Heligmosomoides polygyrus reduces infestation of *Ixodes ricinus* in free-living yellow-necked mice, *Apodemus flavicollis*

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SUMMARY

Free-living animals are usually inhabited by a community of parasitic species that can interact with each other and alter both host susceptibility and parasite transmission. In this study we tested the prediction that an increase in the gastrointestinal nematode *Heligmosomoides polygyrus* would increase the infestation of the tick *Ixodes ricinus*, in free-living yellow-necked mice, *Apodemus flavicollis*. An extensive cross-sectional trapping survey identified a negative relationship between *H. polygyrus* and *I. ricinus* counter to the prediction. An experimental reduction of the nematode infection through anthelmintic treatment resulted in an increase in tick infestation, suggesting that this negative association was one of cause and effect. Host characteristics (breeding condition and age) and habitat variables also contributed to affect tick infestation. While these results were counter to the prediction, they still support the hypothesis that interactions between parasite species can shape parasite community dynamics in natural systems. Laboratory models may act differently from natural populations and the mechanism generating the negative association is discussed.

Key words: *Heligmosomoides polygyrus*, *Ixodes ricinus*, co-infection, host-mediated effects, *Apodemus flavicollis*.

INTRODUCTION

Parasite ecologists have recognized the fundamental role of habitat, seasonality and climate as well as host age, sex and breeding condition in influencing the exposure and susceptibility of hosts to parasitic infections (Wilson *et al*. 2002). There is increasing evidence that interactions between parasites may also play a significant role in affecting susceptibility of hosts to infection (Hochberg and Holt, 1990; Sousa, 1994; Petney and Andrew, 1998; Cox, 2001; Lello *et al*. 2004; Maizels *et al*. 2004; Faulkner *et al*. 2005; Hartgers and Yazdanbakhsh, 2006; Cattadori *et al*. 2007, 2008; Lello and Hussell, 2008). However, while these findings indicate that we should consider the whole community of parasites to understand the dynamics of each component species, results from previous studies are not always consistent (Behnke *et al*. 2001; Poulin, 2001; Behnke, 2008; Graham, 2008; Telfer *et al*. 2008). Studies in controlled laboratory conditions have not only identified strong parasite interactions but also teased apart some of the molecular mechanisms involved (Curry *et al*. 1995; Maizels *et al*. 2004; Edwards *et al*. 2005; Kamal and El Sayed Khalifa, 2006; Graham *et al*. 2007; Bradley and Jackson, 2008; Fenton *et al*. 2008). These experiments are usually undertaken with high parasite doses or parasite and host strains that have been selected for laboratory purposes, and exhibit high responding characteristics. One consequence of these laboratory conditions is that the findings may not be relevant to the epidemiology of free-living natural host-parasite systems. If such interactions are relevant then we would expect to see this reflected in the dynamics of the infra-community of parasites of free-living hosts. Counter to this expectation, comparative studies of field data have found that the variation in the distribution and abundance of parasite infracommunities are mainly the result of variation in host exposure and habitat characteristics (Christensen, 1987; Haukisalmi and Henttonen, 1993*a*, *b*; Lotz and Font, 1994; Sousa, 1994; Behnke *et al*. 2001, 2005, Guègan *et al*. 2005; Behnke, 2008). According to these findings, parasite interactions play a trivial role in free-living systems.

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Parasite interactions can either be synergistic (positive) or antagonistic (negative). A synergistic interaction occurs when a parasite species increases its fitness (life-time, reproductive output) as a consequence of the presence of another species, whereas an antagonistic interaction leads to a fitness reduction of one of the parasite species as a response to the presence of the other species. Such interactions may arise from different mechanisms, ranging from direct competition for resources to indirect host-mediated effects, including the active role of the immune system or the passive effect of parasite produced metabolic compounds made available through the host (Sousa, 1992; Rohde, 1994; Behnke *et al*. 2001; Cox, 2001; Lello *et al*. 2004; Maizels *et al*. 2004; Graham *et al*. 2007; Bradley and Jackson, 2008; Fenton *et al*. 2008).

