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1 **Effects of habitat quality on parasite abundance: do forest fragmentation and food**  
2 **availability affect helminth infection in the Eurasian red squirrel?**

3

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17

18 Short title: **Effects of fragmentation and food on squirrel parasites**

19

20 **Abstract**

21 Habitat quality affects demography, population genetics, space use and phenotypic characteristics of  
22 mammals. However, little is known about the effects of habitat quality, fragmentation and/or food  
23 abundance, on host-parasite interactions. Here we present a first study on the relationships between  
24 the abundance of the dominant gastrointestinal helminth, *Trypanoxyuris (Rodentoxyuris) sciuri*,  
25 infecting the Eurasian red squirrel *Sciurus vulgaris* and three environmental factors: habitat type  
26 (mountain conifer forests vs. lowland mixed deciduous forests), fragmentation (fragmented  
27 woodlands vs. continuous forests) and food availability. Abundance of *T. (R.) sciuri* increased in  
28 fragmented woods. Furthermore, in mountain conifer forests, squirrels were more heavily infected  
29 after a poor Norway spruce seed-crop than in years with medium or high seed production,  
30 indicating that squirrels are less capable of reducing parasite load when food availability is low.  
31 Hence, we suggest that *T. (R.) sciuri* abundance in red squirrels may be determined mainly by  
32 changes in host susceptibility induced by higher stress levels and/or poorer nutritional status, while  
33 in fragments, reduced genetic diversity may also increase host susceptibility to parasite infection.  
34 Although our data do not shed light on the mechanisms generating the observed patterns, results  
35 from other field studies highlighted the effect of stress and nutritional status on parasite infection,  
36 thus suggesting their implication in the changes in the abundance of *T. (R.) sciuri*.

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39

40 **Keywords** body mass; conifer seed-crops; food abundance; habitat fragmentation; helminth  
41 infection; macroparasites; red squirrel; *Sciurus vulgaris*

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43

## 44 **Introduction**

45 Habitat fragmentation (i.e. the reduction of large, continuous habitats into small and more or less  
46 isolated remnants) and spatio-temporal variation in food availability are known to affect distribution  
47 and abundance of different mammals by inducing changes in population dynamics, intra- and  
48 interspecific competition, social behaviour and space use (e.g. Andrén, 1994; Russell & Ruffino,  
49 2012; Bjørneraas *et al.*, 2012) .

50 Although habitat fragmentation and food availability may potentially affect the distribution and  
51 impact of parasites on host populations, the effect of these two parameters on host-parasite  
52 interactions have rarely been investigated. In general, parasite transmission is determined by a  
53 combination of exposure to infective stages and host susceptibility (Anderson & May, 1978). By  
54 altering population density, space use and social structure, both fragmentation and variation in food  
55 availability may thus affect hosts' exposure to parasites. For example, studies on primates have  
56 shown that higher host densities in fragmented populations determine higher parasite infection by  
57 increasing either contact rates between individuals or the abundance of free living infective stages  
58 (Gillespie & Chapman, 2006; Mbora & McPeck, 2009).

59 On the other hand, habitat fragmentation and food shortage may affect host susceptibility,  
60 impairing its immune response by increasing individual stress (Martin, 2009; Busch & Hayward,  
61 2009; Brearley *et al.*, 2013), altering nutritional status (Ezenwa, 2004) or, in the case of  
62 fragmentation, reducing genetic variability (Meyer-Lucht & Sommer, 2005). In particular, high  
63 stress levels may induce the suppression of the host immune response through the action of  
64 glucocorticoid hormones (see Padgett & Glaser, 2003; Martin, 2009) and both habitat fragmentation  
65 and food shortage have been associated with increased individual physiological stress (Busch &  
66 Hayward, 2009; Brearley *et al.*, 2013 and references therein). However, disentangling all these  
67 factors and their effects on host-parasite interactions could be a complex matter. For example,

68 Navarro-Gonzalez *et al.* (2011) found that the degree of fragmentation had no effect on roe deer  
69 (*Capreolus capreolus*) helminth burden and hypothesised that it may be due to individuals living in  
70 agricultural landscape having access to high-quality food that enhances immune response,  
71 compensating for other potentially detrimental effects.

