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PATTERNS AND PROCESS OF PARASITE INFECTION AND TRANSMISSION

A Dissertation in

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by

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ABSTRACT

The major theme of my dissertation research is how the mechanisms that determine infection and transmission are linked. It is important to note that these two questions are intimately related because infection is necessary to generate transmission and transmission is necessary for new infections. The hypotheses I tested to address these questions build on previous evidence that male hosts are more heavily infected across most host and parasite taxa and also can drive transmission of several types of parasites. I set out to test several hypotheses that explain male-biased transmission, 1) Male hosts have higher transmission rates because they have a greater infection rate and produce infectious stages in proportion to their parasite intensity, 2) Male hosts have higher transmission rates because the parasites they harbor are more fecund, thus producing more infectious stages per parasite, 3) Male hosts have higher transmission rates because their behaviors cause infectious stages to more efficiently infect susceptible hosts. While maleness is a particular focus of my research, I believe that the mechanisms driving transmission can apply more generally. I used field experiments to refine and test the hypotheses set out above in a replicated and randomized before-after-control-impact experimental framework. A focus of these experiments was on the mechanisms of exposure that lead to transmission heterogeneities. Specifically, what is the contribution of behaviorally mediated exposure and between-host interactions to generating transmission heterogeneities? To explicitly examine the role of exposure in transmission dynamics, I incorporated network theory into the design and analysis of several of my experiments. The use of network theory provided unique insights into how the role of behaviorally-mediated exposure influences transmission.

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Chapter 1. Introduction to: Patterns and process of parasite infection and transmission

Introduction

My dissertation addresses two main themes in disease ecology:

What are the mechanisms that cause individual hosts to have disproportionately greater parasite infection rates compared to the population?

What are the mechanisms that cause individual hosts to have disproportionately greater transmission rates compared to the population?

The first question receives much attention in the literature because of the general interest in the medical and veterinary fields in individual health. However, the second question is the key for understanding the dynamics of host-parasite systems. A major theme of my dissertation research is how the mechanisms that determine infection and transmission are linked. It is important to note that these two questions are intimately related because infection is necessary to generate transmission and transmission is necessary for new infections. The hypotheses I tested to address these questions build on previous evidence that male hosts are more heavily infected across most host and parasite taxa (Poulin 1996, Zuk & McKean 1996, Zuk 2009) and also can drive transmission of several types of parasites (Perkins et al 2003, Ferrari et al 2004). I set out to test several hypotheses that explain male-biased transmission:

1. **Male hosts have higher transmission rates because they have a greater infection rate and produce infectious stages in proportion to their parasite intensity** (chapters 2, 3, 5). i.e. the same mechanisms that cause heterogeneities in infection rates drive transmission.
2. **Male hosts have higher transmission rates because the parasites they harbor are more fecund, thus producing more infectious stages per parasite** (chapters 2, 3)

3. **Male hosts have higher transmission rates because their behaviors cause infectious stages to more efficiently infect susceptible hosts** (chapters 2, 4)

While maleness is a particular focus of my research, I believe that the mechanisms driving transmission can apply more generally. Males represent an important class of hosts, based on previous research, but there are systems where another host characteristic is associated with greater infection (for example: female chipmunks, see chapter 4) and, potentially, transmission rates. In this general sense, these hypotheses can be summarized as influencing exposure and susceptibility to parasites. Susceptibility includes how likely and how heavily a susceptible host becomes infected, after an infectious contact, as well as how successful the parasite is at producing infectious stages. Important consequences of susceptibility are largely concentrated in the physiological (and evolutionary) interactions between hosts and parasites. Likewise, exposure not only includes the probability of a susceptible host making an infectious contact, but also how efficiently an infected host disseminates parasite infectious stages. The important consequences of exposure result from the ecological interactions between hosts; between hosts and the environment; and between parasites and the environment.

Susceptibility and exposure do not act in isolation or in a simple additive manner to produce infection and transmission. These processes interact, resulting in emergent transmission dynamics that cannot be explained without consideration of both. Host-parasite interactions can have consequences for host-host interactions and vice-versa and susceptibility and exposure are likely to covary in most host-parasite systems. For example, host behavioral changes due to severe disease symptoms (high susceptibility) can reduce between host contacts (low exposure) and reduce transmission (Kiesecker at al 1999). The covariation between sickness behavior and

exposure can even work in the opposite direction and increase transmission from the sickest individuals (Bouwman & Hawley 2010).

My dissertation and approach to testing hypotheses

To address the first question, "**what are the mechanisms that generate heterogeneities in infection?**" I analyzed macroparasite intensity patterns among different classes of hosts (chapters 2, 3, 4) and among parasites with different life histories (chapter 5). In chapter 5, I further examined transmission from the parasite's point-of-view by examining the life-history characteristics of parasites that can lead to aggregation of parasite numbers within a few hosts. My approach to address mechanisms that generate infection heterogeneities was primarily observational. In addition, in chapter 5, I used using a combination of cross-sectional data and simulation modeling to correlate observed patterns of parasite aggregation to host and parasite characteristics. In chapters 2, 3 & 4, I used longitudinal data from experimental controls to examine heterogeneities in infection rates while testing hypotheses that address the second research question: "**do infection heterogeneities translate into transmission heterogeneities?**"

The strength of my dissertation was the field experiments in chapters 2, 3 & 4, which refined and tested the hypotheses set out above in a replicated and randomized before-after-control-impact experimental framework. A focus of these experiments was on the mechanisms of exposure that lead to transmission heterogeneities. Specifically, "**what is the contribution of behaviorally mediated exposure and between-host interactions to generating transmission heterogeneities?"** To explicitly examine the role of exposure in transmission dynamics, I incorporated network theory into the design and analysis of several of my experiments. The use of network theory provided unique insights into the role of exposure in chapter 2 and led me to

design the experiment in chapter 4 to explicitly address how behaviorally-mediated exposure influences transmission.

Titles and brief summary of dissertation chapters

Chapter 2:

Does elevated testosterone result in increased exposure and transmission of parasites?

Grear DA, Perkins SE, & Hudson PJ (2009) *Ecology Letters*, 12, 528-537.

To test how testosterone could increase exposure and transmission, we undertook a longitudinal mark-recapture study where we experimentally elevated testosterone levels in wild male rodents. Individuals in control populations reduced the average number of contacts over the treatment period, while populations with experimentally elevated testosterone levels maintained the number of contacts between hosts. As a result, the transmission potential was higher in testosterone treated populations compared to controls because of a population-level response to high-testosterone individuals. Individual infection intensity with the intestinal parasite *Pterygodermatites peromysci* did not change with testosterone treatment; nor did the prevalence of infection at the population level.

Chapter 3:

Sex-biased transmission of complex life-cycle parasite: why males matter.

Grear DA, Luong LT, Hudson PJ. (2011) Accepted with major revisions, *Journal of Animal Ecology*

Males are hypothesized to produce more onward transmission events than females due to several proposed competing mechanisms, few of which have been empirically tested. We performed a longitudinal field experiment and continuously removed intestinal nematode parasites from male or female mice with anthelmintic treatment and recorded the subsequent

transmission to the non-treated sex of the mouse host. Removing parasites from male mice resulted in lower infection rates among female mice compared to females in control sites. We detected no effect of female-deworming on transmission rates among male mice. We found no difference in prevalence, intensity, or fecundity of *P. peromysci* parasites between sexes in the cross-sectional sample of mice. Without male-biased prevalence, intensity, or parasite fecundity, we concluded that male-biased transmission is unlikely to be created via physiological differences and the parsimonious explanation is that male behaviors spread infective particles in a more successful manner.