In this paper we address 2 broad questions (i) are the dynamics of any one parasite species altered by the presence of other parasites in the infracommunity and (ii) are the effects of any observed interaction relevant to the epidemiology of free-living host-parasite systems? We examined these questions by studying 2 common parasitic species of the yellow-necked mouse (*Apodemus flavicollis*): the gastrointestinal nematode *Heligmosomoides polygyrus* and the ectoparasitic tick, *Ixodes ricinus*. Previous laboratory studies have shown that the immature infective stage of the closely related species *Heligmosomoides bakeri* elicited protective immunity, but adult parasites down-modulated the humoral immune response and may establish chronic infections (Monroy and Enriquez, 1992; Behnke *et al*. 1993, Telford *et al*. 1998; Maizels *et al*. 2004, Cable *et al*. 2006). This immuno-suppressive action permitted greater parasite survival and reproduction but also facilitated the infection by other parasite species (Colwell and Wescott, 1973; Courtney and Forrester, 1973; Jenkins, 1975; Bruna and Xenia, 1976; Jenkins and Behnke, 1977; Behnke *et al*. 1978; Behnke and Ali, 1984; Alghali *et al*. 1985). For example, when laboratory mice were concurrently infected with *H. bakeri* and *Trichinella spiralis* there was a reduction in the acute response against the second nematode (Behnke *et al*. 1978). Field investigations have identified an association between *H. polygyrus* and other gastrointestinal parasites, but failed to establish whether *H. polygyrus* played a role in structuring the parasite community (Behnke *et al*. 2005).

To examine the effect of *H. polygyrus* in natural systems, we monitored the numerical response of *I. ricinus* in free-living yellow-necked mice. We focused on *I. ricinus* because tick abundance can be monitored on serially caught mice without invasive techniques. Secondly, artificial infestations of *I. ricinus* on yellow-necked mice have identified a progressive suppression of the protective response that resulted in increased host susceptibility (Dizij and Kurtenbach, 1995). We first conducted an extensive cross-sectional study in different mouse populations and examined how the relationship between tick infestation and *H. polygyrus* infections were affected by both environmental factors and host characteristics (breeding condition, sex and age). Second, we undertook an experimental manipulation (reduction/increase) of *H. polygyrus* abundance in mice and monitored changes in tick infestation. The hypothesis we tested was that *H. polygyrus* will show suppressive effects on host response, similar to the closely related species *H. bakeri*, and this will result in an increase in *I. ricinus* infestation per host.

MATERIALS AND METHODS

Species description and monitoring

H. polygyrus inhabits the small intestine of yellow-necked mice and has a direct life cycle with infection occurring after the ingestion of third-stage larvae, either with contaminated food or through grooming (Slater and Keymer, 1986; Hernandez and Sukhdeo, 1995). Adult males, as opposed to females, were responsible for the majority of the nematode transmission (Ferrari *et al*. 2004). *H. polygyrus* represented the most common helminth with population prevalences reaching 85% (Rosso *et al*. 2002; Ferrari, 2005). Six additional helminth species were also identified but occurrence was always very low; the second most common parasite was *Hymenolepis fraterna* with a mean prevalence of 34·8% (Rosso *et al*. 2002; Ferrari, 2005).

I. ricinus is found with a high prevalence in the Trentino Province (Chemini *et al*. 1997; Rizzoli *et al*. 2002, 2004; Carpi *et al*. 2008; Rosà *et al*. 2007) and infests a large range of mammalian species although it is the primary tick species found on yellow-necked mice (Perkins *et al*. 2003, 2006). Infestation can be as high as 74 larvae per mouse and the distribution within the host population is aggregated with large body mass breeding males, carrying large infestations (Perkins *et al*. 2003, 2006). There are 3 tick stages: larvae, nymphs and adults (Sonenshine, 1992). In general the larvae, and a smaller proportion of nymphs, feed on small mammals and a meal lasts for 3–5 days (Sonenshine, 1992; Randolph, 1998). Mice reduce tick infestations through self-grooming (Osfeld *et al*. 1993). In yellow-necked mice the immune response toward *I. ricinus* does not provide a protective defence and multiple tick infections may induce immuno-suppression (Dizij and Kurtenbach, 1995).

Yellow-necked mice were sampled in the Dolomitic Alps of the Province of Trentino, Northern Italy, in 2002 (Fig. 1). We used multi-capture live traps (Ugglan type 2, Graham Sweden) located in broadleaf woodlands with mature stands of beech (*Fagus sylvatica*), some Scots pine (*Pinus sylvestris*),

