18-
501 26. doi: 10.1242/jeb.073353.

502 R Core Team, 2018. R: A language and environment for statistical computing. R Foundation for Statistical
503 Computing, Vienna, Austria. URL <https://www.R-project.org/>

504 Réale, D., Festa-Bianchet, M., 2003. Predator-induced selection on temperament in bighorn ewes. *Anim.*
505 *Behav.* 65, 463-470. doi: 10.1006/anbe.2003.2100.

506 Réale, D., Reader, S.M., Sol, D., McDougall, P.T., Dingemanse, N.J., 2007. Integrating animal temperament
507 within ecology and evolution. *Biol. Rev.* 82, 291-318. doi: 10.1111/j.1469-185X.2007.00010.x.

508 Réale, D., Martin, J., Coltman, D.W., Poissant, J., Festa-Bianchet, M., 2009. Male personality, life-history
509 strategies and reproductive success in a promiscuous mammal. *J. Evol. Biol.* 22, 1599-1607.
510 doi: 10.1111/j.1420-9101.2009.01781.x.

511 Rödel, H.G., Meyer, S., 2011. Early development influences ontogeny of personality types in young
512 laboratory rats. *Dev. Psychobiol.* 53, 601-613. doi: 10.1002/dev.20522.

513 Romeo, C., Pisanu, B., Ferrari, N., Basset, F., Tillon, L., Wauters, L.A., Martinoli, A., Saino, N., Chapuis, J-
514 L., 2013. Macroparasite community of the Eurasian red squirrel (*Sciurus vulgaris*): poor species richness and
515 diversity. *Parasitol. Res.* 112, 3527-3536. doi: 10.1007/s00436-013-3535-8.

516 Romeo, C., Wauters, L.A., Cauchie, S., Martinoli, A., Matthysen, E., Saino, N., Ferrari, N., 2014a. Faecal
517 egg counts from field experiment reveal density dependence in helminth fecundity: *Strongyloides robustus*
518 infecting grey squirrels (*Sciurus carolinensis*). *Parasitol. Res.* 113, 3403-3408. doi: 10.1007/s00436-014-
519 4005-7.

520 Romeo, C., Wauters, L.A., Ferrari, N., Lanfranchi, P., Martinoli, A., Pisanu, B., Preatoni, D.G., Saino, N.,
521 2014b. Macroparasite fauna of alien grey squirrels (*Sciurus carolinensis*): Composition, variability and
522 implications for native species. PLoS ONE 9: e88002 doi: 10.1371/journal.pone.0088002.

523 Romeo, C., Ferrari, N., Lanfranchi, P., Saino, N., Santicchia, F., Martinoli, A., Wauters, L.A., 2015.
524 Biodiversity threats from outside to inside: effects of alien grey squirrel (*Sciurus carolinensis*) on helminth
525 community of native red squirrel (*Sciurus vulgaris*). Parasitol. Res. 114, 2621-2628. doi: 10.1007/s00436-
526 015-4466-3.

527 Romeo, C., McInnes, C.J., Dale, T.D., Shuttleworth, C.M., Bertolino, S., Wauters, L.A., Ferrari, N., 2018.
528 Disease, invasions and conservation: no evidence of squirrelpox virus in grey squirrels introduced to Italy.
529 Anim. Cons. doi:10.1111/acv.12433.

530 Santicchia, F., Dantzer, B., van Kesteren, F., Palme, R., Martinoli, A., Ferrari, N., Wauters, L.A., 2018a.
531 Stress in biological invasions: Introduced invasive grey squirrels increase physiological stress in native
532 Eurasian red squirrels. J. Anim. Ecol. 87, 1342-1352. doi: 10.1111/1365-2656.12853.

533 Santicchia, F., Gagnaison, C., Bisi, F., Martinoli, A., Matthysen, E., Bertolino, S., Wauters, L.A., 2018b.
534 Habitat-dependent effects of personality on survival and reproduction in red squirrels. Behav. Ecol.
535 Sociobiol. 72. doi: 10.1007/s00265-018-2546-y.

536 Sato, H., Torii, H., Une, Y., Ooi, H-K., 2007. A new Rhabditoid nematode species in Asian sciurids, distinct
537 from *Strongyloides robustus* in North American Sciurids. J. Parasitol. 93, 1476-1486. doi: 10.1645/GE-
538 1106.1.

