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Macroparasite transmission and dynamics in *Apodemus flavicollis*

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To Giorgio

Πάτροκλος, τὸν ἐγὼ περὶ πάντων τῖον ἑταίρων
ἶσον ἐμῇ κεφαλῇ· τὸν ἀπώλεσα

…Patroklos, whom I honoured above all my
comrades as it were my very self! Him have I lost…
Homer, Iliad

Abstract

This thesis examines the parasite dynamics and the mechanisms affecting parasite load and transmission focalising on the role played by host and habitat heterogeneities. This study is based on the gastrointestinal nematode *Heligmosomoides polygyrus* and the small mammal yellow necked mouse and uses data gathered from experimental field manipulations of parasites intensities and data gathered from trapping monitoring.

Initially the parasite community of yellow-necked mouse (*Apodemus flavicollis)* was explored in North-Eastern Italian Alps with the aim to describe the major patterns and identify the factors affecting parasite community structure. Despite the observed spatial variability it has been found that differences within the host population such age and secondly sex and breeding conditions, were the major factors acting on parasite occurrence and intensity. Habitat differences had a less apparent effect on parasite community structure.

The consequences of *H. polygyrus* infection on other parasite species infections have been analysed, in specific the infestation of the tick *Ixodes ricinus* in populations of *A. flavicollis. H. polygyrus* load and tick infestation were monitored as well as were carried out field manipulations of *H. polygyrus* intensity and were monitored changes in tick infestation. It has been found that *H. polygyrus* load was negatively related to *I. ricinus* infestations. Host factors mediated the *H. polygyrus*-*I. ricinus* interaction such that young and non-breeding mice exhibited higher *I. ricinus* to *H. polygyrus* intensity respect breeding adults.

The role of host sex on parasite abundance was then investigated carrying out a field experiment where the *H. polygyrus* intensity were manipulated in relation to mice gender. In specific, *H. polygyrus* was removed alternately from either sexes and the parasite load was analysed in the untreated sex. It was found that males mice were responsible to drive

parasite transmission in the host population and this was observed in absence of sex-bias in parasite infection, suggesting that this pattern was not a mere consequence of quantitative differences in parasite loads between sexes.

To disentangle the possible mechanism causing this sex bias in parasite transmission mathematical simulations based on parameters obtained for the field experiment were used. Two non mutually exclusive hypotheses causing sex bias in parasite transmission were tested: a- males immune response is less efficient and this causes the development of more successful parasite infective stages or b-males behaviours allow them to be more efficient is spreading in more exposed areas parasite infective stages. Multi-host models were developed and simulations were compared with field results. While it was not disentangled the most dominant mechanism causing sex bias in parasite transmission this study underlined the importance of host sexes in affecting parasite dynamics and host-parasite interaction.

In conclusion this thesis highlighted the importance of considering host and environmental differences when investigating host parasite interactions. This finding could be extremely important when planning measured of disease control or to avoid disease outbreak. Controlling target group of individuals host could avoid economical losses and a more effective measure of intervention.

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Statement of Originality

The work described in this thesis is original research carried out by myself and has not been submitted for consideration previously for a higher degree at this or any other university. Any references henceforth used have been appropriately acknowledged.

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Chapter 1

Introduction: Gastrointestinal parasites of *Apodemus flavicollis*

Introduction

Parasites can have a dramatic effect on host fitness, population dynamics and community structure (Tompkins *et al.,* 2002). Studies on parasite infections have highlighted that host-parasite interactions are dynamical processes and the patterns observed are shaped both by intrinsic factors, associated with host susceptibility (hormones, nutrition, genetics), and extrinsic factors, associated with host exposure (seasonality, habitat, behavior). Eco-epidemiologists have been challenged to disentangle the relative role of these components and how they affect parasite dynamics and the infracommunity structure of parasites (i.e. the community of parasites within each single host). Empirical studies, both in the field and in the laboratory, and theoretical modeling have been immensely useful in revealing the key components and the driving processes involved in such interactions (Roberts *et al*., 2002; Wilson *et al.,* 2002). In this respect, rodents and their parasites have been one of the most studied systems for ecological and parasitological studies both in wild populations and in replicated laboratory experiments (Keymer and Hiorns, 1986; Slater and Keymer, 1986a;b; Lewis, 1987; Scott and Lewis, 1987; Montgomery and Montgomery, 1989; Gregory *et al.*, 1990; Montgomery and Montgomery, 1990; Scott, 1990; 1991; Behnke *et al.,* 1991; Gregory, 1991; Keymer and Tarlton, 1991; Gregory, 1992; Monroy and Enriquez, 1992; Gregory *et al.,* 1992; Quinnell, 1992; Tanguay and Scott, 1992; Abu-Madi *et al.,* 1998; Behnke *et al.*, 1999; Abu-Madi *et al.*, 2000; de Bellocq, 2003).

In this introduction it will be overviewed the current knowledge on host-parasite interaction and the effect of such interactions on parasite and host dynamics, focusing on rodents and their gastrointestinal parasites.

Parasites can be classified according to the relationship they establish with the host in as either microparasites or macroparasites (Anderson and May, 1991). Microparasites

reproduce within the host and they do not have special infective life stages. The generation time is short and they may induce the death of their host or the rise of an immunological response that usually protects the host for the whole life. Therefore, within a host population individuals may be classified as susceptible, infected, infectious and recovered. For example bacteria, virus, protozoa and fungi are typically considered microparasites (Anderson and May, 1991). In contrast, macroparasites, mainly arthropods and helminths, are characterised by more complex life cycles, which involves free living infective stages, sometime intermediate hosts, and they do not reproduce within the host. The more complex antigenic structure involves less effective immune reactions and often chronic infections that lead to morbidity rather than mortality of the host. Indeed, the host's immune system often mediates a large part of interspecific parasite interactions, which are dependent on the integral of past exposure and the competence/maturity of the host's immune response to later infections (Anderson and May, 1991; Woolhouse, 1992).

Macroparasites distribution within the host population

For a better understanding of the impact of macroparasites on host population dynamics, and in particular which individuals undergo the most detrimental parasite mediated effects, it is important to understand how macroparasites are distributed within the host population. The distribution of parasites within the host population is rarely random, but often presents a characteristic aggregated distribution (Shaw and Dobson, 1995; Shaw *et al.,* 1998). This involves the majority of the host population harbouring a low parasite burden while only a low proportion of the host population harbours high parasite burdens. Thus, while parasite aggregation affects few hosts, it also reduces the impact of the parasite on the whole host population, acting to stabilize the host parasite interactions (Anderson and May, 1978; May and Anderson, 1978). More importantly, if parasites are aggregated each individual host will contribute differently to the dynamics

of transmission. In particular if the simultaneous and independent variation in parasite distribution between individual hosts do affect parasite transmission it may introduce a non-linearity that can potentially have large effects on the basic parasite reproduction rate R_0 (Keeling *et al.*, 2003).

These concepts have been elegantly simplified in what is commonly referred to as the 20/80 Rule (Anderson and May, 1991; Woolhouse *et al.*, 1997). This concept, based on theoretical formulations and analyses of different host-parasite systems, suggests that in the majority of cases, 20% of the whole host population is responsible for the transmission of 80% of the parasite population. As such, irrespective of the type of parasite transmission (density dependent or frequency dependent) only a small group of individual hosts is responsible for the majority of parasite persistence (Woolhouse *et al.*, 1997; Perkins *et al.*, 2003). Identifying these host groups and how and when they affect parasite dynamics is an important step for understanding host-parasite interactions.

From an analytical point of view, several methods have been proposed to describe the aggregated distribution of parasites within the host population (for a review Wilson *et al*., 2002). Eco-epidemiologists are now extensively using the negative binomial statistical distribution (Anderson and May, 1978; Shaw and Dobson, 1995). The comparison of more than 300 host-parasite systems found that in 90% of cases a negative binomial distribution provided a more satisfactory statistical fit than the alternative Poisson distribution (Shaw *et al.,* 1998). The negative binomial distribution is defined by two parameters, the mean number of parasite per host (x) and the dispersion parameter (k), which represents an inverse measure of aggregation. When k is small, parasites are more aggregated while as k gets bigger and approaches to infinity the parasite distribution tends to a Poisson distribution with parasites randomly distributed across the host population.

Moreover the *k* parameter describes the parasite "over-dispersion" much better than other measures since it is less sensitive to the number of hosts sampled.

The Heligmosomoides polygyrus- Apodemus flavicollis system

Research on rodent parasites have been important for understanding some of the fundamental interactions between host and parasites and the role of host characteristics in modulating parasite dynamics.

Ecology of Heligmosomoides polygyrus

The nematode, *Heligmosomoides polygyrus* (Dujardin, 1845), a gastrointestinal parasite commonly found in rodent species, has been one of the most successful laboratory models for studying host-parasite interactions (Behnke *et al.,* 1991; Monroy and Enriquez, 1992). *H. polygyrus*, previously known as *Nematospirodes dubius*, is a dioecious nematode belonging to the superfamily of Trichostrongylidae and to the genus of Heligmosomidae (Durette-Desset *et al.,* 1972). To this genus belong two other well-known parasites, which share similar characteristics, *H. glareoli* and *Heligmosomum mixtum,* common in bank vole *Clethrionomys glareolus*. Four subspecies have been identified on the basis of morphological characteristics (Durrette-Desset, *et al.*, 1972). *H. p. polygyrus* and *H. p. corsicus* from European wild rodents, the first from the Northern regions while the second from Mediterranean areas. In North America two species have been isolated, *H. p. americanus* and *H. p. bakeri* (Durette-Desset *et al.,* 1972).

H. polygyrus has a broad infective spectrum having among its hosts *Apodemus* spp., *Mus musculus*, *M. domesticus*, while its occurrence in two species of *Clethrionomys* spp. and in *Peromiscus maniculatus* is debated (Lewis, 1987; Behnke *et al.,* 1991). The life cycle of this nematode can be completed in 13-15 days taking into account differences between subspecies (Keymer, 1985). The parasite infection takes place through the

ingestion of the third stage infective larvae (L3) with contaminated food material or through grooming of infected fur (Slater and Keymer, 1986a; Scott, 1991; Tanguay and Scott, 1992; Hernandez and Sukhdeo, 1995). The ingested larvae colonise the small intestine and develop into adults. The life span of adult worms may last up to 3 months depending on the *H. polygyrus* strain and immunological status of the host (Gregory *et al.*, 1990). Eggs produced by adult females reach the environment within the host faeces and hatch and develop to infective L3 (Anderson, 2000). This phase is strongly affected by temperature and humidity (Anderson, 2000). In natural populations of *A. sylvaticus* the prevalence of *H. polygyrus* ranges between 80% and 100% and mean intensity ranges between 10 and 25 worms per mouse (Gregory, 1992; Abu-Madi *et al.*, 1998).

Ecology of Apodemus flavicollis

The majority of field studies on *H. polygyrus* have focused on common wood mice (*A. sylvaticus*) populations from Central-North Europe. In the North-Eastern Italian Alps the yellow-necked mouse (*A. flavicollis*) is the most common rodent species and represents the most suitable small mammal for field investigations of *H. polygyrus* dynamics (Locatelli and Paolucci, 1998). While the ecology of *A. sylvaticus* has been extensively investigated, the restricted distribution of *A. flavicollis* has produced a smaller number of studies and the ecology of this species is still not completely known.

The adult *A. flavicollis* has a body length ranging from 95 to 120 mm while the tail ranges from 77 to 118 mm, with a body mass up to 45 g.. Although larger in size, *A. flavicollis* is very similar to *A. sylvaticus* and in the field the two species are hardly distinguishable. *A. flavicollis* has paler brown back fur and white under parts. The most distinguishing feature is the yellowish collar on the chest that can occur in a diverse variety of patterns (Corbet and Harris, 1991).

A. flavicollis inhabits long-established woodlands, mainly composed by broadleaf trees, which in the alpine environment are commonly represented by beech woods. The diet is composed primarily of tree seeds (e.g. beechnuts), fruits, buds and invertebrates.

Yellow-necked mouse are mostly nocturnal, during the day inhabiting burrows composed of tunnel systems and nests furnished with vegetable material as bedding (de Mendonça, 2003). In optimum conditions reproduction can occur all year, but usually it is restricted from March to September. Mice that are sexually reproductive undergo changes to the sex organs that can be easily recognised in the field. Males show enlarged testicles, females may alternatively show enlarged abdomen, due to pregnancy, or a perforated vagina indicating the subject is receptive to breeding, or when the breeding has just occurred a whitish copulatory plug is visible in the vagina (Gurnell and Flowerdew, 1990).

The pups are born naked and blind and they quickly develop grey fur. After 2-3 months they reach sexual maturity and progressively they then moult into adult brown pelage (Flowerdew, 1984); if they are born in late summer they are unlikely to breed until the following year. Moulting in the alpine yellow-necked mouse population occurs when the body mass is about 15 g. (±0.7 S.E., n=25) as supported by an analysis on our sampled population in 2001 and confirmed by Barbetta (2003). Once adult moulting is complete then assessing the age of *A. flavicollis* become difficult. As such during the field manipulation individuals have been classified into two discrete age-classes: juveniles (below 15 g. with grey fur) and adult (over 15g. with brown fur) with the later class differentiated into breeding and non-breeding individuals. Alternatively, the eye lens mass, after formalin fixation and stove drying, can be used to determine age (Morris, 1972). Thus, if we know the exact age of some culled individuals (e.g. from a laboratory-

bred population) then we may estimate an appropriate age-eye lens mass line regression, alternatively the eye lens mass could be used as crude relative index of mice age.

In general, the individual life span is very short and only few mice survive more than one year (Flowerdew, 1984). Therefore, the population dynamics of *A. flavicollis* are influenced by periodicity in reproduction which is strongly influenced by seasonal fluctuations, with abundance peaking in autumn and declining over the winter until spring, when the next breeding season starts.

Males and females defend their territory (ca. 0.5 ha.) against individuals of the same sex and home ranges overlap between sexes. Males are polygynous (de Mendonça, 2003) and female fertility is high.

Differences in parasite load between hosts

One of the inferences from previous paragraphs is that only a few infected hosts are responsible for carrying the majority of the parasites, and this will have important consequences for the probability of parasite persistence within a host population. As such, one obvious question is: Why are parasites aggregated within a host population? And, can we identify the key factors responsible for driving such an aggregated pattern?

Several factors have been identified (for a review see Wilson *et al.,* 2002). Indeed, parasite infections are influenced by predisposing host factors including susceptibility, physiological condition, host immunity, genetic diversity, host exposure, behavioural characteristics and extrinsic factors including habitat. Variability in these factors causes changes in the risk of parasitism experienced by individual hosts, and variability in the risk of parasitism is recognised to promote aggregation (Chesson and Murdoch, 1986).

In the next sections there will be an overview of the main factors creating differences in parasite infection.

Host sex

In general, comparative studies on vertebrate hosts have shown that males tend to have higher parasite loads than females (Poulin, 1996; Shalk and Forbes, 1997; Moore and Wilson, 2002). However, laboratory and field studies on the effect of sex on *H. polygyrus* infection in mice have found contrasting results. In fact, while *Apodemus* spp. in natural populations rarely show a sex-bias in parasitism (Gregory, 1992; Gregory, *et al.,* 1992; Abu-Madi *et al.*, 1998; Behnke *et al.*, 1999), laboratory infections with similar doses of *H. polygyrus* infective larvae, show that males have higher burdens than females (Gregory, *et al.*, 1990). Differences in hormonal levels have been suggested to be the most common mechanism giving rise to sex-biased parasitism. Among steroid hormones that influence the immune system, testosterone is known to depress cell-mediated and humoral immunoresponses (Zuk and McKean, 1996) and high testosterone levels have been found to increase the males' susceptibility to parasite infections (Hillgarth and Winfield, 1997).

Differences in behaviour, diet and body size between the sexes can be also important factors generating differential parasite intensity in a population. For instance, studies have suggested that during the breeding period male mice are more exposed to parasites because of the increased activity involved in defending a territory or a mate partner (Ims, 1987; Tinsley, 1989). Vertebrate males tend also to have a greater body size and bigger body surface which enhance food intake compared with females and this can increase the exposure of males to a higher number of infective stages (Arneberg *et al.*, 1998; Moore and Wilson, 2002).

Host Age and Host Immunity

In the majority of macroparasite infections the less effective the immune response against macroparasites the more likely this will give rise to chronic infections. In particular, host age, which reflects the cumulative exposure to parasites, becomes an important factor in understanding changes in parasite intensity (Anderson and Gordon, 1982; Anderson and May, 1991). This is usually examined using the host age-parasite intensity relationship. This relationship can have three main profiles that describe different host-parasite interactions (Hudson and Dobson, 1995). Parasite intensity may increase constantly with host age when infective stages are constantly acquired from the environment and there is lack of a host immune response, vertical transmission or density-dependent parasite mortality within the host. Alternatively, intensity may increase to an asymptote, or may exhibit a convex profile, where parasite intensity increases in the early age classes, peaks and decreases thereafter in the older age individuals. An asymptotic age-intensity profile is typical of density dependent parasite dynamics, where the rates of parasite acquisition and parasite mortality are constant. Mechanisms that determine a convex profile depend on parasite-induced host mortality, host acquired immunity or age related changes in exposure (Hudson and Dobson, 1995; Wilson *et al.*, 2002), what determines the final profile is a balance between these processes (Hudson and Dobson, 1995). Therefore, the host age-parasite intensity profile does differ between different host-parasite systems, but may also change between different populations of the same host species. For example, long term surveys on *H. polygyrus* in *A. sylvaticus* populations in England exhibited asymptotic age-intensity relationships, while mice populations in Ireland showed a convex profile (Gregory, 1992; Quinnell, 1992; Gregory *et al*., 1992) and both parasite-induced host mortality and development of acquired

immunity have been identified for *H. polygyrus* in *A. sylvaticus* (Scott, 1987; Gregory, 1991; Quinnell, 1992).

Woolhouse (1998) investigated the age-intensity/prevalence profiles of several parasites and pathogens in human and animal populations and suggested that changes in acquired immunity with host age can generate different convex profiles. He found that when transmission rate was high, a greater number of individuals became infected quickly at a young age and showed high parasite intensity, whereas when the transmission rate was low the infection rates increased slowly and affected older individuals with lower parasite intensity. Such behaviour is assumed to be caused by a change in acquired immunity with host age and parasite transmission rates. The weaker the immune-response in the young individuals the greater the intensity of infection and, vice-versa, the stronger the immune-response in older individuals, the lower the intensity of infection. Woolhouse termed this phenomenon "peak shift" and suggested that when host immunity influences host susceptibility, we should observe a negative correlation between host age at which the peak occurs and levels of peak infection (Woolhouse, 1998). Occurrence of the peak shift has been demonstrated in infection of macroparasites and microparasites in human populations in Africa (Woolhouse*,* 1998), in a single population of free living rabbits in Scotland (Cattadori *et al.,* 2005a), and in laboratory experiments of mice infected with *H. polygyrus* (Woolhouse, 1998) and represents a clear example of development of acquired immunity with host age.

Comparison of *H. polygyrus* single infection within individuals challenged with repeated infections (i.e. trickle infections) provide evidence of a reduction in parasite *per capita* egg production (Enriquez *et al.,* 1988) and larval establishment success (Keymer and Hiorns, 1986; Enriquez *et al.*, 1988; Keymer and Tarlton, 1991; Scott, 1991) and were interpreted by the authors as a consequence of the development of host acquired

immunity to worm infection. Moreover trickle infections experiments allow to distinguish the density-dependence parasite response from intensity-dependent immune response in particular, studies on *H. polygyrus* evidenced that an increase in its intensity was associated with a higher reduction of parasite fecundity and establishment rates as consequence of immune response (Enriquez *et al.*, 1988).

Host and parasite genetic diversity

The genetic diversity of both the host and parasite strongly influence the distribution of parasites within a host population. Laboratory infections of mice with *H. polygyrus* showed that different strains of mice exhibited different rates of susceptibility to the parasite infection (Scott, 1991). There was also evidence that resistance to parasite infection is a host heritable factor. Trickle infection experiments of mice with *H. polygyrus* found that the variability in parasite load between individual hosts increased from the primary infection to subsequent infections, highlighting the differential contribution of genes to innate and acquired resistance (Gregory *et al.*, 1990). Recently, researchers have suggested that parasite-host interactions are among the main contributors to genetic diversity within host populations (Paterson *et al.*, 1998). In particular, it has been suggested that host-parasite interactions without constraints, like genetic selection or artificial infections, may cause both hosts and their parasites to co-evolve and maintain the reciprocal genetic diversity, as proposed in the *Red Queen* hypothesis (Stenseth and Maynard-Smith, 1984). This hypothesis postulates an arms race between the parasite and the host where each player responds and counter-responds to the evolution of the opposite player in order to maximise its own fitness. Thus, while the outcome may be an unchanged relationship between the host and its parasite, both partners are evolving rapidly (Stenseth and Maynard-Smith, 1984). The contribution of parasites to the genetic

variability of the host have been observed in wild and laboratory mice strains infected with *H. polygyrus*, where mice under natural selection showed higher variability in susceptibility than mice artificially selected (Gregory *et al.,* 1990).

Genetic variability in parasite populations may also influence parasite aggregation within the host population. Laboratory infections of mice with *Trichinella spiralis* and *Trichuris muris* have found that when parasites with diverse genotypes infect the host, then it produces a lower protective response compared to the case when one single parasite genotype infects (Bellaby *et al.*, 1995; Wakelin and Goyal, 1996). Moreover, the recognition of different rates of larval establishment among different *H. polygyrus* strains suggests that natural infections with different compositions of *H. polygyrus* genotypes can generate diverse protective responses and variation in the establishment rate among different strains of this parasite (Quinnell *et al.*, 1991).

Host behaviour

Natural selection should favour those individuals whose behaviour reduces their exposure to parasitic infections through avoidance of infected areas, regular grooming activities or behaviours that can reduce their exposure (Hart, 1994). As highlighted previously, males and females exhibit different hormonal/reproductive strategies but also exhibit different types of interactions during their life. These different behaviours will generate differences in rates of parasite infection and lead to variations in infection intensity. In a laboratory experiment a group of mice was artificially infected with *H. polygyrus* larvae, administered by oral dosage, while a second group of mice acquired infection naturally from the infective substrate (Tanguay and Scott, 1992). The results showed that individuals from the naturally infected groups had higher variance in parasite intensity than artificially infected groups, suggesting the influence of individual behaviour

on the pattern of infection (Tanguay and Scott, 1992). Generally, the effect of behaviour on host susceptibility to infection has been poorly investigated and there are still a large number of questions that need to be addressed.

Host body conditions

Parasitic infection represents an additional energetic cost for the individual host since they require extra resources to fight infection and repair damage sustained during the infection. To cope with the infection, the host invests resources in specific (immune system) and aspecific (fever or inflammatory reactions) defences that under impaired body condition can affect the host response.

The reduced response of a weak mouse to a parasitic infection was particularly evident in laboratory investigations where mice were fed diets deficient in proteins or other essential elements. An increase in *H. polygyrus* burden and an impaired ability to control infections was observed in mice with low protein diets (Slater and Keymer, 1986b; Keymer and Tarlton, 1991). However, disentangling the effect of body condition and diet on the hosts' ability to control an infection is often difficult, since parasites may be the cause rather than the effect of poor body condition. To overcome these methodological pitfalls several approaches have been proposed. For example, one approach is to analyse, for a limited period of time, the co-variation in the rate of infection of different parasite species in relation to host body condition. Specifically, if decreased body condition reduces the host ability to control infections then a related change in intensity is expected when moving from a single to a concomitant infection. When this approach was applied to natural host populations contrasting results were observed. For example, while domestic and feral sheep exhibited a relationship between

body condition and the intensity of different parasite species (Wilson *et al.,* 2002) this association was not observed in rodents (Haukisalmi and Henttonen, 1993).

Finally, the effect of body condition on parasite intensities has also been investigated using morphometric measures together with andrenal gland and thymus mass as an index for corticosterone levels. In two different rodent-parasite systems a positive association between morphometric measures, corticosterone levels, and parasite burdens has been identified (Barnard, *et al.*, 2002; 2003), suggesting body condition impaired host resistance to parasite infection.

Extrinsic factors

Extrinsic variations can also affect host exposure and cause the aggregated distribution of parasites within the host population.

