

1 **Spillover of an alien parasite reduces expression of costly behaviour in native host species**

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13

## 14 Abstract

- 15 1. Understanding effects of invasive alien species (IAS) on native host-parasite relationships is  
16 of importance for enhancing ecological theory and IAS management. When IAS and their  
17 parasite(s) invade a guild, the effects of interspecific resource competition and/or parasite-  
18 mediated competition can alter existing native host-parasite relationships and the dependent  
19 biological traits such as native species' behaviour.
- 20 2. We used a natural experiment of populations of native red squirrels (*Sciurus vulgaris*) that  
21 were colonised by the alien grey squirrel (*Sciurus carolinensis*), comparing repeated  
22 measurements of red squirrel parasite infection and personality with those taken in sites  
23 where only the native species occurred.
- 24 3. We explored two alternative hypotheses: (a) individual differences in personality traits  
25 (activity and/or sociability) of native red squirrel positively affect the probability of  
26 macroparasite spillover and thus the likelihood to acquire the alien's parasitic helminth  
27 (*Strongyloides robustus*); (b) the combined effects of grey squirrel presence and parasite  
28 infection result in a reduction of costly personality traits (activity and/or exploration).
- 29 4. Using data from 323 arena tests across three experimental (native species and IAS) and three  
30 control sites (only native species), we found negative correlations between native species'  
31 activity and infection with *S. robustus* in the sites invaded by the alien species. Activity was  
32 also negatively correlated with infection by its native helminth (*Trypanoxyuris sciuri*) but  
33 only when grey squirrels were present, while in the red-only sites there was no relationship  
34 of *T. sciuri* infection with any of the personality traits. Moreover, individuals that acquired *S.*  
35 *robustus* during the study reduced their activity after infection, while this was not the case  
36 for animals that remained uninfected.
- 37 5. Our results show that parasite-mediated competition is costly, reducing activity in  
38 individuals of the native species, and altering the native host-native parasite relationships.

39

40 **KEYWORDS**

41 Helminths, invasive alien species, macroparasites, Mirror Image Stimulation test, Open Field Test,  
42 personality traits, *Sciurus carolinensis*, *Sciurus vulgaris*

43

44 **1 INTRODUCTION**

45 Species interactions within a guild are often complex and interspecific competition can have  
46 implications for population dynamics, niche partitioning, prey-predator dynamics and host-parasite  
47 interactions (Sih, Cote, Evans, Fogarty, & Pruitt, 2012; Henrichs et al., 2016; Rhoades, Best, &  
48 Stachowicz, 2018). With the world-wide increase of biological invasions, a growing number of  
49 invasive alien species (IAS) and the parasites they carry can change or disrupt species interactions  
50 that were shaped by natural selection, including the existing host-parasite relationships (Hatcher et  
51 al., 2006; Sih et al., 2012; Ezenwa et al., 2016). When new (alien) parasites are transmitted to a  
52 susceptible naive native host species (parasite spillover, Power & Mitchell, 2004) they can affect the  
53 new host's body condition, survival or reproductive success, and even behaviour. Furthermore, in  
54 their new range, invasive species can acquire local parasites. This can result in an extra cost to the  
55 IAS but the IAS can also act as reservoir, amplifying the transmission of parasites to the native  
56 species (parasite spill-back; Kelly, Paterson, Townsend, Poulin, & Tompkins, 2009). These  
57 processes, where parasites mediate native/invader interactions, can play a key role in determining  
58 the outcome of invasions and are referred to as disease-mediated invasions (Strauss et al., 2012;  
59 Lymbery, Morine, Gholipour Kanani, Beatty, & Morgan, 2014).

60 Animal personality, defined as consistent among-individual differences in behaviour (Réale,  
61 Reader, Sol, McDougall, & Dingemanse, 2007; Carter, Feeney, Marshall, Cowlshaw, & Heinsohn,  
62 2013), can influence the probability to encounter infective stages, become infected and,

63 subsequently, spread parasites (Kortet, Hedrick, & Vainikka, 2010; Hawley, Etienne, Ezenwa, &  
64 Jolles, 2011; Ezenwa et al., 2016). Hence, variation in individual personality can result in between-  
65 individual heterogeneities in parasite infection (VanderWaal & Ezenwa, 2016). Conversely,  
66 parasites may influence the expression of behaviour and personality. Some parasites directly  
67 manipulate host behaviour to enhance their transmission (Poulin, 2013; Poirotte et al., 2016;  
68 Petkova, Abbey-Lee, & Løvlie, 2018), while others induce physiological or immunological  
69 changes, which in turn can alter host behaviour (Kortet et al., 2010; Barber & Dingemanse, 2010;  
70 Poulin, 2013). In summary, the interactions between parasites and host behaviour or personality  
71 traits, in particular boldness, activity-exploration or sociability, are complex and parasite  
72 transmission can occur not only between individuals of the same species, but also from one species  
73 to another, potentially influencing the mechanisms of interspecific competition.

74 Here, we use the well-known case of native – alien species competition between the Eurasian red  
75 squirrel (*Sciurus vulgaris*) and the introduced invasive North American grey squirrel (*Sciurus*  
76 *carolinensis*) in Europe to explore the relationships between the native host's personality and  
77 infection by both native and alien (acquired) macroparasites. The two species have similar space  
78 use and activity patterns, and compete for limited food resources (interspecific resource  
79 competition, hereinafter IRC), resulting in smaller body size, and reduced female reproduction and  
80 juvenile recruitment in red squirrels (Wauters, Tosi, & Gurnell, 2002a; Wauters, Gurnell, Martinoli,  
81 & Tosi, 2002b; Gurnell et al., 2004), ultimately causing a decrease in population size and (local)  
82 extinction (Bertolino, Montezemolo, Preatoni, Wauters, & Martinoli, 2014). Furthermore, in areas  
83 where the two species compete, red squirrels had higher sociability scores than in areas without the  
84 alien species (Wauters et al., 2019). In the natural situation (only red squirrels present), the  
85 gastrointestinal macroparasite fauna of red squirrels is dominated by a single species, the pinworm  
86 *Trypanoxyuris sciuri* (Romeo et al., 2013; Santicchia et al., 2015). This species is an oxyurid  
87 nematode with direct faecal-oral transmission: eggs are deposited on the perianal region and  
88 ingested during grooming. Vertical transmission can occur from females to their young in the nest,

89 while horizontal transmission through physical contact or environmental contamination is  
90 considered rare (Anderson, 2000). Because of this mainly vertical transmission and since oxyurids  
91 tend to be low pathogenic (Anderson, 2000), we predict that there will be no relationship between a  
92 red squirrel's personality, considering the traits activity, exploration or sociability, and *T. sciuri*  
93 infection status (being infected or not). In contrast with the natural situation, in woodlands  
94 colonised by grey squirrels, spillover of the grey squirrel's common helminth, *Strongyloides*  
95 *robustus*, from the alien to the native species occurs with about 61% of red squirrels infected by the  
96 alien nematode (Romeo al., 2014a, 2015). This helminth's eggs are shed with host faeces in the  
97 environment where they hatch, the larvae undergo two moults until the infective stage (L3 larvae)  
98 penetrates the skin of a new host, and migrates towards the GI tract. Consequently, the probability  
99 of acquiring *S. robustus* will depend on the exposure to these free-living infective stages (Romeo et  
100 al., 2014a, 2015). Therefore, we predict that infection risk toward the invasive parasite should be  
101 higher for individuals with a more active (considered to forage and/or move more intensively),  
102 explorative (move over longer distances visiting more often areas outside their core-area) or social  
103 personality (use habitat patches shared by more animals), than for red squirrels that are less active  
104 or explorative and less social, since these individuals will move less, over shorter distances and  
105 avoid patches of high squirrel density and contact with other squirrels (Wauters & Dhondt, 1989,  
106 1992; Wauters et al., 2019).