72 In this paper, we use Eurasian red squirrels (*Sciurus vulgaris*) and their dominant helminth,  
73 *Trypanoxyuris (Rodentoxyuris) sciuri* (Romeo *et al.*, 2013), as a model to explore the effect of  
74 habitat fragmentation and food availability on parasite infection.

75 Tree squirrels are ideal model systems to study the diverse effects of forest fragmentation and  
76 spatio-temporal variation in food availability because they occur over a wide range of landscapes  
77 with different proportions of suitable habitat (Andr n, 1994; Koprowski, 2005) and they are  
78 specialised tree-seed predators whose population dynamics and space use strongly depend on  
79 variation in seed abundance (Wauters & Dhondt, 1992; Lurz, Garson & Wauters, 2000; Boutin *et*  
80 *al.*, 2006). Furthermore, they often live in so-called "pulsed resource systems", such as conifer  
81 forests, where food availability may vary greatly between years, as a consequence of variable seed  
82 production by mast-seeding conifer species (Wauters *et al.*, 2008).

83 In particular, Eurasian red squirrels are very sensitive to habitat fragmentation, showing -in most  
84 cases- lower densities (Van Apeldoorn, Celada & Nieuwenhuizen, 1994; Verbeylen, DeBruyn &  
85 Matthysen, 2003) and reduced genetic diversity (Wauters *et al.*, 1994b; Hale *et al.*, 2001) in  
86 fragmented woods than in large forests of similar quality. Furthermore, fragmentation and landscape  
87 structure have different and often complex effects on space use of male and female red squirrels  
88 (Wauters, Casale & Dhondt, 1994a; Verbeylen *et al.*, 2009) and on dispersal patterns and distances  
89 and subsequent choice of settlement habitat (Wauters *et al.*, 2010). Concerning food abundance,  
90 increased tree-seed availability improves red squirrel body condition and increases reproductive rate  
91 and survival, resulting in an increase in population growth and density (Boutin *et al.*, 2006; Wauters

92 *et al.*, 2007, 2008). Moreover, squirrels tend to use larger home ranges in habitats and/or periods  
93 when food resources are poor (Wauters & Dhondt 1992; Lurz *et al.*, 2000; Romeo *et al.*, 2010).

94 As mentioned above, red squirrels in fragmented populations tend to occur at lower densities  
95 than in nearby larger forests of similar habitat quality (Wauters *et al.*, 1994b) and this should result  
96 in lower parasite transmission. However, *T. (R.) sciuri*, like most oxyurids, shed its infective stages  
97 on the perianus of the host, where they are ingested through grooming (Anderson, 2000). Hence,  
98 transmission between individuals occurs mainly through allogrooming, whereas selfgrooming leads  
99 to autoinfection. For a solitary species like the red squirrel, this means that the parasite is likely  
100 acquired during mother-nestling interactions, or, at a later stage, is possibly related with mating  
101 behaviour, and afterwards the infection is maintained through selfgrooming. Previous investigations  
102 (Romeo *et al.*, 2013) showed indeed that the vast majority of individuals are infected by *T. (R.)*  
103 *sciuri* (87% prevalence), hence, intensity of infection will depend only marginally on exposure and  
104 mostly on individual susceptibility that will in turn depend on physiological mechanisms  
105 determined by the interplay of immune system, nutritional status and hormonal stress of the host.