In particular, the small scale spatial arrangement of infective free-living stages is recognised as influencing parasite aggregation in hosts (Keymer and Anderson, 1979). For example, the uneven spatial distribution of free-living infective stages on the ground may be the result of changes in host spatial behaviour, habitat composition or climatic variations in temperature and humidity, which strongly affect the survival and development of the infective larval stages (Gulland and Fox, 1992; Saunders *et al.,* 2000; Cattadori *et al.*, 2005a). Studies have found that temperature and humidity are important in regulating the development and survival of parasites with free-living stages. Nematodes require a warm and moist environment for development and transmission: in general, eggs commence development and migration of larvae onto vegetation when the temperature exceeds 10°C (Saunders *et al*., 2000). Workers have assumed that day degree models (Smith, 1990) provide an adequate model of the development of nematodes infective stages. However, laboratory experiments have shown that such models do not

apply and when there is variability in climatic conditions the development rates of infective stages were faster than predicted by a day degree model. If this is also true for free-living stages in the field then it is important to take into account environmental stochasticity and how this alters parasites infection rates. Moreover, theoretical models need to take these findings into account. At the same time, the seasonal variability in parasite rates of development may influence the susceptibility and exposure of the hosts to subsequent infections and play an important role (Gulland and Fox, 1992; Cattadori *et al.*, 2005b). For example, temporal changes in the occurrence of free-living infective stages (Saunders *et al.,* 2000), coupled with seasonal changes in host behaviour or physiology, may strongly affect susceptibility or exposure and consequently parasite infection distribution (Gregory, 1992).

Parasite community interactions

So far these sections have examined the interaction between one parasite species and its host, however hosts are inhabited by more than one parasite (concomitant infections) that may interact with the host immune response or act directly through competition for resources and so influence host susceptibility as well as parasite community dynamics (Behnke *et al.*, 2001; Cox, 2001; Lello *et al.*, 2004). *A. sylvaticus* is often infected by an entire community of parasites and it is important to consider how these parasites species affect susceptibility and abundance to other parasites (Montgomery and Montgomery, 1989; 1990; Behnke *et al.*, 1999; Abu-Madi *et al.*, 2000; de Bellocq *et al.*, 2003).

Studies, which have examined parasite communities, have done so either by conducting field surveys or undertaking experiments with mixed infections in the lab. Workers that have applied the comparative approach have based their studies on comparisons between observed assemblage patterns (Poulin, 2001; Guègan *et al.,* 2005).

Laboratory experiments tend to concentrate on coupled infections with the aim to disclose quantitative responses and mechanisms involved in parasite interaction (Behnke *et al.,* 2001; Cox, 2001).

Although comparative and laboratory studies sometimes differ in the identification of parasite interactions they share the common conclusion that parasite community interactions should not be underestimated when examining the dynamics of a single parasite species. For example, long term monitoring of the parasite community of *C. glareolus* identified a negative association between the tapeworms *Catenotaenia* spp. and *Paranoplocephala gracilis* with *H. mixtum* and *H. glareoli* respectively (Haukisalmi and Henttonen, 1993), while poor direct evidence of an interaction between parasite species and *H. polygyrus* has been found in wild *Apodemus* spp. (Behnke *et al.,* 2005). In contrast, artificial infections of mice with *H. polygyrus* identified an increase in intensity and survival of *T. spiralis*, *T. muris*, *Hymenolepis diminuta* and *H. citelli* (for a review see Behnke *et al.*, 2001).

Clearly, while we need to identify the interactions within the parasite community we also need to identify the "direction" of these interactions (antagonistic or synergistic) and if such interactions are host mediated. For example, while *H. polygyrus* infections cause an acquired immunoresponse of the mice against larval stages, adult stages generate an immunosuppression response (Monroy and Enriquez, 1992). *H. polygyrus* has a chronic infection strategy that revolves around changing the intestinal environment through the production of immunosuppressive factors that interfere with the T-cell and cytokine-mediated inflammatory response (Monroy and Enriquez, 1992). As such, the activation of different molecular components is strongly affected by the type and intensity of infection. These changes may also influence the infection of other parasites as has been observed for *T. spiralis* and *T. muris* (Behnke *et al.,* 2001).

Thesis aims and structure

The aim of this thesis was to examine parasite dynamics and the mechanisms affecting parasite load and transmission. Particular attention has been given to examining the role played by host characteristics and parasite species interactions in modulating parasite intensity and the rate of parasite transmission.

This general aim has been approached by examining a natural population of yellow-necked mice and their community of macroparasites. Seven yellow-necked mouse populations have been monitored in time and space, coupled with field experiments where parasite intensities were manipulated in order to investigate the causes and consequences of parasite transmission. The nematode *H. polygyrus* has been the focus of this investigation as it is a well known and a common gastrointestinal parasite of *A. flavicollis* in Alpine woodlands.

This thesis is presented as a series of individual chapters covering different aspects of the ecology of *H. polygyrus* in *A. flavicollis* populations in the North-Eastern Alps.

The first chapter is a general introduction on host-parasite interactions and a review of the factors that affect the distribution of macroparasites within rodent populations.

Chapter 2 describes the parasite community in *A. flavicollis* populations in the Trentino province and investigates whether the parasite community of six sampled mouse populations show a similar pattern, and which factors mainly contribute in shaping such structure. While the overall community of parasite was composed of eight species, dominated by *H. polygyrus*, the community structure varied significantly among the host populations at each of the six sample sites. The factors explaining the majority of the pattern observed were host abundance and host age, while habitat composition and parasite interactions had marginal roles.

Chapter 3 investigates the effect of *H. polygyrus* on tick (*Ixodes ricinus*) infestations in mice populations. The relationship between *H. polygyrus* intensity and *I. ricinus* infestation was investigated analysing both extensive field data from six mouse populations (Chap. 2) and through an intensive field experiment where *H. polygyrus* burden was artificially manipulated. Both field observations and experimental manipulations provided evidence that tick infestation was negatively related to *H. polygyrus* intensity. In particular, the analysis of the field observation data revealed a different tick-*H. polygyrus* response in individuals of different age classes and breeding status suggesting a possible role of host immunity.

In Chapter 4 the role of sex in affecting parasite transmission rates was investigated through selective manipulation of *A. flavicollis* populations. In this case, the parasite *H. polygyrus* was removed either from males or females and their contribution to the maintenance and transmission of the parasite in the host population was evaluated. The result highlighted that the removal of *H. polygyrus* from female mice did not affected the rate of males' infection, while the removal of *H. polygyrus* in males caused a decrease of parasite infection in females. This result suggests that host sex is important in affecting parasite transmission rate.

Chapter 5 investigates the role male mice play in driving *H. polygyrus* infection, using a modelling approach. Deterministic mathematical models were applied assuming that the host population is divided into males and females and that the macroparasite has a directly transmitted life-cycle. This approach was used to explore the two biological hypotheses proposed in chapter 4. Results indicate that in order to simulate the patterns observed in the field we have to assume a different spatial arrangement of the free-living infective larvae and a different contribution of host sexes.

Finally, Chapter 6 is an overview discussion of the whole study and how it is related to previous studies. A final conclusion combines the results of the previous chapters suggesting directions for further research.

Project details

This thesis is part of an international project carried out as collaboration between the School of Biological and Environmental Sciences of University of Stirling (Prof. Peter Hudson initially at the Department of Biological Sciences) and the Centro di Ecologia Alpina (CEA) (Dr Annapaola Rizzoli). This scientific research was part of a broader project on the whole community of parasite in mice populations called Ecology of Wildlife Disease project (ECODIS).

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Glossary

In order to avoid misinterpretation generated by different definitions that parasitologists and ecologists may give to words used in this thesis, some important definitions are used as reported below.

Epidemiological terms:

- Abundance. The term abundance is considered according to Bush *et al.* (1997): "the number of particular parasites in/on a single host, regardless of whether or not the host is infected".
- Mean abundance. Mean abundance is defined as: "the total number of particular parasites in a sample of a particular host species divided by the total number of hosts of that species examined (i.e. infected and uninfected host)" (Bush *et al.*, 1997).
- Intensity. The definition is: "the number of individuals of a particular parasite species in a single infected host" (Bush *et al.*, 1997). Intensity differs from abundance because it is relative to only the infected hosts.
- Mean Intensity. Defined as: "the average intensity of a particular species of parasite among the infected members of a particular host species" (Bush *et al.*, 1997).
- Prevalence. Defined as: " the number of hosts infected with one or more individuals of a particular parasite species divided by the number of hosts examined for that parasite species" (Bush *et al.*, 1997).
- Trickle infection. Infection of a host at regular intervals, with a defined dose of infective parasite stages.

Ecology of communities:

- Infrapopulation: According to Bush *et al.* (1997) a parasite infrapopulation is composed of all individuals of a species in an individual host at a particular time.

- Infracommunity. Defined as all the members of all parasite species within a single individual host. The infracommunity is the sum of the infrapopulations of all different parasite species within a single individual host (Bush *et al.*, 1997). The infracommunity richness is thus the number of species a single host harbours.
- Component community. The component community refers to all infrapopulations and infracommunities of parasite species within a host population (Bush *et al.*, 1997). The component community richness is the total number of parasite species recorded in a defined host population.
- Infection and Infestation. Along this thesis the term infection is used to describe the establishment of a physical interaction between a parasite and its host. For macroparasites infection refers to endoparasites. The term infestation refers to a similar process but it is used specifically for ticks, lice, mites and all the ectoparasites.

Apodemus flavicollis classes:

- Adult: yellow-necked mouse with pelage moulted in brown fur and body mass over 15 g. (Barbetta, 2003).
- Juvenile: yellow-necked mouse with grey pelage not yet moulted in brown fur and body mass below 15 g. (Barbetta, 2003).
- Breeding mouse: Individual in sexually reproductive condition which sex organs changed in enlarged testicles, in male, and enlarged abdomen, perforated vagina or copulatory plug in female (Gurnell and Flowerdew, 1990).
- Non-breeding mouse: Individual in sexually non-reproductive conditions that sex organs does not show any physiological change (Gurnell and Flowerdew, 1990).

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Chapter 2

*Community structure of macroparasites in populations of **Apodemus flavicollis**, Trentino, Italy.*

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Abstract

The relative contribution of extrinsic and intrinsic factors on the parasite community structure of the yellow-necked mouse (*Apodemus flavicollis*) was examined in six natural mice populations in the Trentino region (North-Eastern Italian Alps).

A total of 230 mice were sampled in replicated sites in July 2002 and for each individual trapped sex, age and breeding status were recorded. Parasites species were identified and abundance recorded for each individual. The parasites were examined as a whole sample and for each site at three hierarchical levels: component community, infracommunity and single parasite species. Mice abundance, but not sex ratio and host status, was consistently different between sites. Parasite community structure also differed significantly between sampling sites and between individual hosts. *Heligmosomoides polygyrus* was the most common parasite species. Parasite community structure was strongly affected by individual hosts and population characteristics, in particular mouse abundance and age structure were the most significant variables.

Introduction

Wild animal populations are susceptible to a wide range of parasites species and often individual hosts harbor an entire community of parasites, referred to as the infra-community (Petney and Andrew, 1998). Nevertheless, these communities are not stable entities but exhibit variability in their composition of species and intensity of infection in relation to host status and density. There are some interesting differences in the relative importance of hosts (age, sex, immune response, hormones, nutrient status, genetics), extrinsic factors (seasonality, habitat) (Lo *et al.*, 1998; Poulin and Morand, 1999; Sharpilo *et al.*, 2001; Arneberg, 2002; Vidal-Martìnez and Poulin, 2003), and the effect of between-parasite interactions (Behnke *et al.,* 2001a; Poulin 2001), in shaping parasite

community structure. For instance, it has been suggested that the number and intensity of parasite species increase with host density and host body mass (Arneberg, 2002), and that host species with widespread geographical distribution tend to harbor more parasites than the hosts with more restricted geographical ranges (Gregory, 1990).

Studies of macroparasite communities in wild populations of rodents have revealed several interesting patterns. Investigations of *Apodemus* spp. have shown great variability in parasite communities across its range of distribution, with clear differences in parasite species number and infection rate between populations from Central–Northern and Southern Europe (Montgomery and Montgomery, 1989; 1990; Behnke *et al.,* 1999; Abu-Madi *et al.,* 2000; de Bellocq *et al.,* 2003; Milazzo *et al.,* 2005). Interestingly, these studies also suggest that there is high variation in the parasite community between mice populations even at the local scale. These observations indicate that variability in the parasite community may be caused merely by spatial variations in habitat and season, with a marginal role of host characteristics. However, once the spatial effects are taken into account and the parasite community is investigated within a single host population level, then host factors appear to play a key role in shaping parasite distribution and parasite intensity (Haukisalmi and Henttonen, 1999).

Heligmosomoides polygyrus is one of the most common parasites infecting *Apodemus* spp. and interestingly has an important role in influencing the intensity of other parasite infections in laboratory conditions (Behnke *et al.,* 2001a), suggesting that it may play a substantial role in shaping *Apodemus* spp. parasite community structure.

The community of gastrointestinal parasites of yellow-necked mouse (*A. flavicollis*) populations was investigated in six distinct sampling sites in the North-Eastern Italian Alps. Several extrinsic (environmental characteristics) and intrinsic (host components) factors were selected and their relative effects on parasite community structure examined,

in order to answer to the following questions: Is the parasite community structure uniform among the six populations? What are the main factors that shape the parasite community structure?

Methods

Data collection: yellow-necked mouse and macroparasite parasite communities

Data on the community of gastrointestinal parasites in *A. flavicollis* were obtained through extensive trapping of mouse populations in Trentino (the North-Eastern Italian Alps) during July 2002 (Fig. 1). Mice were sampled in broadleaf woodlands, composed mainly of mature beech (*Fagus sylvatica*), with patchy distribution of Scots pine (*Pinus sylvestris*) and spruce (*Picea abies*). Sites were located at an average altitude of 850 m a.s.l. (± 22 m S.E.). Habitat composition was assessed qualitatively by recording the presence and co-occurrence of tree species (Tab. 1).

Individual mice were trapped using multi-capture live traps (Ugglan type 2, Graham Sweden) set for three nights (for a total of 3456 trap nights) in six study sites, each site representing three trapping grids of 64 traps each. The traps were arranged following the small quadrat sampling technique, and the relative abundance of individuals sampled in each of the six sites were estimated accordingly (Myllymäki *et al.,* 1971; Henttonen *et al.,* 1987). The traps were baited with a standardised amount of seeds (a mix of maize, oat, wheat, rice, millet, linseed, rape, vetch, by Zanandrea Sementi producer) and a piece of potato and hay was provided for food and bedding.

Each individual trapped was weighed. When the fur was brown in colour, corresponding to individuals with a body mass exceeding 15 g. (Barbetta, 2003, Chapter 1), the mouse was classified as an adult and euthanasied for post mortem examination of the parasite community (Italian and UK ethical rules applied). The parasites from the

entire alimentary tract were collected and stored. Helminth species were identified and counted following recognised techniques (Durette-Desset, 1968; Quantin, 1971; Mas-Coma and Montoliu, 1978; Meszaros and Murai, 1979; Genov, 1984). Details on host sex and condition of the sexual organs -as indicators of the breeding status- were also recorded. The mass of the eye lens was recorded and used as a relative measure of mouse age (Morris, 1972; Gurnell and Flowerdew, 1990; Gregory *et al*., 1992).

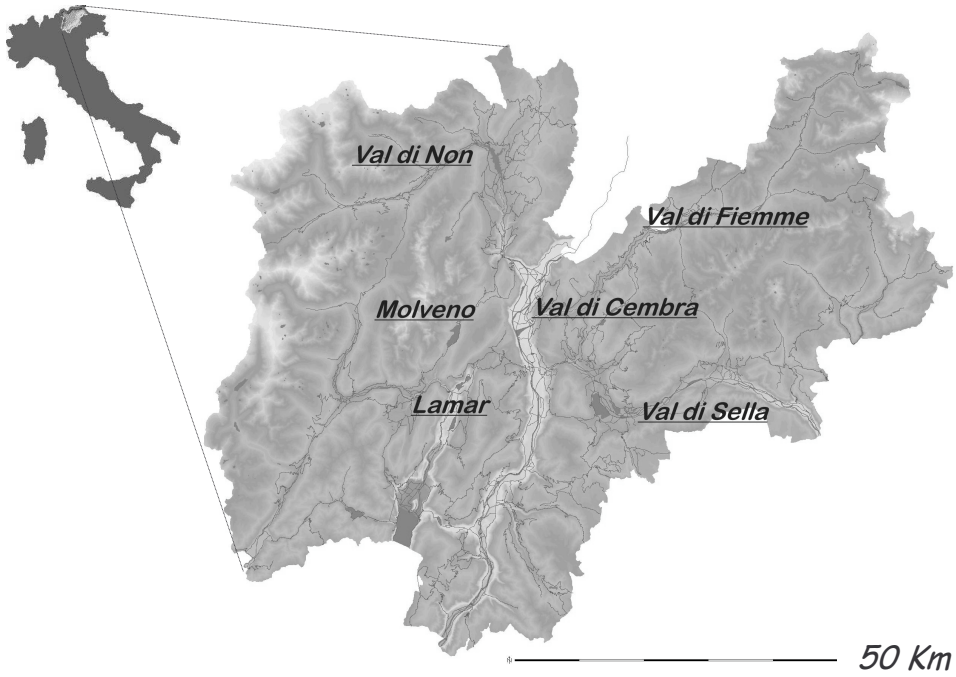

Figure 1 Map of region with the name of the trapping sites indicated

Table 1. Habitat composition and numbers of *A. flavicollis* sampled by study site.

	Cembra	**Lamar**	**Molveno**	**Non**	**Fiemme**	**Sella**
Vegetation	Beech wood with Scots Pine presence Spruce presence	Beech wood with Scots Pine presence Spruce presence	Beech wood with Scots Pine presence	Pure beech wood	Scots Pine and Spruce wood	Pure beech wood
Number of Mice sampled	110	41	32	25	11	11
Males	57	23	21	11	8	5
Females	53	18	11	14	3	6
Breeding	11	25	19	8	7	9
Non-breeding	97	11	13	17	4	2

Parasite community structure

The community of macroparasites was examined at two hierarchical levels: the component community (i.e. all parasites associated with a host population) and the infra-community (i.e. all parasites associated with a single host). The analyses were carried out using the combined data set from the six sites and then repeated within each study site.

Species richness was examined at both hierarchical levels, and was estimated as the total number of parasite species isolated in the mouse populations, or as the average number of parasite species in an individual host (Kennedy and Hartvigsen, 2000). To investigate the community structure, at the population level, the Thul Importance Index (TII, Thul *et al.*, 1985) was computed. This index weights simultaneously the prevalence and abundance of parasites with respect to the whole community, and classifies the parasite species as either dominant (TII $\geq$ 1), co-dominant ($0.01 \leq TII \leq 1$) or subordinate ($0 \leq TII \leq 0.01$). The total number of parasites was estimated within each individual following Kennedy and Hartvigsen (2000). Prevalence and mean abundance of infection of each parasite species was examined within each population (Bush *et al.,* 1997).

To identify if host characteristics (sex, breeding status, age, abundance) and habitat composition (pure mature beech, mature beech with Scot pine and mature beech with spruce wood) were affecting parasite community structure, Generalised Linear Models (GLMs) (or Generalised Linear Mixed Model GLMMs when necessary) with stepwise backward removal were performed using either species richness, total parasite number, prevalence or mean abundance as a response variable and host characteristics or habitat composition with their second order interactions, as independent variables (McCullagh and Nelder, 1983; Crawley, 2000). According to the distribution of the response variables the following error structure and link functions were used: for species richness either Poisson or quasi error structure, and log or identity link functions were selected. Because

of the aggregated distribution of parasites the response variable, total parasite number and mean abundance, were analysed through the modified negative binomial GLM, which provides an estimate of the aggregation parameter, *k*, and log-ratio link function (McCullagh and Nelder, 1983). Prevalence was analysed classifying each mice either positive or negative to the infection of parasite, so logistic regression with binomial error structure and logit link function was used. The remaining variables were modelled as follows: binomial distribution for sex and breeding status, discrete variable for habitat composition and continuous variable for eye lens mass (i.e. mouse age) and mice abundance. The analyses were performed at the population (sum of three grids at each site) and individual level, when possible.

To investigate associations between parasite species, pairwise Spearman correlation tests were performed between parasite species intensities within hosts. To assess the saturation of infracommunities the total number of macroparasites was examined using regression on the number of species per individual. The regression that explained most of the variation was selected among linear, power function and local regression through non parametric smoothing (Loess), and inspected if total number of macroparasites showed a convexity along with number of species per individual. All analyses were conducted with GenStat 6.2.

Results

Mouse populations

The number of mice trapped is significantly different between the six study sites (total *A. flavicollis:* 230, $\chi_5^2 = 178.8$, $P < 0.0001$) (Tab. 1). However, despite this spatial difference in the density of mice, no differences are found between sites on sex (GLM, family =binomial; link = logit: $\chi_5^2 = 5$, $P = 0.41$) eye lens mass (GLM, family=Gaussian,

link=identity; F_5 = 201.17, P = 0.148) and breeding status (GLM, family =binomial; link = logit; χ_5^2 = 3.46, P = 0.628), such that the general structure of the host populations is similar (Fig 2).

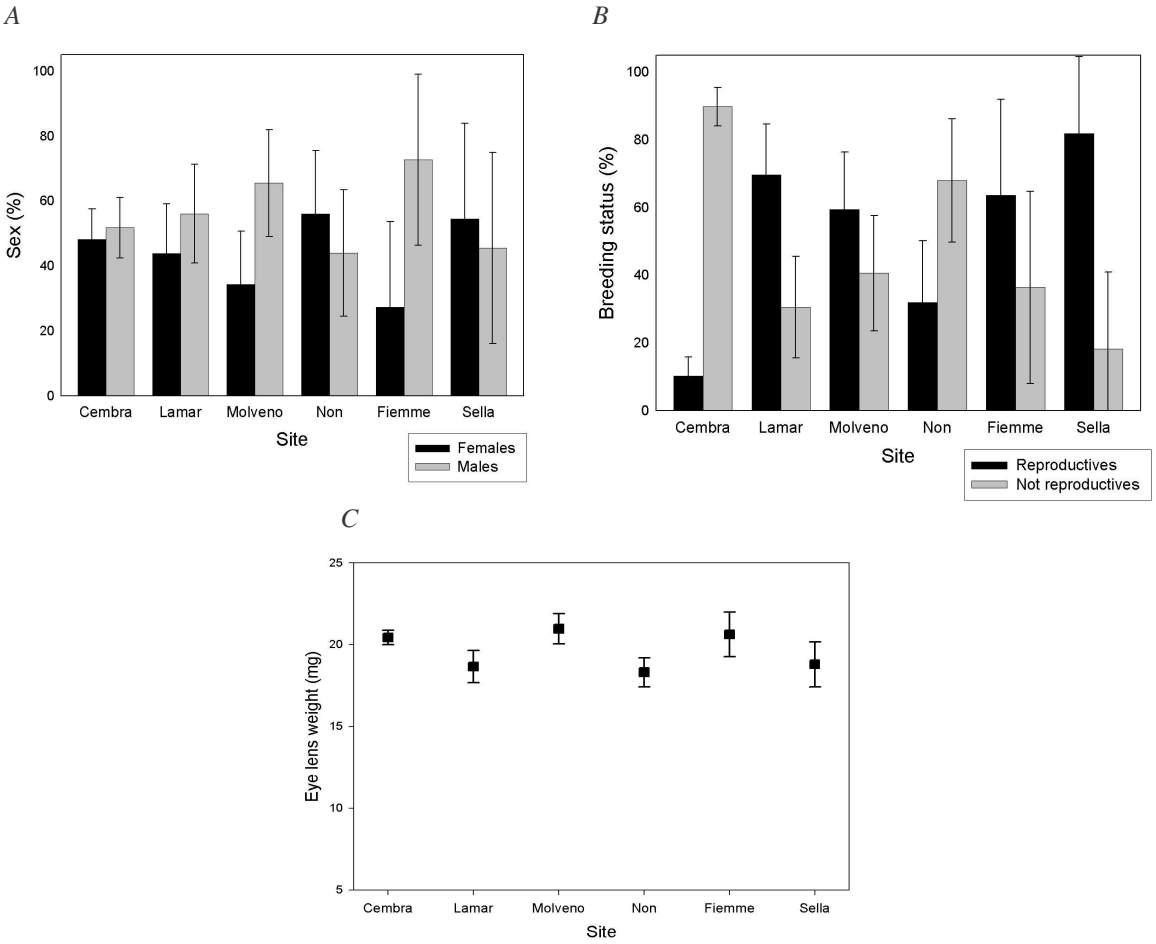

Figure 2. Differences in mouse characteristics by sites. A) Percentages of male and female mice. B) Percentage of breeding and non-breeding mice. C) Mean dry lens mass.