107 Here, we explore two non mutually exclusive hypotheses. (1) Based on the role of the host's  
108 behaviour on the parasites' life cycles described above, we test the hypothesis that the personality  
109 traits of the native species, activity, exploration tendency and sociability, positively affect parasite  
110 infection by the alien helminth *S. robustus* in sites colonised by grey squirrels, but that there is no  
111 relationship between these traits and infection by the common native parasite *T. sciuri*. (2) Since  
112 parasites can also induce physiological changes in their hosts, which in turn can alter host behaviour  
113 or personality (e.g. Poulin, 2013; Ezenwa et al., 2016), we also consider that macroparasite

114 infection induces energetic costs of specific behaviours or personality traits, such as activity and/or  
115 exploration (Hicks et al., 2018), negatively affecting their expression. To test these hypotheses, we  
116 determined whether native red squirrels were infected by the dominant native gastrointestinal  
117 helminth (*T. sciuri*) and/or by the alien helminth (*S. robustus*), acquired through spillover from its  
118 original host, the grey squirrel (Romeo et al., 2014a, 2015), and used 323 arena tests across three  
119 experimental (native species and IAS, n = 152 tests of 79 individuals) and three control sites (only  
120 native species, n = 171 tests of 105 individuals) to quantify host personality and parasite infection  
121 status.

122

## 123 **2 MATERIALS AND METHODS**

### 124 **2.1 Study sites and trapping squirrels**

125 We trapped and monitored squirrels in three red-only sites and in three red-grey sites with  
126 comparable habitat quality (availability of preferred tree-seeds) and squirrel densities (see  
127 Supporting Information S1 and Table S1) (see also Wauters et al., 2019). Trapping was carried out  
128 in two to four periods per year between January 2016 and December 2018 (Supporting Information  
129 Table S1 for details). We used Tomahawk “squirrel” traps (models 201 and 202, Tomahawk Live  
130 Trap, WI, USA), placed on the ground or at breast height against tree trunks, that were more or less  
131 homogeneously distributed over the study site. Trap densities varied among sites in relation to  
132 expected squirrel density (see also Supporting Information Table S1). We pre-baited traps with  
133 hazelnuts 3 to 4 times over a 30 day period, then baited and set for 4-5 days. We checked traps two  
134 to three times per day to reduce the time a squirrel was restrained inside a trap. Each trapped red  
135 squirrel was flushed into a light cotton handling bag (zipper tube) to minimize stress during  
136 handling, and individually marked using numbered metal ear-tags (type 1003 S, National Band and  
137 Tag, Newport, KY, USA). It was weighed to the nearest 5 g using a spring-balance (Pesola AG,

138 Baar, Switzerland) and the length of the right hind foot, a reliable proxy for a squirrel's body size  
139 used in most studies, was measured with a thin ruler ( $\pm 0.5$  mm) (Wauters et al., 2007). Sex, age  
140 class and reproductive condition were determined from external genitalia and body mass, with  
141 juvenile red squirrels weighing less than 250 g (Wauters & Dhondt, 1989). We applied adhesive  
142 tape tests on the perianal region to determine presence/absence of *T. sciuri* since this is a more  
143 reliable method than coprological analysis for oxyurid egg detection (Foreyt, 2011). To detect *S.*  
144 *robustus* eggs, we collected fresh faecal samples from the trap floor (which was cleaned after each  
145 capture event) which were stored dry at 4 °C for later examination. We carried out coprological  
146 analyses within 3 days from sample collection to avoid egg hatching.

147 In the experimental sites (red-grey sites), we removed grey squirrels as part of a red squirrel  
148 conservation project. We euthanized animals by CO<sub>2</sub> inhalation, following the EC and AVMA  
149 guidelines (Santicchia et al., 2018). Doing so, we kept grey squirrel densities controlled to  
150 guarantee persistency of red squirrels in the experimental sites (see also Santicchia et al., 2018).

151

## 152 **2.2 Parasite and personality measures**

153 We examined tape test slides under a microscope ( $\times 40$  or  $\times 100$  magnification) to determine red  
154 squirrels' infection status by *T. sciuri* (1 = infected, 0 = not infected). We analysed faecal samples  
155 quantitatively with McMaster technique and qualitatively with flotation to determine *S. robustus*  
156 intensity of infection (faecal egg count, FEC) and infection status (presence/absence; Romeo et al.,  
157 2014b, 2015) (details of these methods in Supporting Information S2). The FEC method is  
158 commonly used to infer intensity of infection on living hosts (Patterson & Schulte-Hostedde, 2011;  
159 Romeo et al., 2014b), although FEC values are not always linearly related to actual worm burdens.  
160 Romeo et al. (2014b) demonstrated that for grey squirrels, the typical host of *S. robustus*, there was  
161 a significant curvilinear relationship between the number of adult worms and FEC due to

162 suppression of helminth fecundity related to the activation of the host immune response (Paterson &  
163 Viney, 2002).

164 After checking a red squirrel's identity, we released it inside the arena by opening a sliding door (28  
165 × 15 cm, internal opening 12 x 12 cm), allowing the animal to move from the handling bag into the  
166 arena (Mazzamuto et al., 2018). Arena tests consisted of an Open Field Test (OFT) followed by a  
167 Mirror Image Stimulation test (MIS) that were carried out as described elsewhere (Mazzamuto et  
168 al., 2018; Wauters et al., 2019; details in Supporting Information S2). For each experiment we  
169 calculated the time that individuals spent in each behavioural state using the CowLog 3.0.2 software  
170 (Hänninen & Pastell, 2009). Ethogram description and procedures to define the expert-based (EB)  
171 personality traits as in Mazzamuto et al. (2018) and Wauters et al. (2019) (for details see Supporting  
172 Information S2 and Table S2). Applying Factor Analysis (FA) instead of the expert-based approach  
173 did not change our results. All data handling and analysis carried out with R (R Core Team 2019).  
174 To check the assumptions of repeatability of personality traits we repeated both experiments (OFT  
175 and MIS) in different capture-sessions to have at least two arena tests for most individuals. The  
176 minimum time between tests for the same individual was 12 days (mean ± SE = 85 ± 7, range: 12-  
177 734 days).

178

### 179 **2.3 Data handling and statistical analyses**

180 We obtained scores for seven personality traits (Supporting Information Table S2): activity,  
181 exploration and shyness from OFT; sociability, avoidance, alert and other (the latter a combination  
182 of activity and exploration; Mazzamuto et al., 2018) from MIS. Scores were squareroot transformed  
183 before analyses to meet assumptions of normality. Exploratory data analyses revealed that  
184 sociability and avoidance were strongly, negatively correlated (Pearson correlation coefficient  $r = -$   
185 0.89) and had a non-normal error distribution that made it difficult to be fitted in a multivariate

186 MCMCglmm. Therefore, we calculated a new variable that measured the sociability-avoidance  
187 continuum (hence the tendency to react more or less social or amenable to the mirror image) with  
188 the formula:

189 
$$\text{Social tendency} = \log\text{-transformed} [( \text{sociability score} + (1 - \text{avoidance score}) ) / 2]$$

190 Since the definition of personality implies that the measured behaviour must have consistent  
191 between-individual variation over time and under different contexts (e.g. Réale et al., 2007; Carter  
192 et al., 2013), we first estimated the repeatability of the expert-based traits (on all data of both red-  
193 only and red-grey sites) as the between-individual variation divided by the sum of the between-  
194 individual and residual variation, using Bayesian generalized linear mixed effects models based on  
195 a Markov Chain Monte Carlo algorithm with the R package *MCMCglmm* version 2.26 (Hadfield,  
196 2010). Each model had a personality trait as dependent variable and study site, sex, arena test order  
197 (from 1 to a maximum of 6 tests) and year as fixed effects, and squirrel identity as random effect.  
198 Posterior distributions were based on 1050000 iterations with a burnin of 50000 iterations and  
199 thinning of 40, such that 25000 iterations were used to obtain point estimates and 95% credibility  
200 intervals. For the random effects and residual variation an inverse-gamma prior uninformative for  
201 the model was used (Wilson et al., 2010). We found moderate repeatabilities (Table 1,  $R > 0.20$ ; see  
202 also Bell et al., 2009) for activity from OFT and for social tendency and activity-exploration score  
203 from MIS (details in Supporting Information Table S2 and Table S3), which were further used as  
204 personality traits in the MCMCglmm models below.