106 Thus, since the red squirrel is particularly sensitive to habitat fragmentation (Koprowski, 2005)  
107 we may expect *T. (R.) sciuri* parasite infection to be higher in small habitat patches as a  
108 consequence of increased stress levels and reduced genetic diversity that may affect immune  
109 function (Padgett & Glaser, 2003; Meyer-Lucht & Sommer, 2005; Busch & Hayward, 2009). We  
110 may also expect parasite burdens of red squirrels living in pulsed resource systems to vary in  
111 different years as a consequence of changes in food availability and nutritional status of the hosts  
112 (Coop & Kyriazakis, 1999; Ezenwa, 2004). Hence, we made use of roadkilled red squirrels to  
113 explore the effect of habitat fragmentation and variation in food availability on *T. (R.) sciuri*  
114 infection, predicting that: (i) squirrels from fragmented woods are more heavily infected by *T. (R.)*  
115 *sciuri* than animals from larger forests, and (ii) individuals collected from areas and/or in years with

116 higher food availability are less infected than those from areas or years with lower food availability.

117

## 118 **Materials and methods**

119 A total of 92 freshly roadkilled red squirrels were collected between 2002 and 2012 in Northern  
120 Italy in two biogeographic regions: Continental and Alpine as defined by EU habitats Directive  
121 ([http://ec.europa.eu/environment/nature/natura2000/sites\\_hab/biogeog\\_regions/index\\_en.htm](http://ec.europa.eu/environment/nature/natura2000/sites_hab/biogeog_regions/index_en.htm)). Part of these  
122 samples (81) were used by Romeo *et al.* (2013) and another 11 were collected and analysed to  
123 increase our dataset. In this paper, Continental and Alpine regions corresponded to lowland  
124 (deciduous forest or mixed broadleaf-conifer forest) and mountain (mixed conifer forests) habitat  
125 types. In relation to red squirrel ecology, earlier studies on this sciurid consider as non-fragmented,  
126 continuous areas woodlands over 150 ha in size, whereas woodlands below 60 ha and surrounded  
127 by non-forest matrix habitat are considered as fragmented sites (e.g. Wauters & Dhondt, 1992; Van  
128 Apeldoorn *et al.*, 1994; Wauters *et al.*, 1994b; Verbeylen *et al.*, 2009). Based on these studies, and  
129 since no carcasses were collected from areas between 50 and 200 ha in size, we defined areas as  
130 fragmented when size of the woodland where the carcass was found was less than 50 ha. In  
131 contrast, all areas of a size  $> 200$  ha were classified as continuous forests.

132 For the mountain habitat we also estimated food abundance for all sampling years by using cone  
133 counts of Norway spruce (*Picea abies*), the dominant conifer species in most of the Italian Alps  
134 between 1300 and 1800 m a.s.l. (e.g. Salmaso *et al.*, 2009; Lamedica *et al.*, 2011). Cone count data  
135 were taken from other studies on red squirrel population dynamics (see Salmaso *et al.*, 2009) and  
136 from our unpublished data. Cone abundance estimated in August of year  $t$  was considered available  
137 for animals whose carcasses were collected between August year  $t$  and July year  $t+1$ . Subsequently,  
138 we defined 3 abundance classes: cone-crop failure or poor cone-crop (on average  $< 10$  cones/tree);  
139 medium to good cone-crop (from 10 to 200 cones/tree); mast crop ( $> 200$  cones/tree). Each year's

140 cone abundance was thus included in one of these three classes. General trends in cone production  
141 of Norway spruce tend to be synchronous over wide areas (e.g. Mencuccini, Piussi & Zanzi Sulli,  
142 1995; Koenig & Knops, 1998), thus our data collected in the Lombardy Alps and in Valle d'Aosta  
143 could also be used for the carcasses collected in Alpine Regions as Piedmont and Trentino Regions.

144 We stored carcasses in individual plastic bags at -20°C for later examination. For each animal we  
145 recorded sex and body mass, weighed to the nearest gram (Wauters *et al.*, 2007). We searched for *T.*  
146 (*R.*) *sciuri*, by examining the whole gastrointestinal content of each squirrel following standard  
147 parasitological procedures described in Romeo *et al.* (2013, 2014). *T. (R.) sciuri* individuals were  
148 identified morphologically (based on description and measures by Hugot, 1984) and counted.