Component community structure: population level

Eight parasite species are identified: six nematodes *H. polygyrus*, *Syphacia frederici*, *S. stroma*, *Trichuris muris*, *Rictularia proni* and *Aonchotheca murissylvatici*, one cestode *Hymenolepis fraterna* and one trematode *Corrigia vitta*. The parasites *A. murissylvatici H. polygyrus*, *R. proni* and *H. fraterna* are isolated from the small intestine *C. vitta* in the intestine portion adjacent to the pancreas, while *S. frederici*, *S. stroma* and *T. muris* (cieca) are found in the large intestine (Fig. 3).

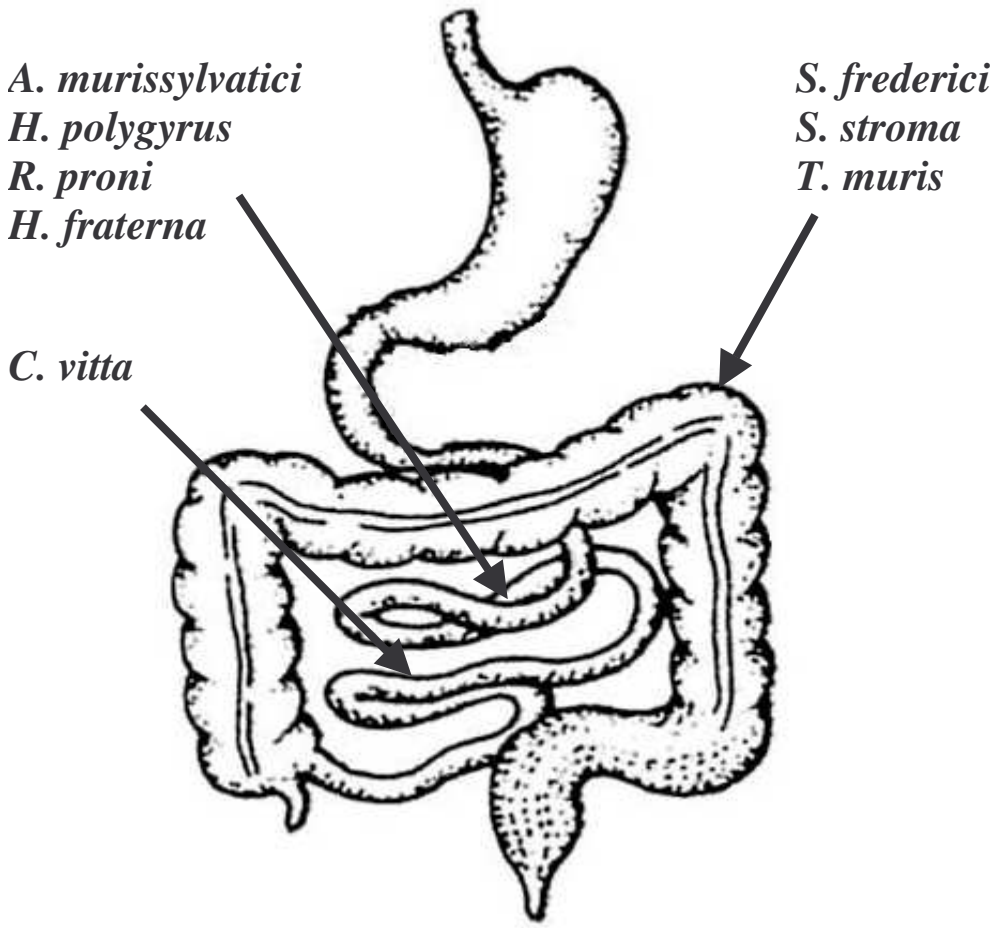

Figure 3. Location of parasite infections, identified in *A. flavicollis*.

The parasite species richness in the six mice populations, from the six study sites, ranges between six and three, and it is positively associated with mouse abundance (GLM, family=quasi, link=identity $F_1 = 3.86$, $P = 0.03$) but not habitat composition. In general, the dominant parasite species are, from the highest to the lowest, *H. polygyrus*, *H. fraterna* and *S. frederici* (Thul index ≥ 1). The parasites *C. vitta*, *T. muris*, *S. stroma* and *R. proni* are mainly present as co-dominat (0.01≤Thul Index≤1) while A. *murissylvatici* is subordinate to the other parasites (0≤Thul Index≤0.01). This pattern is consistent when the analysis is repeated for each study site separately with the exceptions

of *C. vitta* in Lamar and *A. murissylvatici* in Fiemme, where these parasites are dominant (Tab. 2).

Infracommunity structure: individual level

87.4 % of the mice are infected by at least one parasite, and 56.7 % of these individuals harbour multiple parasite species infections (Fig. 4). On average mice harbour 1.6 (±0.06 S.E.) parasite species/mouse.

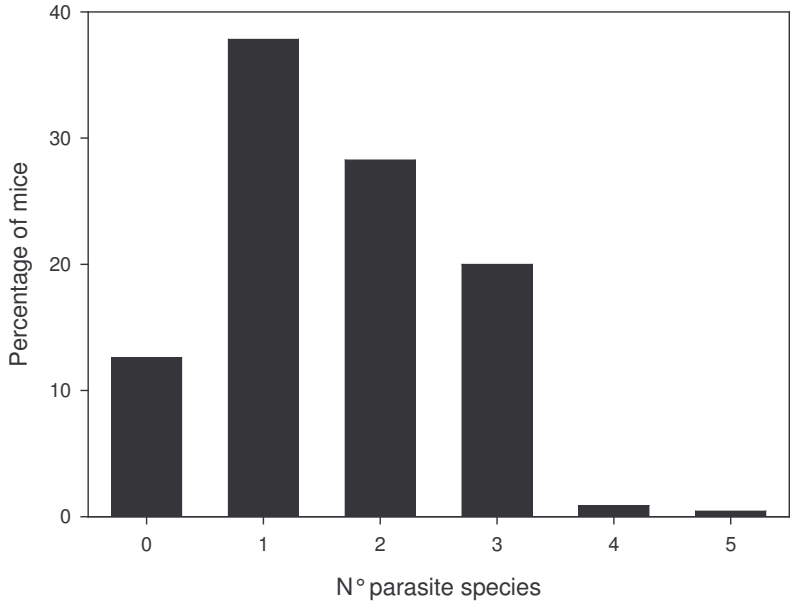

Figure 4. Frequency distribution of infracommunity species richness for the total mice sample.

The mean parasite species richness per mouse differs between study sites (GLM family=Poisson, link=log, $\chi_5^2 = 18.94$, $P = 0.002$) (Tab. 2). A more detailed analysis at the individual level reveals that mean parasite species richness is positively influenced by host age and habitat composition (GLMM, family=Poisson, link=log, random term=sampling site, age effect:$\chi_1^2 = 9.27$, P=0.002, habitat effect: $\chi_2^2 = 9.97$, P=0.007). The geometric mean of total number of macroparasites is 7.07 (±0.54 S.E.)

parasite/mouse and is predominantly due to infection with the three dominant species, *H. polygyrus* (60.7 %), *S. frederici* (22.6%), *H. fraterna* (13.4 %). The total number of parasites per mouse differs significantly among study sites (GLM, family=negative binomial, link=log, Total number of parasite by site, $\chi_5^2 = 36.62$, $P < 0.001$) (Tab. 2) reflecting the spatial difference of abundance of the three dominant species.

While no significant pair-wise correlations are observed between the intensities of the dominant parasite species, the total number of parasites increases progressively with infracommunity species richness (GLMM-IRREML, family=negative binomial, link=log, Total number of parasite by Number of species per mouse $\chi_2^2 = 64.76$, $P < 0.001$). The best fitting line is a second order power function regression (r^2=0.25) which shows a weak tendency to asymptote (Fig. 5).

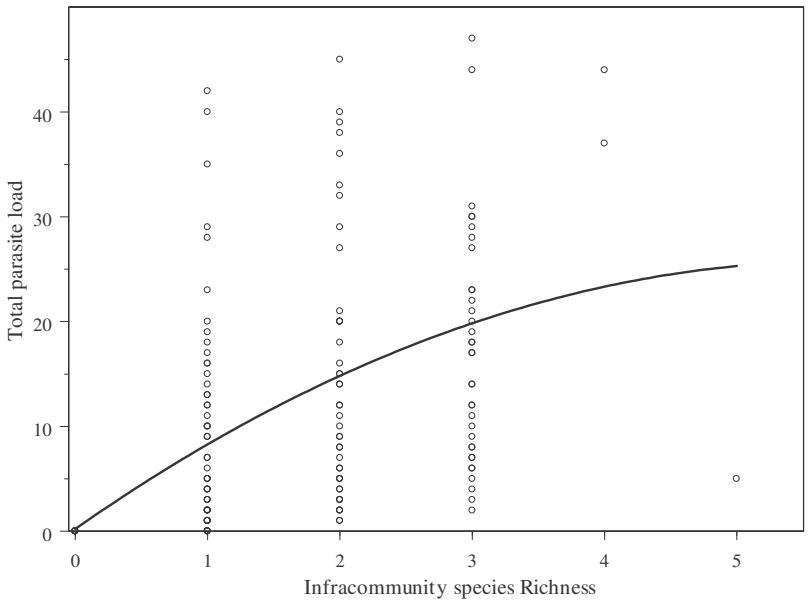

Figure 5. Relationship between total parasite load and infracommunity species richness as described by the best fitting regression ($y=0.22+8.82x- 0.76x^2$).

Table 2 Measure of Component and Infracommunity structure for total population and by population in each of the six study sites (in bold dominant species, in italic codominant and regular subordinate species)

Species	TOT	Cembra	Lamar	Molveno	Non	Fiemme	Sella
Mice sampled	230	110	41	32	25	11	11
Component Community							
Species Thul Importance Index*							
H. polygyrus	**79.243**	**88.791**	**15.356**	**28.755**	**83.569**	**74.258**	**71.428**
S. frederici	**1.027**	**7.603**	**56.291**	**15.834**	**15.016**	**24.114**	**7.407**
H. fraterna	**8.924**	**3.512**	**25.886**	**54.634**	**1.305**		**21.164**
C. vitta	*0.068*		**2.362**	*0.397*	*0.108*		
T. muris	*0.033*	0.004	*0.102*	*0.378*		*0.095*	
S. stroma	*0.023*	*0.065*					
R. proni	*0.015*	*0.026*					
A. murissylvatici	0.004					**1.531**	
Total n° of helminth species identified	8	6	5	5	4	4	3
Infracommunity							
Mean species richness (± S.E.)	1.62 (±0.06)	1.82 (±0.08)	1.24 (±0.17)	2.01 (±0.16)	0.96 (±0.17)	1.27 (±0.23)	1.27 (±0.19)
Mean total parasite load[§] (± S.E.)	7.07 (±0.54)	10.81 (±1.04)	4.40 (±0.77)	7.72 (±1.39)	2.78 (±0.64)	8.16 (±3.68)	3.32 (±1.00)

* Species are classified dominant when Thul index ≥ 1 (in bold), as codominat when 0.01≤Thul Index≤1 (in italic) and subordinate when 0≤Thul Index≤0.01.

[§] ***Geometric mean***

Parasite species

Prevalence

Prevalence of *H. polygyrus* differs significantly between sites (GLMM, family=binomial, link=logit; $\chi_1^2 = 11.73$, $P < 0.001$; Appendix I).

In general, the factors that shows to affect significantly the majority of parasite species infections are host age, host abundance, followed by host sex, habitat composition and breeding status. For *H. polygyrus* and *H. fraterna,* infection is positively related to host age, while for *S. frederici* infection increases with host abundance and habitat composition. Infection of *C. vitta* increases with host age, in male hosts, but not in breeding individuals or in wood with presence of Scot's pine. Infection of *T. muris* increases with host age, while decreases with host abundance and in males. The results for each parasite species are summarised in Appendix I. Due to low prevalence, *S. stroma*, *A. murissylvatici* and *R. proni* are not analysed.

Table 3. Prevalence (% infected ± S.E.) of helminth for the total population and by population in each of the six study sites.

Species	TOT	Cembra	Lamar	Molveno	Non	Fiemme	Sella
H. polygyrus	66.1 (±0.03)	85.4 (±0.03)	31.7 (±0.07)	62.5 (±0.08)	48.0 (±0.09)	72.7 (±0.01)	45.4 (±0.15)
S. frederici	32.6 (±0.03)	38.2 (±0.04)	39.0 (±0.07)	28.1 (±0.07)	12.0 (±0.06)	36.4 (±0.14)	9.1 (±0.08)
H. fraterna	34.8 (±0.03)	33.6 (±0.04)	39.0 (±0.07)	59.4 (±0.08)	12.0 (±0.06)		45.4 (±0.15)
C. vitta	3.5 (±0.01)		9.7 (±0.04)	9.4 (±0.05)	4.0 (±0.03)		
T. muris	3.9 (±0.01)	1.8 (±0.01)	4.9 (±0.03)	12.5 (±0.05)		9.1 (±0.08)	
S. stroma	1.7 (±0.008)	3.6 (±0.01)					
R. proni	1.7 (±0.008)	3.4 (±0.01)					
A. murissylvatici	0.4 (±0.004)					9.1 (±0.08)	

Abundance of infection

The distribution of each parasite species is aggregated in the entire mouse population (Tab. 4). Mean parasite abundance is significantly different between sites for *H. polygyrus* and *H. fraterna* (GLMM, family=negative binomial, link=log, $\chi_1^2 = 63.17$, $P < 0.001$, $\chi_1^2 = 16.1$, $P < 0.001$, respectively; Appendix II). Host age and habitat composition are the most common variable to affect mean abundance of parasite species, host abundance, sex and breeding status are also important (Appendix II). Host age has a negative effect on *H. polygyrus* and *H. fraterna* and positive on *C. vitta* and *T. muris.* Mouse abundance shows a significant effect on *T. muris* and *C. vitta* while in *H. polygyrus* and in *H. fraterna,* mouse abundance interacts with sex, age and breeding status (i.e. the nematode decreases in abundance in non-breeding, older males) for the first, and for the latter with habitat composition (i.e. the nematode increases in abundance in pure beech woods and when spruce is presence). Host sex influences *H. polygyrus*, with a bias towards males, and *T. muris,* towards females. Finally habitat composition influences the abundance of all species directly and interacting with other factors. The detailed results are summarised in Appendix II.

Table 4. Parameter values for species aggregation* and mean parasite abundance[§] for the total population and by population in each of the six study sites.

Species	*k**	TOT[§]	Cembra[§]	Lamar[§]	Molveno[§]	Non[§]	Fiemme[§]	Sella[§]
H. polygyrus	0.359	7.06 (±0.81)	11.95 (±1.48)	1.12 (±0.57)	2.38 (±0.64)	2.56 (±0.89)	8.82 (±3.26)	2.45 (±1.70)
S. frederici	0.135	2.63 (±0.39)	2.29 (±0.43)	3.34 (±0.94)	2.91 (±1.41)	1.84 (±1.30)	5.73 (±2.95)	1.27 (±1.27)
H. fraterna	0.199	1.56 (±0.21)	1.21 (±0.22)	1.54 (±0.39)	4.75 (±1.07)	0.16 (±0.09)		0.73 (±0.30)
C. vitta	0.015	0.13 (±0.06)		0.56 (±0.28)	0.22 (±0.16)	0.04 (±0.04)		
T. muris	0.116	0.05 (±0.02)	0.03 (±0.02)	0.05 (±0.03)	0.16 (±0.08)		0.09 (±0.09)	
S. stroma	0.006	0.09 (±0.05)	0.19 (±0.11)					
R. proni	0.012	0.04 (±0.02)	0.08 (±0.05)					
A. murissylvatici	0.005	0.07 (±0.07)					1.45 (±1.45)	

* *k* (negative binomial exponent) calculated using maxim likelihood method of the whole sample

[§] Mean abundance and S.E. estimated according Bush *et al.* 1997

Discussion

The study of the gastrointestinal parasites of the yellow-necked mouse in Trentino region showed (North-Eastern Italian Alps) a community composed of eight parasite species. The parasite community structure varied significantly among the host populations of the six sample sites. Parasite distribution was mainly affected by host abundance and host age while the effect of habitat composition and parasite interactions was less apparent. The differences found between the six sampled populations were consistent at each level investigated namely: parasite component community, infracommunity, and prevalence and mean abundance of single parasite species.

Among the eight parasite species isolated *H. polygyrus*, *S. frederici* and *H. fraterna* respectively, were the most common and can be considered as core species (*sensu* Hanski, 1982) since they occurred with high intensity in the majority of the sites. However, *H. polygyrus* quantitatively prevailed over the other two species confirming the predominant role of Heligmosomidae in the European muridae (Haukisalmi *et al.,* 1988; Montgomery and Montgomery, 1990; Abu-Madi *et al.,* 1998; Behnke 1999; Abu-Madi *et al.,* 2000; Behnke *et al.,* 2001b; Barnard *et al.,* 2003).

These results on the spatial difference of the parasite community in populations of *A. flavicollis,* in the North-Eastern Italian Alps, are in line with the findings from other *Apodemus* spp. populations from Europe (Montgomery and Montgomery, 1989; 1990; Abu-Madi *et al.,* 2000; de Bellocq *et al.,* 2002; 2003). The positive effect of mice abundance on community species richness and parasite prevalence and abundance suggests a density dependent effect of host abundance on parasite transmission such that higher host densities allow the establishment of those parasite species that require higher transmission rates for the maintenance of a viable parasite population (Arneberg, 2002),

increasing the probability that a host encounters an infective stage (Anderson and May 1978; Arneberg *et al.,* 1998).

Host characteristics had a strong influence on parasite community within populations. In particular, parasite species distribution was strongly affected by host age and secondly by sex and host breeding status. These findings are consistent with previous studies on wood mouse in Northern Europe (Gregory 1992; Gregory *et al.,* 1992; Abu Madi *et al.,* 1998; Behnke *et al.,* 1999). The age-intensity profiles for *H. polygyrus* and *H. fraterna* exhibited a pattern where parasite intensity declined in older age individuals. This pattern may have been generated by parasite-induced mortality or acquired immunity or even age-related changes in susceptibility to parasite infections (Wilson *et al*., 2002). Unfortunately, no information was available to disentangle between these possible causes. Previous studies on *H. polygyrus* have identified both host mortality effects in inbred mice (Scott, 1990), and the development of immunity in laboratory mice (Monroy and Enriquez, 1992). While further analyses are needed to identify the parsimonious mechanism taking place in these alpine mice populations, previous investigations ascribed the *H. polygyrus* convex age-intensity profile to acquired immunity, as was also observed in wild mice population even in the absence of parasite-induced host mortality (Gregory *et al.,* 1990; 1992).

Despite the spatial variability observed, host characteristics have been thought to play a fundamental role in shaping parasite species distribution (Wilson *et al.,* 2002) and parasite community structure. An investigation of wild populations of bank vole, *Clethrionomys glareolus,* found that the aggregated parasite distribution observed was more a consequence of differences in host exposure/susceptibility rather than large scale spatial variability (Haukisalmi and Hentonnen, 1999). Moreover, differences among individuals of a host population play an important role in the dynamics of parasite

infections, in fact macroparasites usually exhibit an aggregated distribution within the host population and often a limited proportion of the whole host population is responsible for most of the parasite transmission (Woolhouse *et al.,* 1997; Ferrari *et al.,* 2004 i.e. Chapter 4). Therefore the identification of host differences in parasite load is crucial for understating the dynamics of parasite infection within these mouse populations.

These *A. flavicollis* populations showed that the parasite species had low intensities, low number of co-infections with limited niche overlap, low correlation between species intensities, and with a limited saturation of the infra-community intensities. However, the complex characteristics of parasite interactions (Lello *et al.*, 2004) suggest that additional investigations are required for identifying any possible parasite interaction (Poulin, 2001). In this respect, previous studies on other microtine species in wild populations have suggested that parasite species associations are a limited phenomenon in natural populations, often occurring on a particular season and site (Montgomery and Montgomery, 1990; Haukisalmi and Henttonen, 1993;Behnke *et al.*, 2005).

This study was based on mice populations sampled during a single trapping month. While three replicates for each population have been used these data still represent a restricted period of observation on the parasite community in mice. As such, it does not provide information on temporal changes in mice populations and parasite distribution that usually occurs in wild population (Haukisalmi *et al.,* 1988; Montgomery and Montgomery, 1989). Therefore, these conclusions could not be generalised to other parts of the year. Moreover, it must be stressed that component community species richness could be affected by sample size (Walther *et al.,* 1995; Arnebeg, 2002) and while in the analyses population abundance was standardised by the trapping effort and area, some of our populations exhibited low host numbers which may have affected the final conclusions.

In conclusion, the analysis of gastrointestinal parasite of *A. flavicollis* in the North-Eastern Italian Alps confirmed the high spatial differences between different rodent populations, suggesting that the parasite community of each host population can be considered unique. Moreover, host characteristics occurred to be a key component in affecting parasite community structure within each mice population and highlighted their role in influencing parasites dynamics.