205

### 206 **2.3.1 MCMCglmm models on parasite infection status – personality relationships**

207 Since we were interested in testing possible interactions of area-type (red-only vs. red-grey) on the  
208 personality – parasite relationships, we performed two multivariate MCMCglmm models, one for  
209 each area-type. All data were used for each area-type, hence sample sizes are 171 for 105

210 individuals in red-only, and 152 for 79 individuals in red-grey area-type. In the red-only model, the  
211 personality traits (activity, social tendency and activity-exploration, see Table 1) and infection status  
212 by *T. sciuri* were the dependent variables. In the red-grey model, the same personality traits, and  
213 infection status by *T. sciuri* and by *S. robustus* were the dependent variables. We then tested  
214 explicitly for any differences in the various correlations between personality traits and *T. sciuri*  
215 infection between the area-types by comparing the slopes of the posterior distributions from the two  
216 separate models.

217 The expert-based personality traits (activity, social tendency and activity-exploration) were treated  
218 as dependent variables after centering and scaling  $[(x_i - \text{mean } x)/SD \ x]$  with a Gaussian residual  
219 error distribution, while *T. sciuri* and *S. robustus* infections were treated as binomial dependent  
220 variables (0 not infected, 1 infected). Assumption of normality of the personality traits was  
221 supported by their QQ-plots. In addition, sex, body mass, year and arena test order were added as  
222 fixed effects; the latter accounted for possible habituation effects. We did not include pregnant  
223 females to avoid a bias due to extra weight of developing embryos. In both models, the effect of  
224 arena test order was set to zero for *T. sciuri* and *S. robustus* infection. As repeated observations were  
225 present, individual was added as a random effect. Also study site was added as random effect (as a  
226 heterogeneous identity matrix) to avoid pseudoreplication problems during the parameter estimation  
227 process. For both the residual and between-individual variation, an unstructured variance-  
228 covariance matrix was modelled, allowing the estimation of correlations among the dependent  
229 variables (covariance divided by the square root of the product of the variances). Posterior  
230 distributions were based on 1050000 iterations with a burnin of 50000 iterations and thinning of 40,  
231 such that 25000 iterations were used to obtain point estimates and 95% credibility intervals. For all  
232 fixed effects related parameters (i.e., intercept, slopes, differences), the prior distribution was  
233 Gaussian with zero mean and variance equal to  $10^8$  (default setting in MCMCglmm). For the  
234 random effects and residual variation and inverse Wishard prior was set with diagonal elements

235 equal to 0.5, 0.5 and 0.1 for the residual (i.e., within-individual variation), between-individual and  
236 nested study site effect respectively. The believe parameter was set to 0.01. We applied the Gelman-  
237 Rubin statistic (Gelman & Rubin, 1992) and Geweke diagnostic (Geweke, 1992) which confirmed  
238 model consistency and convergence. Full model outputs are provided in Supporting Information,  
239 Table S4 and S5.

### 241 **2.3.2 GLMM on changes *S. robustus* infection status and changes in activity**

242 In the red-grey area-type, we had two consecutive measures of the activity score from OFT for a  
243 subset of 24 samples. In 14 cases the squirrels changed from a status of non-infected to infected by  
244 *S. robustus* (group 1). In the other 10 cases, the animal remained non-infected both at first and  
245 second measure of activity (group 2). If becoming infected was the cause for the decrease in activity  
246 expression observed in the MCMCglmm model (see results), we predict a significant decrease in  
247 activity scores in group 1 [hence a negative value for the difference between the second (infected)  
248 minus first (non-infected) activity measure], but no difference between second and first measure in  
249 group 2. We used a GLMM with the difference in amount of activity between second and first  
250 measure as dependent variable (hereinafter  $\Delta$  activity), and group as fixed effect. We added arena  
251 test order (first or subsequent), sex and *T. sciuri* infection status as potentially confounding factors,  
252 and squirrel id as random effect since three individuals were used twice (occurred once in group 1  
253 and once in group 2). We used a stepwise backward model selection, removing the non-significant  
254 (partial  $p > 0.10$ ) fixed effects.

### 256 **2.3.3 GLMM on activity – intensity of infection by *S. robustus***

257 FEC were obtained from 62 samples of 43 individual red squirrels for which we also had measures  
258 of personality traits. This sample size proved too small to run multivariate models as above and

259 resulted in convergence problems in an overparametrized model. We therefore, used centered and  
260 scaled activity scores as response variable in a GLMM to explore whether activity (the only trait  
261 significantly related to infection status, see Results) decreased with intensity of infection by *S.*  
262 *robustus*. FEC values were centered and scaled after Log FEC transformation and used as fixed  
263 effect. We added infection status of *T. sciuri*, squirrel sex, body mass and arena test order (first vs  
264 subsequent tests) as fixed effects and the interaction of FEC by *T. sciuri* infection status to account  
265 for a potential effect of multi-species infection. Squirrel id and study sites were added as random  
266 effects to avoid pseudoreplication problems during the parameter estimation and significance  
267 testing.

268

## 269 **3 RESULTS**

### 270 **3.1 Grey squirrel presence and parasite infection**

271 In red-only sites, 116 on 171 tape tests were positive for *T. sciuri* (estimate  $\pm$  SE =  $68 \pm 4\%$ ) against  
272 113 on 152 ( $74 \pm 4\%$ ) in red-grey sites. Prevalence of infection (an individual was scored as  
273 infected when at least one test was positive) by *T. sciuri* was  $69 \pm 4\%$  (72 on 105 red squirrels) in  
274 red-only sites and  $78 \pm 5\%$  (62 on 79 individuals) in red-grey sites. The alien parasite, *S. robustus*,  
275 was not found in red-only sites, whereas in the red-grey sites, 95 on 152 ( $63 \pm 4\%$ ) faecal samples  
276 were positive for its eggs, for a prevalence of  $75 \pm 5\%$  (59 on 79 individuals). In the red-grey sites,  
277 138 out of 152 samples ( $91 \pm 7\%$ ) had at least one parasite species; of these 43 were positive only  
278 for *T. sciuri* ( $28 \pm 4\%$ ), 25 only for *S. robustus* ( $17 \pm 3\%$ ) while 70 ( $46 \pm 4\%$ ) were from red  
279 squirrels infected by both *S. robustus* and native *T. sciuri*. Faecal egg counts (FEC) varied from 1 to  
280 945 ( $n = 62$ ; median 53; 25-75% quartiles 9 – 316). On average (mean  $\pm$  SE), an infected red  
281 squirrel had  $201 \pm 36$  *S. robustus* eggs in the McMaster slide.

282

### 283 3.2 Personality and parasitism

284 We explored effects of a squirrel's sex and body mass, and of year and arena test order on the traits  
285 activity, social tendency and activity-exploration and on infection status of *T. sciuri* (both area-  
286 types) and of *S. robustus* (only red-grey) infection status.

287 In the red-only sites, there were strong and positive correlations between the three personality  
288 traits, suggesting a behavioural syndrome of more active-explorative squirrels being also more  
289 social (Table 2, all  $r > 0.85$ ). The correlations of activity, activity-exploration or social tendency  
290 with *T. sciuri* infection were not significant (Table 2), indicating that a red squirrel's personality was  
291 not related with the probability of infection with *T. sciuri* in red-only sites. Among the relationships  
292 between the fixed effects and the dependent variables, the traits activity-exploration and social  
293 tendency were expressed more strongly in 2017 and 2018 than in 2016 while in 2018 fewer red  
294 squirrels were infected by *T. sciuri* than in 2016 (Supporting Information Table S4).