539 Shaw, D.J., Grenfell, B.T., Dobson, A.P., 1998. Patterns of macroparasite aggregation in wildlife host
540 populations. Parasitology 117, 597-610.

541 Sheldon, B., Verhulst, S., 1996. Ecological immunology: costly parasite defences and trade-offs in
542 evolutionary ecology. Trends Ecol. Evol. 11, 317-321. doi: 10.1016/0169-5347(96)10039-2.

543 Sih, A., Bell, A.M., Johnson, J.C., Ziemba, R.E., 2004. Behavioral syndromes: an integrative overview. Q.
544 Rev. Biol. 79, 241-277. doi: 10.1086/422893.

545 Smith, B.R., Blumstein, D.T., 2008. Fitness consequences of personality: a meta-analysis. *Behav. Ecol.*
546 19, 448-455. doi: 10.1093/beheco/arm144.

547 Stoffel, M.A., Nakagawa, S., Schielzeth, H., 2017. rptR: Repeatability estimation and variance
548 decomposition by generalized linear mixed-effects models. *Methods Ecol. Evol.* 8, 1639-1644.
549 doi: 10.1111/2041-210X.12797.

550 Tompkins, D.M., Hudson, P.J., 1999. Regulation of nematode fecundity in the ring-necked pheasant
551 (*Phasianus colchicus*): not just density dependence. *Parasitology* 118, 417–423.

552 Tompkins, D.M., Sainsbury, A.W., Nettleton, P., Buxton, D., Gurnell, J., 2002. Parapoxvirus causes a
553 deleterious disease in red squirrels associated with UK population declines. *Proc. R. Soc. B* 269, 529-533.
554 doi: 10.1098/rspb.2001.1897.

555 VanderWaal, K.L., Ezenwa, V.O., 2016. Heterogeneity in pathogen transmission: mechanisms and
556 methodology. *Funct. Ecol.* 30, 1606-1622. doi: 10.1111/1365-2435.12645.

557 Villanúa, D., Pérez-Rodríguez, L., Gortázar, C., Höfle, U., Viñuela, J., 2006. Avoiding bias in parasite
558 excretion estimates: the effect of sampling time and type of faeces. *Parasitology* 133, 251-259. doi:
559 10.1017/S003118200600031X.

560 Viney, M.E., Lok, J.B., 2015.. The biology of *Strongyloides spp.* In: *Wormbook*, ed. The *C. elegans*
561 Research Community, WormBook. doi/10.1895/wormbook.1.7.1, <http://www.wormbook.org>

562 Wauters, L.A., Dhondt, A.A., 1989. Body weight, longevity and reproductive success in red squirrels
563 (*Sciurus vulgaris*). *J. Anim. Ecol.* 58, 637-651. doi: 10.2307/4853.

564 Wauters, L.A., Dhondt, A.A., 1990. Nest-use by red squirrels (*Sciurus vulgaris* Linnaeus, 1758). *Mammalia*
565 54, 377-389. doi: 10.1515/mamm.1990.54.3.377.

566 Wauters, L.A., Tosi, G., Gurnell, J., 2002a. Interspecific competition in tree squirrels: do introduced grey
567 squirrels (*Sciurus carolinensis*) deplete tree seeds hoarded by red squirrels (*S. vulgaris*)? *Behav. Ecol.*
568 *Sociobiol.* 51, 360-367. doi: 10.1007/s00265-001-0446-y.

569 Wauters, L.A., Gurnell, J., Martinoli, A., Tosi, G., 2002b. Interspecific competition between native Eurasian
570 red squirrels and alien grey squirrels: does resource partitioning occur? *Behav. Ecol. Sociobiol.* 52, 332-341.
571 doi: 10.1007/s00265-002-0516-9.

572 Wauters, L.A., Githiru, M., Bertolino, S., Molinari, A., Tosi, G., Lens, L., 2008. Demography of alpine red
573 squirrel populations in relation to fluctuations in seed crop size. *Ecography* 31, 104-114. doi:
574 10.1111/j.2007.0906-7590.05251.x.

575 Wetzel, E.J., Weigl, P.D., 1994. Ecological implications for flying squirrels (*Glaucomys* spp.) of effects of
576 temperature on the in vitro development and behavior of *Strongyloides robustus*. *Am. Midl. Nat.* 131, 43-54.
577 doi: 10.2307/2426607.