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Appendix I

Significant factors affecting the prevalence of parasites of the gut helminths of the *A. flavicollis*. Generalized Linear Mixed model used with binomial family and logit link.

a) *H. polygyrus* prevalence. Model Type: GLMM (Random Terms = Site $\chi_1^2 = 11.73$, $P < 0.001$)

Model Term	Effect	S.E.	Wald Statistic (χ^2)	d.f.	*P*
Host sex			0.80	1	0.371
Female	0	0			
Male	-0.03	0.009			
Host Age	0.07	0.036	3.97	1	0.046
Host Abundance	0.03	0.010	3.09	1	0.079
Sex * Host Abundance			10.38	1	0.001
Female	0	0			
Male	-0.03	0.009			

b) *S. frederici* prevalence. Model Type: GLMM (Random Terms = Site $\chi_1^2 = 2.73$, $P = 0.098$)

Model Term	Effect	S.E.	Wald Statistic (χ^2)	d.f.	*P*
Host Abundance	0.01	0.007	4.07	1	0.044
Habitat composition			10.49	2	0.005
Pure beech wood	1.52	0.521			
Scot's Pine presence	0	0			
Spruce presence	1.21	0.443			

c) *H. fraterna* prevalence. Model Type: GLMM (Random Terms = Site $\chi_1^2 = 1$, $P = 0.228$)

Model Term	Effect	S.E.	Wald Statistic (χ^2)	d.f.	*P*
Habitat composition			3.80	2	0.150
Pure beech wood	0.61	0.619			
Scot's Pine presence	0	0			
Spruce presence	-0.12	0.643			
Host abundance	-0.04	0.017	0.26	1	0.607
Host age	0.16	0.038	13.00	1	<0.001
Habitat composition * Host abundance			13.50	2	0.001
Pure beech wood	0.03	0.016			
Scot's Pine presence	0	0			
Spruce presence	0.06	0.016			

d) *C. vitta* prevalence. Model Type: GLMM (Random Terms = Site $\chi_1^2 = 1.78$, $P = 0.182$)

Model Term	Effect	S.E.	Wald Statistic (χ^2)	d.f.	*P*
Habitat composition			**10.33**	**2**	0.006
Pure beech wood	-5.77	2.057			
Scot's Pine presence	0	0			
Spruce presence	-1.66	2.862			
Host sex			6.9	1	0.009
Female	0	0			
Male	7.21	2.743			
Breeding status			8.15	1	0.004
Non-breeding	8.22	2.873			
Breeding	0	0			
Host abundance	0.71	0.295	5.96	1	0.015
Host age	0.46	0.106	19.47	1	<0.001
Host sex* Host Age			9.32	1	0.002
Female	0	0			
Male	2.19	0.718			

e) *T. muris* prevalence. Model Type: GLMM (Random Terms = Site $\chi_1^2 = 0.5$, $P < 0.469$)

Model Term	Effect	S.E.	Wald Statistic (χ^2)	d.f.	*P*
Host age	0.38	0.083	21.18	1	<0.001
Host sex			9.85	1	0.002
Female	0	0			
Male	-1.94	0.617			
Host abundance	-0.02	0.007	7.47	1	0.006

Appendix II

Significant factors affecting the Abundance of parasites of the gut helminths of the *A. flavicollis* Linear Mixed model used with negative binomial family and log link.

a) *H. polygyrus* abundance. Model Type: GLMM (Random Terms = Site $\chi_1^2 = 63.17$, $P < 0.001$)

Model Term	Effect	S.E.	Wald Statistic (χ^2)	d.f.	*P*
Host sex			4.45	1	0.035
Female	0	0			
Male	0.51	0.241			
Breeding status			0.01	1	0.920
Non-breeding	-0.01	0.366			
Breeding	0	0			
Habitat composition			6.43	2	0.040
Pure beech wood	-0.04	0.367			
Scot's Pine presence	0	0			
Spruce presence	0.69	0.290			
Host age	-0.02	0.042	9.03	1	0.003
Host abundance	0.04	0.013	1.00	1	0.318
Breeding status* Host Age			7.95	1	0.005
Non-breeding	0.16	0.056			
Breeding	0	0			
Host sex * Host abundance			9.98	1	0.002
Female	0	0			
Male	-0.02	0.006			
Breeding status* Host abundance			8.48	1	0.004
Non-breeding	-0.03	0.009			
Breeding	0	0			
Age*Host abundance	-0.002	0.0007	12.34	1	<0.001

b) *S. frederici* abundance. Model Type: GLMM (Random Terms = Site $\chi_1^2 = 0.5$, $P = 0.479$)

Model Term	Effect	S.E.	Wald Statistic (χ^2)	d.f.	*P*
Habitat composition			10.39	2	0.006
Pure beech wood	1.31	0.430			
Scot's Pine presence	0	0			
Spruce presence	1.29	0.442			

c) *H. fraterna* abundance. Model Type: GLMM (Random Terms = Site $\chi_1^2 = 16.10$, $P < 0.001$)

Model Term	Effect	S.E.	Wald Statistic (χ^2)	d.f.	*P*
Host age	-0.04	0.077	14.90	1	<0.001
Host abundance	-0.02	0.014	0.01	1	0.930
Habitat composition			3.57	2	0.168
Pure beech wood	0.87	0.498			
Scot's Pine presence	0	0			
Spruce presence	0.06	0.594			
Habitat composition * Host Age			7.54	2	0.023
Pure beech wood	0.20	0.085			
Scot's Pine presence	0	0			
Spruce presence	0.07	0.095			
Habitat composition * Host Abundance			14.36	2	<0.001
Pure beech wood	0.01	0.014			
Scots Pine presence	0	0			
Spruce presence	0.06	0.015			

d) *C. vitta* abundance. Model Type: GLMM (Random Terms = Site $\chi_1^2 = 0.99$, *P* =0.3197)

Model Term	Effect	S.E.	Wald Statistic (χ^2)	d.f.	*P*
Host abundance	0.10	0.041	6.32	1	0.012
Host Age	0.38	0.075	24.61	1	<0.001

e) *T. muris* abundance. Model Type: GLMM (Random Terms = Site $\chi_1^2 = 1.32$, *P* <0.2506)

Model Term	Effect	S.E.	Wald Statistic (χ^2)	d.f.	*P*
Habitat composition			8.98	2	0.011
Pure beech wood	0.71	0.710			
Scot's Pine presence	0	0			
Spruce presence	3.0	1.008			
Host sex			14.32	1	<0.001
Female	0	0			
Male	-1.81	0.478			
Host Age	0.28	0.053	27.20	1	<0.001
Host abundance	-0.03	0.012	8.54	1	0.003

Chapter 3

*Tick-nematode interactions in a yellow-necked mouse (**Apodemus flavicollis**) population*

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Abstract

There is increasing evidence that within a host, parasite species interact and these interactions are important in shaping parasite community structure and have an influence on host population dynamics. When parasites infect different parts of the host body, direct competition is unlikely and parasites may interact indirectly through the host immune response even when the parasites are not closely related. This study investigated whether changes in the gastrointestinal nematode, *Heligmosomoides polygyrus,* affected the infestation of the same individual yellow-necked mice, *Apodemus flavicollis,* with the tick *Ixodes ricinus.* Extensive monitoring as well as experimental manipulation was used. The nematode infection of yellow-necked mice was manipulated and changes in the nematode abundance, tick infestation and host biometrics were subsequently recorded. Changes in *H. polygyrus* negatively affected tick infestation and these changes were more apparent in non-breeding individuals and in young age mice than breeding adults. The interaction between *H. polygyrus* and ticks seemed to be modulated by the host immune response and it is suggested that gastrointestinal parasites play an important role in affecting parasite community structure.

Introduction

Free-living animal populations are usually inhabited by a community of parasitic species that form an infra-community within each individual host (Petney and Andrews, 1998). These communities are dynamically structured, changing over time in relation to current levels of infection that alter either host exposure and/or susceptibility. However, there is still no general consensus about the role of inter-specific interactions between parasites and how these interactions influence the dynamics of constituent parasite species within the host (Roberts *et al*., 2002; Guègan *et al.,* 2005). Workers who have applied the

comparative approach have found that parasite communities are simply little more than random assemblage of species that depend on variation in host exposure (Poulin, 2001; Guègan *et al.,* 2005). In contrast, laboratory studies on concomitant infections have invariably identified the importance of species interactions and have stressed the important role of the host in mediating these interactions and influencing susceptibility to infection (Behnke *et al.*, 2001; Cox, 2001). When parasite interactions have been observed, two types of relationship have been recorded: parasites may interact with each other synergistically (positively) or antagonistically (negatively). A synergistic interaction occurs when the second parasite species increases its fitness (increase in fecundity, establishment rate or survival) as a consequence of the presence of the first species. An antagonistic interaction leads to a fitness reduction of the second parasite species directly or indirectly for food or space, with the first species. There is growing evidence that the majority of these parasite interactions are mediated by the host immune response, either through cross-immunity (negative) or through immuno-suppressive (positive) effects (Behnke *et al.*, 2001; Cox, 2001). For example, mice challenged with *Trichinella spiralis* showed resistance to the challenge of a second parasite species, *Trichuris muris,* suggesting that the immune response developed to *T. spiralis* also induced protection against *T. muris* (Lee *at al.*, 1982).

Nevertheless, other host characteristics may interact with the immune response and modify the pattern observed. Differences between individual hosts, like differences between sexes, status or age, can have a significant effect on parasite dynamics and parasite-parasite interactions, and generate further complexities in the system (Perkins *et al.*, 2003; Lello *et al.*, 2004). A consequence of these differences is often a highly aggregated distribution of parasites within the host population, such that the majority of individuals harbour very few parasites while the minority of hosts carry large parasite

loads. Indeed, if the hosts that become more susceptible to a first parasite species are also more susceptible to the co-infection with a second parasite species, these two components covary and introduce non linearities into the system that affect parasite aggregation and community structure within the host population.

While there is a relatively good literature on the interspecific interactions between gastrointestinal parasites (for reviews see Christensen *et al*., 1987; Behnke *et al.*, 2001), with some evidence of interactions between nematodes and microparasites (for a review see Cox, 2001) there is little known about the relationship between nematodes and ectoparasites (Schwarzenbach *et al.,* 2004). This study examined whether changes in the gastrointestinal nematode *Heligmosomoides polygyrus* affected the infestation of the tick *Ixodes ricinus* on yellow-necked mice, *Apodemus flavicollis*.

Previous studies have shown that *H. polygyrus* establishes a chronic infection in mice which allows the parasite to survive and reproduce. Specifically, the nematode down-modulates the host intestinal inflammatory reaction by impairing the mucosa mast cell response (Monroy and Enriquez, 1992). These changes then facilitate the infection by other parasites, for example when mice were concurrently infected with *H. polygyrus* and *T. spiralis* a reduction in the acute response against the second nematode was recorded, suggesting that *H. polygyrus* caused an immuno-suppressed reaction in mice (Behnke *et al*., 1993).

I. ricinus is a tick that commonly infects rodent species during the early stages of its life cycle, and there is no evidence of acquired resistance in *A. flavicollis* as observed when repeatedly exposed to *I. ricinus* larvae (Dizij and Kurtenbach, 1995). In general, Ixodid tick infestations cause a complex immune response in individual hosts involving antigen presentation cells (APC), lymphocytes T and B, and a number of bioactive molecules that the tick manages to successfully avoid (Willadsen, 1980; Wikel, 1996;

Brossard and Wikel, 1997; Willadsen and Jongejan, 1999). Therefore, when *H. polygyrus* and *I. ricinus* co-infect a mouse host two possible alternative patterns may be expected: first the nematode infection has a positive host-mediated effect on tick infestation, where the immunosuppressive characteristics of the nematode infection facilitates tick infestation, or second, the nematode has no effect on the *I. ricinus*, because the two parasites interact with the host via independent immune mechanisms. To test between these two mutually exclusive hypotheses two field activities were carried out, first an extensive sampling of yellow-necked mouse populations and second an intensive experimental manipulation of *H. polygyrus* abundance and the monitoring of changes in tick infestation in individual yellow-necked mice. Recent studies of host-parasite interactions in yellow-necked mice have found that adult males carry higher tick infestations and have higher transmission rates of *H. polygyrus* than females (Perkins *et al.*, 2003; Ferrari *et al.*, 2004 i.e. Chapter 4). Hence, the effect of host sex and host status on nematode-tick interaction was also investigated.

Methods

Species description and monitoring

The interaction between the nematode *H. polygyrus* and the tick *I. ricinus* in yellow-necked mouse populations was examined using monitoring data from an extensive study undertaken in the summer of 2002 (see Chapter 2). Data were also collected from an intensive field manipulation carried out from spring to autumn 2002 (Fig. 1). The trapping sites were located in the North-eastern Italian Alps at a mean altitude of 850 m a.s.l. (± 22 m S.E.) in broadleaf woodlands, mainly mature stands of beech (*Fagus sylvatica*) with patchy presence of Scots pine (*Pinus sylvestris*), spruce (*Picea abies*) and with sparse

under-storey and little ground flora that represents the typical habitat of the yellow-necked mouse in its Italian range of distribution (Locatelli and Paolucci, 1998).

Both male and female yellow-necked mice are territorial with home ranges overlapping between sexes. Males are polygynous (de Mendonça, 2003), and reproduce mainly between March and September. The dynamics of *A. flavicollis* populations show strong seasonal fluctuations with abundance peaking in autumn and declining over the winter.

H. polygyrus is a nematode with a direct life cycle, with a larval stage spent on the ground and adult stage infecting into host's small intestine. This nematode is the most common gastrointestinal parasite found in yellow-necked mice in Italy (Rosso *et al*., 2002; this study). Additionally, this nematode has been extensively used as laboratory model and its infection dynamics are well known (Slater and Keymer, 1986; Gregory *et al.,* 1990; Scott, 1990; Gregory, 1991; Quinnell, 1992). *H. polygyrus* down-regulates the host immune response both at the local level of the intestine inflammatory response, and at systemic level influencing antibody production (Monroy and Enriquez, 1992; Behnke *et al*., 1993; Telford *et al.*, 1998). Moreover, *H. polygyrus* infection is known to affect various behavioural attributes of the mouse host, such as social status, predator avoidance and spatial learning (Freeland, 1981; Kavaliers and Colwell, 1995; Barnard *et al.*, 1998; Kavaliers *et al*., 1998).

The tick *I. ricinus* infects a wide number of mammalian hosts. The larvae, and to a less extent the nymph, feed preferably on small mammals. The life cycle of *I. ricinus* is characterised by three stages (larvae, nymph and adult) and in the Alps each one lasts about a year so taking three years for a tick to complete its life cycle. The degree of *I. ricinus* infestation can be very high and is often aggregated in the host population, such that very few individuals carry large infestations and this has been suggested to be

affected by host characteristics, for instance sex, host status or body conditions (Perkins *et al.*, 2003). The time a tick spends feeding on a mouse is relatively short, usually just a few days.

Mice were caught using multi-capture live traps (Ugglan type 2, Graham Sweden) baited with hay, a standardised amount of seeds (a mix of maize, oat, wheat, rice, millet, linseed, rape, vetch, by Zanandrea Sementi producer, Italy) and a piece of potato. Details of sex, pelage colour, breeding status (breeding *vs.* non-breeding) and body mass were recorded for each mouse trapped (more details in Ferrari *et al.,* 2004 - see Chapter 4).

The extensive trapping was performed for three nights (for a total of 3456 trap nights) in the month of July at six sites, where each site had three trapping grids and 64 multi-capture live traps per grid, following the small quadrat sampling technique (Myllymäki *et al.,* 1971). This technique allowed us to estimate the relative abundance of individuals sampled in each of six sites (Myllymäki *et al.,* 1971; Henttonen *et al.,* 1987). Adult mice, that is, mice with brown pelage and a body mass above 15 g. (Barbetta, 2003, see Chapter 1), were euthanised (UK and Italian ethical rules applied) and *H. polygyrus* parasites were identified and isolated using the filtration-sedimentation technique (Euzeby, 1982), and abundance estimated for each mouse individual. Eye lens mass was used as relative measure of mouse age (Morris, 1972; Gregory *et al.*, 1992). Total number of ticks and identification of their life stages (larvae, nymphs and adults) was systematically recorded for each mouse.

The intensive field manipulation was carried out for two nights every other week from May to August (for a total of 5376 trap nights) in a site structured in six trapping grids of 64 multi-capture traps each (i.e. 8x8 traps at 15m inter-trap interval covering an area of 1.1 ha). To minimise any possible movement of mice from one grid to the next, grids were set at more than 500 m apart and with natural and artificial barriers (road,

rivers etc.) between them. The yellow-necked mouse population was monitored using capture-mark-recapture, where every new individual was identified with an implantable subcutaneous passive induced transponder tag (Trovan ID 100, Ghislandi & Ghislandi, Italy). Adult individuals were selectively manipulated as follow: 4 trapping grids were selected at random and half of the mice population on each grid was orally treated with an anthelmintic to artificially reduce *H. polygyrus* load (anthelmintic Pyrantel pamoate - Gellini pharmaceutical; dose: 100 mg/Kg -Wahid and Behnke, 1996; Quinell, 1992). The remaining animals were orally infected to increase *H. polygyrus* load, dosing the mice with infective third larvae stage of *H. polygyrus* (Keymer and Hiorns, 1986). The last 2 trapping grids were used as control and no treatments were performed. Each dosed mouse was systematically treated following the same procedure every other week and for the entire duration of the experiment. The faeces collected from each trapped mouse were analysed using the Mc Master technique to estimate the number of *H. polygyrus* eggs per gram of faeces (EPG), no faeces were gathered when traps contained more than one individual; for a complete description of the methodology see Ferrari *et al.,* 2004 (i.e. Chapter 4).

Tick abundance and life stages were recorded for each individual trapped.

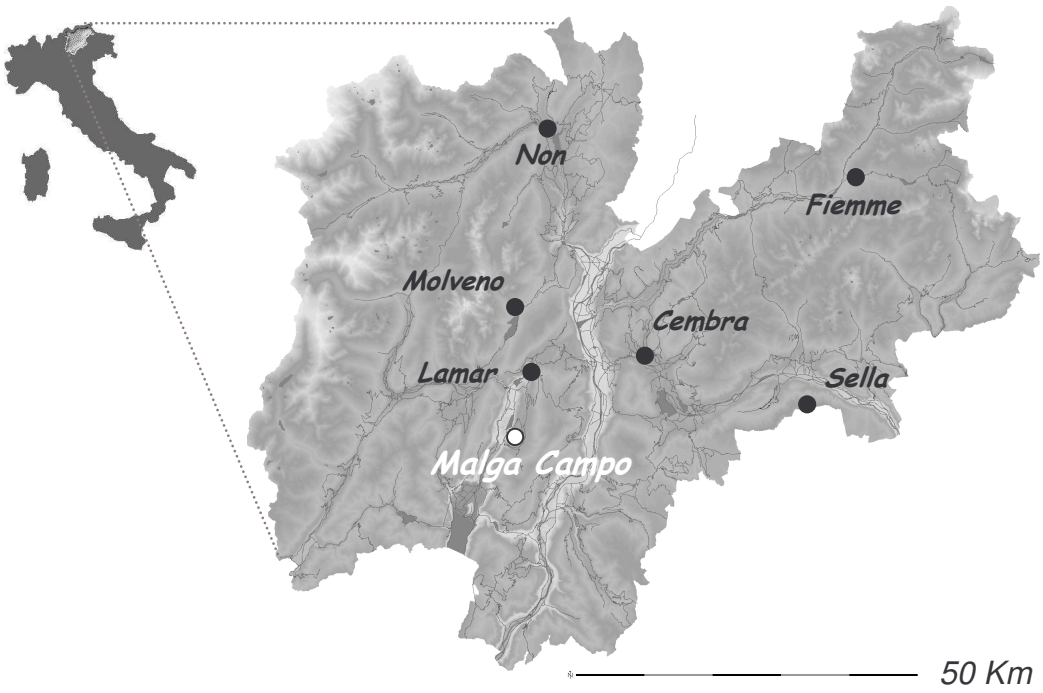

Figure 1. Location of the study sites. Black points: extensive trapping grids and White point: intensive trapping grids.

Statistical analysis

To examine whether *H. polygyrus* loads affected the abundance of *I. ricinus* in the yellow-necked mouse, Generalised Linear Models with negative binomial errors (GLM) were performed on the extensive data set. Specifically, the analyses tested if *H. polygyrus* abundance, host characteristics (sex, age, and breeding status), and habitat composition (pure mature beech, mature beech with Scot pine and mature beech with spruce wood) explained changes in total tick infestation between individual hosts. The minimal adequate model was then selected using stepwise backward deletion routine, the maximal model including all factors and their second order interactions (Crawley, 2002).

This relationship was also investigated using the intensive trapping data set. A Generalised Linear Mixed Model with negative binomial errors (GLM-IRREML) was undertaken using total tick number per individual host as the response variable and parasite treatments (increase *H. polygyrus*, decrease *H. polygyrus* and control) as the

independent variable. Since, the tick blood meal lasts 4-5 days (Sonenshine, 1992; Randolph, 1998) each count of ticks on mice trapped every other week can be considered independent. However, to deal with pseudo-replication due to autocorrelation for capture-recapture of same individuals during a trapping week, the weekly recaptures were not considered and each individual was treated as a random factor. Finally, to account for temporal variation in the longitudinal trapping the trapping week was entered as an additional random factor.

Results

Extensive trapping

The number of yellow-necked mice trapped is significantly different between the 6 trapping sites (total individuals trapped 230, $\chi_5^2 = 178.8$, $P < 0.0001$) with numbers of mice caught ranging from 11 to 110 individuals (for detailed description of the mouse sample see Chapter 2). In general, the prevalence of *H. polygyrus* is 66.1 % and the mean abundance is 10.7 (± 1.1 S.E.) worms/host. Ticks are found on 50.4 % of the animals and mean infestation is 8.8 (± 1.1 S.E.) ticks/host, 96.4% of these ticks are larvae and the remainder nymphs and occasionally adult stages. The minimal adequate model that describes the tick-nematode interaction suggests that total number of ticks per host is negatively affected by *H. polygyrus* load, host breeding status, their interaction and occurrence of spruce woodland. In contrast the host age-*H. polygyrus* interaction and beech woodland habitat (modelled as a discrete variable) have a positive effect (Tab. 1). Specifically, total tick infestation is higher in breeding than non-breeding mice (mean ± S.E.: 11.2 ± 1.7 *vs* 5.4 ± 0.9, respectively), and the model suggests that total tick infestation has a greater decrease with an increase of *H. polygyrus* load in non-breeding young mice than in adult hosts (Fig. 2).

Table 1. Extensive monitoring data set: minimal adequate GLM model between total tick number by mouse, as response, and host characteristics, *H. polygyrus* load and habitat conditions, as explanatory variables.

Model terms	Coefficients	Deviance	d.f.	*P*
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	-0.064	14.062	1	<0.001
H. polygyrus abundance * Mouse Age	0.012	6.834	1	0.008

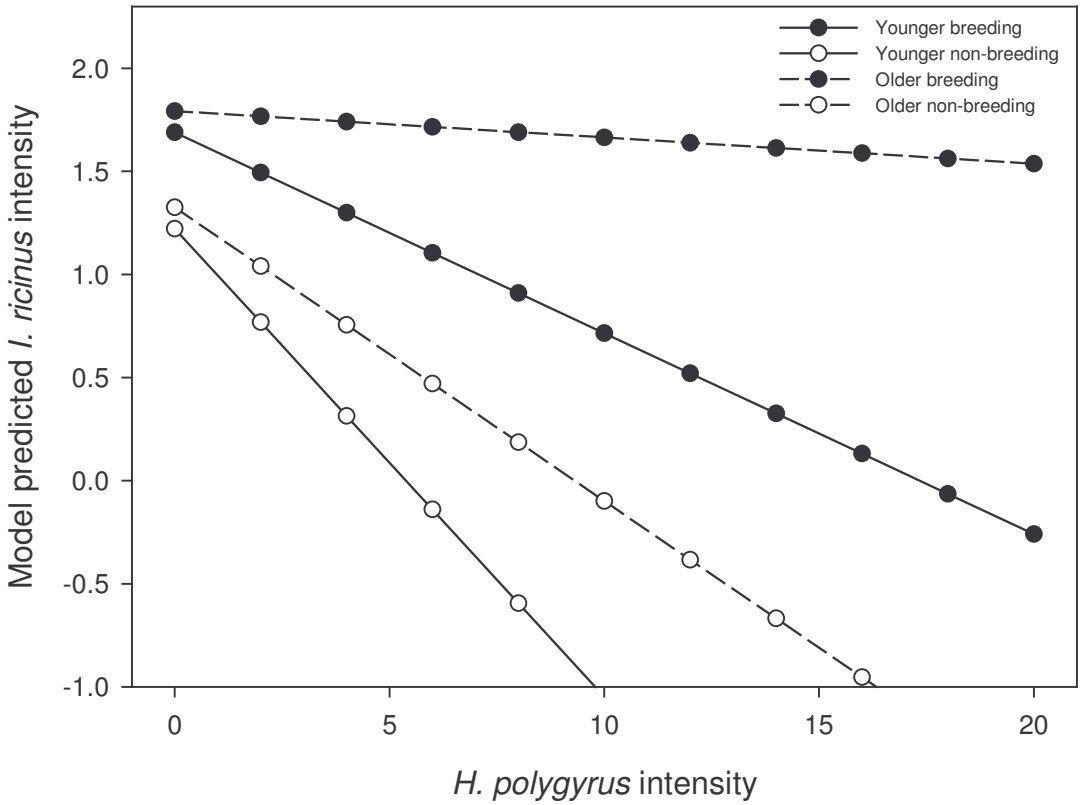

Figure 2. Relationship between *I. ricinus* infestation and *H. polygyrus* abundance in breeding and non-breeding mice of different age from model predictions by the minimal adequate GLM model on extensive trapping data set. Model based on the subset of mice of the 1st and 3rd quartiles, representing younger and older, of the relative measure of age (i.e. eye lens mass).

Intensive trapping

87 yellow-necked mice (45 males and 42 females; 41 breeding and 46 non-breeding) trapped from the end of May to the end of August (equal to 167 captures) do not evidence significant differences in the total number of mice trapped between grids (χ_5^2=8.25, *P*=0.143) or between treatment groups (χ_2^2=5.85, *P*=0.054). On the total of 143 faecal samples (equal of 73 individuals) collected and any significant differences between grids (χ_5^2=8.54, *P*=0.129) or between treatments (χ_2^2=3.13, *P*=0.209) is observed.

The manipulation of *H. polygyrus* load and the associated changes in ticks infestation on mice shows significant results (GLMM-IRREML, family=negative binomial, link=log, comparison between treatments; response = Ticks: χ_2^2 = 6.87, *P* = 0.03; response = EPG: χ_2^2 = 9.12, *P* = 0.01). However, the *a posteriori* pairwise comparison between treatment groups reveals that the infection treatment proves to be not entirely effective in increasing the *H. polygyrus* load and no changes in tick abundance are recorded (Tab. 2, Fig. 3). In contrast the anthelmintic treatment causes a significant decrease of *H. polygyrus* abundance coupled with a significant tick increase (Tab. 2, Fig. 3). Further analysis of animals dosed with anthelmintic reveals that after treatment, tick infestation is higher for males and individuals in breeding condition (geometric mean ± S.E.: males 6.11 ± 3.04 *vs* females 1.8 ± 0.5, *P*> 0.05; breeding 4.4 ± 1.8 *vs* non-breeding 1.9 ± 0.7 *P*>0.05). Further analyses to identify an effect of host characteristic on tick-nematode interaction do not reveal any apparent significant pattern.

Table 2. Comparisons between treatment groups (control, infection and anthelminthic): A- *I. ricinus* infestation (ticks/host) and B- *H. polygyrus* load (EPG). Comparison based on *a posteriori* pairwise Tukey test on GLMMs on the text.