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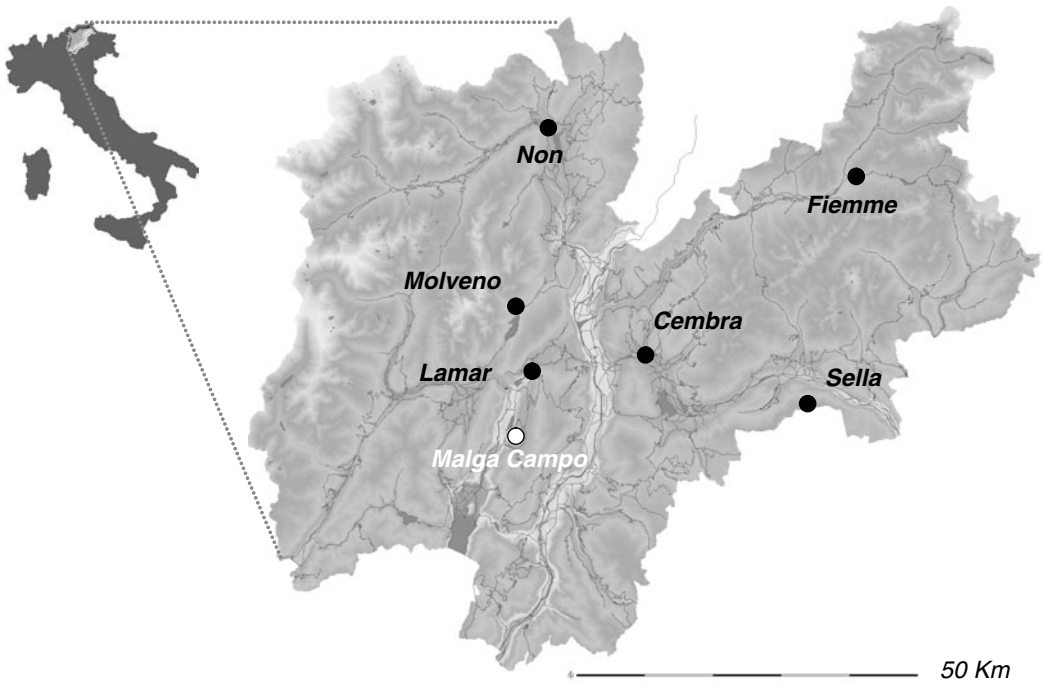

Fig. 1. Location of the extensive and intensive study areas: black points identify the 6 sites of the cross-sectional study and white points the area of the intensive experimental study (the individual grids are too close to be appreciated at this scale).

spruce (*Picea abies*) and a sparse under-storey (mean altitude ± S.E.: 850 ± 22 m a.s.l.), the typical habitat of yellow-necked mice in the Italian Alps (Locatelli and Paolucci, 1998).

Extensive cross-sectional study

The extensive cross-sectional trapping of yellow-necked mice was performed in July 2002 using multi-capture live traps at 6 study sites for 3 nights, with a total effort of 3456 trap nights (Fig. 1). Each site comprised of 3 trapping grids of 64 multi-capture live traps, each set up following the procedures established by Myllymäki *et al*. (1971) and Henttonen *et al*. (1987). For each mouse trapped we recorded sex, body mass, breeding condition and tick infestation. Individuals were classified in breeding condition when we observed descended testes for males and perforated vagina or pregnancy for females (Gurnell and Flowerdew, 1990). Mice with body mass above 15 g were euthanized and stored in a single plastic bag, following the animal procedures of the European Commission Directive 86/609/EEC implemented by Italy. Mice below 15 g often carry very low or no infections and since we were interested in identifying a clear *I. ricinus-H. polygyrus* interaction they were released (Perkins *et al*. 2003; Ferrari *et al*. 2004). In the laboratory, mice were carefully inspected: the number of each tick stage recorded, the gastrointestinal tract removed and *H. polygyrus* extracted, using the filtration-sedimentation technique, and the total number counted (Euzeby, 1982). Eye lenses were collected and the mass of both eye lenses was used as relative measure of mouse age (Morris, 1972; Gregory *et al*. 1992).

Experimental manipulation of H. polygyrus

The experimental reduction or increase of *H. polygyrus* abundance in yellow-necked mice was undertaken through an intensive live trapping program over 2 nights, every other week from May to August 2002, for a total of 5376 trap nights. Animals were trapped in 6 grids of 64 multi-capture live traps each (i.e. 8 × 8 traps at 15 m inter-trap interval covering an area of 1·1 ha, Fig. 1). Grids were located in the same valley and habitat, but more than 500 m apart with natural and artificial barriers (road, open field etc.) between them to minimize movements of individuals between grids. For every trapped individual we recorded sex, body mass, breeding condition and tick number and life-stages. Additionally, each individual was identified with an implantable subcutaneous passive induced transponder tag (PIT tag; Trovan ID 100, Ghislandi & Ghislandi, Italy) that allowed us to monitor the course of the infections in the mouse population using capture-mark-recapture techniques.