295 In the red-grey sites, the correlation between the traits activity and activity-exploration was  
296 significant (Table 2). In contrast with the red-only sites, there was a significant but negative  
297 correlation between *T. sciuri* infection and activity, but no such relationship was found with the  
298 other two traits (Table 2). Similarly, expression of activity was also negatively correlated with *S.*  
299 *robustus* infection status, while the relationships of probability of infection by *S. robustus* with the  
300 other two traits was not significant (Table 2). The probabilities of being infected by *T. sciuri* and by  
301 *S. robustus* were not correlated suggesting that there was no significant tendency for red squirrels  
302 that were infected by *T. sciuri* to be infected also by *S. robustus* ( $r = 0.53$ , 95%  $CI = -0.19$  to  $1.00$ ;  
303 Table 2). Among the fixed effects, sex affected social tendency and probability of infection by *S.*  
304 *robustus*: males tended to behave less social, and were also less infected with *S. robustus* than  
305 females (Supporting Information Table S5). Activity was expressed more strongly in 2017 than in  
306 2016 and had lowest values in 2018 (Supporting Information Table S5).

307

### 308 **3.3 Changes in *S. robustus* infection and in activity**

309 On the subset of 24 samples with consecutive measures of activity scores, 14 changed from being  
310 non-infected to infected by *S. robustus* (group 1, see methods), while 10 were non-infected at both  
311 measures (group 2). The effects of arena test order ( $t_{19} = 0.59$ ;  $p = 0.59$ ), *T. sciuri* infection status  
312 ( $t_{20} = 1.39$ ;  $p = 0.18$ ) and sex ( $t_{21} = 1.50$ ;  $p = 0.15$ ) on  $\Delta$  activity were not significant and removed  
313 during stepwise model selection. The  $\Delta$  activity in group 1 was negative and significantly different  
314 from that in group 2 ( $\Delta$  activity group 1, mean  $\pm$  SE =  $-0.182 \pm 0.049$ ;  $\Delta$  activity group 2, mean  $\pm$  SE  
315 =  $0.009 \pm 0.083$ ; group effect:  $t_{22} = 2.22$ ;  $p = 0.037$ ). Thus, red squirrels in group 1 showed a  
316 significant decrease in activity from non-infected to infected by *S. robustus* while there was no  
317 difference between second and first measure in group 2 (Fig. 1; for individual changes in activity  
318 see Fig S1a, b in Supporting Information), suggesting that becoming infected by *S. robustus* was the  
319 cause for the observed negative correlation between activity and *S. robustus* infection in the global  
320 MCMCglmm model.

321

### 322 **3.3 Expression of activity and intensity of *S. robustus* infection estimated by FEC**

323 Since in the MCMCglmm models above, only activity of red squirrels was related with *S. robustus*  
324 infection status, we only analysed activity as dependent variable. Red squirrels expressed more  
325 activity in the first (22 samples) than in subsequent (40 samples) arena tests (estimate  $0.79 \pm 0.23$ ;  
326  $t_{55} = 3.46$ ;  $p = 0.001$ ) and there was a significant FEC by *T. sciuri* infection status interaction ( $-0.59$   
327  $\pm 0.26$ ;  $t_{55} = 2.26$ ;  $p = 0.028$ ). Effects of sex and body mass were not significant (partial  $p > 0.30$ )  
328 and removed from the model. To allow interpretation of the interaction, we tested relationships  
329 between FEC and activity, correcting for the arena test order effect, for animals infected by *T. sciuri*  
330 (44 cases) and not infected by *T. sciuri* (18 cases) separately. When already infected with their  
331 native parasite, red squirrels showed lower activity with increased shedding of *S. robustus* eggs (-

332  $0.35 \pm 0.12$ ;  $t_{41} = 2.86$ ;  $p = 0.007$ ; Fig 2a). In contrast, when they were not infected by *T. sciuri*,  
333 there was no relationship between their activity and *S. robustus* egg shedding ( $0.21 \pm 0.22$ ;  $t_{15} =$   
334  $0.94$ ;  $p = 0.36$ ; Fig. 2b).

335

#### 336 **4 DISCUSSION**

337

338 Relationships between host behaviour and/or personality and infection by native parasites have been  
339 widely studied (Barber & Dingemanse, 2010; Poulin, 2013; Ezenwa et al., 2016). In contrast,  
340 studies on the role of invasive parasites on native hosts or native species communities, have  
341 focussed on highly pathogenic parasite species, often microparasites, and their role in influencing  
342 disease outbreaks in the invaded ecosystem and/or native species (Tompkins et al., 2002;  
343 Goedknecht et al., 2016). For example, a paradigm of disease-mediation invasions is the red-grey  
344 squirrel in Great Britain, with spillover of squirrelpox virus (SQPV) of the alien to the native  
345 squirrel. Grey squirrels are apparently unaffected by the infection, while infected red squirrels have  
346 extremely high mortality rates (Tompkins et al., 2002; Fiegna et al., 2016): hence the virus  
347 facilitates replacement of native red by alien grey squirrels (Tompkins, White & Boots, 2003).  
348 In contrast with most microparasites, the majority of macroparasites have more subtle, non-lethal  
349 effects on their hosts, that may include changes in behaviour and/or energy allocation (Anderson &  
350 May, 1979; Mathot & Dingemanse, 2015; Hicks et al., 2018).

351 Here we investigated, to our knowledge for the first time, relationships of both native and alien  
352 macroparasites, the latter acquired through spillover, with potential changes in the behaviour or  
353 personality of the native host. Through a large-scale field study conducted in North Italy, we found  
354 that relationships of personality traits of native red squirrels with macroparasite infections differed  
355 between red-only sites and sites colonised also by the alien grey squirrel (red-grey sites). In the  
356 former, there was no relationship between red squirrel activity and *T. sciuri* (its common helminth)

357 infection, while in red-grey sites, infection of red squirrels by *T. sciuri* and by *S. robustus*, an alien  
358 helminth acquired through parasite spillover from grey squirrels, was negatively correlated with  
359 host activity. Moreover, in the red-grey sites, individuals that changed infection status (from non-  
360 infected to infected) of *S. robustus* reduced activity after becoming infected, while no such trend  
361 was observed among red squirrels that remained uninfected.

#### 362 363 **4.1 Interactions between host personality and native macroparasite infection**

364  
365 Measuring personality traits and macroparasite infection of native red squirrels in (control) study  
366 sites without alien competitors or alien parasites and in (experimental) study sites that were  
367 colonised by alien grey squirrels carrying the alien helminth *S. robustus*, allowed us to investigate  
368 potential changes in native host – parasite relationships caused by parasite spillover. In the red-only  
369 sites, there was no relationship between a red squirrel's activity, exploration tendency or social  
370 tendency and *T. sciuri* infection status, confirming our first prediction which was based on this  
371 oxyurid's transmission route characterised mainly by direct and vertical (mother – young)  
372 transmission. However, where native squirrels co-occurred with alien squirrels, the infection status  
373 of *T. sciuri* was negatively correlated with the expression of the personality trait activity; in other  
374 words, red squirrels infected by *T. sciuri* had lower activity than non-infected animals. This  
375 difference of the correlations of activity with *T. sciuri* infection between the red-only and the red-  
376 grey sites was significant, and suggests a cost to red squirrels of being infected with their common  
377 gastrointestinal helminth, but only when their woodland has been invaded by grey squirrels.  
378 We can only give a tentative explanation of possible pathways that can explain this trend. In the red-  
379 grey sites, grey squirrels have been found to act as a stressor on co-occurring red squirrels,  
380 increasing the concentration of glucocorticoids (stress hormones) in individuals of the native  
381 species (Santicchia et al., 2018). One of the effects of long-term increased glucocorticoid levels  
382 (chronic stress) is a suppression of the immune system (decrease in immune efficiency, e.g.