Treatments	Differences between treatment (± 95% confidence limits)	d.f.	*P*
A: *I. ricinus* (ticks/host)			
Control *vs* Infection	0.258 (±1.599)	120	0.751
Control *vs* Anthelmintic	1.150 (±0.919)	120	0.015
Infection *vs* Anthelmintic	0.829 (±1.742)	120	0.328
B: *H. polygyrus* EPG			
Control *vs* Infection	0.331 (±2.949)	67	0.822
Control *vs* Anthelmintic	4.934 (±3.600)	67	0.009
Infection *vs* Anthelmintic	5.265 (±4.214)	67	0.017

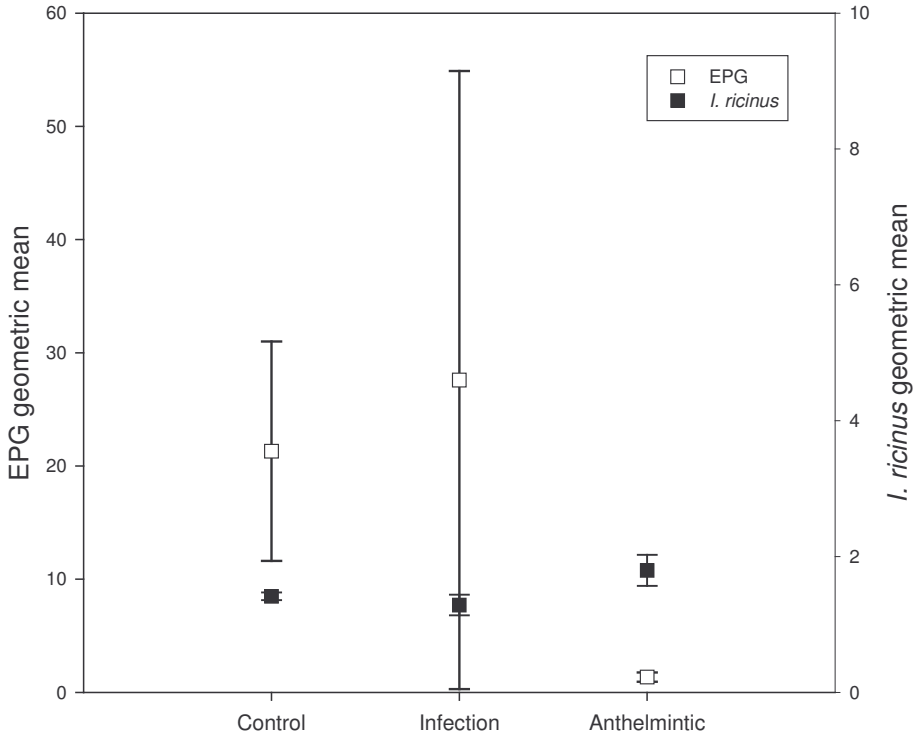

Figure 3. Geometric mean *I. ricinus* infestation (ticks/host) and number of eggs per gram (EPG) of *H. polygyrus* by treatment.

Discussion

Field observations and experimental manipulations were used to look for evidence of an interaction between *H. polygyrus* and *I. ricinus* in yellow-necked mice and results revealed that tick infestation was negatively related to changes in *H. polygyrus* abundance. Moreover, field observations showed a faster decrease in tick infestation with increasing *H. polygyrus* abundance in non-breeding individuals and younger mice.

Changes in tick infestation on mice has been suggested to be the result both of environmental factors (Randolph and Storey, 1999; Randolph *et al*., 2000) and host characteristics associated with habitat components (Perkins *et al*., 2003). The present study confirmed the role of host characteristics and environmental variables, but also suggested that a co-infection with the gastrointestinal nematode *H. polygyrus* is important in affecting tick infestation. Both initial hypotheses that 1- *H. polygyrus* infection down regulates host condition to make it more suitable to ectoparasites, or 2- that no interaction is taking place between the two parasite species, were not supported by these field results. In contrast a negative association between *H. polygyrus* load and *I. ricinus* infestations was found.

The immunological properties that characterise the infection of both parasites and the lack of direct interaction between the tick and the gastrointestinal nematode seem to suggest a role for mediation by the host immune response. We know that tick salivary fluid is composed of multiple elements with compound effects on the host, ranging from anti-haemostatic, anti-inflammatory to immunological properties (Wikel, 1996). The complex immunoreactions involve both Th1 cell mediated response and Th2 humoral response (Willadsen and Jongejan, 1999) that may result in either the development of immuno-suppression or acquired resistance according to the tick-host species systems involved (Dizij and Kurtenbach, 1995). As such, the influence of the host immune system

on tick infestation suggests that the antagonistic tick-nematode interaction could have been caused by an immune response of the host directed toward different parts of this system. With respect to the Th1/Th2 dichotomy response (Behnke *et al.*, 2001; Cox, 2001) the *H. polygyrus* may have down modulated the host Th2 defences towards the nematode infection and redirected it to fight the tick infestation developing a Th1 response.

However other non immunological mechanisms could have generated the same pattern. Indeed, metabolic toxic products may have been produced by one species against another as a response to limited nutritional resources or simply as a discharge of toxic components by one species which affected the other species (Behnke *et al.,* 2001). Differences in host behaviours may have affected the pattern of infection, assuming that *H. polygyrus* can be acquired through the fur by grooming (Hernandez and Sukhdeo, 1995), a behaviour also used to control tick infestation (Mooring and Hart, 1997). Individual variation in such behaviour may lead to the observed negative association, where mice that are grooming heavily will have low tick intensities, but will potentially ingest many infective larvae, leading to high intensities of *H. polygyrus*. However, the tick infestation change subsequent to the artificial reduction of *H. polygyrus* load suggests this behavioural mechanism could be considered negligible.

The extensive trapping showed that host status, sex and age are important in affecting nematode-tick interactions, suggesting a possible role of host hormonal components. In this respect, it is possible that higher steroid hormonal levels during the host breeding phase affected the ability of the immune system to down regulate the tick-nematode interaction. As such, the high tick infestations observed in breeding mice may have been caused by high steroids levels which reduced both innate and acquired immunity to feeding ticks (Hughes and Randolph, 2001). In contrast, a lower hormonal concentration

in non-breeding mice may have led to a weaker immunosuppressive response that allowed the *H. polygyrus*-tick interaction.

The extensive monitoring gave a snap-shot of the relationship between ticks and *H. polygyrus* and the experimental treatment supported these observations despite the experimental procedure of infection which failed to increase nematode abundance, probably due to an inappropriate dosage in relation to host conditions (like: status, sex, hormonal levels, etc.). While these results are not conclusive, there is evidence of a negative interaction between *I. ricinus* and *H. polygyrus* which suggests that host immune-mediated effect and host characteristics play an important role in regulating this relationship. The mechanism of interaction is still obscure and future work should be able to disentangle the underling processes involved. This is a fundamental step if we want to understand parasite interactions and parasite community dynamics.

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Chapter 4

The role of host sex in parasite dynamics: Field experiments on the yellow-necked mouse *Apodemus flavicollis*

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Abstract

The role of host sex in parasite transmission has been investigated asking the question: "Is host sex important in influencing the dynamics of infection in free living animal populations?" The helminth community was experimentally reduced in either males or females in a yellow-necked mouse (*Apodemus flavicollis*) population using an anthelmintic, in replicated trapping areas, and subsequently monitored the prevalence and intensity of macroparasites in the untreated sex. The study focussed on the dominant parasite *Heligmosomoides polygyrus* and found that reducing parasites in males caused a consistent reduction of parasitic intensity in females, estimated through faecal egg counts, but the removal of parasites in females had no significant influence on the parasites in males. This finding suggests that males are responsible for driving the parasite infection in the host population and females may play a relatively trivial role. The possible mechanisms promoting such patterns are discussed.

Introduction

The predisposition of macroparasites to aggregate within their host populations is generated by the presence of one or more heterogeneities in the transmission or establishment process (Wilson *et al*., 2002). Exhaustive empirical studies have shown that variability in parasite infection arise as a consequence of individual differences in exposure and susceptibility to infection (Keymer and Anderson, 1979; Hudson and Dobson, 1995; Shaw and Dobson, 1995). While the importance of the two mechanisms in the generation of aggregation appears to vary from one host species to the next (Gregory and Woolhouse, 1993), there is evidence from mammal species that susceptibility is the dominant process and that variation between individuals is associated with sex, age and

body size as well as individual behaviour and diet (Poulin, 1996a; 1996b; Schalk and Forbes, 1997; Wilson *et al.,* 2002).

Recently, a number of comparative studies have investigated sex-biased parasitism and reached the conclusion that within vertebrate hosts, males tend to have significantly higher parasite prevalence and intensity than females (Poulin, 1996a; 1996b; Schalk and Forbes, 1997; McCurdy *et al.,* 1998; Moore and Wilson, 2002). Furthermore, experimental manipulations have shown that male-biased infection was greater when hosts were artificially infected with standard numbers of infective stages than in naturally infected hosts (Schalk and Forbes, 1997). While this evidence is not conclusive for all groups of hosts and parasites, the direction is consistent: males harbour higher levels of infection than females. However, these findings provide no insight into the consequences of this disparity on the dynamics of the infection.

One implication of sex biased parasitism in vertebrate hosts is that heavily infected males may drive the parasite dynamics even though successful transmission will depend on other mechanisms, such as the behaviour of susceptible hosts and the spatial distribution of infective stages. Counter to this hypothesis, some authors have suggested that since the sex bias observed is often relatively small (usually <5%) this is unlikely to have a high effect on parasite dynamics (Wilson *et al*., 2002). Either way, disentangling which functional group is important in driving the infection process could be crucial to the understanding of how parasites flow through a host population.

This study investigated the role of host sex in parasite transmission asking the question: "Is host sex important in influencing the dynamics of infection in free living animal populations?" The helminth community of either males or females of yellow-necked mice (*Apodemus flavicollis*) was experimentally reduced using an anthelmintic, in replicated trapping areas, and subsequently monitored the prevalence and intensity of

macroparasites in the untreated sex. This study focussed the attention on the trichostrongylid nematode *Heligmosomoides polygyrus,* a species that exhibits a direct life cycle and that is known to infect a large number of microtine and murine rodents (Lewis, 1987; Gregory, 1992). In the sibling host species, *A. sylvaticus,* infections are generally sex biased with females carrying 5 % fewer parasites than males (Gregory, 1992; Gregory *et al.*, 1992). The route of infection is either through ingestion of larvae with contaminated food or through the grooming of fur contaminated with infective stages (Hernandez and Sukhdeo, 1995). The yellow-necked mice-*H. polygyrus* system represents an ideal system to investigate heterogeneities in host parasites dynamics, partly because previous studies have examined this relationship in detail (e.g. Keymer and Hiorns, 1986; Gregory, 1992; Gregory *et al.*, 1992) but also because *H. polygyrus* prevalence is reasonably high and mice can be regularly trapped and sampled.

Methods

Study area and rodent monitoring

The yellow-necked mouse is a widely distributed rodent in the woodlands of the Italian Alps, and was the most abundant small mammal species in the study area: Malga Campo (Trentino). A long term study of the yellow-necked mouse population was previously undertaken and estimated a mean local density of 2.8 mice/ha (±0.5 S.E.) during the year of this study (2001).

Intensive live trapping of yellow-necked mice was carried out in a mixed broadleaf woodland of mainly mature stands of beech with scattered alder and pine and sparse under-storey and little ground flora (Dolomitic Alps, 650-950 m. a.s.l., 1652050E 5093750N). A set of nine trapping grids (each consisting of 49 traps, 7x7 at 15m inter-trap interval, covering an area of 0.81 ha) was established using multi-capture live traps

(Ugglan Type 2, Graham - Sweden). The woodland area was selected as representative of the yellow-necked mouse habitat, and the replicated trapping grids were positioned in woodlands with similar vegetation composition and structure. To minimise possible movement of individuals between grids, each grid was more than 500 m from the neighbouring grid with natural or artificial barriers (rock falls, roads, etc.) between them.

Live trapping was undertaken for two nights, every other week for a total of 14,994 trap nights from February until mid- September. Traps were baited with a standardised amount of seeds (a mix of maize, oat, wheat, rice, millet, linseed, rape, vetch - Zanandrea Sementi, Italy), and with potatoes as a source of water and hay for bedding. Special care was taken not to over-feed or to attract transient individuals. Each mouse trapped was individually tagged with a subcutaneous passive induced transponder (Trovan ID 100, Ghislandi & Ghislandi, Italy). Faecal samples were collected from each trap (no faeces were gathered when traps contained more than one individual) during each trapping session. Details on body condition, mass and breeding status were also recorded. Mice were classified in juveniles, individuals with a body mass below 15g. and a grey pelage - that indicated the post-juvenile moult had not occurred yet- and adults, mice with a body mass greater than 15. g and brown adult pelage (Barbetta, 2003, see Chapter 1). Adults were further classified as individuals in breeding conditions (descended testes for males and perforated vagina or pregnant for females, Gurnell and Flowerdew, 1990) or non-breeding conditions.

Parasite manipulation

The yellow-necked mouse population was monitored every two weeks from February to March, and from the first week of April until the middle of September and adult individuals were selectively treated with the anthelmintic Ivermectin (IVOMEC-plus

Merial) by injecting a subcutaneous dose of 10 mg/kg (Wahid *et al*., 1989). Juveniles were not treated since they were not yet infected. Out of nine trapping grids, three were randomly selected and all females caught were treated with anthelmintic. In a second random group of three trapping grids only males were treated and the three remaining grids were used as controls where no individuals were treated. The drug efficacy was assumed to last 11-15 days (Wahid *et al*., 1989) and the prepatency period of *H. polygyrus* from egg to egg was 13-15 days long (Keymer, 1985) so each individual was treated once a month. *H. polygyrus* egg production follows a 24h cycle fluctuation (Brown *et al.,* 1994b) and to avoid a possible temporal effect in faecal collection the order of collection of faeces samples was randomised between the trapping grids and the grids themselves.

Bank vole *Clethrionomys glareolus* was the second most abundant small rodent in the study area, and to circumvent any confounding effect caused by inter-species transmission of gastrointestinal parasites (Mèszàros, 1978; Lewis, 1987), all bank voles trapped were treated with anthelmintic for the entire experiment.

Parasite identification and count

While the community of gastrointestinal parasites was monitored in the faeces (detailed parasite list in Rosso *et al*., 2002), particular attention was given to the helminth *H. polygyrus.* This helminth is one of the most common parasites of the genus *Apodemus* and has a relevant impact on host population (Scott, 1987). Moreover this parasite has been extensively used as a laboratory model. Nevertheless, the entire community was checked of gastrointestinal parasites for any unusual patterns and found that the helminth community in the mice population was characterised by a relatively low prevalence and intensity of infection (Rosso *et al.,* 2002).

Faeces collected from each individual were stored at 4° C overnight in Petri dishes on damp blotting paper to standardise the humidity content. Each sample was then weighed and a flotation technique performed to assess the presence of *H. polygyrus* eggs to provide prevalence estimates (Sloss and Kemp, 1978). To quantify the amount of parasite's eggs per gram of faeces (EPG), the McMaster technique was used on faecal samples more than 0.4 grams in mass (Keymer and Hiorns, 1986). One gram of faeces was diluted in 10 ml of flotation solution (saturated NaCl) that allowed a minimum resolution of 33 *H. polygyrus* eggs per gram of faeces. For both techniques, samples were inspected under microscope magnification of 100X and every *Heligmosomoides* spp. egg was classified as *H. polygyrus* since this is the only species of this genus found in *A. flavicollis* in our study areas (Rosso *et al.,* 2002).

Statistical analysis

To investigate if prevalence of *H. polygyrus* in yellow-necked mice was significantly affected by host characteristics and environmental variables a Generalised Linear Mixed Model (GLMM, Genstat 3.2) with binomial errors and logit link function was performed. Presence/absence of infection was used as the response variable and a series of fixed explanatory variables and their interactions (i.e. anthelmintic treatment, host sex, breeding status, grid and period of trapping, etc.) were selected to identify the model that best explained the variance observed. This procedure was repeated using GLMM with iterative reweighted linear model (IRREML; with negative binomial errors) based on EPG as response and host population and environmental characteristics as explanatory components.

To overcome autocorrelations in the multiple trapping data points and therefore non-independence of data, the transponder code, which represented each animal's unique

identity tag, was entered into GLMM models as a random effect. The variance explained by each explanatory factor and its significance were calculated using stepwise backwards deletion and Wald test (Crawley, 2002). An *a posteriori* multiple comparison Tukey test was carried out between treatment groups to identify which sex treated component caused the pattern observed.

Results

Rodents monitoring

A total of 143 yellow-necked mice (73 males and 70 females) were trapped between February and September (403 captures) and 46% of these individuals were trapped only once. There are no significant differences in the total number of individuals trapped or the sex ratio between the three treatment types (for all: $P>0.05$). No significant temporal variation in sex ratio is observed during the experiment ($P>0.05$).

A total of 319 faeces samples were collected of which 315 samples (83.5 % of these samples were from multi-captured individuals) were analysed using the flotation technique and 243 samples (87.2% of these were multi-captured individuals) were selected for the McMaster analysis. While we are aware that a percentage of these samples (16.5% for flotation and 12.8% for McMaster) is from individuals trapped once, these cases are added together with the control groups since no significant differences in parasite prevalence and EPG are observed between these transient individuals and the control individuals (for all: $P>0.05$).

Parasite manipulation

Prior to anthelmintic treatment, host sex ratio does not differ between grids as well as parasite prevalence and EPG intensity (for all: $P>0.05$).

Following the selective treatment of either males or females, no significant temporal changes are observed in parasite prevalence and EPG intensity in the control areas ($P>0.05$), in contrast *H. polygyrus* prevalence shows a significantly different response between the two treated sexes. In trapping grids where females are manipulated parasite prevalence is 73% in untreated males and 10% in the treated females, while in trapping grids where males are manipulated prevalence is 25% in untreated females and 6.7% in treated males. In the control grids, prevalence is 60.5% in males and 58.3% in females (Fig. 1). The interaction between sex and treatment significantly contributes to the variation in *H. polygyrus* prevalence in the host population (GLMM: Sex-Treatment $\chi_2^2=8.24$, $P=0.016$) but sex or treatment alone does not significantly explain this variation ($P>0.05$). The *a posteriori* pairwise comparison between treatment groups reveals a significant decrease in prevalence in females where males are treated compared with females in control grids ($P<0.038$; Tab. 1).

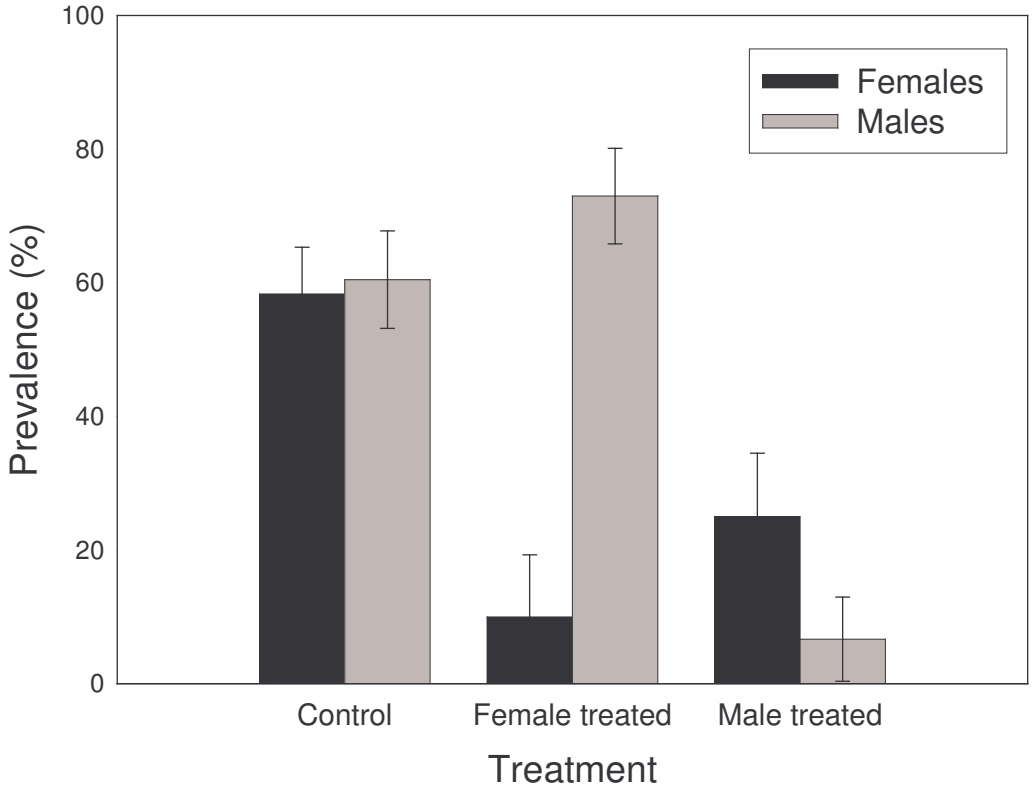

Figure 1. Prevalence of *Heligmosomoides polygyrus* by sex and treatment.

Table 1. Pairwise comparison Tukey test between control and treatments by sex A- analysis based on prevalence B- analysis based on EPG.

Groups tested	Differences between effects (±S.E.)	d.f.	*P*
A: Prevalence			
Female Control *vs* Male Control	0.193 (0.578)	166	0.38
Female Control *vs* Female Untreated	1.357 (0.749)	166	0.038
Male Control *vs* Male Untreated	0.731 (1.404)	166	0.305
Female Control *vs* Female Treated	2.448 (1.240)	166	0.026
Male Control *vs* Male Treated	2.850 (1.399)	166	0.022
B: EPG			
Female Control *vs* Male Control	0.2149 (1.069)	191	0.42
Female Control *vs* Female Untreated	2.794 (1.266)	191	0.014
Male Control *vs* Male Untreated	0.825 (2.079)	191	0.346
Female Control *vs* Female Treated	4.574 (1.721)	191	0.004
Male Control *vs* Male Treated	13.356 (26.805)	191	0.310

In the grids where females are treated, the geometric mean of the *H. polygyrus* eggs per gram of faeces (EPG) is 28.9 (±3.1 S.E.) in males and 0.5 (±1.0 S.E.) in females, while in grids where males are treated EPG geometric mean is 1.3 (±1.2 S.E.) in females and 0.0 (±1.0 S.E.) in males (Fig. 2). In the control grids, EPG geometric mean is 18.9 (±3.2 S.E.) in females and 32.2 (±5.5 S.E.) in males. Both sex alone and the interaction between sex and treatment significantly contribute to the variation of *H. polygyrus* EPG in the host population (GLMM-IRREML: EPG by Sex: χ_1^2=5.30, P=0.021; EPG by Sex and Treatment: χ_2^2=6.17, P=0.045); treatment alone does not show any apparent effect (P>0.05). *H. polygyrus* EPG is similar between sexes within control grids. In accordance with the a *posteriori* prevalence analysis, a significant decrease in EPG is found between females where males are treated compared to control females (P<0.014) (Tab. 1).

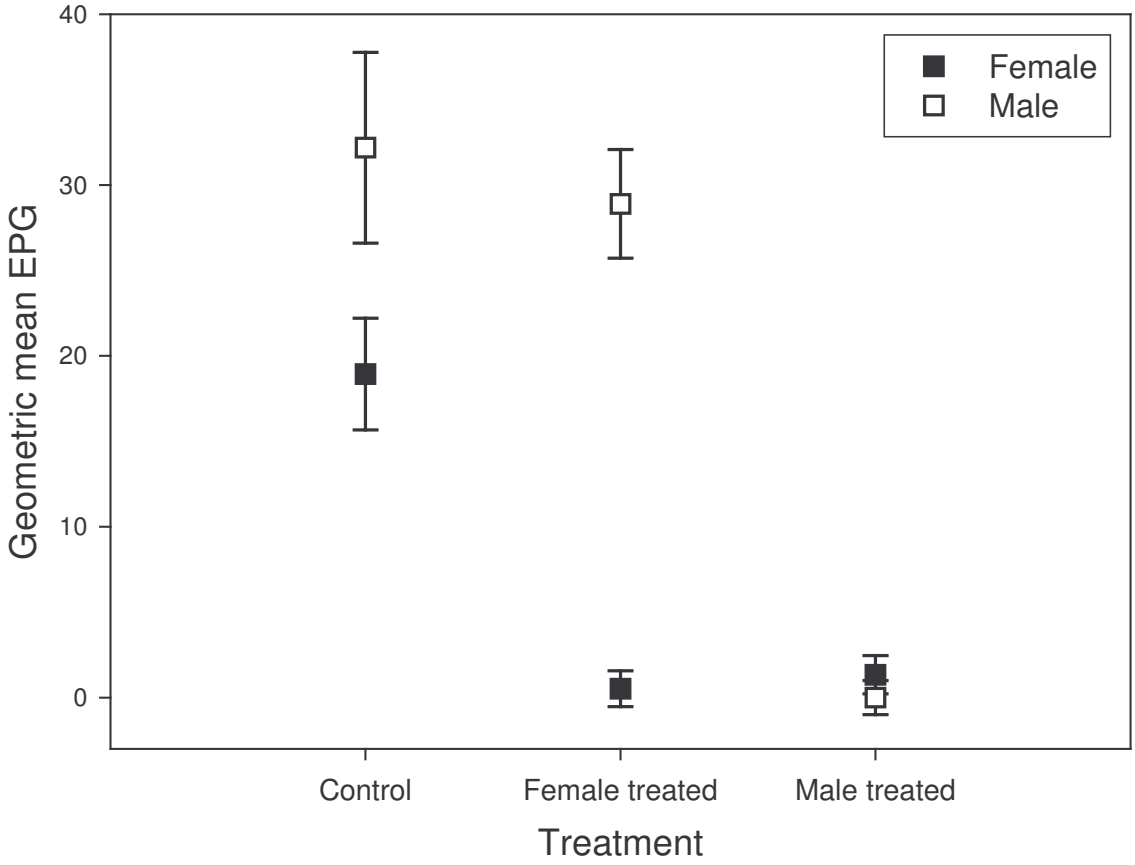

Figure 2. EPG (eggs per gram of faeces) of *Heligmosomoides polygyrus* by sex and treatment.