Chapter 4:

Feeding and foraging behaviors interact to influence the parasite community of the eastern chipmunk (Tamias striatus)

Host behavior is an important determinant of the spread of and exposure to infectious stages, but is difficult to measure and often ignored by disease ecologists. We examined two aspects of host foraging behavior that influence parasite transmission: the availability and spatial distribution of food in relation to parasites with fecal-oral transmission routes. We employed an experimental approach and added supplemental food, in uniform or clumped spatial distributions, to natural populations of the eastern chipmunk and monitored the response of chipmunk foraging behavior and infection levels of their gastro-intestinal helminth community using replicated longitudinal capture-mark-recapture techniques. Providing a supplemental food source, regardless of spatial distribution, decreased the transmission of a trophically-transmitted parasite, i.e. transmission that requires ingestion of invertebrates. The contact networks appeared to become sparser in response to either spatial distribution of supplemental food compared to controls, but was confounded by biased trapability in response to treatment. The prevalence of

two directly transmitted gastro-intestinal nematode parasites was strongly correlated to network in-degree, while the prevalence of trophically transmitted parasites was not. We concluded that the relative availability of different food sources is an important influence on the transmission of parasites and the specific transmission route, relative to the type of available food, is a key factor in determining transmission and community composition of parasites.

Chapter 5:

The dynamics of macroparasite host-self-infection: a study of the patterns and processes of pinworm (Oxyuridae) aggregation

Grear DA & Hudson PJ (2011) *Parasitology*, 138, 619-627.

Oxyuridae parasites infect a wide range of mammalian hosts and have a unique reproductive strategy that involves conventional horizontal transmission, as well as re-infection of an already infected host. We asked the question, do the unique aspects of pinworm life-history explain an exception to the widely observed patterns of aggregation of parasite populations? We empirically examined the differences among Oxyuridae (genus: *Syphacia*) compared with other helminth (genus: *Heligmosomoides*) parasite aggregation in two rodent hosts with similar ecology. To investigate the effects of pinworm life-history characteristics on generating aggregation, we present a stochastic model that explores aggregation under a range of host-self-infection, parasite death, and transmission scenarios. Oxyuridae parasites had consistently greater aggregation compared to other nematodes regardless of host or parasite species identity and pinworm aggregation exceeded the range of macroparasite aggregation described previously. Our simulations demonstrated that host-self-infection, on its own, is sufficient to generate greater than predicted aggregation values.

Chapter 6: *Summary & Conclusions*

Appendix A: Luong LT, Grear DA, & Hudson PJ (2010) Male host are responsible for the transmission of a trophically transmitted parasite, *Pterygodermitites peromysci*, to the intermediate host in the absence of sex-biased infection. *International Journal for Parasitology*, 39, 1263-1268.

REFERENCES

Bouwman, K. M. & Hawley, D. M. (2010) Sickness behaviour acting as an evolutionary trap? Male house finches preferentially feed near diseased conspecifics. *Biol. Lett.*, 6, 462-465.

Ferrari, N., Cattadori, I.C., Nespereira, J., Rizzoli, A., & Hudson, P.J. (2004). The role of host sex in parasite dynamics: field experiments on the yellow-necked mouse *Apodemus flavicollis*. *Ecol. Lett.*, 7, 88-94.

Kiesecker, J. M., Skelly, D. K., Beard, K. H., & Preisser, E. (1999) Behavioral reduction on infection risk. *Proc. Nat. Acad. Sci*., 96, 9165-9168.

Perkins, S.E., Cattadori, I.C., Tagliapietra, V., Rizzoli, A.P., & Hudson, P.J. (2003). Empirical evidence for key hosts in persistence of a tick-borne disease. *Parasitology*, 33, 909-917.

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

Zuk, M. & McKean, K. A. (1996) Sex differences in parasite infections: patterns and processes. *Int. J. Parasit.,* 26, 1009-1024.

Zuk, M. (2009) The sicker sex. *PLoS Pathogens*, 5, e1000267.doi:10.1371/ journal.ppat.1000267.

Chapter 2. Does elevated testosterone result in increased exposure and transmission of parasites?

Daniel A Grear, Sarah E Perkins, Peter J Hudson

ABSTRACT

Male-biased infection is a common phenomenon in vertebrate-parasite systems and male-biased transmission has been experimentally demonstrated. One mechanism that is hypothesized to create male-biased transmission is the immunosuppressive effect of testosterone because it increases susceptibility to infection. Testosterone also influences host behavior and, consequently, may increase exposure to parasites. To test how testosterone could increase exposure and transmission, we undertook a longitudinal mark-recapture study where we experimentally elevated testosterone levels in wild male rodents. Individual infection intensity with the intestinal parasite *Pterygodermatites peromysci* did not change with testosterone treatment; nor did the prevalence of infection at the population level. Individuals in control populations reduced the average number of contacts over the treatment period, while populations with experimentally elevated testosterone levels maintained the number of contacts between hosts. This was a surprising result because all individuals, including females and untreated males, in treated populations had higher number of contacts compared to control individuals at the end of the experiment. As a result, the transmission potential was higher in testosterone treated populations compared to controls because of a population-level response to high-testosterone individuals. Our results indicated that males with high testosterone levels alter the population level contacts, producing different social networks and increasing transmission potential compared to those where testosterone is at background levels.

Keywords: Social network, testosterone, contact rates, *Peromyscus leucopus*, sex-biased transmission

INTRODUCTION

Males are more likely to be infected with parasites and to carry a greater intensity of infection than females of the same species in vertebrate-parasite systems (Zuk & McKean 1996). Extensive comparative analyses have found that, across a range of mammalian species, this pattern is particularly pronounced for macroparasitic infections (Poulin 1996; Moore & Wilson 2002). Male-biased infection rates are usually attributed to either ecological factors, such as behaviors that increase exposure of males to infective stages, or physiological factors, usually based on hormonal differences between males and females (Grossman 1989). Indeed, one established hypothesis that explains the empirically observed male-biased infection rates is that males are more susceptible to parasites because the male hormone testosterone is immuno-suppressive (Folstad & Karter 1992). However, not all attempts to test this hypothesis have found evidence to support it (Roberts *et al.* 2004; Nunn *et al*. 2008).

In addition to the effect of testosterone on host susceptibility, testosterone also impacts host behaviors that influence rates of infectious contact, so leading to male-biased parasitism. For example, an experimental study on red grouse (*Lagopus lagopus scoticus*) found that testosterone implanted males not only had significantly higher infection levels of the caecal nematode *Trichostrongylus tenuis*, but also took larger territories and were more likely to be bigamous than controls (Seivwright *et al.* 2005). Elevated testosterone is also associated with physiological changes such as mass gain and breeding status, which may interact with factors that influence exposure and susceptibility. Behaviorally, males with elevated testosterone tend to exhibit greater space use (Chandler *et al.* 1994; Seivwright *et al*. 2005), more aggressive behavior (Salvador *et al.* 1997; Martinez-Sanchis *et al.* 2003) and higher copulation frequency (Alatalo *et al.* 1996). Such behaviors can increase contact rates and spread of parasite infective stages, and so increase parasite transmission.