383 Romero, 2004) which could, in our case, reduce the red squirrel's immune response to contrast *T.*  
384 *sciuri* infection. In general, oxyurid infections tend to be low pathogenic and without overt  
385 symptoms. However, heavy pinworm burdens might negatively affect the intestinal structure and/or  
386 function (Taffs, 1976) and the humoral or cell-mediated immune response is essential for  
387 maintaining the host-oxyurid parasite equilibrium (e.g. lab mice, Taffs, 1976; primates, Sorci et al.,  
388 2003). An alternative, but not mutually exclusive, explanation for the negative relationship between  
389 *T. sciuri* infection status and amount of activity in experimental sites could be a worse nutritional  
390 status, inducing either a higher host susceptibility to parasite infection or a reduced capability to  
391 cope with it. However, despite the habitat differences between control and experimental sites, they  
392 were of comparable quality in terms of availability of high-energy food resources (see Supporting  
393 Information S1 and Table S1). Moreover, previous studies did not reveal differences in prevalence  
394 or in intensity of infection by *T. sciuri* between conifer and deciduous woods (Santicchia et al.,  
395 2015). Hence, our results suggest that in sites invaded by grey squirrels, the typical equilibrium that  
396 exists between red squirrels and oxyurid infection is disrupted, possibly though a less efficient  
397 immune response that might be related directly to increased physiological stress caused by the  
398 occurrence of the alien squirrel or, indirectly, to the spillover of the alien parasite *S. robustus*.  
399 Indeed, previous research highlighted a significant increase in *T. sciuri* prevalence in areas co-  
400 inhabited by grey squirrels, suggesting a reduced capability of the native species of coping with its  
401 native infection (Romeo et al., 2015).

402

#### 403 **4.2 Effects of *S. robustus* spillover**

404

405 In the red-grey sites, we found a negative correlation between expression of activity by red squirrels  
406 and their infection status with the alien nematode *S. robustus*. Hence, infected animals showed  
407 lower activity during OFT than non-infected ones. These data support our second hypothesis that

408 infection by the alien macroparasite induces energetic costs of specific behaviours or personality  
409 traits, in this case activity, negatively affecting its expression. However, we did not find a  
410 significant correlation between activity-exploration measured in the arena with *S. robustus*  
411 infection: the trend was negative, but credibility intervals were large and included 0. This could be  
412 related to squirrels having low amounts of exploration behaviours (head dip, sniff and scratch, Table  
413 S2) in the arena test, and/or a poor agreement between the energetic costs of this trait as measured  
414 in the arena and exploration in a natural environment.

415 We have three lines of concurring evidence that suggest an influence of the alien parasite on the  
416 expression of the personality trait activity among the red squirrels occurring in study sites colonised  
417 by invasive grey squirrels (red-grey sites). First and foremost, we found a significant negative  
418 correlation between *S. robustus* infection status and amount of activity. Second, the directionality of  
419 this result (parasite infection causes reduction in expression of a personality trait) was confirmed by  
420 a subset of data where activity expression was reduced in animals tested both before and after  
421 infection by *S. robustus*, but not in a control group of squirrels that remained uninfected in each of  
422 the two tests (Fig. 1). Third, among red squirrels infected by both *T. sciuri* and *S. robustus*, higher  
423 intensity of infection by the alien nematode reduced activity more strongly than low infections. This  
424 relationship was not found among animals not infected by their common endoparasite *T. sciuri*. The  
425 latter result suggests a cumulative effect of parasite coinfections (e.g. Fenton, Viney & Lello, 2010;  
426 Ezenwa, 2016; Rothenburger et al., 2019) and that multi-species infections of native and alien  
427 parasites could exacerbate the effects of each parasite species on host behaviour or personality. This  
428 might be especially true for those host species which have a naturally poor parasite community in  
429 terms of species richness and which, therefore, may be less capable of coping with multiple  
430 infections. This is the case of red squirrels, that are rarely infected by gastro-intestinal parasites  
431 other than *T. sciuri* and a few coccidian protozoan (Romeo et al., 2013, Hoffmannova et al., 2016).  
432 Finally, similarly to a previous study (Romeo et al., 2015), we found no significant association

433 between *T. sciuri* and *S. robustus* infection, indicating that co-infection by the other parasite had no  
434 effect on the probability of infection of red squirrels by either of the two helminths.

435 We admit that we did not have direct measures of intensity of infection (worm counts in the  
436 intestine), but used the McMaster count of the number of *S. robustus* eggs expelled with the host's  
437 faeces (FEC). Despite this caveat, low and high FEC values are likely a good index of, respectively,  
438 low and high worm burdens. Moreover, FEC from red squirrels in this study suggest higher  
439 intensities of infection by *S. robustus* than in the original host (this study from 1 to 945 eggs, mean  
440  $\pm SE = 201 \pm 36$ ; grey squirrels in Romeo et al., 2014b from 1 to 55; and in Romeo et al. submitted  
441 from 1 to 43). This data strongly suggests a lower capability of the naive host of responding, and  
442 controlling, *S. robustus* infection.

443

#### 444 **4.3 Critical considerations on directionality of host personality – parasite infection** 445 **relationships**

446

447 Our two non-mutually exclusive hypotheses (personality affects parasite infection vs. parasite  
448 infection affects personality expression) are based on the direction (positive vs negative,  
449 respectively) of the correlations among the dependent variables in the multivariate MCMCglmm  
450 models. Hence, one could argue that the negative correlations of the amount of activity expressed  
451 during OFT and the infection status of *T. sciuri* and *S. robustus* is caused by low activity increasing  
452 the probability of becoming infected by each of the two helminths. There are several reasons why  
453 this is extremely unlikely. First, it is hard to find a biological explanation of how low activity could  
454 increase the likelihood of encountering infective stages of either parasite. Indeed, other studies on  
455 rodents addressing host personality – parasite relationships found positive effects of activity,  
456 exploration and/or boldness on infection by ecto- or endoparasites. In eastern chipmunks (*Tamias*

457 *striatus*), bolder males had higher endoparasite loads than shy ones (Paterson & Schulte-Hostedde,  
458 2011), while in least chipmunks (*Tamias minimus*), more explorative animals hosted a greater  
459 abundance of ectoparasites compared to less exploratory individuals (Bohn et al., 2017). Finally, in  
460 the native grey squirrel host, probability of infection by *S. robustus* was higher for bolder, more  
461 explorative individuals than shy, less explorative ones (Santicchia et al., 2019). However, since all  
462 these studies are correlational, caution is needed with the interpretation of the direction of the  
463 relationships. Boyer et al. (2010) tried to address directionality using path analysis, a statistical  
464 approach, while an experimentally induced infection with a brain trematode (*Diplostomum phoxini*)  
465 was used in a study on Eurasian minnows (*Phoxinus phoxinus*) (Kekäläinen et al., 2014).  
466 Second, if low activity increased the probability of infection by *T. sciuri*, it should do so  
467 independently of the presence of grey squirrels, but this pattern was not supported by our data that  
468 showed a lack of correlation between activity and *T. sciuri* infection in the red-only sites. Third, this  
469 hypothesis cannot explain the significant decrease in within-individual expression of activity  
470 observed in the subset of red squirrels that became infected by the alien parasite between  
471 consecutive arena tests; a decrease not observed among animals that remained uninfected. As  
472 already mentioned above, this is the strongest result - although on a small sample - that it is indeed  
473 becoming infected with *S. robustus* that drives the reduction in activity and not *vice versa*. Fourth,  
474 the fact that also intensity of infection by *S. robustus* negatively affects the amount of activity  
475 expressed, but only among red squirrels co-infected by both helminths, confirms that infection by  
476 an alien parasite is costly to the naive native host and causes red squirrels to reduce behaviours or  
477 traits (in our case activity) that require high energy expenditure.

478

#### 479 **4.4 Conclusions**

480

481 As predicted based on its typical oxyurid life-cycle with mainly direct transmission, we found no  
482 relationship of the personality traits activity, activity-exploration tendency, or social tendency of red

483 squirrels with *T. sciuri* infection status under natural conditions (in sites without the alien species).  
484 This pattern changed in sites colonised by the alien competitor (red-grey sites) where infection with  
485 *T. sciuri* reduced the expression of the trait activity, but not of the other two traits. Likewise,  
486 spillover of the alien helminth *S. robustus* from grey squirrels to native red squirrels reduced the  
487 amount of activity expressed by the latter and this was confirmed by comparing levels of activity of  
488 animals that were tested before and after infection by *S. robustus*. Finally, also intensity of infection  
489 by *S. robustus*, estimated by faecal egg counts, was negatively related to the expression of activity,  
490 but only in the case of co-infection with both helminths. These results strongly suggest that  
491 infection by the alien parasite is costly to the naive native host species, in particular when  
492 individuals are already infected by a common helminth, and is indeed the causal factor behind the  
493 reduction of energetically costly personality traits, such as activity.