Despite the treatment, a low percentage of re-infected positive cases are recorded in the treated individuals (less then 10 %). This is not surprising, since some individuals could become re-infected before being treated again (see Methods). However since these mice are treated almost immediately, the effect of these few individuals is probably negligible on the final result.

Finally, the pattern of distribution of EPG in the control group is investigated and this does not differ from the negative binomial distribution for both males (k=0.156, *P*=1.00) and females (k=0.131, *P*=1.00). It is important to observe that the top 20% of the most

infected hosts accounts for 73% of the total *H. polygyrus* eggs expelled and are collected from males in 62% of cases (Fig 3).

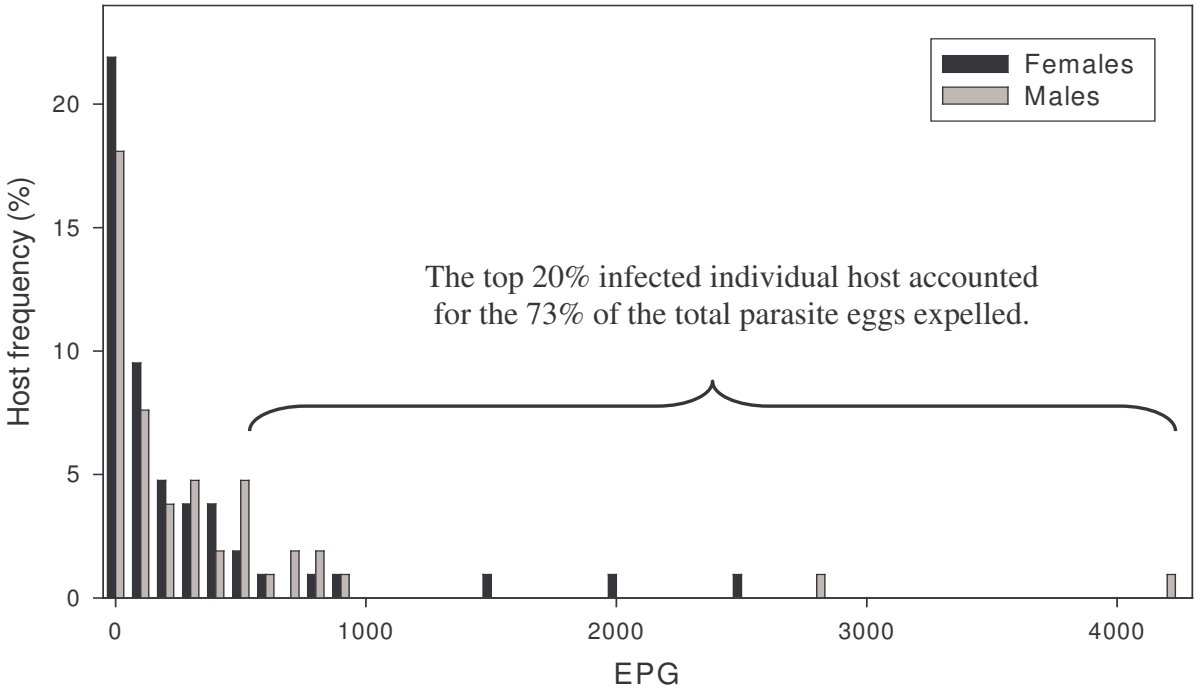

Figure 3. Aggregated distribution of EPG from the control population of males and females *A. flavicollis*.

Discussion

The parasite load of a natural population of yellow-necked mice was experimentally manipulated with respect to sex and results showed that after reducing the parasite intensity of males a decrease in the parasites intensity of females was observed. In contrast a similar effect was not observed on males when females were treated for parasites. This result suggests that males have a dominant role to play in driving the dynamics of parasite transmission in this system while females have a relatively trivial role.

Faecal analysis was used to investigate the dynamics of parasite transmission in a host population. While there is evidence that this is a reliable technique (Scott, 1988) the

slightly different results found between the flotation and the Mac Master method was probably caused by both a different amount of faecal samples available for the two tests and differences in test sensitivity. Ideally total number of parasites per individual would have been recorded but this would have meant euthanising mice and would have led to immigration of new untreated mice. Parasites were also removed with a systemic anthelmintic drug that would have affected the whole community of parasites and their interactions, including ticks (Wahid *et al*., 1989). This may have had an influence on the nature of the results nevertheless, the difference observed in parasite reduction between the sexes was clear.

So far, variations in parasite distribution, such as sex biased parasitism, have been associated with differences in host susceptibility and, accordingly, difference in their parasite load. Little attention has been given to the role of sex bias in parasite transmission; in effect the male bias in parasite rates does not explain the different abilities of sexes in maintaining the infection. In the absence of empirical evidence, some authors have tended to emphasize the role of sex bias while others have dismissed it as unimportant (Poulin, 1996a; Wilson *et al.,* 2002). Few studies have highlighted the importance of identifying the functional groups responsible for transmission in the population or have been able to disentangle their contribution in the maintenance of parasite populations (Anderson and May, 1991; Woolhouse *et al*., 1997; Perkins *et al.,* 2003). The experiments undertaken here demonstrate that host sex effects vary not only in the ability to modulate parasite establishment but also in their contribution to parasite transmission dynamics, with males playing a dominant role in successful parasite infection.

These findings lead us to question which mechanisms could be associated with the role of males in causing differences in subsequent infection levels of females and other

males. The experiments do not reveal what the mechanism could be but this could arise as a consequence of differential behaviour between the sexes that would lead to increased exposure of one sex to transmission. For example, transmission can occur through grooming and differences in allogrooming between sexes could result in increased rate of transmission from one sex to the next (see: Hernandez and Sukhdeo, 1995). Furthermore, infected males have been observed to have larger territories than uninfected males and this could influence contact rates between infected males and susceptible hosts (Brown *et al.,* 1994a). In this respect, Ims (1987a) found that while spatial distribution of reproductive female microtine rodents was determined by food availability, the spatial strategy in reproductive males reflected the availability of fertilizable females. Therefore reproductive females show a stronger site-specific organisation, which could explain low rate of transmission, whereas males' home ranges tended to extensively overlap at high density and decrease at low densities (Ims, 1987b), which could explain the higher rate of transmission.

Another possible mechanism may act through sexual differences in the immunological response of hosts such that worms in males produce fertile eggs at a higher rate than in female hosts. In fact, immunological differences between the sexes may contribute by modulating parasite egg fertility, parasitic worm size, and the rate of development and survival of infective stages with males providing a better environment for parasite growth and reproduction than females (Poulin, 1996b; Finkelman *et al.,* 1997). However, as Tompkins and Hudson (1999) noted, density dependence in worm size may cause different responses in the development and fecundity of the worms. In this regard, a gastro-intestinal analysis was carried out on 111 individuals collected from an area near to the study site in the summer of 2001 and found no significant differences in prevalence and mean intensity of infection of *H. polygyrus* between sexes (prevalence

35.2% males *vs* 29.8 % females, mean intensity 11.2 males *vs* 7.5 females, for all P>0.05, unpublished data).

Interestingly, when the distribution of EPG among sexes was examined 20% of the most infected individuals (represented by 62% of males) accounted for the 73% of the total eggs expelled. This finding is in line with other observations on yellow-necked mice by Perkins *et al.* (2003) that investigated ticks distribution and occurrence of tick borne encephalitis virus in a population of yellow-necked mice near our study area. These researchers found that more than 90% of the potential transmission of tick borne encephalitis comes from male yellow-necked mice. These heterogeneities can play a very important role in influencing the size of the parasite basic reproduction number (R_0) and have important implications in the selective treatment of individuals in control programs.

In conclusion this study identified a male bias in parasite transmission and evidence that this had important consequences for parasite dynamics. Not only are there differences in sex susceptibility to parasite infection but also in the sex competence to modulate transmission such that even a relatively small proportion of the host population can be responsible for the majority of the transmission. This finding improves the knowledge on parasite dynamic and may be useful for planning parasite control programmes when the identification of functional groups is practicable.

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Chapter 5

The role of sex in parasite dynamics: model simulations on transmission of Heligmosomoides polygyrus ***in yellow-necked mouse populations.***

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Abstract

The mechanism causing sex-biased parasite transmission of *Heligmosomoides polygyrus* in *Apodemus flavicollis* was investigated using a modelling approach. Two hypotheses that were not mutually exclusive were examined: 1. sex-biased parasite transmission is caused by differences in immunity between sexes, with reduced male immunity leading to greater production of infective parasite stages, 2- sex-biased parasite transmission is caused by differences in host behaviour, with males more successful at disseminating parasite infective stages in areas commonly used by both sexes. Model simulations were performed using different levels of complexity and model outputs compared with results from a field manipulation of *H. polygyrus* in populations of *A. flavicollis,* previously presented by Ferrari *et al.* (2004, i.e. Chapter 4). Model simulations predicted sufficiently well the experimental field results, and both hypotheses explained the pattern observed. Males can drive the infection if their immune response decreases, and this allows a higher production of successful infective larvae. Alternatively, males can drive the infection if their behavioural characteristics allow them to spread infective larvae in areas more frequently used by the individual hosts. These results highlight the role of sex in affecting parasite transmission and suggest further directions to disentangle the intrinsic mechanism causing sex-bias in parasite transmission.

Introduction

Macroparasites tend to exhibit an aggregated distribution in the majority of their vertebrate hosts with most individuals harbouring low number of parasites and only few individuals carrying high parasites intensities (Shaw and Dobson, 1995; Shaw *et al*., 1998). Comparative studies have shown how such distributions could be generated by differences in susceptibility (including host sex, genetics, age) as well as in exposure (e.g.

habitat, climate or behaviour) to infectious stages (Wilson *et al.,* 2002). For example, an uneven spatial distribution of the free-living stages of *Hymenolepis diminuta* was found to influence the degree of parasite aggregation within host populations of flour beetle (*Tribolium confusum)* (Keymer and Anderson, 1979).

A number of empirical studies have suggested that host sex is important in generating differences in parasite intensities, with males usually carrying higher parasite intensities than females (Poulin, 1996; Wilson *et al.,* 2002). Males are also important in maintaining most of the parasite transmission, despite the parasite's distribution in the host population (Perkins *et al.,* 2003; Ferrari *et al.,* 2004 i.e. Chapter 4). In this respect, Ferrari *et al.* (2004 i.e. Chapter 4) investigated whether the transmission of the gastrointestinal parasite *Heligmosomoides polygyrus* was related to a particular sex of the host, the yellow-necked mouse (*Apodemus flavicollis*). They selectively manipulated either one of the sexes in a field experiment and found that when *H. polygyrus* was removed from yellow-necked male mice the parasite load (measured as egg production) significantly decreased in females, while when *H. polygyrus* was removed in females, they did not see any apparent effect on the parasite load in males. These results were observed in the absence of significant sex-bias in parasite load or in the number of parasite eggs expelled within the host's faeces. This is an important point that underlines two significant aspects of the dynamics of *H. polygyrus* infection in *A. flavicollis*. First, male mice drive the infection, in essence they are more efficient in transmitting parasites than females and second, this pattern does not necessarily imply sex-bias parasitism, and therefore differences in susceptibility and exposure between host sexes (Skorping and Jensen, 2004).

The mechanism responsible for sex-biased parasite transmission should seek to distinguish between the processes that make males more successful in expelling parasite eggs that have a greater success of transmission with processes that lead the males to drop

these eggs in strategic places or favourable habitats that subsequently lead to higher transmission. In this respect two biological hypotheses are proposed to explain sex-biased parasite transmission: 1- Immunity in males is less successful in fighting the parasite infection, and this leads to a higher egg hatching rate and free-living larvae survival of the male's worms, and 2- Spatial behaviour of males results in the eggs coming from their parasites have a greater transmission success (Ferrari *et al.,* 2004 i.e. Chapter 4).

In order to examine the relative contribution of these two non-mutually exclusive hypotheses, mathematical models are used to explore the dynamics of parasite infection for a macroparasite with a direct life cycle. Mathematical modelling is a valuable tool that allows us to define the conditions under which different mechanisms may operate and so identify critical experiments that can be undertaken.

The definitive model for studying host-macroparasite interactions was initially developed by Anderson and May (1978) and subsequently modified to include different levels of complexity and several biological components (Dobson and Hudson, 1992; Hudson *et al.,* 1992; Woolhouse, 1992; Roberts *et al.,* 1995; White *et al.,* 1996; Rosà and Pugliese, 2002). In particular, some models have focused on multiple host-parasite systems, either systems where one host species carries multiple parasite species, or one parasite is shared by more than one host species (Dobson, 1985; Hochberg and Holt, 1990; Schmitz and Nudds, 1994; Tompkins *et al.,* 2000; Greenman and Hudson, 2000).

In this paper, the multi-host model is modified focusing on a system where one parasite species is shared by the two sexes of one host species. The models are tailored to the population dynamics of *H. polygyrus* in the host *A. flavicollis,* and the parameters are estimated using field data (Ferrari *et al.*, 2004 i.e. Chapter 4) and published literature on *H. polygyrus* and *A. flavicollis.*

Initially, the *A. flavicollis-H. polygyrus* interaction is examined using a simple model (*Model 1*) that does not consider host sex. This model provides a baseline against which to compare the subsequent models that examined interactions between the infection processes and host sex. As such, *Model 1* was then extended to a multi-host system where the two-host compartment is represented by the two sexes of the host species (*Model 2* and *3*). *Models 2* and *3* allow for differences in immunity or behavioural characteristics between males and females hosts, respectively. Then *Models 2* and *3* were used to simulate the parasite removal of the field experiment (Ferrari *et al.,* 2004 i.e. Chapter 4), and the model predictions were compared with the field results to identify the conditions under which a single sex is responsible for the transmission of the parasite population in the absence of sex-biased parasitism.

Model descriptions

To examine the interaction between *H. polygyrus* and *A. flavicollis*, the macroparasite model, with direct life-cycle transmission following Anderson and May (1978), was applied. This is a deterministic model that assumes that time is a continuous variable and that processes of reproduction, birth and death, occur continuously. These models explore the dynamics of host population size, the adult parasite population size and the abundance of free-living infective stages. In accordance with the observed frequency distribution of *H. polygyrus* in *A. flavicollis* in Trentino (Ferrari *et al*., 2004 i.e. Chapter 4), adult parasites are assumed to be distributed among individual hosts following a negative binomial distribution, and numerically represented by the exponent *k* that gives a measure of the extent of parasite aggregation within the host population (Shaw *et al.,* 1998). Due to the high dimensionality of the multi-host models which make complete algebraic

analysis difficult (Begon and Bowers, 1995; Greenman and Hudson, 2000) the 2-sex-host-parasite system was investigated only through numerical simulations.

Model 1. No sex effect

Initially, the whole population of hosts and parasites is considered. The flow chart of this basic model is shown in Figure 1 where the various birth, death and transmission processes are summarised.

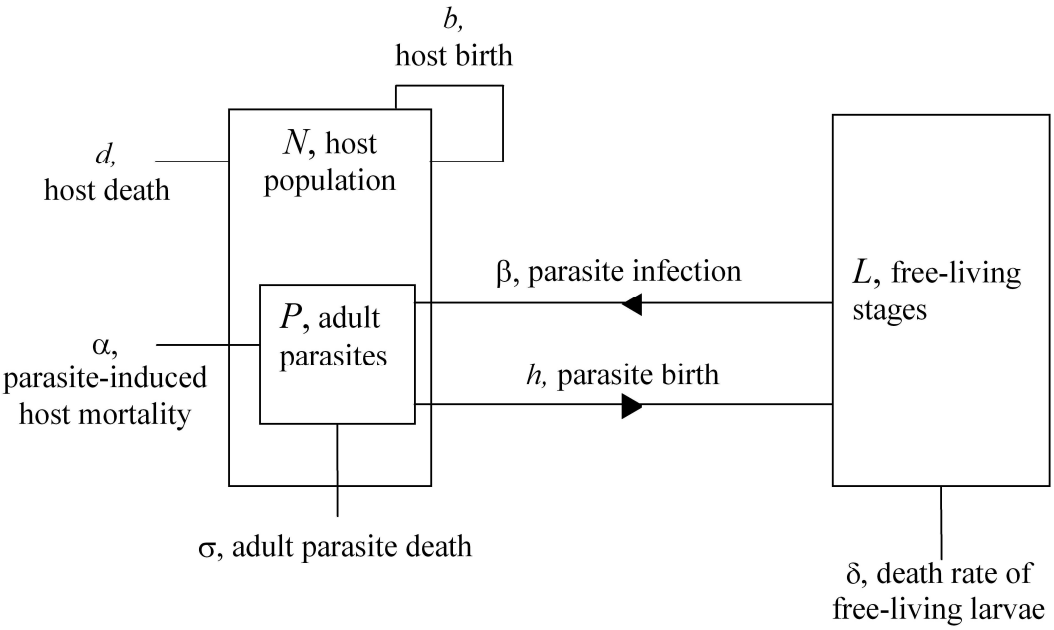

Figure 1. Flow chart of the direct life cycle of *H. polygyrus* in *A. flavicollis* population.

The resulting model consists of the three coupled differential equations:

$$\frac{dN}{dt} = N\,[b - d - (b - d)N / K - \alpha x] \qquad (1)$$

$$\frac{dx}{dt} = x\,[-\sigma - b - \alpha(x / k + 1)] + \beta\psi L \qquad (2)$$

$$\frac{dL}{dt} = hNx - \delta L - \beta NL \qquad (3)$$

A full list of parameters with their biological interpretation is given in Table. 1. The equation was derived for the mean parasite intensity $x(t)$ ($P(t)/N(t)$) rather than the total parasite population $P(t)$, since $x(t)$ is more easily related to field measurements. The host population N, in equation (1), increases with birth rate b and decreases with death rate d. A linear density dependence is assumed in the growth rate of host population with K being the *A. flavicollis* carrying capacity. The host population is reduced by the effect of the parasite on host survival (the parameter α represents the parasite-induced host mortality) while the effect by *H. polygyrus* on host fecundity is assumed to be negligible (Scott, 1990). A linear relationship between parasite burden and parasite-induced host mortality is assumed, such that the total death rate of a host, carrying i parasites, is given by $d+\alpha i$. The mean parasite burden x, in equation (2), increases with the rate of larval ingestion β, is reduced by a proportion that fail to develop into an adult stage ψ, and decreases due to the combined effects of parasite death rate σ and host death rate d, both natural and parasite-induced. Infective free-living larvae, in equation (3), are produced by adult parasites at rate h (this would be the rate of egg production multiplied by the egg hatching rate and the survival probability from egg to infecting larval stage). Free-living larvae decrease through both natural mortality δ and ingestion by hosts β.

Model 2. Two host sexes and a common free-living infective larvae pool

To examine the effect of host sex on the dynamics of *H. polygyrus*, the dynamics of male and female hosts were investigated, respectively. The host population compartment of *Model 1* was split into two compartments corresponding to males, M, and females, F. Consequently, the adult parasite pool was also split in two subpopulations: x_M, the adult parasites harboured by males and x_F those harboured by females. However, males and females are assumed to share a common free-living infective pool, L. This model allows

us to explore the consequences of different parasites dynamics in relation to host sex, such as differences in parasite production of infective larvae (*h*), and also the consequences of behavioural differences in the exposure of the two sexes to free-living infective stages (β). The life-cycle of the two parasite subpopulations sharing a common free-living infective pool is illustrated in Figure 2:

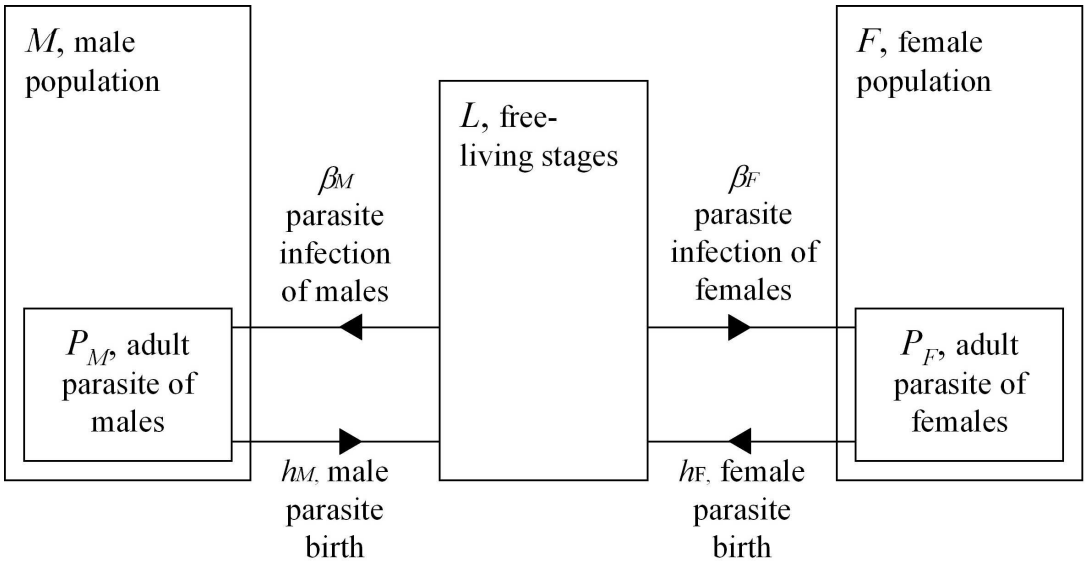

Figure 2. Flow chart of life cycle of two-host sexes and common free-living infective pool.

Model 2 is described by the following five coupled differential equations:

$$\frac{dM}{dt} = bF + M[-d_M - (b - d_M)(M + F)/K - \alpha_M x_M] \quad (4)$$

$$\frac{dF}{dt} = F[b - d_F - (b - d_F)(M + F)/K - \alpha_F x_F] \quad (5)$$

$$\frac{dx_M}{dt} = x_M[-\sigma_M - b - \alpha_M (x_M / k_M + 1)] + \beta_M \psi_M L \quad (6)$$

$$\frac{dx_F}{dt} = x_F[-\sigma_F - b - \alpha_F (x_F / k_F + 1)] + \beta_F \psi_F L \quad (7)$$

$$\frac{dL}{dt} = h_M M x_M + h_F F x_F - \delta L - \beta_M ML - \beta_M FL, \quad (8)$$

Equations (4-8) describe the temporal variations in the abundance of male and female hosts, indicated respectively with M and F, their mean adult parasite burden, x_M and x_F, and the size of the common free-living infective pool, L. Here, it is assumed that newborn hosts are proportional only to female abundance, and the sex ratio of the offspring is set to 1:1. While more complex functions could be used, relating the number of newborns to the abundance of total adults individuals (Iannelli, *et al.,* 2005), this choice is more relevant to the parasite system and the problem considered in this study. All other aspects concerning the dynamics of male and female hosts (M and F) and their mean parasites burden (x_M and x_F) are modelled following *Model 1* but taking into account the sex specific parameters. For the common free-living pool the gain terms are estimated as the sum of the production of infective larvae by male and female hosts, h_M and h_F, and the losses are due to the ingestion of larvae by both sexes, β_M and β_F. In the two-host-sexes model the number of parameters in *Model 2* is doubled respect to *Model 1* except for host birth rate and the death rate of common free-living larvae (Tab. 1).