The aim of this study was to address the question of how increased testosterone can potentially influence transmission of parasites via changes in exposure within a natural host population. Recent applications of network theory to ecology and epidemic modeling have provided a novel way to approach questions about the transmission of diseases (Proulx *et al*. 2005; May 2006). Conventional methods for evaluating heterogeneities in contacts in natural animal populations have been limited to estimating contact probability from home range overlap or observing individual behaviors (Revilla & Palomares 2002; Weihong *et al*. 2005; Schauber *et al.* 2007). The former is usually measured at a spatial and temporal scale that is too coarse to accurately estimate potential transmission and the latter is too time and labor intensive to collect the amount of data to make general conclusions. Graph theory and network analysis have been applied successfully to several human disease systems in an effort to quantify heterogeneous infectious contacts (see review of May 2006) and has, only recently, been applied to wildlife-parasite systems including African buffalo – tuberculosis (Cross *et al*. 2004) and rodent-nematode systems (Perkins *et al.* 2008, Perkins *et al.* 2009).

We addressed the question: How does elevated testosterone influence contact rates between hosts and, in turn, what are the implications for parasite transmission? We experimentally manipulated testosterone in wild male rodents (*Peromyscus leucopus*, Rafinesque; the white-footed mouse) and recorded differences in contact rates by constructing network graphs specific to the transmission of parasites. We predicted that males treated with increased testosterone would have a higher average contact rate compared to control individuals. We also predicted that the consequences of increased contact rates of testosterone treated males would increase transmission potential at a population level.

METHODS

Data collection

Wild rodents (*P. leucopus*) were live-captured on 8 replicated open trapping grids, consisting of 64 traps in a square 8 x 8 array at 10 meter intervals (Ugglan #2 multiple capture traps, Grahnab AB, Sweeden). Five grids received the experimental treatment and 3 grids were untreated population-level controls. All grids were located 20km south of State College, Pennsylvania, USA in open forested habitat and separated by at least 500m. No individuals were recorded on more than one grid. We recorded trap location, body length, body mass, sex, and breeding condition for each mouse capture. Males were considered in breeding condition if their testes were descended. Females were considered in breeding condition if they had a perforated vagina, were lactating, or were pregnant. Mice were individually marked using a passive induced transponder tag (EIDAP, Sherwood Park, Alberta, Canada) and trapped bi-weekly beginning May 2007 with 2 trap nights per trap session, for a total of 12 weeks. Blood was collected at each capture and was stored on ice for 1-4 hours until the serum and the red blood cells could be separated by centrifugation and frozen at -20^{o} C. Serum testosterone levels were determined using a salivary testosterone enzyme immunoassay kit (Salimetrics LLC, State College, USA) by adapting the procedure for rodent sera following the methods of Washburn *et al.* (2006). During the final trap session of the experiment, we euthanized all male mice captured on the treatment site that had been treated. We dissected these individuals to identify and count all nematodes in their intestinal tracts. We also collected feces from all mice captured during the final trap session and performed fecal egg counts using a McMasters egg floatation for all fecal samples greater than 0.06g (Sloss and Kemp 1978): 1g feces mixed with 10ml saturated sucrose solution, giving a minimum resolution of 37 eggs per gram feces (epg). All animal handling was

approved by the Institutional Animal Care and Use Committee at Penn State University (IACUC #23268).

Testosterone treatment

Fifty percent of male *P. leucopus*, selected randomly from all captures of sub-adult and adult males during a single week in July 2007, were treated with a sub-cutaneous testosterone implant on the five treatments grids. The remaining males, not randomly selected for testosterone treatment, received a control treatment of an empty testosterone delivery device. Testosterone treatment capsules were constructed by filling sterile silastic tubing (12mm length x 1.95mm diameter, Helix Medical, Carpinteria, CA) with testosterone propionate (Sigma-Aldrich, St. Louis, MO) and sealed with medical grade silicone adhesive. The dose was determined by a preliminary laboratory trial, where a 12mm implant increased testosterone by 2 standard deviations above a previously observed population mean, and remained elevated compared to controls for 3-4 weeks (Grear, unpublished). Males on the three additional grids were monitored, but did not receive any treatment.

Node and edge definitions and network analysis

Each individual mouse was defined as a node and had attributes defined for sex (male or female), and treatment (testosterone, sham, or none). Undirected edges approximated a potential infectious interaction based on proximity of capture in time and space. An edge between node *i* and node *j* was defined if *j* was captured in the same or adjacent trap to *i* during the same or the subsequent trap session or vice-versa. As such, an edge between two nodes approximates a transmission event for a directly transmitted microparasite (i.e. bacteria or virus) with a short infectious period (1-2 weeks) or a macroparasite with a 1-2 week free-living infectious stage. Two networks with undirected edges were created for each of the trapping grids: one

representing all trapping sessions up to and including the treatment session and the second for all subsequent trapping sessions.

We analyzed the networks using two metrics: degree and clustering coefficient. The degree of a node is the number of edges that connect that node to other nodes and represents a measure of contact rate. The clustering coefficient measures of how connected the neighbors of a node are and is calculated by dividing the number of edges connecting nodes that share an edge with a focal node by the total possible connections among these neighboring nodes. A clustering coefficient of 0 indicates that there are no connections between the neighbors of a given node and a clustering coefficient of 1 indicates that all of the neighbors of a given node are connected. The network statistics were calculated using the package 'Statnet' in R (Handcock *et al.* 2003; R Development Core Team 2008) and the clustering coefficients using package i-network (Wang 2007).

Statistical Analysis

Testosterone levels of individual males were analyzed using a linear-mixed-model with individual treatment (testosterone, sham, not-treated), time since treatment, the interaction between treatment and time since treatment, and mass as fixed effect explanatory variables with individual and population as random variables. The relationship between testosterone treatment and body mass, as well as testosterone and breeding status, was analyzed using models of the same structure with the response body mass or a binomial response of breeding condition, respectively. 95% confidence intervals were created around the coefficients of each factor-level to evaluate its effect on the response variable.

Intensity of infection from end-of-experiment dissections of male mice was analyzed with generalized linear models with the number of parasites per host as a response with a Poisson

error structure. Due to the low recapture rate of individuals at the termination of the experiment, the GLM did not account for between site variation and contained explanatory variables for individual treatment and mass. We performed a similar GLM analysis for prevalence (binomial response) of infection and parasite egg shedding rate (eggs per gram feces) from fecal samples collected from all individuals during the last trapping session, including sex, mass, and treatment as explanatory factors.

We characterized the degree distributions of each of our networks by fitting the observed degrees per node to a Poisson lognormal discrete (random) distribution and to a Waring discrete (exponential) distribution using the package 'Degreenet' in R (Handcock 2003). The Waring distribution is a flexible form of an exponential distribution that has been used to describe several types of empirical networks (Handcock & Morris 2007). To choose the best-fitting distribution to each of our networks we used Akaike's information criterion corrected for small sample size (AIC_C) (Burnham & Anderson 2002).

The network degree statistic was analyzed using a Poisson generalized linear-mixed-model (GLMM) with the individual node and replicate population as random error variables. Fixed effect variables included average mass during the treatment period, network size (number of nodes), time period (before or after treatment), and individual node attribute (testosterone-treated males, sham-implanted males, non-treated males, females). The population-level degrees were compared among all populations using the same GLMM structure with the additional dependent variable of population treatment. The clustering coefficient was analyzed using the same Poisson GLMM structure with the count of edges among neighbors of each node as the response and the total possible edges among neighbors as an offset.