149

## 150 **Statistical analysis**

151 Before analysis, we checked data for spatial and temporal autocorrelation (Zuur, Ieno & Elphick,  
152 2010) to exclude potential biases from non randomized data sampling.

153 We analysed *T. (R.) sciuri* abundance (number of worms/host) through a Generalised Linear  
154 Model (GLM) with negative binomial error structure in order to account for the aggregate  
155 distribution of parasites within host population (Shaw, Grenfell & Dobson, 1998). We explored the  
156 effects of habitat fragmentation (fragmented vs. continuous forests), habitat type (mountain conifer  
157 forests vs. lowland mixed forests), sex and body mass and all their two-way interactions on  
158 helminth abundance. Body mass was considered as a measure of structural development (age) and  
159 condition (e.g. Wauters *et al.*, 2007). Since there were few juveniles in our sample, and body mass  
160 is age-class dependent, we did not include age as a factor in our models (see also Romeo *et al.*,  
161 2013).

162 To test the effect of food availability on parasite abundance, we run a separate GLM on the  
163 subset of animals collected in mountain habitat, adding seed-crop size class to the abovementioned

164 factors.

165 Finally, we run two GLMs with normal distribution to investigate variation in host body mass: a  
166 first model on the whole dataset testing the effect of fragmentation, habitat type, sex, season and  
167 their second order interactions and a second one on the subset of mountain animals testing the effect  
168 of food availability, sex, season and their interactions. Residuals of the model did not deviate from a  
169 normal distribution (Shapiro-Wilk test,  $p > 0.05$ ).

170 In all the cases, we first fitted saturated models including all fixed effects and their second order  
171 interactions. Subsequently, model selection was carried out using the Bayesian Information  
172 Criterion (BIC, Schwarz, 1978; Burnham & Anderson, 2004). Results are reported as mean ( $\pm 1$   
173 SE). All the statistical analysis were done using SAS/STAT 9.4 software (Copyright © 2011, SAS  
174 Institute Inc., Cary, NC, USA).

175

## 176 **Results**

177 Overall, 89 out of 92 squirrels were infected by *T. (R.) sciuri* (prevalence:  $97\% \pm 3\%$ ). Mean  
178 abundance of *T. (R.) sciuri* was  $460 \pm 99$  worms/host, with intensity of infection ranging from 1 to  
179 5227 worms/ infected host. Thirty-seven carcasses were from fragmented habitats, 55 from  
180 continuous forests. Among habitat types, 54 were from mountain and 38 from lowland areas.

181 Minimum selected models showed that *T. (R.) sciuri* abundance in red squirrels was affected by  
182 habitat fragmentation, while the partial effects of habitat type or body mass were not significant  
183 (second and third best model, Table 1). On average, squirrels from fragmented woods had a higher  
184 parasite abundance than those from continuous forests (Fig. 1). There was no effect of sex on *T. (R.)*  
185 *sciuri* abundance ( $P > 0.05$  in all models) in our dataset and no significant interactions between  
186 fragmentation (fragmented vs continuous forest) and habitat type.

187 The second analysis showed that, in the mountain habitat, infection by *T. (R.) sciuri* was affected

188 by food availability and increased with increasing body mass (body mass estimate  $0.017 \pm 0.008$ ,  
189 best model in Table 2). Parasite abundance was higher in years following a Norway spruce seed-  
190 crop failure or poor seed-crop size than in years with medium to good or mast cone-crops ( $P <$   
191  $0.001$ ). There was no difference in parasite abundance/host between years with medium to good and  
192 years with mast cone-crops ( $P = 0.54$ ; Fig. 2).

193 Finally, the two models investigating variation in host body mass showed that neither sex, nor  
194 season, fragmentation, habitat type or food availability had any effect on the dependent variable (all  
195  $P > 0.05$ ).