494

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502

#### 503 **AUTHORS' CONTRIBUTIONS**

504 FS, LAW, NF and AM developed the hypotheses and study design. LAW, APP and CR collected  
505 data, SVD, DP and FS analysed output data, LAW and FS led the writing of the manuscript. All  
506 authors contributed critically to the drafts and gave final approval for publication.

507

508 **DATA ACCESSIBILITY**

509 We confirm that, should the manuscript be accepted, the data supporting the results will be archived  
510 in an appropriate public repository (zenodo) and the data DOI will be reported at the end of the  
511 article.

512

513 **REFERENCES**

514 Anderson, R.C. (2000). Nematode parasites of vertebrates: their development and transmission (2nd  
515 ed.). Wallingford, UK: CABI Publishing.

516 Anderson, R.C., & May, R. (1979). Population biology of infectious diseases: Part I. *Nature*, 280,  
517 361–367. doi:10.1038/280361a0

518 Barber, I., & Dingemanse, N. J. (2010). Parasitism and the evolutionary ecology of animal  
519 personality. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 365, 4077-  
520 4088. doi:10.1098/rstb.2010.0182

521 Bell, A. M., Hankinson, S. J., & Laskowski, K. L. (2009). The repeatability of behaviour: a meta-  
522 analysis. *Animal Behaviour*, 77, 771-783. doi:10.1016/j.anbehav.2008.12.022

523 Bertolino, S., Montezemolo, N. C., Preatoni, D. G., Wauters, L. A., & Martinoli, A. (2014). A grey  
524 future for Europe: *Sciurus carolinensis* is replacing native red squirrels in Italy. *Biological*  
525 *Invasions*, 16, 53–62. doi:10.1007/s10530-013-0502-3

526 Bohn, S.J., Webber, Q.M.R., Florko, K.R.N., Paslawski, K.R., Peterson, A.M., Piche, J.E., Menzies,  
527 A.K., & Willis, C.K.R. (2017). Personality predicts ectoparasite abundance in an asocial sciurid.  
528 *Ethology*, 123, 761-771. doi: 10.1111/eth.12651.

529 Boyer, N., Réale, D., Marmet, J., Pisanu, B., & Chapuis J-L. (2010) Personality, space use and tick

530 load in an introduced population of Siberian chipmunks *Tamias sibiricus*. *Journal of Animal*  
531 *Ecology*, 79, 538-547.

532 Brehm, A.M., Mortelliti, A., Maynard, G.A., & Zydlewski J. (2019). Land-use change and the  
533 ecological consequences of personality in small mammals. *Ecology Letters*, doi: 10.1111/ele.13324

534 Carter, A. J., Feeney, W. E., Marshall, H. H., Cowlshaw, G., & Heinsohn, R. (2013). Animal  
535 personality: what are behavioural ecologists measuring? *Biological Reviews*, 88, 465-475.  
536 doi:10.1111/brv.12007

537 Ezenwa, V.O. (2016). Helminth–microparasite co-infection in w  
538 rodents and rabbits. *Parasite Immunology*, 38, 527-534. doi.org/10.1111/pim.12348

539 Ezenwa, V. O., Archie, E. A., Craft, M. E., Hawley, D. M., Martin, L. B., Moore, J., & White, L.  
540 (2016). Host behaviour–parasite feedback: an essential link between animal behaviour and disease  
541 ecology. *Proceedings of the Royal Society of London B: Biological Sciences*, 283, 2015-3078.  
542 doi:10.1098/rspb.2015.3078

543 Fenton, A., Viney, M.E., & Lello J. (2010). Detecting interspecific macroparasite interactions from  
544 ecological data: patterns and process. *Ecology Letters*, 13, 606-615. doi: 10.1111/j.1461-  
545 0248.2010.01458.x

546 Fiegna, C., Dagleish, M.P., Coulter, L., Milne, E., Meredith, A., Finlayson, J., Di Nardo, A., &  
547 McInnes, C.J. (2016). Host-pathogen dynamics of squirrelpox virus infection in red squirrels  
548 (*Sciurus vulgaris*). *Veterinary Microbiology*, 182, 18–27. doi: 10.1016/j.vetmic.2015.10.012.

549 Foreyt, W. J. (2011). *Veterinary parasitology reference manual* (5th ed.). Iowa, USA: Wiley-  
550 Blackwell.

551 Gelman, A., & Rubin, D. B. (1992). Inference from iterative simulation using multiple sequences.

552 *Statistical Science*, 7, 457–472. doi:10.1214/ss/1177011136.

553 Geweke, J. (1992). Evaluating the accuracy of sampling-based approaches to the calculation of  
554 posterior moments. In J. M. Bernardo, A. P. Berger, A. P. Dawid, & A. F. M. Smith (Eds.) *Bayesian*  
555 *Statistics* 4, pp. 169–193. Oxford University Press, Oxford. doi: 1176289

556 Goedknecht, M. A., Feis, M. E., Wegner, K. M., Luttkhuizen, P. C., Buschbaum, C., Camphuysen,  
557 K. C., ..., Thieltges, D. W. (2016). Parasites and marine invasions: Ecological and evolutionary  
558 perspectives. *Journal of Sea Research*, 113, 11–27. doi: 10.1016/j.seares.2015.12.003

559 Gurnell, J., Wauters, L. A., Lurz, P. W., & Tosi, G. (2004). Alien species and interspecific  
560 competition: effects of introduced eastern grey squirrels on red squirrel population dynamics.  
561 *Journal of Animal Ecology*, 73, 26–35. doi:10.1111/j.1365-2656.2004.00791.x

562 Hadfield, J. D. (2010). MCMC Methods for Multi-Response Generalized Linear Mixed Models:  
563 The MCMCglmm R Package. *Journal of Statistical Software*, 33, 1–22. doi:10.18637/jss.v033.i02.

564 Hänninen, L., & Pastell, M. (2009). CowLog: open-source software for coding behaviors from  
565 digital video. *Behavior Research Methods*, 41, 472–476. doi:10.3758%2FBRM.41.2.472

566 Hatcher, M. J., Dick, J. T. A., & Dunn, A. M. (2006). How parasites affect interactions between  
567 competitors and predators. *Ecology Letters*, 9, 1253–1271. doi:10.1111/j.1461-0248.2006.00964.x

568 Hawley, D. M., Etienne, R.S., Ezenwa, V. O., & Jolles, A. E. (2011). Does animal behavior underlie  
569 covariation between hosts' exposure to infectious agents and susceptibility to infection?  
570 Implications for disease dynamics. *Integrative and Comparative Biology*, 51, 528-539.  
571 doi:10.1093/icb/icr062

572 Henrichs, B., Oosthuizen, M. C., Troskie, M., Gorsich, E., Gondhalekar, C., Beechler, B. R.,  
573 Ezenwa, V. O., & Jolles, A. E. (2016). Within guild co-infections influence parasite community

574 membership: a longitudinal study in African Buffalo. *Journal of Animal Ecology*, 85, 1025-1034.  
575 doi:10.1111/1365-2656.12535

576 Hicks, O., Burthe, S.J., Daunt, F., Newell, M., Butler, A., Ito, M., Sato, K., & Green, J.A. (2018).  
577 The energetic cost of parasitism in a wild population. *Proceedings of the Royal Society of London*  
578 *B: Biological Sciences*, 285, 20180489. doi: 10.1098/rspb.2018.0489

579 Hofmannová, L., Romeo, C., Štohanzlová, L., Jirsová, D., Mazzamuto, M.V., Wauters, L.A.,  
580 Ferrari, N., & Modrý, D. (2016). Diversity and host specificity of coccidia (Apicomplexa:  
581 Eimeriidae) in native and introduced squirrel species. *European journal of protistology*, 56, 1-14.  
582 doi: 10.1016/j.ejop.2016.04.008

583 Kelly, D.W., Paterson, R.A., Townsend, C.R., Poulin, R., & Tompkins, D. M. (2009). Parasite  
584 spillback: a neglected concept in invasion ecology? *Ecology*, 90, 2047–2056. doi:10.1890/08-  
585 1085.1

586 Kekäläinen, J., Lai, Y-T., Vainikka, A., Sirkka, I., & Kortet R. (2014). Do brain parasites alter host  
587 personality? – Experimental study in minnows. *Behavioral Ecology and Sociobiology*, 68, 197-204.