Model 3. Two host sex and two free-living infective pools

In the final model, a further distinction in parasite transmission is introduced where two separate compartments of free-living infective larvae are considered and they correspond to the larvae produced by adult parasite harboured by males, L_M, and females, L_F respectively (Fig. 3).

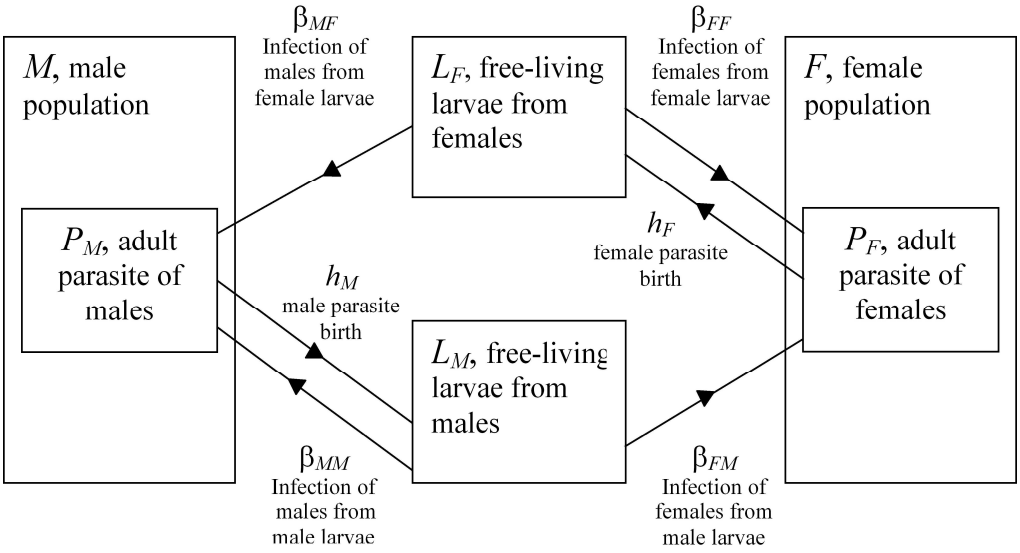

Figure 3. Flow chart of life cycle with two-host sexes and two free-living infective pools

The resulting model is defined by six coupled differential equations describing the changes in male and female hosts, their mean adult parasite burden, and the free-living stages produced by the hosts of different sex (L_M, L_F).

$$\frac{dM}{dt} = bF + M[-d_M - (b - d_M)(M + F)/K - \alpha_M x_M] \quad (9)$$

$$\frac{dF}{dt} = F[b - d_F - (b - d_F)(M + F)/K - \alpha_F x_F] \quad (10)$$

$$\frac{dx_M}{dt} = x_M[-\sigma_M - b - \alpha_M(x_M/k_M + 1)] + \beta_{MM}\psi_M L_M + \beta_{MF}\psi_M L_F \quad (11)$$

$$\frac{dx_F}{dt} = x_F[-\sigma_F - b - \alpha_F(x_F/k_F)] + \beta_{FF}\psi_F L_F + \beta_{FM}\psi_F L_M \quad (12)$$

$$\frac{dL_M}{dt} = h_M M x_M - \delta_M L_M - \beta_{MM} M L_M - \beta_{FM} F L_M \quad (13)$$

$$\frac{dL_F}{dt} = h_F F x_F - \delta_F L_F - \beta_{FF} F L_F - \beta_{MF} M L_F, \quad (14)$$

The dynamics of male and female hosts is modelled as in *Model 2*. Mean parasite intensities for each host sex (x_M, x_F) increase according to the sum of ingested larvae from each larval compartment (L_M, L_F). When the ingestion rate of the free-living larvae from

the two different infective pools is equal for males and females ($\beta_{MM}=\beta_{FM}$, $\beta_{FF}=\beta_{MF}$) the *Model 3* behaves exactly as *Model 2*.

Parameter estimation

Most of the parameters for the model simulations are derived from the field experiment by Ferrari *et al.* (2004 i.e. Chapter 4) and unpublished data on population dynamics of *A. flavicollis* in Trentino (Italy). The mean lifespan of *A. flavicollis* is considered to be 270 days and number of offspring is assumed to be six per year so the daily host mortality *d* is 0.0037/day and host birth rate *b* 0.00821/day. The observed sex ratio in the field is close to 1:1 and here it is assumed it to be 1:1. The carrying capacity *K* is set to 50 mice per hectare according to the maximum density observed during the field experiment (unpublished data).

The adult *H. polygyrus* life span in *Apodemus* spp. is assumed to be 76 days, and the mortality rate (σ) as 0.013/day after Gregory *et al*. (1990). The lifespan of infecting larvae L_3 is assumed to be 2 months with a mortality rate (δ) of 0.016/day for Trychostrongilidae worms (Dobson and Hudson, 1992; Fernàndez *et al.,* 2001). Laboratory experiments estimated the parasite-induced mortality by a single parasite on a host (α) to be 0.00024/day (Keymer and Hiorns, 1986). The proportion of infective free-living stages that develop to adult stages (ψ) in laboratory mice varies from 80% to 8%, the lowest values is selected since wild mice show higher resistance to infection (Slater and Keymer, 1988; Enriquez *et al.*, 1988; Gregory *et al.,* 1990). *H. polygyrus* exhibit an aggregated distribution in *A. flavicollis* natural populations that do not significantly differ from a negative binomial distribution (deviance=63.5, df=104, *P*=0.9, *k*=0.36) (Chapter 1), this aggregated pattern justifies the model assumptions.

Uncertain parameters

Sensitivity analysis is used to estimate the unknown parameters of larval ingestion (β) and fecundity rates (*h*) in *Model 1* to show how the variables of the system, such as parasite intensity ($\bar{x}$) or mice density ($\overline{N}$), are sensitive to the progressive changes of the unknown parameters.

Specifically, according to *Model* 1 (see Appendix), the equilibrium density ($\overline{N}$) of the hosts depends only on the carrying capacity (*K*), the parasite-induced host mortality (α), the maximum host growth rate (*b*-*d*) and the average parasite load ($\bar{x}$), while indirectly it is affected by the larval ingestion (β) and fecundity rates (*h*). Based on the independent parameter estimates, the predicted value for $\overline{N}$ is 23.3 mice/ha, very close to the observed value. An equation for the equilibrium of the mean parasite burden ($\bar{x}$) (see Appendix) where we know all the parameters values except β and *h* is then generated. The solutions of equation A2 (see Appendix), using the observed value of 10 worms per mice (Rosso *et al.*, 2002), are shown in Figure 4.

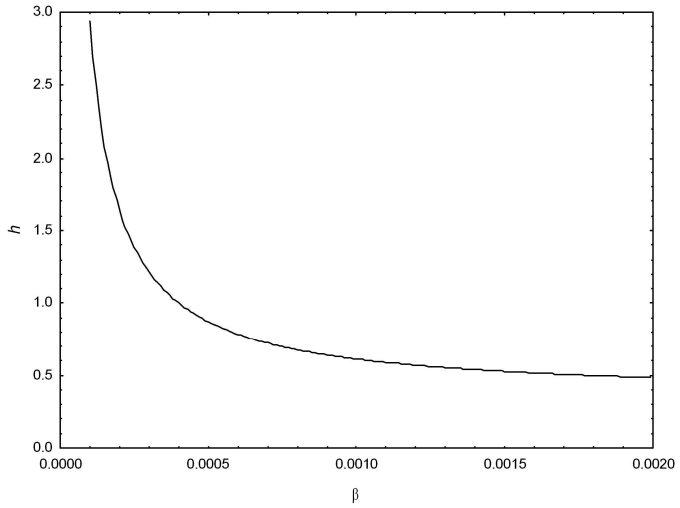

Figure 4. Values of *h* as a function of the parameter β (see eq. A2 in Appendix) needed to fit a mean parasite burden of 10 worms/mice

Based on other studies on *H. polygyrus* infection (Tanguay and Scott, 1992), simulations are based on the values of 0.0004 for β and 1 for *h*. To check the robustness of this choice the sensitivity of parasite intensity ($\bar{x}$) to the selected parameters values are evaluated. All the other parameters are held at the fixed values shown in Table 1 while β and *h* are alternatively allowed to vary. Figure 5 shows the changes in mean parasite intensity by varying β (Fig. 5 A) and *h* (Fig. 5 B).

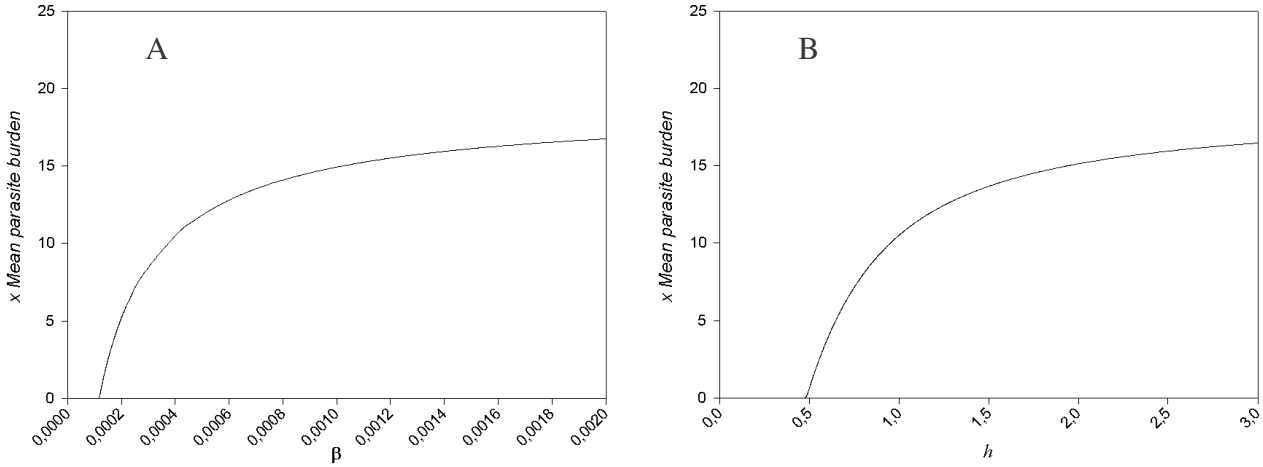

Figure 5. Sensitivity analysis on β and *h* over mean parasite burden. When β varies and *h*=1 (A), when *h* varies and β=0.004 (B). The parameter values are fixed as in Table 1

The full list of parameter values is given in Table 1 where the time is measured in days and densities in hectares^{-1} (ha^{-1}).

Simulation of host-parasite interaction, using *Model 1* and the parameter values in Table 1, in order to check their reliability, exhibit damped oscillations for host and parasite populations before reaching a stable coexistence at the equilibrium (Fig. 6).

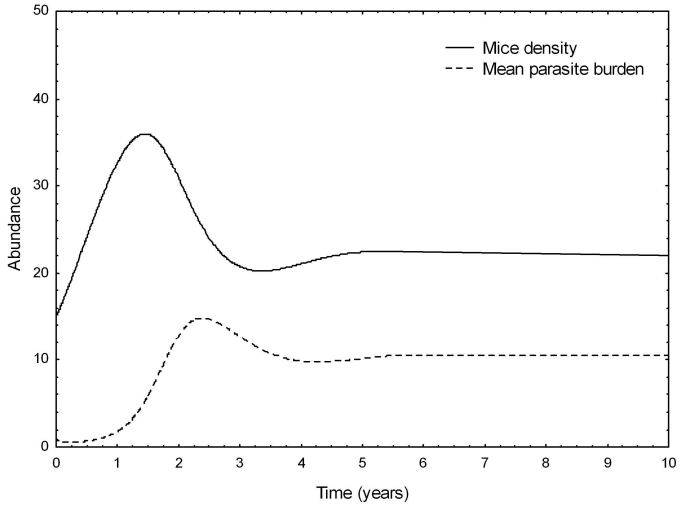

Figure 6. Simulations for the whole mice population and their mean parasite burden. The parameter values are from Table 1.

Table 1. Numerical values and biological interpretation of population parameters for *A. flavicollis* and *H. polygyrus*.

Parameter	Value (units)	Description
N (F,M) *	Variable	Size of host population
x	Variable	Mean adult parasite burden
L	Variable	Number of free-living infective larvae
K	50	Host population carrying capacity
*d**	$3.7 \cdot 10^{-3}$ (day $^{-1}$)	Instantaneous death rate of host due to all causes except parasite
b	$8.21 \cdot 10^{-3}$ (day $^{-1}$)	Instantaneous birth rate of hosts
σ*	$1.3 \cdot 10^{-2}$ (day $^{-1}$)	Instantaneous death rate of adult parasite
*h**	1 (day $^{-1}$)	Instantaneous rate of production of infective parasite larvae
ψ*	$8 \cdot 10^{-2}$	Proportion of ingested infective larvae that develop to the adult stages
α*	$2.4 \cdot 10^{-4}$ (worm^{-1} day $^{-1}$)	Instantaneous death rate of host due to parasite
β*	$4 \cdot 10^{-4}$ (host $^{-1}$ day $^{-1}$)	Instantaneous rate of ingestion of free-living infective larvae
*k**	0.36	Aggregation parameter of the Negative Binomial distribution (for *H. polygyrus* in *A. flavicollis)*
δ*	$1.6 \cdot 10^{-2}$ (day $^{-1}$)	Instantaneous death rate of infective free-living larvae

$^{*}_{M, F}$ in *Model 2* and *3* refer to parameter values for specific host sex

Simulations

To explore the two hypotheses: 1- male immunity is less successful and this results in a higher egg hatching rate, coupled with increased free-living larvae survival, and 2- male behaviour influences the dissemination of parasite infective stages, leading to increased rates of infection, mathematical simulations are used assuming that the rate of production of infective larvae (h) or the ingestion rate of infective larvae (β) varied between sexes while all the remaining parameters are assumed constant.

Differences in immunity between sexes

Initially the condition where changes in immunity with host sex affect the hatching rate of parasite eggs and the survival of the free-living larval stage are simulated. Specifically, eggs expelled by males have a higher hatching rate and larval survival than eggs expelled by females (i.e. $h_M > h_F$). The parameter values of h_M and h_F are chosen in order to obtain, in natural conditions, equilibrium values similar to those from *Model 1*. Simulations based on *Model 2* show that the *H. polygyrus-A. flavicollis* system reaches, through damped oscillations, a stable equilibrium with no sex-bias in parasite burden and abundance between the two host sexes (Fig. 7).

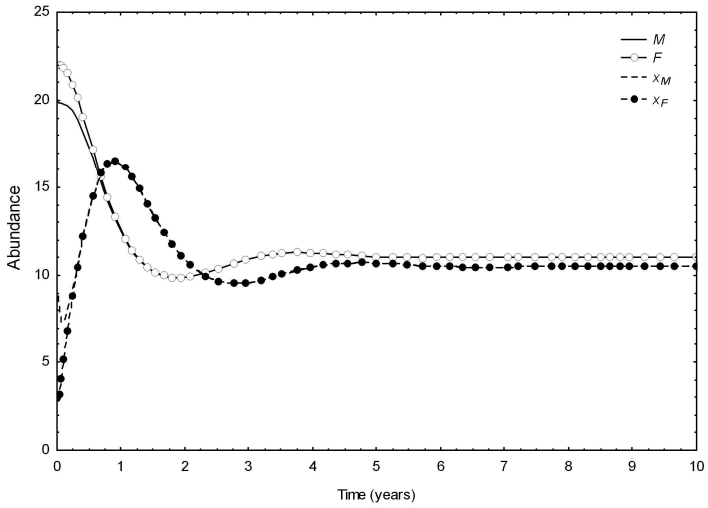

Figure 7. Temporal dynamics of the mouse population and mean parasite intensity in natural conditions (i.e. no treatment is applied) according to *Model 2* when males (—) and females (-○-) mice share a common free-living larvae pool and parasites harboured by males (- - -) have higher eggs hatching rate and free-living larvae survival than those in females (-●-) (i.e. h_M=1.67, h_F=0.33). When four lines are not visible this due to overlap of male and female lines and overlap of male and female parasite lines. Parameters values are those of Table 1.

The selective reduction of parasite burden in each sex is then simulated following the experimental protocol of Ferrari *et al.* (2004 i.e. Chapter 4), by increasing, in males or females respectively, the instantaneous death rate of adult parasites (i.e. σ) by fifty fold, that represent a reduction in survival sufficient to decrease the parasite intensity, but at less than 100% efficient as observed by Ferrari *et al.* (2004 i.e. Chapter 4). Changes in parasite intensity in the non-treated sex are then monitored. In this case the simulations are run for a short period (one year) in order to have the same time-scale of the field experiment, that was carried out in six months (Ferrari *et al*., 2004 i.e. Chapter 4).

When the simulated anthelmintic treatment is applied to female mice, parasite intensity decrease slightly in males and decrease almost to zero in treated females (Fig. 8 A). A strong increase in female abundance is observed as a result of the sharp decrease in parasite intensity and, consequently a reduction in parasite-induced host mortality (Fig. 8 A). Counter to this, when the anthelmintic treatment for male mice is simulated, parasite

intensity decreases progressively in the untreated females to almost zero in the treated males (Fig. 8 B). Moreover, host abundance increase but the effect is less apparent than when females are treated, this is because of the assumption that birth rate depends only on female density.

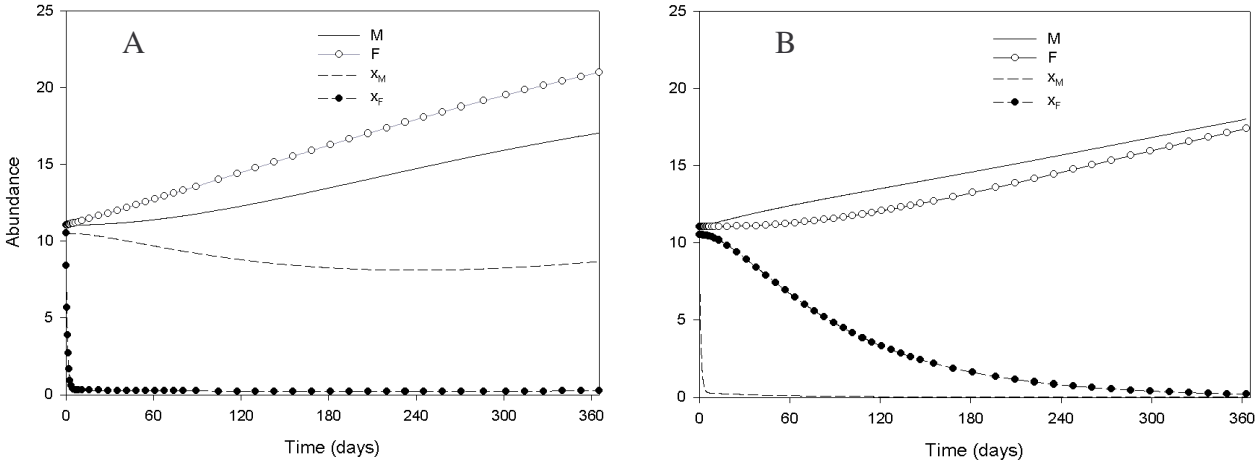

Figure 8. *Model 2: s*imulations of the effect of selective anthelmintic treatment by host sex. Mice abundance and mean parasite intensity are reported. Males hosts are assumed to have higher eggs hatching rates and free-living larvae survival and sexes are assumed to share a common free-living larvae pool(h_m= 1.67, h_f =0.33). Anthelmintic treatment is applied to females (A) and males (B). Male mice numbers are labelled as (M: —) and females as (F: -○-) while mean parasites intensities of males as (x_M: - - -) and those of females as (x_F:-●-). When four lines are not visible this due to overlap of male or female parasite lines upon Time axes.

Differences in host behaviour between sexes

Using *Model 2,* sex-biased differences in host behaviour are investigated at different rate of ingestion of free-living larval stage for each sex (i.e. different β). In this case, the model predicts a sex-bias in parasite intensity and host abundance (Fig. 9), which is not observed in the experimental results of Ferrari *et al.* (2004 i.e. Chapter 4).

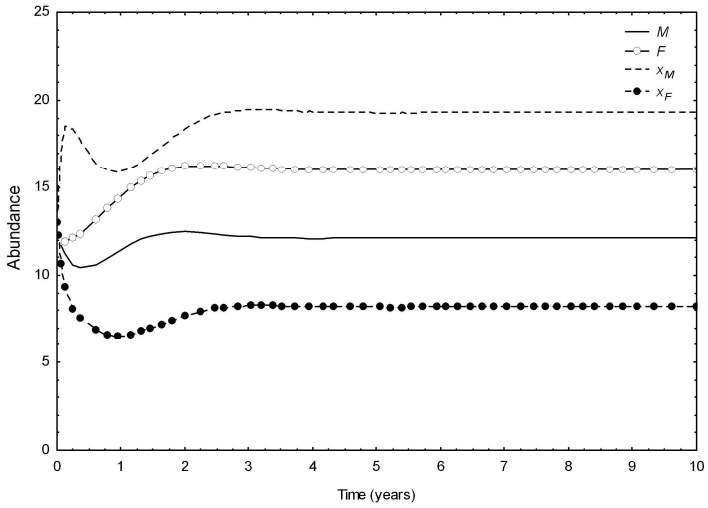

Figure 9. Temporal dynamics of mouse population abundance and mean parasite intensity (i.e. no treatment applied) according to *Model 2* where host sexes share a common free-living larvae pool and the encounter rate between hosts and free-living stages for males is higher than for females (β_M=0.0006 > β_F=0.0002). The other parameters values are those of Table 1. Male mice abundance is labelled as (M: —) and female as (F: -○-) while mean parasites intensities of males as (x_M:- - -) and those of females as (x_F: -●-).

To mimic the condition where males mainly contribute to the transmission of parasites in the host population but no sex-bias in parasite burden is taking place, simulation from *Model 2* to *Model 3* are carried out where the free larvae ingestion rates are set higher in the larval compartment of males hosts than in the larval compartment of females hosts (i.e. $\beta_{MM}>\beta_{MF}$ and $\beta_{FM}>\beta_{FF}$) while keeping the overall larval intake equal for the two sexes (i.e. $\beta_{MM}+\beta_{MF} = \beta_{FM}+\beta_{FF}$).

This inequality may cause a symmetrical contribution of the two larval compartments (i.e. $\beta_{MM}=\beta_{FM}$ and $\beta_{FF}=\beta_{MF}$) to each sex. However, under natural conditions it is likely that females are exposed more to their own infective pool ($\beta_{FF} >\beta_{MF}$) and males to their infective pool ($\beta_{MM}>\beta_{FM}$) despite the overall contribution of the male larval compartment being the main pool (i.e. $\beta_{MM}>\beta_{MF}$ and $\beta_{FM}>\beta_{FF}$). In the absence of estimates of the free-living larvae ingestion rates arbitrary values are set: β_{MM}= 0.0007 host $^{-1}$ day $^{-1}$, β_{MF}=0,0001 host $^{-1}$ day $^{-1}$, β_{FF}= 0.0002 host $^{-1}$ day $^{-1}$, β_{FM}=0.0006 host $^{-1}$ day $^{-1}$. These βs

are selected to obtain, under natural conditions, similar equilibrium values to those seen in *Model 1,* hence, when no treatment is performed, the system reaches a stable equilibrium with no significant sex-biased parasitism or differences in host abundance (Fig. 10).

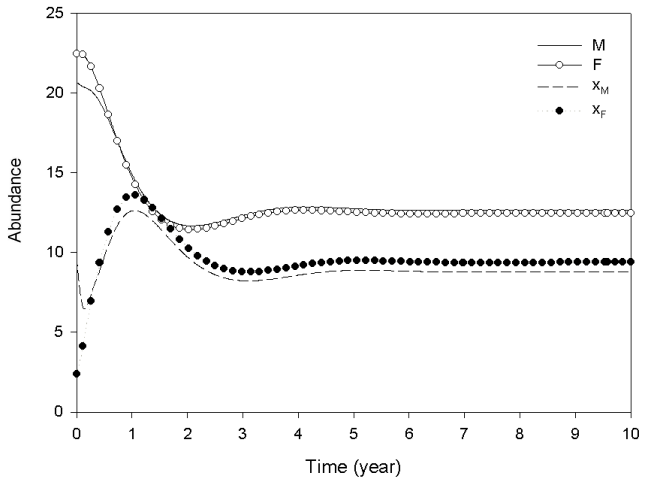

Figure 10. Temporal changes for mouse population abundance and mean parasite intensity (i.e. no treatment is applied) according to *Model 3* when the free-living infective larvae are split in two subpopulations and mice are more exposed to larvae coming from male mice (β_{MM}= 0.0007, β_{MF}=0.0001, β_{FF}= 0.0002, β_{FM}=0.0006). Male mice abundance is labelled as (M: —) and female as (F: -○-) while mean parasites intensities of males as (x_M:- - -) and those of females as (x_F: -●-).