H. polygyrus was manipulated in mice weighing above 15 g. The parasite was removed from every other individual caught in 4 grids, through oral treatment with the anthelmintic Pyrantel pamoate (Gellini pharmaceutical; dose: 100 mg/Kg). We selected Pyrantel pamoate since it is active on

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gastrointestinal nematodes but is not systemic and does not affect the ectoparasite infestation (Wahid and Behnke, 1996; Quinnell, 1992). The mice caught that were not anthelmintic treated, were orally infected with an average number of 30, third-stage infective *H. polygyrus* larvae to increase nematode abundance (Keymer and Hiorns, 1986; Gregory *et al.* 1990). Infective *H. polygyrus* larvae were obtained from eggs collected from yellow-necked mice faeces from the study area. Eggs were developed to third-stage larvae and passed through 2 yellow-necked mice (trapped in the study area and treated with anthelminthic); the pure worm culture was then used for the field infection (Keymer and Hiorns, 1986).

The implanted ID tag code allowed us to identify and re-treat each individual every 2 weeks and for the duration of the experiment. The pre-patent period of *H. polygyrus* is 13–15 days (Keymer, 1985) so a 15-day period elapsed between treatments to guarantee the success of the manipulation. Individuals caught from the remaining 2 trapping grids were used as controls. Since *H. polygyrus* manipulation affects nematode transmission and abundance in mice sharing the same habitat (Ferrari *et al.* 2004), control mice were located in untouched grids close to the treatment sites. An *a priori* analysis of tick infestation and nematode abundance data (based on eggs per gram of mouse faeces, EPG) in mice trapped before the onset of the experimental manipulation, confirmed the similarity between the control and treatment sites both for tick infestation ($\chi_2^2 = 0{\cdot}68$, $P = 0{\cdot}711$) and *H. polygyrus* infection ($\chi_2^2 = 1{\cdot}67$, $P = 0{\cdot}432$). Mouse faeces were removed from the traps of single caught individuals and the EPG of *H. polygyrus* estimated using the McMaster technique. No faeces were gathered when traps contained more than 1 individual.

Data analysis

To identify the relationship between *H. polygyrus* abundance and *I. ricinus* infestation in mice from the cross-sectional trapping survey we used Generalised Linear Models (GLM, with negative binomial errors). The total tick infestation per host was used as a response variable, while *H. polygyrus* abundance, host characteristics (sex, age and breeding condition), and habitat composition (3 habitats: pure mature beech, mature beech with Scots pine and mature beech with spruce wood) were included as independent factors. The minimal adequate model was selected using stepwise backward deletion from the maximal initial model including all factors and their second order interactions (Crawley, 2002). To test the relative contribution of *I. ricinus* and host properties on *H. polygyrus* abundance the analysis was repeated using *H. polygyrus* abundance as the response variable, and tick infestation and host characteristics as independent factors.

To investigate the response of *I. ricinus* infestation to the experimental manipulation of *H. polygyrus* abundance we used Generalised Linear Mixed Models (GLMM-IRREML). Total number of ticks per host, as a response variable, was examined in relation to *H. polygyrus* treatment (*H. polygyrus* infection, *H. polygyrus* removal and control with no host manipulation), host sex, age and breeding condition, as independent factors. This analysis was based on mice recaptured following the initial treatment. Since the intensive longitudinal monitoring of the mouse population was performed every 2 weeks and because the blood meal of a tick lasts 3–5 days, the number of ticks counted on mice trapped between weeks can be considered an independent measure. However, to deal with pseudo-replication due to autocorrelation for capture-recapture of the same individuals in the same trapping week, we removed the weekly recaptures and treated the individuals as a random factor. To account for temporal variation in the longitudinal trapping we entered the trapping week as an additional random factor. An *a posteriori* multiple comparison Tukey test was conducted on the between-treatment effects computed by the GLMM, to identify which group contributed the most to the pattern observed. To statistically confirm the experimental success of *H. polygyrus* treatments a GLMM analysis was repeated using EPG as response and treatment as explanatory variable, an *a posteriori* Tukey test was then performed to highlight which treatment mainly contributed to the between-treatment differences. All statistical analyses were performed using Genstat 6th edition (Lawes Agricultural Trust, 2002) and the cut off for statistical significance was fixed at a probability $P < 0{\cdot}05$. Different models were tested and the best minimum models are presented and discussed. Data are presented as means $\pm$ 1 standard error.