To determine a final statistical model we began with a GLMM with the additive fixed effects of treatment period and node attribute. We included additional fixed effect variables if a drop-in-deviance X^2 test comparing a full and reduced model was significant ($p < 0.05$). We first added body mass, then network size, and then the node attribute-treatment period interaction. To evaluate fixed effects coefficients of the final GLMM, Bayesian confidence intervals were produced by taking 10,000 samples from the posterior distribution of fitted GLMMs using Markov-Chain-Monte-Carlo (MCMC) methods and creating a highest posterior density (HPD) interval. We fitted GLMMs and created HPD confidence intervals using the package 'lme4' in R (Bates *et al.* 2008). Coefficients were considered significant if their confidence interval did not overlap zero.

We also calculated an assortativity index (r) for each network that estimated the mixing of sexes based on shared contacts (Newman 2003). The r values range from -1 to 1 where, if r = 1, the mixing is completely assortative and edges are only shared among nodes of the same sex. When r = -1, the mixing is completely dissassortative and edges are shared only among different sexes. Intermediate values near zero represent random or neutral mixing. We used a linear-mixed-model with replicate population as a random error to test for the effect of treatment period (before or after) and population treatment on r.

We estimated the transmission potential of our observed contact rates by estimating R_0, the basic reproductive number of a disease, modified to incorporate the effects of network structure (Aparicio & Pascual 2007):

$$R_0 = \langle k \rangle \rho ,$$

where <k> is the average degree of a network with a random degree distribution and ρ is the probability of transmission per contact. We calculate R_0 for our observed contact rates over a range of ρ values from very low (0.01) to high transmissibility (1).

RESULTS

We treated 25 males with testosterone and 26 received an empty control implant over the five treatment sites. We monitored 45 males from untreated sites. We recaptured 18/25 of the testosterone treated males, 19/26 of the control implanted males, and 39/45 of the untreated males at least once subsequent to the treatment trapping session. We also captured a small number of males from treated sites (non-treated males) after the treatment was applied and these individuals were included in statistical models, but not explicitly reported in the subsequent results.

Infection levels

We recaptured 14 treated male mice during the final trapping session: 6 testosterone-treated males and 8 sham-treated males. We identified 2 nematode species from the intestinal-tract dissections: *Pterygodermatites peromysci* was the most common, found at 79% prevalence (11/14), and *Syphacia peromysci* was uncommon, found at 21% prevalence (3/14). All subsequent parasite data refers to only *P. peromysci*. There was an insufficient recapture of treated males to evaluate variation between sites, thus analysis was pooled by treatment only. There was no detectable difference in intensity of infection between the testosterone-treated and sham-treated males (95% CI [-0.13, 1.53]) (Fig 1).

We collected a sufficient quantity of feces from 46 individuals for fecal egg flotation and calculated an overall prevalence of 39% (18/46). There was no detectable difference in prevalence by mass (95% CI [-0.35, 0.08]), between any treatment group (sham 95% CI [-2.7,

1.3], testosterone 95% CI [-3.2, 3.5] and sex (male compared to female 95% CI[-0.52, 6.20] (Table 1). Of the infected individuals identified from the fecal egg counts, there was no difference in the intensity of fecal egg shedding by mass (95% CI [-0.06, 0.02]), between any treatment group (sham 95% CI [-0.45, 0.24], testosterone 95% CI [-0.25, 0.85] and sex (male compared to female 95% CI [-0.10, 0.38]; with mean log egg count per gram = 0.67 +/- 0.13 SE (Table 1).

Sera testosterone levels

The best fit GLMM to describe sera testosterone levels in males included the interaction of treatment and time since treatment ($X^2 = 18.27$, $df = 9$, $P = 0.03$) and mass ($X^2 = 3.57$, $df = 1$, $P = 0.05$). Testosterone-treated males had significantly increased testosterone during the first trap session following treatment (2-weeks) compared to control-males (95% CI [0.10, 2.71]). At the first trap-session, the testosterone levels in sham-treated males was not different from controls (95% CI [-1.76, 0.79]). There were no further differences in the time since treatment among any treatment group. There was no significant relationship between mass and testosterone levels (95% CI [-0.001, 0.11]).

The effect of testosterone on body mass

In the period before treatment, over all individuals, female body mass was smaller compared to males (95% CI [-4.54, -0.90]) and there was no difference between testosterone-treated males (95% CI [-2.00, 2.54]) and sham-treated males (95% CI [-2.79, 1.51]) compared to control-site males. We analyzed body mass relationships independently in males and females for remaining analyses. The body mass of females on all sites increased after treatment was applied (95% CI [0.32, 0.61]) and male body mass increased after treatment in all sites (95% CI [0.37,

0.60]), with no evidence of an interaction between time after treatment and individual treatment ($X^2 = 1.63$, $df = 3$, $P = 0.65$).

Female body mass was no different on treated sites compared to untreated sites (95% CI [-1.0, 2.56]) and there was no evidence for an interaction between female mass and corresponding site-treatment ($X^2 = 2.07$, $df = 1$, $P = 0.15$). There was no difference between the mass of treated males or sham-treated males compared to control males (*treated-males* 95% CI [-0.37, 3.10]; *sham-treated* 95% CI [-1.79, 1.61]) and confidence interval overlap of control-treated and testosterone-treatment males indicated there was no difference between treatments.

The effect of testosterone on female breeding condition

In female mice, there was evidence for an interaction between the proportion of females in breeding condition in treated sites and the time since treatment ($X^2 = 3.62$, $df = 1$, $P = 0.05$), with a positive relationship between the proportion of females in breeding condition in treated sites and the time since treatment compared to females in untreated sites (95% CI [0.02, 0.70]). There was no change in the proportion of females in breeding condition with time after treatment in all sites (95% CI [-0.24, 0.30]), nor any overall difference in the proportion of females in breeding condition in treated sites compared to untreated sites (95% CI [-2.2, 0.41]).

The effect of testosterone on male breeding condition

The proportion of males in breeding condition from all sites increased with time during the experiment (95% CI [0.23, 0.51]) and there was no evidence for an interaction of this relationship and any treatment group ($X^2 = 3.94$, $df = 3$, $P = 0.40$). There was no difference between the proportion of treated-males or sham-treated males and control males in breeding condition (*treated-males* 95% CI [-0.56, 0.95]; *sham-treated* 95% CI [-1.29, 0.15]) and

confidence interval overlap of control-treated and testosterone-treatment males indicates there was no difference between treatments.

Network degree distributions

We created a total of 16 networks from our 8 trapping sites, one network from the trapping sessions previous to and including treatment, and a second from the trapping sessions following treatment. The degree distributions of all networks were described better by a random distribution compared to an exponential distribution with average degrees ranging from 2.18 - 6.91 (Table 2).

Network degree of individuals on treated sites

Treated-males and sham-treated males had a higher degree (i.e. number of contacts) compared to females (*sham-treated males* 95% CI [0.12, 0.51]; *treated-males* 95% CI [0.04, 0.47]). The confidence interval overlap of control-treated and testosterone-treated males indicated no difference between these treatments. The average mass of an individual had a weak positive correlation with individual degree (95% CI [0.02, 0.04]). The best fit GLMM describing individual network degree from the testosterone treated sites included individual treatment, treatment period, average mass of an individual ($X^2 = 52.34$, $df = 1$, $P < 0.01$), and network size ($X^2 = 53.1$, $df = 1$, $P < 0.001$). There was no evidence of an interaction between treatment period and individual treatment ($X^2 = 0.77$, $df = 3$, $P = 0.86$). The network degree did not significantly change from the time before treatment to after treatment (95% CI [0.02, 0.20]) and there was no significant correlation between average mass and degree (95% CI [-0.02, 0.001]).