578 Wilkes, C.P., Thompson, F.J., Gardner, M.P., Paterson, S., Viney, M.E., 2004. The effect of the host
579 immune response on the parasitic nematode *Strongyloides ratti*. *Parasitology* 128, 661–669. doi:
580 10.1017/S0031182004005062

581 Wolf, M., van Doorn, G.S., Leimar, O., Weissing, F.J., 2007. Life-history trade-offs favour the evolution of
582 animal personalities. *Nature* 447, 581-584. doi: 10.1038/nature05835.

583

584

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

Study area	Squirrel population size*	Squirrel density(ind/ha**)	<i>Strongyloides robustus</i> 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

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

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

591

592 **Table 2.** Pearson's correlation between foot length, body mass and PC1 in grey squirrels. Sample size
593 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

594

595

596 **Table 3.** Selected models explaining observed variation in infection by *Strongyloides robustus* in grey
 597 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

598 [†]females held as reference level

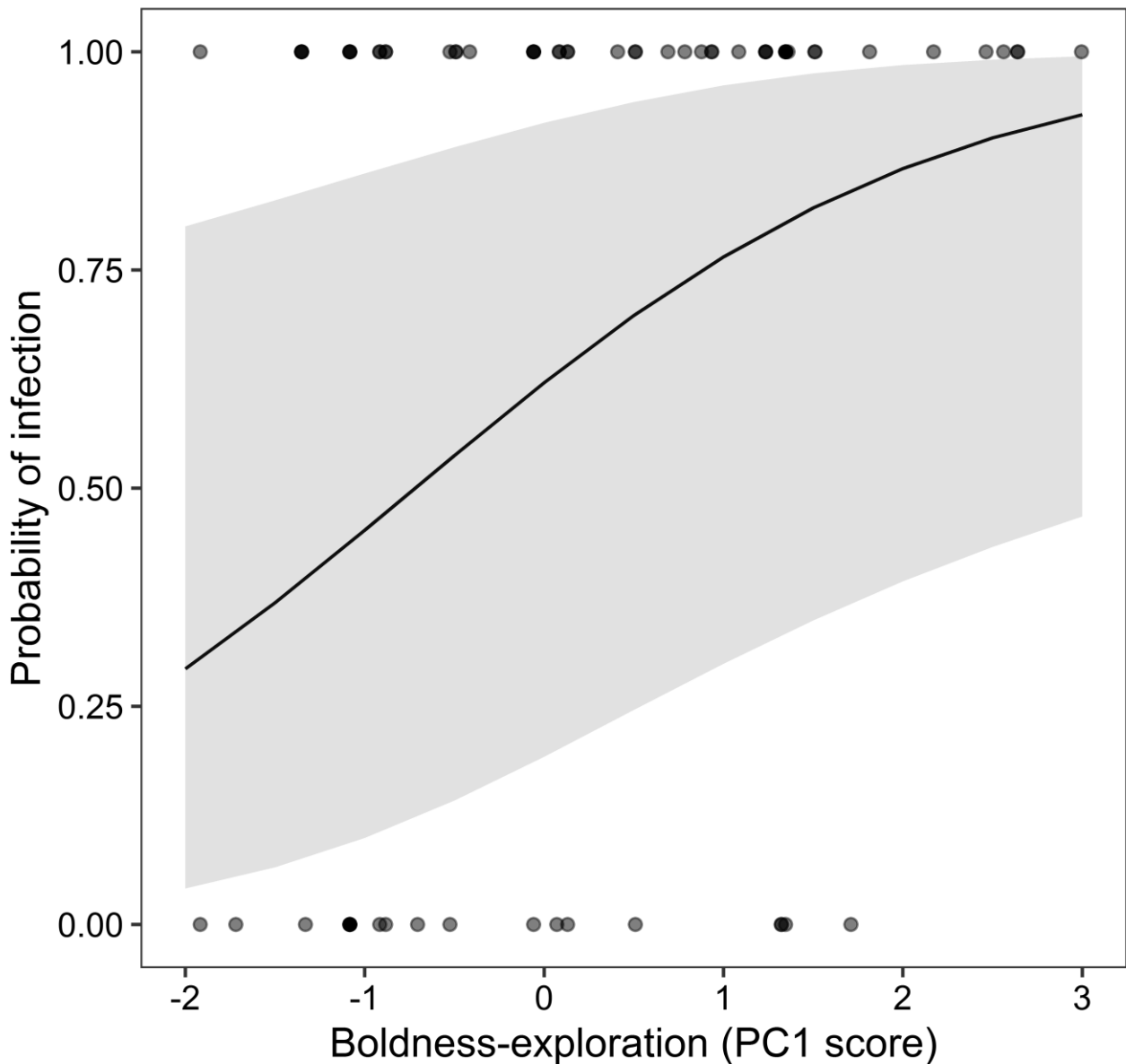
599

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

603

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

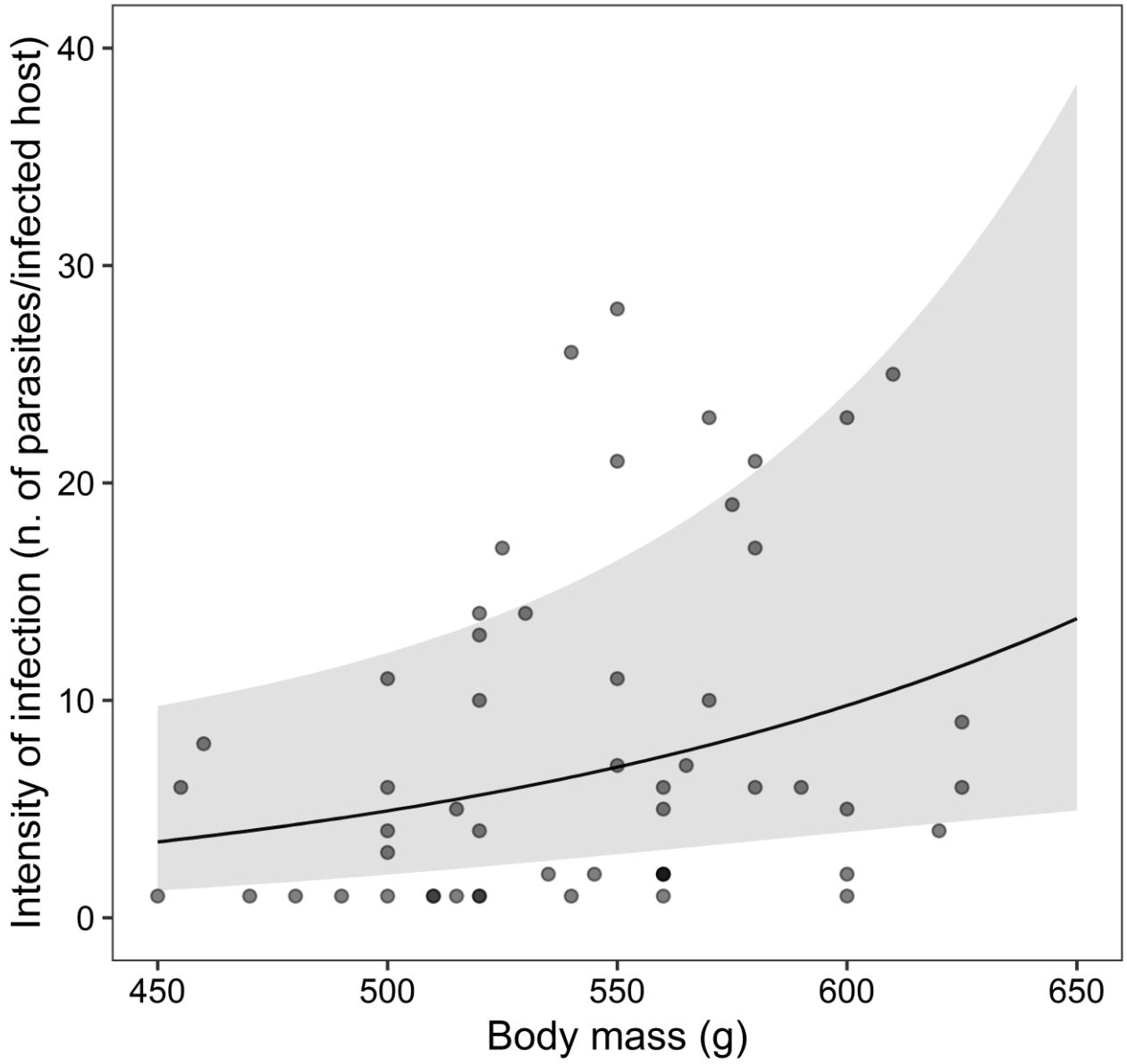
607



608

609

610



611