The simulations of the anthelmintic treatment show that when *H. polygyrus* is removed from female mice, the parasite intensity decreases moderately in the non-treated males and decreases almost to zero in females (Fig. 11 A); on the other hand, when *H. polygyrus* is removed from males, parasite intensity decreases considerably in females (Fig. 11 B). Interestingly, host abundance increases in anthelmintic treated males and females.

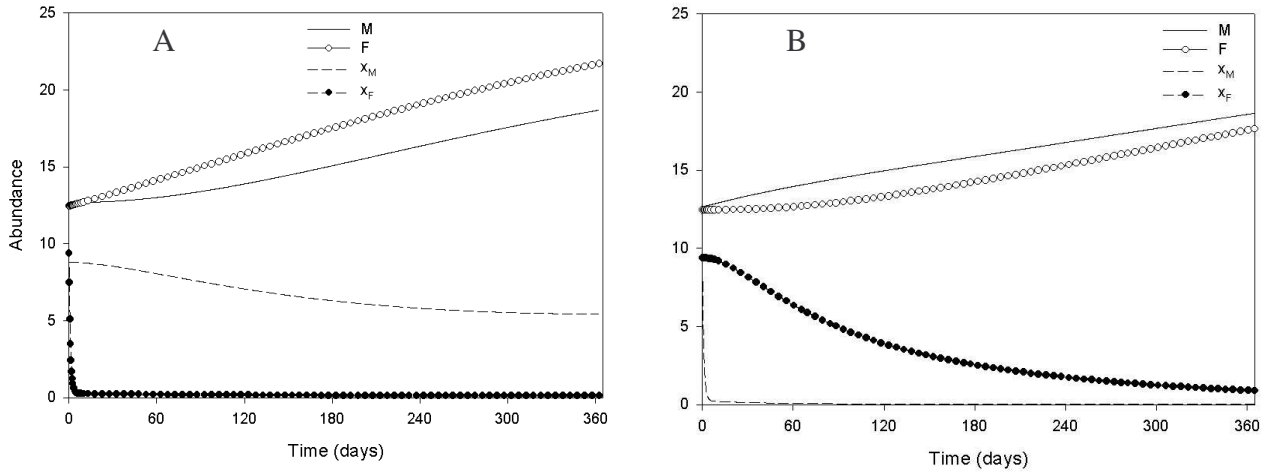

Figure 11. *Model 3* simulations of the effects of anthelmintic treatment on mice abundance and mean parasite intensity when the free-living infective larvae are divided into two groups and mice are more exposed to those coming from males (β_{MM}=0.0007, β_{FM}=0.0001, β_{FF}=0.0002, β_{MF}=0.0006). Anthelmintic treatment is applied to females (A) and males (B). Male mice abundance is labelled as (M: —) and female as (F: -○-) while mean parasites intensities of males as (x_M:- - -) and those of females as (x_F: -●-). When four lines are not visible this due to overlap of male or female parasite lines upon Time axes.

Finally, *Model 3* is run assuming sex differences in both the egg hatching rate and free-living larvae survival and in mouse behaviour and it is evidenced that males increase their role only slightly in driving parasite infection relative to when a single mechanism is considered (i.e. *Model 2* or *Model 3*, respectively). Females confirm their secondary role showing that when their parasites are removed the infection of males do not depend on the rate they ingest the free-living infective larvae (i.e. β's).

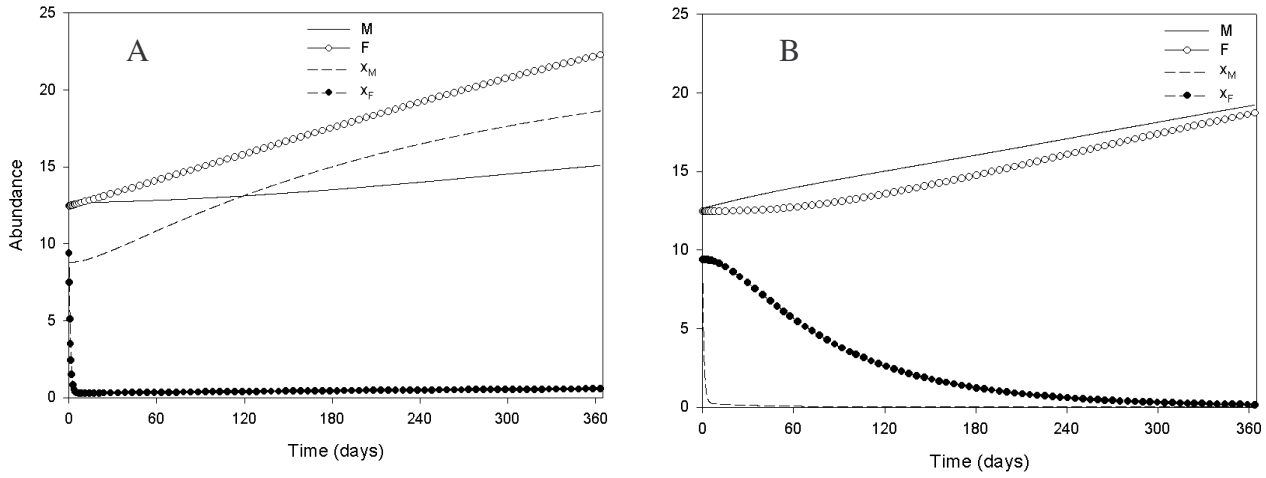

Figure 12. *Model 3* simulation of the effect of anthelmintic treatment on mice abundance and mean parasite intensity when the free-living infective larvae are divided in two groups, males have a higher rate of production of infective larvae. (h_m=1.67, h_f =0.33) and mice are more exposed to larvae coming from males (β_{MM}=0.0007, β_{FM}=0.0001, β_{FF}=0.0002, β_{MF}=0.0006). Anthelmintic treatment applied to females (A) and applied to males (B) to males. Male mice abundance is labelled as (M: —) and female as (F: -○-) while mean parasites intensities of males as (x_M:- - -) and those of females as (x_F: -●-). When four lines are not visible this due to overlap of male or female parasite lines upon Time axes.

Discussion

The previous study empirically showed that male *A. flavicollis* drive *H. polygyrus* infection while females do not seem to have a relevant impact (Ferrari *et al.,* 2004 i.e. Chapter 4). In this study biological mechanisms causing a sex-bias in parasite transmission, was examined using a modelling approach and two not mutually exclusive hypotheses were compared: 1- sex-biased parasite transmission is caused by differences in immunity between sexes or 2- is due to behavioural differences between males and females. Model simulations predicted sufficiently well the experimental field results however both hypotheses provided some explanation, and to account for the patterns observed it is necessary to postulate two distinct larval spatial arrangements.

An increasing number of studies have identified the role of gender in affecting parasite dynamics, however little evidence exists on whether sex can influence other traits of host-parasite interactions. Field studies have provided evidence that gender influences parasite intensity and transmission, with males being responsible for driving the infection (Perkins *et al.,* 2003; Ferrari *et al.,* 2004 i.e. Chapter 4). While it seems likely that the hosts that harbour more parasites are also the individuals with higher parasite transmission rates, the field manipulation of *H. polygyrus* in *A. flavicollis* did not identify a sex bias in parasite load, suggesting that the mechanism that affects parasite transmission must reside in sex difference in host-parasite interaction rather than quantitative parasite load differences (Skorping and Jensen, 2004).

Sex differences in immune responses of the host can affect the parasite's egg hatching rate or the survival of infective stages (Finkelman *et al.,* 1997). In such conditions even if males and females harbour the same number of parasites and expel the same number of eggs per nematode, the qualitative/quantitative contribution to successful infective stages will be greater if they arise from hosts with a weak immune response. For example, males with reduced immunity, induced by testosterone, can distribute more successful free-living infective stages (Folstad and Karter, 1992). Model simulations suggest that the contribution of males to the pool of free-living infective stages is more apparent that the contribution from females.

Sex differences in behaviour may also have an important role and influence the spatial distribution of infective free-living larvae. For example, male territorial behaviour may lead them to defecate in more exposed areas that mark a well defined territory, or have more contacts with a large number of females (Ims, 1987). In contrast, females may have more hygienic habits and tend to defecate away from the nesting areas or interact only with a small number of males (de Mendonça, 2003). The results of these behaviours

can be theoretically represented by two distinct free-living infective pools, which differ in their origin, from males or females, and the encounter probability, where faeces from males are more exposed that those from females. These simulations suggest that once parasites are removed from males there is a reduction in the infective larval pool and, consequently, the probability for a female to encounter the more exposed males' faeces decreases. Vice-versa, this does not seem to occur when parasites are removed from females since their faeces are spread in areas that are not used or rarely used by the host population.

While quantitative comparisons can not be done between simulation outputs and field experiment results without more accurate parameter estimation, these simulations allowed us to investigate the potential mechanisms responsible for sex bias in parasite transmission. The comparison of patterns predicted with those observed clearly suggests that sex bias in parasite transmission is not just related to an overall difference in host susceptibility/exposure. In fact simulations with no free infective larvae sub-pools generated a bias in parasite intensity that was not observed in the field experiment (*Model 2*, Fig. 9). Moreover, the discrepancy from model prediction on anthelmintic treatment and field results on the mice sex ratio underlines the field limits in detecting any sex ratio changes in case they occurs. In fact this discrepancy while could refute the model prediction, at the same time could be ascribed to the inability of the field condition to reveal change in mice abundance since animals were free to move and thus reproduction strategies may have balanced the sex ratio (Ims, 1987)

As it has been pointed out previously the two proposed mechanisms are not mutually exclusive and in natural conditions it is likely they may occur together. Moreover, it is possible that other mechanisms may have been involved beside those analysed here. For example, these models assume that host sexes do not differ in their biological traits except

for transmission rates (β) and parasite fertility (h). However, in wild conditions sexes may differ in their demographic traits, such as different lifespan, or mechanism of host-parasite interaction, such as parasite-induced host mortality, or differences in the proportion of larvae that fully develop to adult parasites or even differences in adult parasite lifespan. While these aspects have been deliberately omitted in the modelling, in order to focus on host-parasite interactions, preliminary simulations suggest that their introduction would cause a sex-bias in parasite load, which again has not been observed in the field experiment (Ferrari *et al.,* 2004 i.e. Chapter 4). In addition, these models do not include other important biological aspects, such as seasonality in the force of infection or host reproduction that is known to have an important effect on the dynamics of *A. flavicollis* and *H. polygyrus* (Gregory, 1992). These flexible models can be easily modified to add these further levels of complexity in the future.

In conclusion, model simulations suggest that sexes can differ in their ability of spread the free-living parasite stages either through changes in host immunity or behaviour. This study is a further step towards an understanding of the mechanisms causing sex-biased parasite transmission and suggests further experimental directions.

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Appendix

The values of *N*, *x* and *L* at an equilibrium point of *Model* 1 were studied. Setting the right hand sides of (1)-(3) equal to 0, we obtain:

$$N = K[1 - \alpha x/(b-d)]$$

$$\beta\psi L = x(\sigma + b + \alpha(x/k + 1)]$$

$$L = hNx/(\delta + \beta N)$$

The first equation yields *N*, once *x* is known. Substituting the third into the second, and using *N* as given by the first, one obtains the following equation:

$$\beta\psi hK[1 - \alpha x/(b-d)] = [\sigma + b + \alpha(x/k + 1)][\delta + \beta K(1 - \alpha x/(b-d))], \qquad \text{(A1)}$$

from which the equilibrium value of *x* can be found, hence those of *N* and *L*.

Going in the other direction, if we know the equilibrium values of (1)-(3), the parameter values have to be such that equation (A1) is satisfied.

In particular, if we wish to estimate β and *h*, we find:

$$h = \frac{[\sigma + b + \alpha(x/k + 1)][\delta + \beta K(1 - \alpha x/(b-d))]}{\beta\psi K[1 - \alpha x/(b-d)]}. \qquad \text{(A2)}$$

Chapter 6

General Discussion: Parasite heterogeneities, from causes to consequences

In the general introduction to this thesis the importance in assessing the differences that characterise the host-parasite systems and their role in affecting host and parasite dynamics has been underlined. Empirical studies have provided evidence that the macroparasite distribution within a host population is aggregated in a few heavily infected individuals and such a pattern must be the result of a combination of host characteristics and extrinsic factors (Shaw *et al.*, 1998; Wilson *et al.*, 2002). Theoretical formulations based on the analysis of different host-parasite systems suggest that pathogen transmission is driven by a restricted group of hosts (Woolhouse *et al.,* 1997). The aggregated parasite distribution within the host population, coupled with the uneven contribution of individuals to parasite transmission may produce non-linearities in the host-parasite interaction, which may in turn, have large effects on the dynamics of infection. Therefore, the identification of such components and how they act on a host-parasite system is crucial if we wish to prevent the spread of an infection in a population or if we need to control disease.

In this thesis the interaction between a host and its parasites has been examined, with the aim of identifying the most important intrinsic and extrinsic factors and how they affect parasite load and transmission. To address these topics the investigations were focused on the host, the yellow-necked mouse *Apodemus flavicollis,* and its most common gastrointestinal parasite the nematode, *Heligmosomoides polygyrus*. This parasite infects a broad spectrum of wild hosts and has been extensively used in laboratory and field studies, providing an extensive knowledge about its biology and epidemiology (Slater and Keymer, 1986a; 1986b; Keymer and Hiorns, 1986; Lewis, 1987; Scott and Lewis, 1987; Montgomery and Montgomery, 1989; Abu-Madi *et al.,* 1998; Gregory *et al.*, 1990; Montgomery and Montgomery, 1990; Scott, 1990; 1991; Behnke *et al.,* 1991; Gregory, 1991; Keymer and Tarlton, 1991; Gregory,1992; Gregory

et al., 1992; Monroy and Enriquez, 1992; Quinnell, 1992; Tanguay and Scott, 1992; Behnke *et al.*,1999; Abu-Madi *et al.*, 2000; de Bellocq, 2003). For instance, we know its effects on host population dynamics (Scott, 1990; Gregory, 1991; Quinnell, 1992) and its immunological properties (Keymer and Tarlton, 1991; Monroy and Enriquez, 1992; Behnke *et al.*, 1993; Telford *et al.,* 1998).

In the north-eastern Italian Alps, the area where the field studies are based, the most abundant host of *H. polygyrus* is the yellow-necked mouse, a small mammal usually characterised by high densities and short life span that lead to population dynamics with rapid turn over (Locatelli and Paolucci, 1998). The features of the nematode *H. polygyrus* and the small mammal *A. flavicollis* make this host-parasite system a suitable model for investigations of host-parasite interaction and the mechanisms driving the dynamics of infection.

To investigate the factors influencing this system were used data collected from extensive and intensive field monitoring, as well as from field manipulation experiments. These approaches allowed to identify patterns occurring in nature, but also allowed to perturb the system and test the hypotheses previously defined.

A number of factors have been identified to act on the *A. flavicollis-H. polygyrus* system. In the first instance (Chapter 2) a number of relevant spatial differences have been identified to affect parasite community structure among *A. flavicollis* populations sampled in different areas of the Trentino province. The differences were apparent at each parasite level investigated: the parasite species, infra-community and component community, suggesting that both environmental and host characteristics were affecting the pattern observed.

Despite the recognition of spatial differences, which characterise several rodents' parasite communities, the role of host characteristics was also identified. Host age, sex

and host breeding condition were particularly influential in shaping the parasite community of a single mouse population. As Haukisalmi and Hentonnen (1999) pointed out, the parasites' aggregated distribution is more imputable to individual differences in host exposure and susceptibility rather than to spatial diversity. The consequences of these observations are particularly meaningful because they highlight the key role of host characteristics in parasite dynamics, in particular their role in driving the infection at the population level. The group of hosts holding the majority of parasites in the host population was identified, and these highly infectious individuals are more exposed/susceptible to parasite infections and parasite transmission. From a management point of view, these individuals represent the group on which we should focus parasite control strategies. In contrast, differences in local factors have a higher probability in promoting dissimilarities between populations from different sites.

Of particular importance was how the extensive monitoring confirmed (Chapter 2) that *H. polygyrus* is the most abundant parasite species in this part of North-Eastern Alps as observed in other parts of Europe and in other rodents species (Haukislami *et al.*, 1988; Montgomery and Montgomery, 1989; 1990; Abu-Madi *et al.*, 1998; Behnke *et al.*, 1999; Abu-Madi *et al.*, 2000; Behnke *et al.*, 2001b; de Bellocq *et al.*, 2003; Milazzo *et al.,* 2005). The dominant role of *H. polygyrus* and its recognised immuno-modulatory properties stimulated a more accurate investigation of the occurrence of host-mediated parasite interactions (Keymer and Tarlton, 1991; Monroy and Enriquez, 1992; Behnke *et al.*, 1993; Telford *et al.,* 1998). Indeed, there is growing evidence that parasite interactions are often mediated by the host immune response (Behnke *et al.*, 2001a; Cox, 2001). Moreover, the recognition that parasite interactions affect host susceptibility to secondary infections stimulated further the study of how *H. polygyrus* affects concomitant infections (Poulin, 2001). A field experiment was carried out where *H. polygyrus* load in

free-ranging mice was manipulated (Chapter 3). The aim of this experiment was to test whether *H. polygyrus* immune characteristics affected the infestation of the ectoparasite *Ixodes ricinus*, and whether such interaction was synergistic or antagonistic. The results found that high *H. polygyrus* burdens were associated with low *I. ricinus* infestation. This pattern was apparent both in the extensive field monitoring and in the intensive experimental manipulation.

While no clear conclusion could be drawn on the immunological host-mediated mechanisms generating this pattern, the principal outline from this result is the demonstration of how parasite interactions may influence the host susceptibility to concomitant infections proving that parasite infections represent a further source of variability in host-parasite interactions. In particular, it is important to note that the strength of parasite interactions differed with host age and breeding condition, suggesting that in specific groups of individual hosts, like young and non-breeding mice, *H. polygyrus* have a stronger influence on *I. ricinus* infestation. This result implies that the *I. ricinus* infestation on mice can be affected by co-infection by other parasites rather than simply host characteristics and environmental factor (Randolph and Storey, 1999; Perkins *et al*., 2003).

The observation that differences among *A. flavicollis* hosts affect *H. polygyrus* infection leads to the question: what is the restricted group of hosts that are responsible for the maintenance of parasite infections? Sex of the rodent was selected as host factor since it is known to affect host susceptibility to infection (Schalk and Forbes, 1997; McCurdy *et al.,* 1998; Moore and Wilson, 2002). An extensive review among host and parasite taxa identified a general male bias in parasite rates (Poulin, 1996). However, what could be the consequences of such bias on parasite transmission remains unresolved (Poulin 1996; Wilson *et al.,* 2002). A field experiment was carried out where the *H.*

polygyrus intensity was manipulated in a wild population of mice testing the hypothesis that males, rather than females, were responsible for driving parasite transmission rate (Chapter 4). The experimental selective removal of *H. polygyrus* from each sex provided evidence that while the parasite burden in females did not affect the burden in males, the removal from males significantly decreased the parasite intensities of females. Importantly, it must be remarked that this result was obtained in the absence of a large sex-bias in parasite load, suggesting that the pattern did not emerge as a consequence of quantitative sex differences in parasite production. This empirical result generated two considerations concerning the role of host sex on parasitism. First, the new observation that males drive the parasite transmission has added to the currently well-known differences in susceptibility between sexes, and stresses the importance of the sexes in their ability to transmit/maintain parasite infection. Second, this result raises the point that we now need to examine which mechanisms generate such a pattern: is this pattern the consequence of differences in behaviour between sexes or is it because of physiological (hormones, immune response, etc) mediated effects upon host-parasite interactions?

To explore the possible mechanisms that make males such an important group of hosts in driving a parasite infection, two hypotheses were analysed through mathematical modelling. Two non-mutually exclusive hypotheses were tested (Chapter 5): sex bias parasite transmission is caused by 1- differences in behavioural characteristics between males and females and 2- differences in the immune response between the two sexes. The models were based on the field experiment previously carried out and described in Chapter 4. Models parameters were based on data obtained from the field manipulation experiment (Chapter 4) and the relative fit of the model was tested by comparing the predictions from both hypotheses with the results observed in the field experiment. The simulations were not conclusive, in that the results could not disentangle the relative role

of the two mechanisms. In some respect both outcomes can potentially occur in wild populations of mice; however this study provided useful suggestions. For example, each sex may have different ways of quantitatively and qualitatively depositing their faeces, which may result in differences in the spatial distribution of free-living infective stages. In addition, researchers have suggested that the variation of infective stages at the small spatial scale influence the host's exposure experience (Keymer and Anderson, 1979). As such differences in sex behaviour and physiological characteristics may have promoted the small-scale heterogeneous spatial assemblage of infective stages causing the observed sex-bias in parasite transmission.

This thesis investigated the role of environmental and host characteristics in influencing parasite dynamics and host-parasite interactions. In particular it recognises the important role of host sex and parasite interactions in the context of spatial and temporal variability in host dynamics. Studies on host-parasite interactions are usually based on comparative approaches. They provide broad information of the extent and pattern of the aggregation of parasites and how this is related to host and extrinsic factors. In contrast, we have relatively poor information on the epidemiological consequences of such aggregation. The path to understand the fundamental mechanisms of such variation in host-parasite dynamics is not easy. Comparative analysis is unlikely to prove sufficient to address the issues and more structured protocols involving long-term monitoring or experimental perturbation of the system are needed, properly supported by mathematical simulations. It is apparent that future development in understanding host-parasite interactions should go through a more detailed understanding of the consequence of such mechanisms if we are to understand the dynamics of infection.

For instance, the evidence of parasitological spatial differences between mice populations sampled in different areas may raise questions about host-parasite co-

evolution. Additionally, we can question if spatial differences in parasite community structure may reflect the geographical structure of the alpine environment and the meta-population structure of the yellow-necked mouse populations. In this respect further analyses are needed to clarify whether spatio-temporal differences in parasite community structure are a consequence of changes in host population dynamics and/or host genetic structure and whether this is related to changes in environmental components.

The recognition of the negative association between the *H. polygyrus* intensity and tick infestation raise questions concerning the physiological mechanisms involved in the interaction between ecto- and gastrointestinal parasites. To explain the within-host mechanisms responsible of such patterns more accurate analyses are needed on the role of host-mediated immune response (i.e. specific antibodies) in determining infection status. From an eco-parasitological point of view the interaction between *H. polygyrus* and ticks could be approached analysing similar host- parasite systems such as the interaction between ticks and Heligmosomoidae parasites of the bank vole *Clethrionomys glareous.* Moreover, this analysis can be extended to investigate the interaction between mice and tick-borne diseases like tick-borne encephalitis (TBE) or Lyme disease.

The observation that male mice are driving a parasite infection suggests that effective disease control should target these individuals. In order to do this we must answer the question: how many males do we have to treat in order to reduce parasite transmission and prevent disease persistence? We can approach this question through field experiments and treat different percentages of infected individuals and monitor the parasite reduction in the host populations, or we can apply mathematical modelling and estimate Ro, the basic reproductive number of parasite population, and the threshold of infected host needed to maintain the infection. Clearly, to estimate these parameters we need to identify more accurately the biological mechanisms that generated such sex-biases in parasite

transmission. The use of mathematical simulations, to explore alternative mechanisms causing such patterns (Chapter 5), has been useful for directing further investigations. For example, to test for any difference in parasite egg quality between male and female mice the hatching rate of *H. polygyrus* eggs collected from the faeces of the two sexes can be measured at different time of the year to account for seasonal changes in host and parasite characteristics. Radio tracking of mice could be use to monitor differences in the habitat use during different activities by the two sexes. This study should be also integrated with more accurate investigations on differences the social behaviour of yellow-necked mouse sexes.

In summary, this thesis has investigated the interaction between the yellow-necked mouse and its parasite *H. polygyrus*, with the aim of identifying intrinsic and extrinsic factors affecting host-parasite interactions, parasite abundance and transmission.

This work provides evidence that variations in host characteristics, such as host sex, age, and variations in habitat structure and composition, play an important role in the dynamics of parasite transmission. To further understand host-parasite interactions greater effort should focus in revealing the consequence, rather than only on the causes, of such host differences on host-parasite dynamics and on dynamics of infection.

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