196

## 197 **Discussion**

198 Our data showed that red squirrels have a higher parasite load in fragmented woods than in large  
199 forests, while there was no significant difference between habitat types (mixed deciduous lowland  
200 vs. mountain conifer forests). Moreover, in mountain conifer forests, parasite load increased in  
201 years following a poor seed-crop indicating that low food abundance resulted in higher parasite  
202 burdens.

203 Why do squirrels have larger nematode loads in fragments? Physiological stress responses are  
204 functional to ensure future survival and reproduction. However, the response to chronic stress may  
205 have immunosuppressive effects (e.g. Hanssen, Folstad & Erikstad, 2003; Moller & Saino, 2004),  
206 thereby increasing susceptibility to parasite infections and vulnerability to disease (Martin, 2009).  
207 The magnitude of the stress response can be modulated by environmental changes, such as habitat  
208 deterioration and/or fragmentation (Busch & Hayward, 2009; Martin, 2009). Hence, habitat  
209 fragmentation can potentially reduce immunocompetence, resulting in increased parasite burdens.  
210 Up to date, relatively few studies have tested how habitat loss and fragmentation influence parasite  
211 infections in mammals, and most of them have been carried out on primates, small rodents and

212 some marsupials (Chapman *et al.*, 2006; Püttker, Meyer-Lucht & Sommer, 2008; Mbora &  
213 McPeck, 2009; Mbora, Wiczowski & Munene, 2009). These studies highlight that the higher  
214 parasite burdens found in fragments than in nearby large forests are determined mainly by three  
215 factors: higher host density, reduced access to high-quality food and higher basal stress levels  
216 (Chapman *et al.*, 2006; Mbora & McPeck, 2009).

217 Abundance of autoinfective parasites that lack free living stages, such as *T. (R.) sciuri* which  
218 colonises the host mainly through self-grooming, should be affected mainly by host susceptibility  
219 rather than exposure. The increased *T. (R.) sciuri* abundance in fragments may be therefore related  
220 to red squirrels' increased individual stress levels induced by higher overlap of core-areas and more  
221 frequent intraspecific interactions among individuals than in large forests (Wauters *et al.*, 1994a,  
222 Verbeylen *et al.*, 2009). Such patterns have been found, for example, in Red Colobus monkeys,  
223 where fecal cortisol levels were positively correlated with nematode burden and average basal stress  
224 levels were much higher in fragments, than in a nearby large forest (Chapman *et al.*, 2006).

225 Coupled with the effect of fragmentation on parasitism, our findings on the effect of food  
226 availability lend further support to the idea that *T. (R.) sciuri* abundance is influenced mainly by  
227 changes in host susceptibility. Evidence from domestic and wild herbivores shows that nutrition  
228 plays an important role in a host's ability, through its immune response, to regulate parasite  
229 establishment, growth, and fecundity (i.e. resistance). Many of the negative effects of GI parasitism  
230 observed in herbivores are exacerbated by dietary deficiencies caused by reduced access to high-  
231 quality food resources (Coop and Kyriazakis, 1999; Ezenwa, 2004). Thus, poor availability of  
232 primary food resources (energy and protein rich conifer tree seeds in the case of red squirrels,  
233 Gurnell, 1987; Wauters *et al.*, 1992) is expected to increase parasite abundance.

234 The stress-induced suppression of immune response may even explain the positive effect of body  
235 mass on parasite abundance in squirrels living in mountain conifer forests. Body mass of squirrels is

236 related to their dominance rank (e.g. Wauters & Dhondt, 1989), with heavier individuals having  
237 larger home-ranges and higher reproductive success than subordinates. Differences in mating  
238 behaviour and space use induced by dominance rank are more marked in coniferous than in  
239 deciduous habitats (Wauters & Dhondt, 1989; Wauters, Dhondt & De Vos, 1990). Hence, in  
240 mountain forests, body mass determines stronger differences among individuals in space use and  
241 social interactions (i.e. with heavier animals having larger home ranges and more contacts with  
242 conspecifics) and, potentially, in stress levels. However, we must admit that our results concerning  
243 body mass could have been biased by the opportunistic sampling of road-killed animals, preventing  
244 us from drawing definitive conclusions on the matter.