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

590 Levine, J. M., Bascompte, J., Adler, P. B., & Allesina, S. (2017). Beyond pairwise mechanisms of  
591 species coexistence in complex communities. *Nature*, 546, 56–64. doi:10.1038/nature22898

592 Lymbery, A. J., Morine, M., Gholipour Kanani, H., Beatty, S. J., & Morgan, D. L. (2014). Co-  
593 invaders: The effects of alien parasites on native hosts. *International Journal for Parasitology:*  
594 *Parasites and Wildlife*, 3, 171–177. doi:10.1016/j.ijppaw.2014.04.002

595 Mathot, K.J., & Dingemanse, N.J. (2015). Energetics and behavior: unrequited needs and new

596 directions. *Trends in Ecology & Evolution*, 30, 199–206. doi:10.1016/j.tree.2015.01.010.

597 Mazzamuto, M. V., Cremonesi, G., Santicchia, F., Preatoni, D. G., Martinoli, A., & Wauters, L. A.  
598 (2018). Rodents in the arena: a critical evaluation of methods measuring personality traits. *Ethology*,  
599 *Ecology and Evolution*, 31, 38-58. doi:10.1080/03949370.2018.1488768

600 Paterson, S., & Viney, M.E. (2002). Host immune responses are necessary for density dependence  
601 in nematode infections. *Parasitology*, 125, 283–292. doi: 10.1017/s0031182002002056

602 Patterson, L.D., & Schulte-Hostedde, A.I. (2011). Behavioural correlates of parasitism and  
603 reproductive success in male eastern chipmunks, *Tamias striatus*. *Animal Behaviour*, 81, 1129-1137.  
604 doi:10.1016/j.anbehav.2011.02.016

605 Petkova, I., Abbey-Lee, R. N., & Løvlie, H. (2018). Parasite infection and host personality: *Glugea*-  
606 infected three-spined sticklebacks are more social. *Behavioral Ecology and Sociobiology*, 72, 173.  
607 doi:10.1007/s00265-018-2586-3

608 Poirotte, C., Kappeler, P. M., Ngoubangoye, B., Bourgeois, S., Moussodji, M., & Charpentier, M. J.  
609 E. (2016). Morbid attraction to leopard urine in *Toxoplasma*-infected chimpanzees. *Current Biology*,  
610 26, R83–R101. doi:10.1016/j.cub.2015.12.020

611 Poulin, R. (2013). Parasite manipulation of host personality and behavioural syndromes. The  
612 *Journal of Experimental Biology*, 216, 18-26. doi:10.1242/jeb.073353

613 Power, A.G., & Mitchell, C.E. (2004). Pathogen spillover in disease epidemics. *The American*  
614 *Naturalist*, 164(5), S79–S89. doi:10.1086/424610

615 R Core Team (2019). R: A language and environment for statistical computing. R Foundation for  
616 Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>

617 Réale, D., Reader, S. M., Sol, D., McDougall, P. T., & Dingemanse, N. J. (2007). Integrating animal

618 temperament within ecology and evolution. *Biological Reviews*, 82, 291-318. doi:10.1111/j.1469-  
619 185X.2007.00010.x

620 Rhoades, O. K., Best, R. J., & Stachowicz, J. J. (2018). Assessing Feeding Preferences of a  
621 Consumer Guild: Partitioning Variation Among versus Within Species. *The American Naturalist*,  
622 192, 287-300. doi:10.1086/698325

623 Romeo, C., Pisanu, B., Ferrari, N., Basset, F., Tillon, L., Wauters, L. A., Martinoli, A., Saino, N., &  
624 Chapuis, J.-L. (2013). Macroparasite community of the Eurasian red squirrel (*Sciurus vulgaris*):  
625 poor species richness and diversity. *Parasitology Research*, 112, 3527-3536. doi:10.1007/s00436-  
626 013-3535-8

627 Romeo, C., Wauters, L. A., Ferrari, N., Lanfranchi, P., Martinoli, A., Pisanu, B., Preatoni, D. G., &  
628 Saino, N. (2014a). Macroparasite fauna of alien grey squirrels (*Sciurus carolinensis*): Composition,  
629 variability and implications for native species. *PloS ONE*, 9, e88002.  
630 doi:10.1371/journal.pone.0088002

631 Romeo, C., Wauters, L. A., Cauchie, S., Martinoli, A., Matthysen, E., Saino, N., & Ferrari, N.  
632 (2014b). Faecal egg counts from field experiment reveal density dependence in helminth fecundity:  
633 *Strongyloides robustus* infecting grey squirrels (*Sciurus carolinensis*). *Parasitology Research*, 113,  
634 3403-3408. doi:10.1007/s00436-014-4005-7

635 Romeo, C., Ferrari, N., Lanfranchi, P., Saino, N., Santicchia, F., Martinoli, A., & Wauters, L. A.  
636 (2015). Biodiversity threats from outside to inside: effects of alien grey squirrel (*Sciurus*  
637 *carolinensis*) on helminth community of native red squirrel (*Sciurus vulgaris*). *Parasitology*  
638 *Research*, 114, 2621–2628. doi:10.1007/s00436-015-4466-3

639 Romero, L.M. (2004). Physiological stress in ecology: lessons from biomedical research  
640 *Trends in Ecology & Evolution*, 19, 249–255. doi: 10.1016/j.tree.2004.03.008

641 Rothenburger, J.L., Himsworth, C.G., Nemeth, N.M., Pearl, D.L., Treuting, P.M., & Jardine, J.M.  
642 (2019). The devil is in the details—Host disease and co -infections are  
643 pathogen carriage in Norway rats (*Rattus norvegicus*). *Zoonoses and Public Health*, 66, 622-635.  
644 doi: 10.1111/zph.12615

645 Santicchia, F., Romeo, C., Martinoli, A., Lanfranchi, P., Wauters, L.A., & Ferrari, N. (2015). Effects  
646 of habitat quality on parasite abundance: do forest fragmentation and food availability affect  
647 helminth infection in the Eurasian red squirrel? *Journal of Zoology, London*, 296, 38-44. doi:  
648 10.1111/jzo.12215

649 Santicchia, F., Dantzer, B., van Kesteren, F., Palme, R., Martinoli, A., Ferrari, N., & Wauters, L.A.  
650 (2018). Stress in biological invasions: introduced invasive grey squirrels increase physiological  
651 stress in native Eurasian red squirrels. *Journal of Animal Ecology*, 87, 1342–1352.  
652 doi:10.1111/1365-2656.12853

653 Santicchia, F., Romeo, C., Ferrari, N., Matthysen, E., Vanlauwe, L., Wauters, L. A., & Martinoli, A.  
654 (2019). The price of being bold? Relationship between personality and endoparasitic infection in a  
655 tree squirrel. *Mammalian Biology*, 97, 1–8. doi:10.1016/j.mambio.2019.04.007

656 Sih, A., Cote, J., Evans, M., Fogarty, S., & Pruitt, J. (2012). Ecological implications of behavioural  
657 syndromes. *Ecology Letters*, 15, 278-289. doi:10.1016/j.anbehav.2017.09.008

658 Sorci, G., Skarstein, F., Morand, S., & Hugot, J-P. (2003). Correlated evolution between host  
659 immunity and parasite life histories in primates and oxyurid parasites. *Proceedings of the Royal*  
660 *Society of London B: Biological Sciences*, 270, doi: 10.1098/rspb.2003.2536

661 Strauss, A., White, A., & Boots, M. (2012). Invading with biological weapons: the importance of  
662 disease-mediated invasions. *Functional Ecology*, 26, 1249–1261. doi: 10.1111/1365-2435.12011