Clustering coefficients of individuals on treated sites

Sham-treated males and treated-males had lower clustering coefficients compared to females (*sham-treated males* 95% CI [-0.19, -0.12]; *treated-males* 95% CI [-0.31, -0.09]). The

confidence interval overlap of sham-treated males and treated-males indicated there was no difference between these treatments. The best fit GLMM to describe the clustering coefficient from the testosterone treated sites included individual treatment, treatment period and the average mass of an individual ($X^2 = 31.34$, $df = 1$, $P < 0.001$). There was no evidence of an interaction between individual treatment or sex and treatment period ($X^2 = 0.63$, $df = 3$, $P = 0.89$), nor evidence that the network size was correlated with clustering coefficient in the treated populations ($X^2 = 1.20$, $df = 1$, $P = 0.27$). There was no evidence of a difference in clustering coefficient during the period before or after treatment (95% CI [-0.14, 0.05]) and there was no significant relationship between mass and clustering coefficient (95% CI [-0.003, 0.003]).

Network degree of individuals on untreated sites

There were no differences in degree between control-males and females (95% CI [-0.02, 0.31]) and degree was significantly greater during the period before treatment (95% CI [0.35, 0.71]). There was a weak positive correlation between mass and network degree (95% CI [0.01, 0.04]) and network size also had a weak positive correlation with individual degree (95% CI [0.02, 0.05]). The best fit GLMM to describe individual network degree from the untreated sites included sex, treatment period, average mass of an individual ($X^2 = 5.44$, $df = 1$, $P = 0.02$) and the network size ($X^2 = 5.34$, $df = 1$, $P = 0.02$). There was no evidence for an interaction between sex and treatment period ($X^2 = 0.56$, $df = 1$, $P = 0.45$).

Clustering coefficient of individuals on untreated sites

There was a weak negative correlation between mass and clustering coefficient (95% CI [-0.02, -0.01]). The clustering coefficient of individuals in untreated populations did not differ in control-males compared to females (95% CI [-0.27, 0.03]) and was not different in the periods before and after treatment (95% CI [-0.20, 0.23]). The best fit GLMM to describe clustering

coefficient from the untreated sites included sex, treatment period and average mass of individuals ($X^2 = 4.48$, $df = 1$, $P = 0.03$). There was no evidence of an interaction between sex and treatment period ($X^2 = 0.22$, $df = 1$, $P = 0.64$) and there was no evidence that network size was correlated with clustering coefficient in the untreated populations ($X^2 = 2.35$, $df = 1$, $P = 0.13$).

Site level network degree

Male treatment status was not included in models comparing population degree or clustering coefficient because there was no evidence of individual differences in degree among male treatments. While there was some difference between the network degree of males and females, including sex as a fixed effect in the GLMM of site-level network degree did not improve model fit to the data ($X^2 = 1.24$, $df = 1$, $P = 0.27$) and, thus, the following results are reported as average degree of all individuals in a network. The degree in treated populations and non-treated populations was not different in the time period before treatment and, while the degree did not change in treated populations, it decreased significantly in treated populations (*treatment period:site treatment* $X^2 = 16.40$, $df = 1$, $P < 0.001$, Fig 2b). The best fit GLMM to describe site-level patterns of network degree included site treatment, treatment period, average mass of individuals ($X^2 = 7.21$, $df = 1$, $P = 0.007$), and network size ($X^2 = 16.40$, $df = 1$, $P < 0.001$). There was a weak positive correlation between network size and degree (95% CI [0.02, 0.04]) and there was no relationship between mass and degree (95% CI [-0.001, 0.010]).

Site level clustering coefficient

Clustering coefficient increased in treated populations after treatment (Fig 2c), but there was no evidence for an interaction between site treatment and treatment period ($X^2 = 0.13$, $df = 1$, $P = 0.71$). The best fit GLMM to describe site-level clustering coefficient included site

treatment, treatment period, average mass of an individual ($X^2 = 36.73$, $df = 1$, $P < 0.001$), and network size ($X^2 = 4.95$, $df = 1$, $P = 0.02$). There was a weak trend of clustering coefficient to be higher in treated populations compared to non-treated populations (95% CI [0.002, 0.004]) and of clustering coefficient to be lower during the period before treatment compared to the period after treatment over all populations (95% CI [-0.076, -0.002]). There was a weak positive correlation between network size and clustering coefficient (95% CI [0.001, 0.018]) and there was no correlation between average mass and clustering coefficient (95% CI [-0.004, 0.001]).

Assortitivity by sex

The assortativity index of networks by sex did not vary among site treatment (compared to null model: $X^2 = 1.42$, $df = 1$, $P = 0.23$) or treatment period (compared to null model: $X^2 = 2.00$, $df = 1$, $P = 0.16$) and there was no evidence for an interaction between treatment and treatment period ($X^2 = 3.82$, $df = 3$, $P = 0.28$). The assortativity index was consistently negative and near a value of zero (95% CI [-0.21, -0.06]), indicating that the populations mixed randomly according to sex.

R_0 estimation

We compared the estimate of R_0 for networks with the observed average degree of our testosterone treated sites ($\langle k \rangle = 4.89$) and untreated sites ($\langle k \rangle = 2.90$) during the period after treatment and found that across a range of transmission probabilities, the basic reproductive number, R_0, was 1.7 times higher in sites where males received the testosterone treatment (Fig 3).

DISCUSSION

We were able to document how sex-hormone levels in individual males can have a profound population-level effect on transmission dynamics. We did not find that contact rates

among treatment groups or sex changed relative to any other group in response to the testosterone treatment, but we did identify that all individuals in treated sites had higher contact rates, leading to a potentially important increase in the ability of a parasite to spread. We did not detect any individual differences in clustering, either among individual treatment, sex, or in response to site-treatment. Regardless, the influence of high-testosterone males is to increase overall contact rates and these results provide evidence that testosterone may contribute to male-biased transmission via a population-scale mechanism, in addition to the postulated individual physiological mechanisms.

The fact that we found evidence to support our hypothesis of an influence on population-level transmission, but no evidence to support our individual-level hypothesis, leads to the question, how are contacts being altered? In the untreated sites, the relative contact rate of males and females was similar to treated sites with the only change being that the overall contact rate was reduced. We addressed how males and females interacted and found the mixing patterns to be constant among our sites with no response to treatment. This finding is similar to a network analysis of male-female mixing in yellow-necked mice, *Apodemus flavicollis,* in northern Italy, where male-female assortativity was consistently invariant (Perkins *et al.* 2008) and a study of multiple live-captures that reported a random mixing of sex from trapping data of *P. leucopus* in southern Illinois, USA (Feldhamer *et al*. 2008). While other observational and network approaches have reported contacts assorted within sex more often than between sexes in white-tailed deer (*Odocoilus virgianus*, observations: Nixon *et al.* 1991) and bottle-nosed dolphins (*Tursiops spp*., networks: Lusseau & Newman 2004), random mixing by sex may be more characteristic than previously assumed in some rodent species.