RESULTS

Extensive cross-sectional sampling

A total of 230 yellow-necked mice were trapped from the 6 sampling sites, the mean capture was 38·3 (95% CL 9–68)15·1 mice per site. Over the 6 sites, the prevalence of *H. polygyrus* was 66·1% (95% CL 59·9–72·2%), and the geometric mean abundance 10·7 (95% CL 8·9–11·2) worms/host. *I. ricinus* was found on 49·6% (95% CL 43·1–56·0%) of the individuals, the geometric mean infestation was 8·8 (95% CL 7·9–10·0) and 96·4% of ticks were in the larval stage while the remaining were nymphs with a very occasional adult stage. We found that 67·8% (95% CL 59·4–76·2%) of mice infested with *I. ricinus* were co-infected with *H. polygyrus*.

The minimal model that best described the variation in tick infestation across the 6 study sites

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Table 1. Extensive, cross-sectional sampling of yellow-necked mice for *Ixodes ricinus*

(Generalized Linear Model between total tick number per host, as a response, and *H. polygyrus* abundance, host breeding condition and age and habitat, as explanatory variables.)

I. ricinus vs	Coefficients	Deviance	D.F.	*P* value
Habitat vegetation		56·318	2	<0·001
Pure beech wood	0·485			
Scot's Pine presence	0			
Spruce presence	−0·995			
Breeding status		15·250	1	<0·001
Breeding	0			
Non-breeding	−0·233			
H. polygyrus abundance	−0·331	5·633	1	0·017
Mouse age	0·014	0·370	1	0·542
H. polygyrus abundance * Mouse breeding status		14·062	1	<0·001
Breeding	0			
Non-breeding	−0·064			
H. polygyrus abundance * Mouse age	0·012	6·834	1	0·008

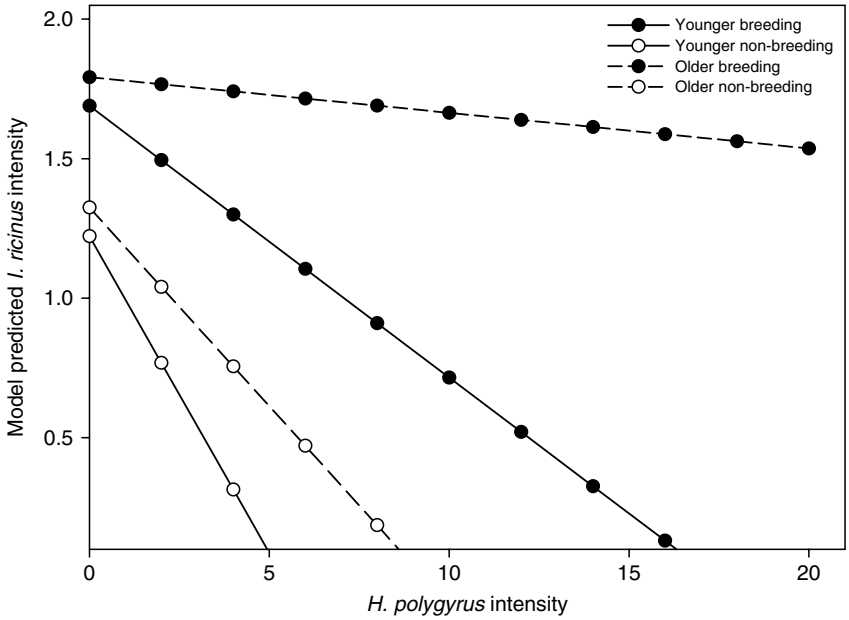

Fig. 2. Model prediction of the relationship between *Ixodes ricinus* infestation and *Heligmosomoides polygyrus* abundance in breeding and non-breeding mice of different ages. Predictions are based on mice in the 1st and 3rd quartiles, representing younger and older individuals, respectively.

showed that the total number of ticks per host was negatively related to *H. polygyrus* abundance, the individual hosts not in breeding condition, the presence of spruce vegetation and the combined effect of *H. polygyrus* abundance and non-breeding condition of mice (Table 1). In contrast, pure beech woodland habitat and the combined effect of host age and *H. polygyrus* abundance had a positive effect on the tick number (Table 1). In particular the model showed that host breeding condition and age interacted with *H. polygyrus* abundance such that non-breeding mice as well as younger hosts exhibited a more pronounced reduction of tick infestation with increasing *H. polygyrus* abundance compared to older and breeding hosts (Fig. 2). To examine which of the two parasites had a stronger effect on the dynamics of the other species, we repeated this analysis using *H. polygyrus* as a response variable, and *I. ricinus* and host characteristics as independent components. The results suggested that host breeding status, sex and pure beech woodland exhibited a positive effect on nematode infection while tick infestation had a negative influence and only when interacting with host characteristics (Table 2).