663 Taffs, L.F. (1976). Pinworm infections in laboratory rodents: a review. *Laboratory Animals*, 10, 1-

664 13. doi: 10.1258/002367776780948862

665 Tompkins, D.M., Sainsbury, A.W., Nettleton, P., Buxton, D., & Gurnell, J. (2002). Parapoxvirus  
666 causes a deleterious disease in red squirrels associated with UK population declines. *Proceedings of*  
667 *the Royal Society of London B: Biological Sciences*, 269, 529–533. doi: 10.1098/rspb.2001.1897

668 Tompkins, D.M., White, A.R., & Boots, M. (2003). Ecological replacement of native red squirrels  
669 by invasive greys driven by disease. *Ecology Letters*, 6, 189–196. doi:10.1046/j.1461-  
670 0248.2003.00417.x

671 VanderWaal, K. L., & Ezenwa, V. O. (2016). Heterogeneity in pathogen transmission: mechanisms  
672 and methodology. *Functional Ecology*, 30, 1606-1622. doi:10.1111/1365-2435.12645

673 Wauters, L. A., & Dhondt, A. A. (1989). Body weight, longevity and reproductive success in red  
674 squirrels (*Sciurus vulgaris*). *Journal of Animal Ecology*, 58, 637-651. doi:10.2307/4853

675 Wauters, L. A., & Dhondt, A. A. (1992). Spacing behaviour of red squirrels, *Sciurus vulgaris*:  
676 variation between habitats and the sexes. *Animal Behaviour*, 43, 297-311. doi: 10.1016/S0003-  
677 3472(05)80225-8

678 Wauters, L., Tosi, G., & Gurnell, J. (2002a). Interspecific competition in tree squirrels: do  
679 introduced grey squirrels (*Sciurus carolinensis*) deplete tree seeds hoarded by red squirrels (*S.*  
680 *vulgaris*)? *Behavioral Ecology and Sociobiology*, 51, 360–367. doi:10.1007/s00265-001-0446-y

681 Wauters, L., Gurnell, J., Martinoli, A., & Tosi, G. (2002b). Interspecific competition between native  
682 Eurasian red squirrels and alien grey squirrels: does resource partitioning occur? *Behavioral*  
683 *Ecology and Sociobiology*, 52, 332–341. doi:10.1007/s00265-002-0516-9

684 Wauters, L. A., Vermeulen, M., Van Dongen, S., Bertolino, S., Molinari, A., Tosi, G., & Matthysen,  
685 E. (2007). Effects of spatio-temporal variation in food supply on red squirrel *Sciurus vulgaris* body

686 size and body mass and its consequences for some fitness components. *Ecography*, 30, 51–65.

687 doi:10.1111/j.2006.0906-7590.04646.x

688 Wauters, L. A., Mazzamuto, M. V., Santicchia, F., Van Dongen, S., Preatoni, D. G., & Martinoli, A.

689 (2019). Interspecific competition affects the expression of personality traits in natural populations.

690 *Scientific Reports*, 9, 11189. doi:10.1038/s41598-019-47694-4

691 Wilson, A.J., Réale, D., Clements, M.N., Morrissey, M.M., Postma, E., Walling, C.A., Kruuk,

692 L.E.B., & Nussey, D.H. (2010). An ecologist's guide to the animal model. *Journal of Animal*

693 *Ecology*, 79, 13–26.

694

695 **Table 1.** The squareroot transformed proportions of time red squirrels were engaged in behaviours  
696 related to the different personality traits defined by the expert-based approach during OFT and MIS.  
697 Data grouped by situation (study sites with only red squirrels = red-only; study sites with both red  
698 and grey squirrels = red-grey). Repeatability (R) estimated with the MCMCglmm model (see  
699 methods).

Personality trait	red-only (n=171)		red-grey (n=152)		Repeatability	
	Mean	SD	Mean	SD	R	95% CI
OFT						
Activity	0.57	0.16	0.52	0.17	0.39	0.23 – 0.52
Shyness	0.73	0.17	0.77	0.17	0.41	0.24 – 0.58
Exploration	0.21	0.10	0.20	0.11	0.05	0.01 – 0.29
MIS						
Sociability	0.18	0.28	0.36	0.36	0.20	0.04 – 0.34
Avoidance	0.69	0.31	0.60	0.36	0.10	0.002 – 0.25
Alert	0.30	0.19	0.27	0.17	0.02	0.004 – 0.18
Activity-exploration	0.37	0.20	0.31	0.18	0.46	0.31 – 0.61
Social score <sup>1</sup>	-2.01	1.17	-1.61	1.33	0.27	0.12 – 0.41

700 <sup>1</sup> Calculated with formula social score = log-transformed [(sociability score + (1-avoidance score))/2]

701

702 **Table 2.** Correlations (95% credibility intervals between brackets) between the dependent variables  
703 derived from the MCMCglmm models by area-type (red-only or red-grey), and the differences  
704 between posterior slopes of the correlation estimates for red-only and red-grey area-type (red-only –  
705 red-grey: mean  $\pm$  *SD* and 95% *CI*). *T. sciuri* is estimated probability of infection by *T. sciuri*; *S.*  
706 *robustus* is estimated probability of infection by *S. robustus*. Estimates of the between-individual  
707 and within-individual variances for the three personality traits in the red-only and the red-grey sites  
708 and the calculated differences between sites are detailed in the Supporting Information (Table S6).

709

<b>Variables</b>	<b>Red-only</b>	<b>Red-grey</b>	<b>Difference</b>
Activity – social	<b>0.86 (0.60 – 0.99)</b>	0.20 (-0.44 – 0.82)	<b>0.66 <math>\pm</math> 0.35 (-0.004 – 1.39)</b>
Activity – Activity-exploration	<b>0.88 (0.66 – 0.99)</b>	<b>0.72 (0.32 – 0.99)</b>	0.16 $\pm$ 0.23 (-0.23 – 0.72)
Social – Activity-exploration	<b>0.85 (0.56 – 0.99)</b>	0.35 (-0.28 – 0.98)	0.48 $\pm$ 0.38 (-0.16 – 1.32)
Activity – <i>T. sciuri</i>	0.20 (-0.26 – 0.64)	<b>-0.69 (-0.99 – -0.33)</b>	<b>0.89 <math>\pm</math> 0.30 (0.26 – 1.44)</b>
Activity-exploration – <i>T. sciuri</i>	0.08 (-0.51 – 0.67)	-0.30 (-0.79 – 0.22)	0.37 $\pm$ 0.40 (-0.42 – 1.15)
Social – <i>T. sciuri</i>	0.17 (-0.55 – 0.88)	0.07 (-0.45 – 0.59)	0.10 $\pm$ 0.46 (-0.83 – 0.96)
Activity – <i>S. robustus</i> <sup>1</sup>		<b>-0.66 (-0.99 – -0.004)</b>	
Activity-exploration – <i>S. robustus</i> <sup>1</sup>		-0.52 (-0.99 – 0.21)	
Social – <i>S. robustus</i> <sup>1</sup>		-0.27 (-0.99 – 0.53)	
<i>T. sciuri</i> – <i>S. robustus</i> <sup>1</sup>		0.53 (-0.19 – 1.00)	

710 <sup>1</sup> Relationships only occurring in Red-grey sites. Significant results in bold.

711

712 |

713 **FIGURE 1** Difference in the squareroot transformed values of proportion activity expressed during  
714 OFT ( $\Delta$  activity; see methods and results for details) in two groups of red squirrels. In group 1,  
715 animals were not infected during the first activity measure, but infected during the second measure.  
716 In group 2, animals were uninfected at both measures of activity. Boxplots show median (horizontal  
717 line), mean (black diamond) and 1<sup>st</sup> (25%) and 3<sup>rd</sup> (75%) quartiles.

718

719 **FIGURE 2** Relationship between intensity of infection by *S. robustus*, estimated using faecal egg  
720 counts (FEC), and a standardized score of amount of activity expressed by red squirrels during the  
721 OFT for: a) red squirrels also infected by *T. sciuri*; b) red squirrels not infected by *T. sciuri*.  
722 Observed values (grey dots) and values predicted by the model (line). Grey band indicates 95% *CI*.

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