One challenge for any study that tries to quantify exposure and transmission through contact rates in natural systems is recording a contact which would result in transmission. Our method for measuring spatial and temporal associations may not characterize who infects whom or have the resolution to capture fine-scale social associations. However, this approach can provide a reasonable proxy of potential transmission given our assumption of a contact represents associations in space and time that are necessary, but not automatically sufficient, to produce an infectious contact.

We assumed that individual contact rates are not influenced by infection status such that contact rates of infected individuals are distributed similarly to uninfected individuals. A number of studies have identified a positive correlation between testosterone levels and parasite prevalence and intensity (see reviews Poulin 1996; Zuk & McKean 1996). However, we found no difference in intensity or prevalence of infection among treatments or sexes, albeit with a low recapture rate of treated males and infection with a parasite with a transmission cycle that is much longer than the treatment lasted (Luong, unpublished). Further, studies of red grouse have experimentally identified that elevated testosterone leads to increased parasite intensity (Mougoet *et al*. 2005) and leads to behaviors that increase exposure (Seivwright *et al*. 2005). Research in captive deer mice (*Peromyscus maniculatus*), has shown that experimental infections with macroparasites does not change feeding rates (Schwanz 2006), but reduces activity (Poirier *et al*. 1995). Yet, studies in wild wood mice (*Apodemus sylvaticus*) observed that males infected with the nematode parasite *Heligmosomoides polygyrus* had larger territories than uninfected males (Brown *et al*. 1994). Given that the effect we observed influenced contact rates of all individuals, and not just the testosterone-treated males, the consequence of increased transmission potential may be robust to any interaction between infection status and behavior.

Based on the data that we could analyze from our network graphs, we cannot identify the mechanism that caused the population-wide change in contact rates. Indeed, the relative difference in network degree in response to treatment was because of a reduction of contact rates in the control sites (Fig 2b). We believe this reflects a seasonal change in breeding behavior, where breeding is practically halted during the time corresponding to our treatment in *P. leucopus* (Terman 1998; Vandegrift *et al*. 2008). Similar seasonal patterns in network contact rates in *A. flavicollis* have also been observed (Perkins *et al.* 2009) and could potentially lead to seasonal differences in parasite transmission. One possible mechanism is that rodent scent-signaling is linked to testosterone levels (Sawyer 1980) and provides a signaling route between high-testosterone males and the rest of the population, without necessarily increasing direct contact rates. Having even some males with high testosterone, may maintain intrasexual competition in males (Roberts 2007) and responses of females that are elicited during the peak of breeding (Drickamer 2007).

Nonetheless, the consequence of having high testosterone males in a rodent population is a potential increase in transmission because of increased contact rates. Our estimated R_0, the basic reproductive capacity of a parasite to spread in a susceptible population, increased 1.7 fold when males were treated with testosterone (Fig 3). The estimation of R_0 from network contact structure has been derived for networks with contact distributions that range from regular to highly aggregated and the variation in contacts per individual is important to accurately estimating R_0 (Aparicio & Pascual 2007). We observed networks with consistently random (Table 2) contact distributions and, as such, the estimation of R_0 is simple because the variation in contacts scales directly with the average. Other network properties such as local clustering (Petermann & De Los Rios 2004) and assortative mixing (Morris 1995) also influence R_0

estimation. Greater clustering generally decreases R_0 for a given network degree (Keeling 1999) and the slowing of epidemic spread is greatest with very high clustering. Our observed networks had clustering coefficients (0.4 – 0.7, Fig 2c) that were consistently below levels that would affect our estimation of R_0 (Keeling 1999). Assortative mixing can also influence R_0 with disease spreading quickly within groups but slowly between groups when mixing of node type is assortative (Eames 2007). Perkins *et al*. (2008) demonstrated that assortative mixing in rodent populations was not sufficient to predict empirical parasite dynamics and we found no evidence for assortment of contacts by sex, thus assortativity has negligible influence of our estimation of R_0. Even with more complex contact rate distributions and network topology, the estimation of R_0 is made very practical by the application of contact network methodology (Aparicio & Pascual 2007; Bansal *et al*. 2007).

In addition, our estimation of R_0 assumes that there is homogeneous contribution to transmission from all classes of individuals in the population. Previous research has identified that testosterone can have a direct effect on parasite intensity and shedding rate in red grouse (Mougeot *et al.* 2005) and that males can be responsible for driving parasite transmission in yellow-necked mice (Ferrari *et al.* 2004). Here, we present evidence that individual heterogeneity in male sex-hormones can drive population contact rates and may compound the physiological effects of high testosterone levels on individual contributions to transmission; possibly leading our estimate of R_0 to be conservative.

This work represents an important contribution to the growing recognition of the importance of studying individual-level host heterogeneities in the context of parasite transmission. Few studies have empirically addressed heterogeneities in contact rates and, while the importance of contact structure has been recognized theoretically (Meyers *et al.* 2005; Bansal

et al. 2007), this study explicitly addresses contact rates in the context of disease transmission. In relation to male-biased transmission, we documented a mechanism by which a transmission-network wide increase in contact rates is driven by an individual heterogeneity. The role of testosterone as an individual-level heterogeneity that can increase parasite intensity and shedding has been demonstrated as a physiological effect (Hughes & Randolph 2001; Mougeot *et al.* 2005) and observed to have a potentially important effect of individual behavior that can influence exposure and transmission (Martinez-Sanchis *et al.* 2003; Seivwright *et al.* 2005). Our experiment demonstrates an additional mechanism whereby testosterone levels can lead to increased transmission potential that is not linked to susceptibility and not revealed at the individual level. The generality of our results in natural host-parasite systems needs further testing that considers the host, as well as the parasite, and employing a network theory to quantify transmission risk is a promising way to address any number of hypotheses about transmission dynamics.

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REFERENCES

Alatalo, R.V., Hoglund, J., Lundberg, A., Rintamaki, PT., & Silverin, B. (1996). Testosterone and male mating success on the black grouse leks. *Proc. R Soc. B Biol. Sci.,* 263, 1697-1702.

Aparicio, J.P. & Pascual, M. (2007). Building epidemiological models from R0, an implicit treatment of transmission networks. *Proc. R Soc. B Biol. Sci.,* 274, 505-512.

Bansal, S., Grenfell, B.T., & Meyers, L.A. (2007). When individual behaviour matters: homogeneous and network models in epidemiology. *J. R. Soc. Interface*, 4, 879-891.

Bates, D., Maechler, M., & Dai, B. (2008). lme4: linear-mixed effects models using S4 classes. R package version 0.999375-27. http://lme4.rforge.r-project.org/.

Brown, E.D., Macdonald, D.W., Tew, T.E., & Todd, I.A. (1994). *Apodemus sylvaticus* infected with *Heligmosomoides polygyrus* (Nematoda) in arable ecosystems: epidemiology and effects of infection on the movement of male mice. *J. Zool., Lond.*, 234, 623-640.

Burnham, K.P. & Anderson, D.R. (2002). Model selection and inference: a practical information-theoretic approach. 2nd edition, Spring-Verlag, New York.

Chandler, C.R., Ketterson, E.D., Nolan, V., & Ziegenfus, C. (1994). Effects of testosterone on spatial activity in free-ranging male dark-eyed juncos, *Junco hyemalis*. *Anim. Behav.*, 47, 1445-1455.