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Table 2. Extensive cross-sectional sampling of yellow-necked mice for *Heligomosomoides polygyrus*

(Generalized Linear Model between *H. polygyrus* abundance per host, as a response, and tick abundance, host breeding condition, sex and age and habitat, as explanatory variables.)

H. polygyrus vs	Coefficients	Deviance	D.F.	*P* value
Habitat vegetation		9·117	2	0·0104
Pure beech wood	0·288			
Scot's Pine presence	0			
Spruce presence	−0·134			
Mouse sex		2·181	1	0·139
Females	0			
Males	0·252			
Breeding status		24·715	1	<0·001
Breeding	0			
Non-breeding	0·559			
Total tick number	−0·255	0·665	1	0·414
Mouse age	0·089	0·370	1	0·003
Total tick number * Breeding status		9·506	1	0·002
Breeding	0			
Non-breeding	−0·052			
Total tick number * Mouse sex		6·595	1	0·010
Females	0			
Males	−0·325			
Total tick number * Habitat vegetation		13·430	2	0·001
Pure beech wood	−0·305			
Scot's Pine presence	0			
Spruce presence	0·080			

Experimental manipulation of H. polygyrus

Overall, a total of 87 yellow-necked mice were trapped (45 males and 42 females; 41 in breeding and 46 in non-breeding status) and marked with PIT tags between May and August 2002. Of these individuals, 25% were recaptured twice, 9% 3 times and 17% more than 3 times; the mean time-period between the first and the last capture was 5 weeks.

The level of tick infestation was affected by treatment (GLMM-IRREML, Wald = 6·87, D.F. = 2, P = 0·03) while host characteristics (age, sex and breeding condition) did not significantly contribute to the pattern observed (Fig. 3). The experimental infection/removal of *H. polygyrus* from mice was successful in that there was a significant change in EPG between treatments (GLMM-IRREML, Wald = 9·12, D.F. = 2, P = 0·01). The *a posteriori* pairwise comparison between treatments revealed that the anthelmintic treatment caused a significant decrease in *H. polygyrus* EPG (Table 3B), coupled with a significant increase in tick infestation (Table 3A). However, the infection with *H. polygyrus* was not sufficiently effective in increasing the nematode abundance compared to the control, which also caused no apparent change in tick infestation (Table 3). In fact, we found a large variation in EPG between mice, suggesting that the infection was successful for some individuals but not others (Fig. 3).

DISCUSSION

We undertook an extensive cross-sectional monitoring of populations and an intensive experimental manipulation of individuals of free-living yellow-necked mice and examined the hypothesis that the increase in the abundance of *H. polygyrus* would increase the infestation of the co-infecting *I. ricinus*. We selected *H. polygyrus* because experiments with the laboratory model *H. bakeri* have identified suppression of immune-mediated mechanisms by the parasite adult stages, which should result in a positive effect on other co-infecting parasites (Colwell and Wescott, 1973; Courtney and Forrester, 1973; Jenkins, 1975; Bruna and Xenia, 1976; Jenkins and Behnke, 1977; Behnke *et al.* 1978; Behnke and Ali, 1984; Alghali *et al.* 1985; Monroy and Enriquez, 1992; Behnke *et al.* 1993; Telford *et al.* 1998; Maizels *et al.* 2004). We made the assumption that *H. polygyrus* would behave similarly to *H. bakeri*. We also selected *I. ricinus* because previous work identified that infestation levels were negatively related to host susceptibility (Dizij and Kurtenbach, 1995) and we could monitor the response of this ectoparasite

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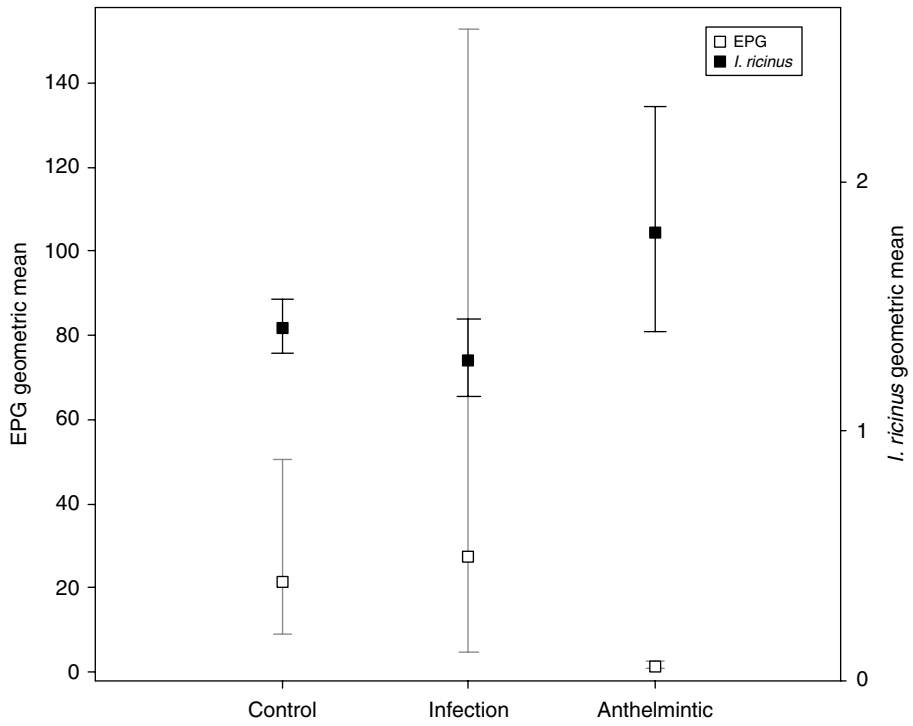