245 Furthermore, data collected in mountain habitat shows that *T. (R.) sciuri* burden increases in  
246 years with poor seed-crop size. Such relationship between poor food availability and increased  
247 levels of parasite infection were found also in studies on Soay sheep, snowshoe hare and primates  
248 (Gulland, 1992; Murray, Keith & Cary, 1998; Chapman *et al.*, 2006), suggesting that low food  
249 availability leads to poor nutritional status, allowing parasites to take advantage of a decline in the  
250 animal's immune system. In our case, the higher abundance of *T. (R.) sciuri* in years with poor seed-  
251 crop size was not accompanied by a reduction in body mass of the host, suggesting that red squirrels  
252 have physiological and/or behavioural mechanisms to compensate for a reduced food quantity.  
253 Indeed, in periods of poor tree-seed abundance, squirrels shift their diet, including larger amounts of  
254 less nutritional food such as buds, shoots, lichens and fungi (Gurnell, 1987; Wauters, Swinnen &  
255 Dhondt, 1992). This shift can have significant effects, since shortage of even a few nutritional  
256 components can highly impair host resistance to infections (Coop & Kyriazakis, 1999; Ezenwa,  
257 2004).

258 These results arise from a field study which primary aim was to investigate potential parasitism-  
259 related patterns occurring in the wild. For the moment, our explanations for the observed

260 differences in nematode abundance between fragmented and continuous forests remain speculative.  
261 So far, we gave particular emphasis to the potential role of stress and nutritional status in impairing  
262 squirrels' resistance to parasite infections. However, other mechanisms are also likely to alter  
263 susceptibility of red squirrels to parasites, such as reduced genetic diversity in fragmented areas  
264 (Wauters *et al.*, 1994b; Hale *et al.*, 2001; Behnke *et al.*, 2003) or differences in grooming behaviour  
265 that may affect transmission (Ferrari *et al.*, 2009).

266 In order to fully disclose the real mechanisms undergoing the observed patterns of parasite load  
267 variation, specific studies on genetic variation and stress levels assessment of hosts under diverse  
268 situations will be needed. However, in a context of generalised poor awareness of the potential  
269 impact of habitat degradation on parasitism, this study contributes in adding evidence of another  
270 potential negative impact of anthropogenic habitat alterations on animal fitness and consequently on  
271 wild populations demography and distribution.

272

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411 Table 1. Minimum selected models (best 3 models) exploring effects of host characteristics and  
412 environmental variables on *Trypanoxyuris (Rodentoxyuris) sciuri* abundance (no. worms/host) in  
413 Eurasian red squirrels (*Sciurus vulgaris*) using the entire dataset.

Model BIC	$\Delta$ BIC	Model parameters	$\chi^2$	df	P
1224.31	0.00	Habitat fragmentation	11.32	1	0.0008
1228.42	4.11	Habitat fragmentation	5.70	1	0.017
		Habitat type	0.40	1	0.53
1232.45	8.14	Habitat fragmentation	4.03	1	0.045
		Habitat type	0.64	1	0.42
		Body mass	0.52	1	0.47

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417 Table 2. Minimum selected models (best 3 models) exploring effects of host characteristics and  
 418 environmental variables on *Trypanoxyuris (Rodentoxyuris) sciuri* abundance (no. worms/host) in  
 419 Eurasian red squirrels (*Sciurus vulgaris*) using the mountain dataset.