Cross, P.C., Lloyd-Smith, J.O., Bowers, J.A., Hay, C.T., Hofmeyr, M., & Getz, W.M. (2004). Integrating association data and disease dynamics in a social ungulate: bovine tuberculosis in African buffalo in the Kruger National Park. *Ann. Zool. Fenn.,* 41, 879-892.

Drickamer, L.C. (2007). Acceleration and delay of reproduction in rodents. In: *Rodent Societies: an Ecological and Evolutionary Perspective* (eds. JO Wolff & PW Sherman). University of Chicago Press, Chicago, pp. 106-114.

Eames, K.T.D. (2007). Contact tracing strategies in heterogeneous populations. *Epidiol. Infect.*, 135, 443-454.

Feldhamer, G.A., Rodman, L.B., Carter, T.C., & Schauber, E.M. (2008). Multiple capture of white-footed mice (*Peromyscus leucopus*): evidence for social structure? *Am. Midl. Nat.*, 160, 171-177.

Ferrari, N., Cattadori, I.C., Nespereira, J., Rizzoli, A, & Hudson, P.J. (2004). The role of host sex in parasite dynamics: field experiments of the yellow-necked mouse *Apodemus flavicollis*. *Ecol. Lett.*, 7, 88-94.

Folstad, I. & Karter, A.J. (1992). Parasites, bright males, and the immunocompetence hypothesis. *Am. Nat.*, 139, 603-622.

Grossman, C. (1989). Possible underlying mechanisms of sexual dimorphism in the immune response, fact and hypothesis. *J. Steroid Biochem.,* 34, 241-251.

Handcock, M.S. (2003). degreenet: models for skewed count distributions relevant to networks. Version 1.0, URL: http://statnetproject.org.

Handcock, M.S., Hunter, D.S., Butts, C.T., Goodreau, S.M., & Morris, M. (2003). Statnet: A suite of R packages for the Statistical Modeling of Social Networks. Version 2.0, URL http://www.csde.washington.edu/statnet.

Handcock, M.S., & Morris, M. (2007). A simple model for complex networks with arbitrary degree distribution and clustering. In: *Statistical Network Analysis: Models, Issues, and*

New Directions (eds: Airoldi E, Blei DM, Fienberg SE, Goldenberg A, Xing EP and Zheng AX). Springer-Verlag, Berlin, pp. 103-114.

Hughes, V.L. & Randolph, S.E. (2001). Testosterone increases the transmission potential of tick-borne parasites. *Parasitology,* 123, 365-371.

Lusseau, D. & Newman, M.E.J. (2004). Identifying the role that animals play in their social networks. *Biol. Letters*, 271, S477-S481.

Keeling, M.J. (1999). The effects of local spatial structure of epidemiological invasions. *Proc. R Soc. B Biol. Sci.,* 266, 859-867.

Martinez-Sanchis, S., Arnedo, M.T., Salvador, A., Moya-Albiol, L., & Gonzalez-Bono, E. (2003). Effects of chronic administration with high doses of testosterone propionate on behavioral and physiological parameters in mice with differing basal aggressiveness. *Aggressive Behav.,* 29, 173-189.

May, R.M. (2006). Network structure and the biology of populations. *Trends Ecol. Evol.*, 7, 394-399.

Moore, S.L. & Wilson, K. (2002). Parasites as a viability cost of sexual selection in natural populations of mammals. *Science*, 297, 2015-2018.

Morris, M. (1995). Data driven network models for the spread of infectious disease. In: *Epidemic Models: Their Structure and Relation to Data* (ed. Mollison, D.). Cambridge University Press, Cambridge, pp. 302-322.

Mougeot, F., Redpath, S.M., Piertney, S.B., & Hudson, P.J. (2005). Separating behavioral and physiological mechanisms in testosterone-mediated trade-offs. *Am. Nat.,*166, 158-168.

Meyers, L.A., Pourbohloul, B., Newman, M.E.J., Skowronski, D.M., & Brunham, R.C. (2005). Network theory and SARS: predicting outbreak diversity. *J. Theor. Biol.*, 232, 71-81.

Newman, M.E.J. (2003). Mixing patterns in networks. *Physical Review E*, 67, 026126.

Nixon, C.M., Hansen, L.P., Brewer, P.A., & Chelsvig, J.E. (1991). Ecology of white-tailed deer in an intensively farmed region of Illinois. *Wildlife Monographs*, 118, 1-77.

Nunn, C.L., Lidenfors, P., Pursall, E.R., & Rolff, J. (2008). On sexual dimorphism in immune function. *Proc. R Soc. B Biol. Sci.,* 364, 61-69.

Perkins, S.E., Ferrari, M.F., & Hudson, P.J. (2008). The effect of social structure and sex-biased transmission on macroparasite infection. *Parasitology*, 135, 1561-1569.

Perkins, S.E., Cagnacci, F., Stradiotto, A., Arnoldi, D., & Hudson, P.J. (2009). A comparison of social networks derived from ecological data: implications for inferring infectious disease dynamics. *J. Anim. Ecol.,* in press.

Petermann, T. & De Los Rios, P. (2004). Role of clustering and gridlike ordering in epidemic spreading. *Physical Review E*, 69, 06616.

Poirier, S.R., Rau, M.E., & Wang, X. (1995). Diel locomotory activity of deer mice (*Peromyscus maniculatus*) infected with *Trichinella nativa* or *Trichinella pseudospiralis*. *Can. J. Zool*., 73, 1323-1334.

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

Proulx, S.R., Promislow, D.E.L., & Phillips, P.C. (2005). Network thinking in ecology and evolution. *Trends Ecol. Evol.*, 6, 345-353.

R Development Core Team (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Revilla, E. & Palomares, F. (2002). Spatial organization, group living and ecological correlates in low-density populations of Eurasian badgers, *Meles meles*. *J. Anim. Ecol.*, 71, 497-512.

Roberts, M.L., Buchanan, K.L., & Evans, M.R. (2004). Testing the immunocompetence handicap hypothesis: a review of the evidence. *Anim. Behav.*, 68, 227-239.

Roberts, S.C. (2007). Scent marking. In: *Rodent Societies: an Ecological and Evolutionary Perspective* (eds. Wolff, J.O. & Sherman, P.W.). University of Chicago Press, Chicago, pp. 255-266.

Salvador, A., Veiga, J.P., Martin, J., & Lopez, P. (1997). Testosterone supplementation in subordinate, small male lizards: consequences for aggressiveness, color development, and parasite load. *Behav. Ecol.*, 8, 135-139.

Sawyer, T.F. (1980). Adrogen effects on responsiveness to aggression and stress-related odors of male mice. *Physiol. Behav.*, 25, 183-187.

Schauber, E.M., Storm, D.J., & Nielsen, C.K. (2007). Effects of joint space use and group membership on contact rates among white-tailed deer. *J. Wildl. Manage.*, 71, 155-163.

Schwanz, L.E. (2006). Schistosome infection in deer mice (*Peromyscus maniculatus*): impacts on host physiology, behavior and energetics. *J. Exp. Biol.*, 209, 5029-5030.

Seivwright, L.J., Redpath, S.M., Mougeot, F., Lecki, F., & Hudson, P.J. (2005). Interactions between intrinsic and extrinsic mechanisms in a cyclic species: testosterone increases parasite infection in red grouse. *Proc. R Soc. B Biol. Sci.*, 272, 2299-2304.

Sloss, M. W., and R. L. Kemp. 1978. Veterinary Clinical Parasitology. Iowa State University Press, Ames, Iowa, USA

Terman, C.R. (1998). Early-summer reproductive curtailment in wild white-footed mice and reproductive recovery in the laboratory. *J. Mammal.*, 79, 320-325.