Fig. 3. Changes in the geometric mean of *Ixodes ricinus* infestation (ticks/host) and number of *Heligmosomoides polygyrus* eggs per gram of host faeces (EPG) in relation to the experimental treatment. The 95% confidence limits are reported.

without invasive techniques. If *H. polygyrus* influences tick infestation, this can potentially have a significant impact on the dynamics of tick-borne diseases in the mouse populations. For example, *I. ricinus* is the vector of the zoonotic infection that causes tick-borne encephalitis and changes in the tick dynamics could have a major effect on the dynamics of the viral infection (Labuda *et al*. 1997; Randolph, 2000).

In our cross-sectional study we found that there was a negative relationship between the two parasite species and the experimental removal of *H. polygyrus* resulted in an increase in *I. ricinus*. These findings were counter to the prediction based on *H. bakeri* studies; indeed our field manipulation showed that this was a consequence of *H. polygyrus* infection influencing *I. ricinus* rather than the reverse. The comparative field study revealed that habitat and host breeding condition explained 24% and 8%, respectively, of the variation in tick infestation; nematode infection alone explained 2% but its effect was further increased to 4% when interacting with host breeding condition and to 3% with age. Despite the low statistical contribution, compared to habitat characteristics, *H. polygyrus* appeared to have a significant impact on tick infestation.

Previous studies on free-living mouse populations found that *H. polygyrus* infection is strongly influenced by host age, but habitat can play an important role in causing spatial differences in the intensity of infection among host populations (Gregory *et al*. 1992; Behnke *et al*. 1999; Behnke 2008). Detailed large-scale studies and modelling also showed how weather and vegetation are crucial for the distribution and abundance of questing ticks (Randolph and Storey, 1999; Randolph, 2000). Our extensive cross-sectional results confirm the importance of environmental characteristics on the distribution of both parasites and also support the hypothesis of a possible effect of the nematode on the spatial distribution and level of tick infestation among different sampling sites of Trentino.

Younger non-breeding individual mice exhibited the strongest *H. polygyrus-I. ricinus* interaction, *I. ricinus* decreased with increasing *H. polygyrus* abundance and this was more apparent in non-breeding than breeding mice, and between young rather than old individuals. Host breeding conditions and age could have altered the nematode-tick interaction and further affected the dynamics of co-infection. Indeed we found that *H. polygyrus* abundance was mainly driven by host breeding condition, age and habitat, while *I. ricinus* had a marginal contribution. This finding suggests that *H. polygyrus* may act as a dominant species in the infra-community of parasites and probably modulates the dynamics of the other parasite species.

The negative relationship between *H. polygyrus* and *I. ricinus* was identified in the extensive cross-sectional study and the anthelmintic manipulation experiment confirmed that this relationship occurs as a cause and effect mechanism, i.e. *H. polygyrus* causes

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Table 3. Average values (±95% confidence limits) for *Heligmosomoides polygyrus* eggs per gram (EPG) and *Ixodes ricinus* by treatment and *a posteriori* pairwise Tukey test based on GLMM estimates

(Comparisons between treatment groups (control, infection and anthelminthic) for: A-*I. ricinus* infestation and B-*H. polygyrus* number (EPG).)