Model BIC	$\Delta$ BIC	Model parameters	$\chi^2$	Df	P
634.51	0.00	Food abundance	20.7	2	< 0.0001
		Body mass	4.02	1	0.045
638.50	4.01	Food abundance	20.3	2	<0.0001
		Body mass	3.77	1	0.052
		Habitat fragmentation	0.01	1	0.97
641.86	7.35	Food abundance	16.6	2	0.0003
		Body mass	4.18	1	0.041
		Habitat fragmentation	0.62	1	0.43
		Hab frag * body mass	0.62	1	0.43

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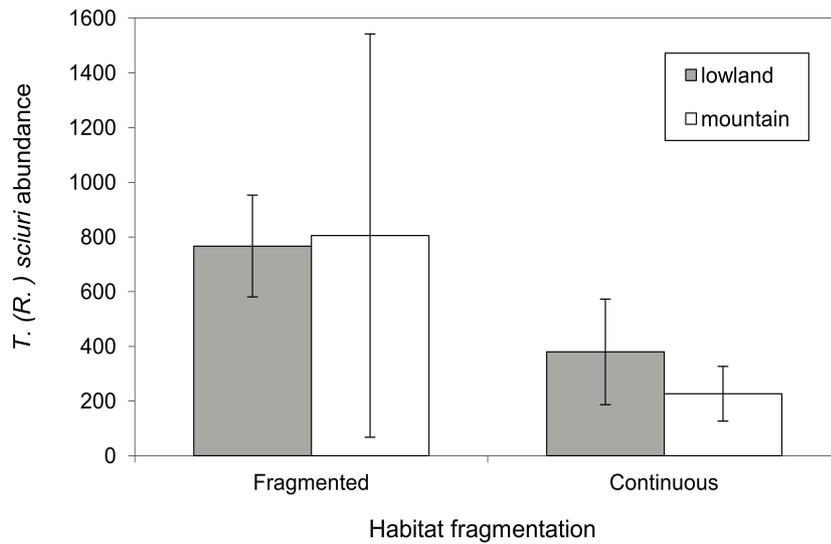
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431 Fig. 1. Mean abundance (no. of worms/host) of the nematode *Trypanoxyuris (Rodentoxyuris) sciuri*  
432 infecting Eurasian red squirrels (*Sciurus vulgaris*) in fragmented and continuous forests and in  
433 different habitat types (mountain conifer forests or lowland mixed deciduous woods). Bars  
434 represent standard error.

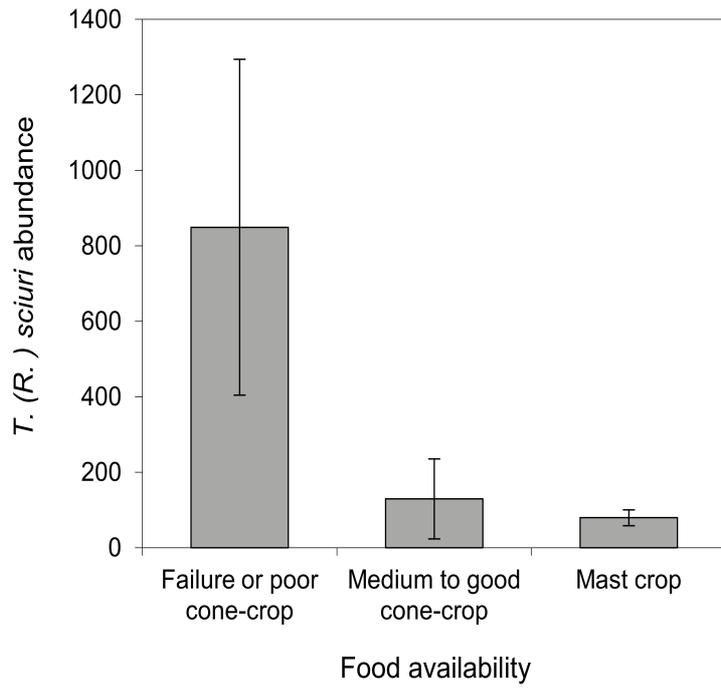


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438 Fig. 2. Mean abundance (no. of worms/host) of the nematode *Trypanoxyuris (Rodentoxyuris) sciuri*  
439 infecting Eurasian red squirrels (*Sciurus vulgaris*) in relation to food availability (*Picea abies* seed  
440 production in mountain habitat). Bars represent standard error.



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