Treatments	*H. polygyrus* EPG Geometric mean (95% CL)	*I. ricinus* Geometric mean (95% CL)
Control	21·30 (8·98–50·56)	1·41 (1·30–1·52)
Infection	27·59 (4·97–152·91)	1·28 (1·14–1·44)
Anthelmintic	1·35 (0·74–2·44)	1·79 (1·40–2·30)

	Differences between predicted effects (±S.E.)	D.F.	*P* value
(A) *I. ricinus* (ticks/host)			
Control *vs* Infection	0·258 (±0·816)	120	0·751
Control *vs* Anthelmintic	1·150 (±0·496)	120	0·015
Infection *vs* Anthelmintic	0·829 (±0·889)	120	0·328
(B) *H. polygyrus* EPG			
Control *vs* Infection	0·331 (±1·505)	67	0·822
Control *vs* Anthelmintic	4·934 (±1·837)	67	0·009
Infection *vs* Anthelmintic	5·265 (±2·150)	67	0·017

changes in the *I. ricinus* population. Unfortunately, the design of the experiment did not provide the opportunity to clarify the mechanisms generating the pattern observed. We can exclude the hypothesis that this was caused by direct competition for space since these two parasites use different parts of the host body. We also exclude the possibility of direct competition for resources since ticks and *H. polygyrus* depend on different trophic elements, the first specialized as bloodsuckers and the second feeding on intestinal tissues (Bansemir and Sukhdeo, 1994). We can also rule out alternative explanations of a potential role of host behaviour on parasite interactions. *H. polygyrus* can be ingested through fur grooming and grooming is also used to control tick infestation (Hernandez and Sukhdeo, 1995; Osfeld *et al.* 1993). Therefore, mice that groom heavily will have fewer ticks and will potentially ingest more *H. polygyrus* infective larvae, than less active individuals. It is also possible that differences in ranging behaviour between male and female as well as during their life cycle (breeding *vs* non-breeding or dispersing young *vs* territorial adults) may have contributed to changes in exposure (Randolph, 1997; Stradiotto, 2008). However, the evidence of a cause and effect mechanism, as by the manipulation of *H. polygyrus* load on tick infestation, suggests that parasite interaction and host behaviour are independent mechanisms.

Our initial assumption that *H. bakeri* and *H. polygyrus* behave in the same manner may not be correct. This difference may be because *H. bakeri* has been inadvertently selected to be more immunosuppressive than its natural counterpart or because the larval stages stimulate an immune response that overrides the adult immuno-suppressive abilities. This possible difference warrants further investigation. One hypothesis is that *H. polygyrus* and *I. ricinus* interact through the host, either through the immune response or the release of toxic products that may potentially affect the other species (Behnke *et al.* 2001; Bradley and Jackson, 2008; Fenton *et al.* 2008). At the moment we do not have evidence to tease apart these hypotheses. We can, however, recognize that the interaction is modulated by the host reproductive status and age; indeed we found that non-breeding mice and young individuals had fewer ticks but more *H. polygyrus*. Reproduction and age can both be associated with the strength of the host immune response (Schalk and Forbes, 1997; Woolhouse, 1998). Previous studies on other systems found a trade-off in energy allocation between the immune system and host reproduction, with a deprivation of resources and a more efficient immune response during the breeding season (Apanius, 1991). Age may also affect host susceptibility and time of exposure to infective stages, and contribute to the development of an acquired immune response (Woolhouse, 1998). The trickle bi-weekly infections with *H. polygyrus* larvae may also have enhanced the immune response against this nematode in some individuals, and this may have caused the failure of the worm dosing and the large variability in worm EPG observed among the mice. In this sense, the absence of a significant change in tick infestation in trickle-infected mice may be the result of a large variability in the immune response between individual hosts. This conclusion is supported by previous experimental laboratory infections, where different mouse strains trickle-dosed with *H. polygyrus* exhibited different responses, with some becoming completely

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resistant to re-infections as a consequence of a full protective immune response (Brailsford and Behnke, 1992; Behnke *et al*. 2003).

In conclusion, both laboratory experiments and field observations leave part of the question on the role of co-infections on the dynamics of a single parasite species unresolved, and are therefore inadequate to provide a full picture of the consequences of parasite interactions in the real world. The approach used in the present study was aimed to overcome some of these limitations, by providing simultaneously a large-scale comparative approach and an experimental manipulation in natural conditions. Despite the fact that the manipulation of *H. polygyrus* abundance in mice was effective only at reducing *H. polygyrus* infection, we found clear evidence that changes in *I. ricinus* infestation are related negatively to changes in *H. polygyrus* abundance, and that these changes occur as a cause effect process. Different mechanisms, i.e. host immunity, release of parasite toxic products, may have caused this pattern and we do not exclude that their relative role may have changed throughout the time of the infection according to host and environmental condition. The testing of this system in the laboratory will allow us to disentangle the underlying processes affecting the interaction between *I. ricinus* and *H. polygyrus*.

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