Vandegrift, K.J, Raffel, T.R., & Hudson, P.J. (2008). Parasites prevent summer breeding in white-footed mice, *Peromyscus leucopus*. *Ecology*, 89, 2251-2258.

Wang, S.C. (2007). inetwork: network analysis and plotting. Version 1.2, URL: http://www.r-project.org.

Washburn, B.E., Millspaugh, J.J., Morris, D.L., Schulz, J.H., & Faaborg, J. (2006). Using a commercially available enzyme immunoassay to quantify testosterone in avain plasma. *The Condor,* 109, 181-186.

Weihong, J.I, White, P.C.L., & Clout, M.N. (2005). Contact rates between possums revealed by proximity data loggers. *J. Appl. Ecol.*, 42, 595-604.

Zuk, M. & McKean, K.A. (1996). Sex differences in parasite infections: patterns and processes. *Int. J. Parasit*., 26, 1009-1024.

Table 2.1 - Prevalence and intensity of *P. peromysci* fecal egg shedding, sampled from the final trapping session of the experiment. There were no significant differences in prevalence or intensity of egg shedding among groups.

	infected	total	prevalence	SE	mean(*log* epg)[a]	SE[b]
Untreated sites						
Females	3	8	0.38	0.17	0.73	0.05
Males	3	6	0.50	0.20	0.58	0.15
Treatment sites						
Females	2	9	0.22	0.14	0.42	NA
Testosterone-males	1	2	0.50	0.35	1.21	NA
Sham-males	2	6	0.33	0.19	0.60	NA
Untreated-males	7	15	0.47	0.13	0.71	0.15

[a] Intensity of fecal egg count was calculated from only infected individuals
[b] Standard error only calculated with >2 infected samples

Table 2.2 – Summary of the network degree distribution for each replicate trapping site during the time period before treatment (May – July) and after treatment (July-August)

	Site	Nodes	Time Period	Average Degree	Standard Error*	AIC_C † Lognormal Poisson	AIC_C † Waring (exponential)
Untreated Sites	I	29	Before Treatment	3.24	0.36	**125.22**	132.32
		11	After Treatment	2.18	0.45	**46.31**	51.59
	VI	26	Before	3.54	0.37	**175.54**	181.35
		22	After	2.36	0.33	**116.17**	132.59
	III	43	Before	6.7	0.4	**225.25**	240.69
		28	After	4.14	0.38	**114.23**	126.86
Treated Sites	II	35	Before	4.74	0.5	**122.27**	124.11
		27	After	5.48	0.45	**77.27**	83.66
	IV	25	Before	3.5	0.57	**237.17**	254.61
		19	After	3.26	0.41	**143.65**	159.46
	V	44	Before	6.91	0.54	**106.71**	115.48
		33	After	6.55	0.45	**75.91**	80.89
	VII	31	Before	4.9	0.58	**162.03**	167.73
		21	After	3.9	0.43	**84.31**	94.06
	IX	43	Before	5.72	0.59	**240.04**	243.44
		31	After	5.1	0.41	**147.18**	148.69

*Standard error of average degree calculated from generalized linear mix models. See results for model details.

† Akaike's information criteria corrected for small sample size. Lower values indicate a superior model fit to observed data.

Figure 2.1

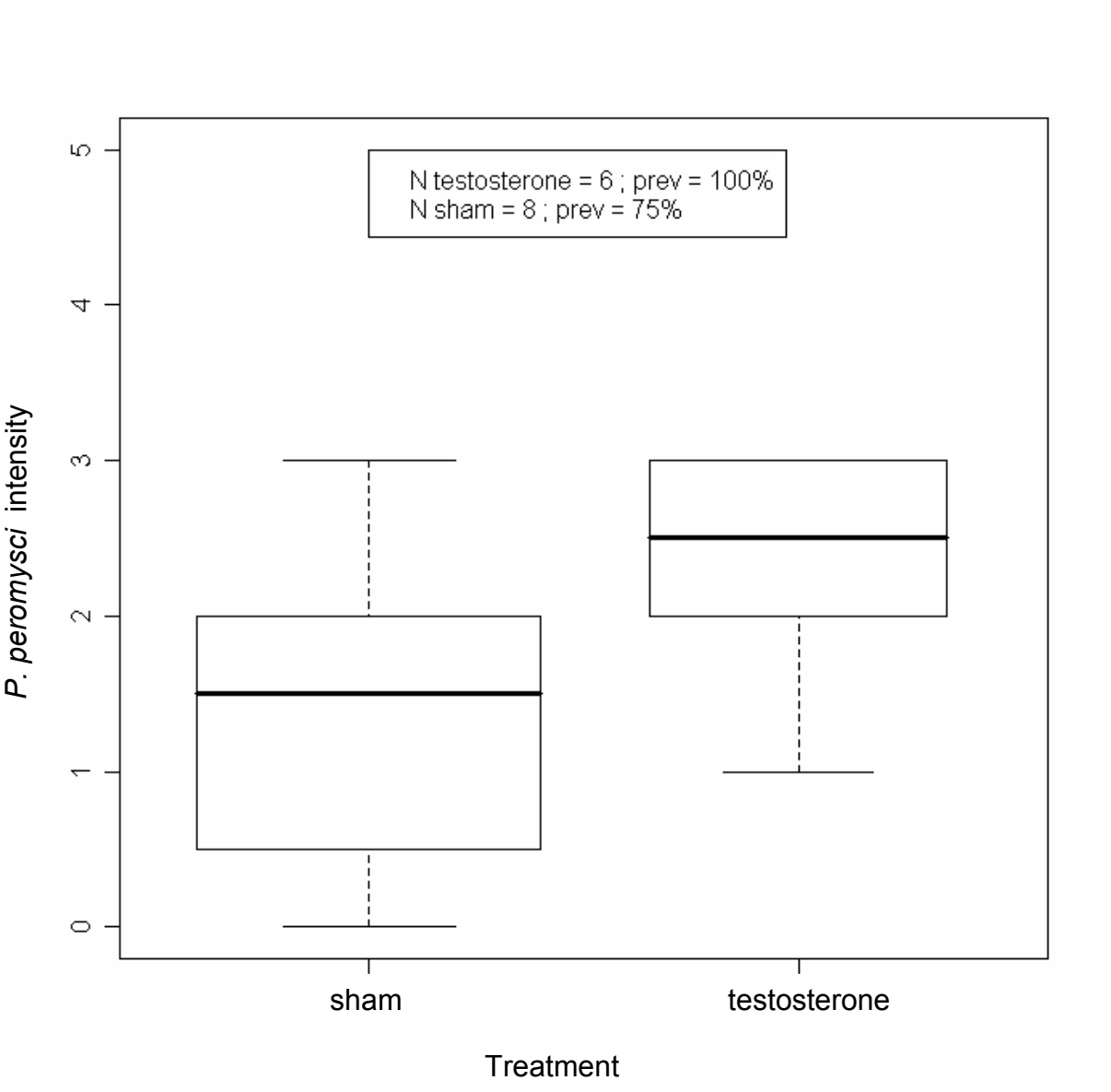
N testosterone = 6 ; prev = 100%
N sham = 8 ; prev = 75%
P. peromysci intensity
0
1
2
3
4
5
sham
testosterone
Treatment

Figure 2.2

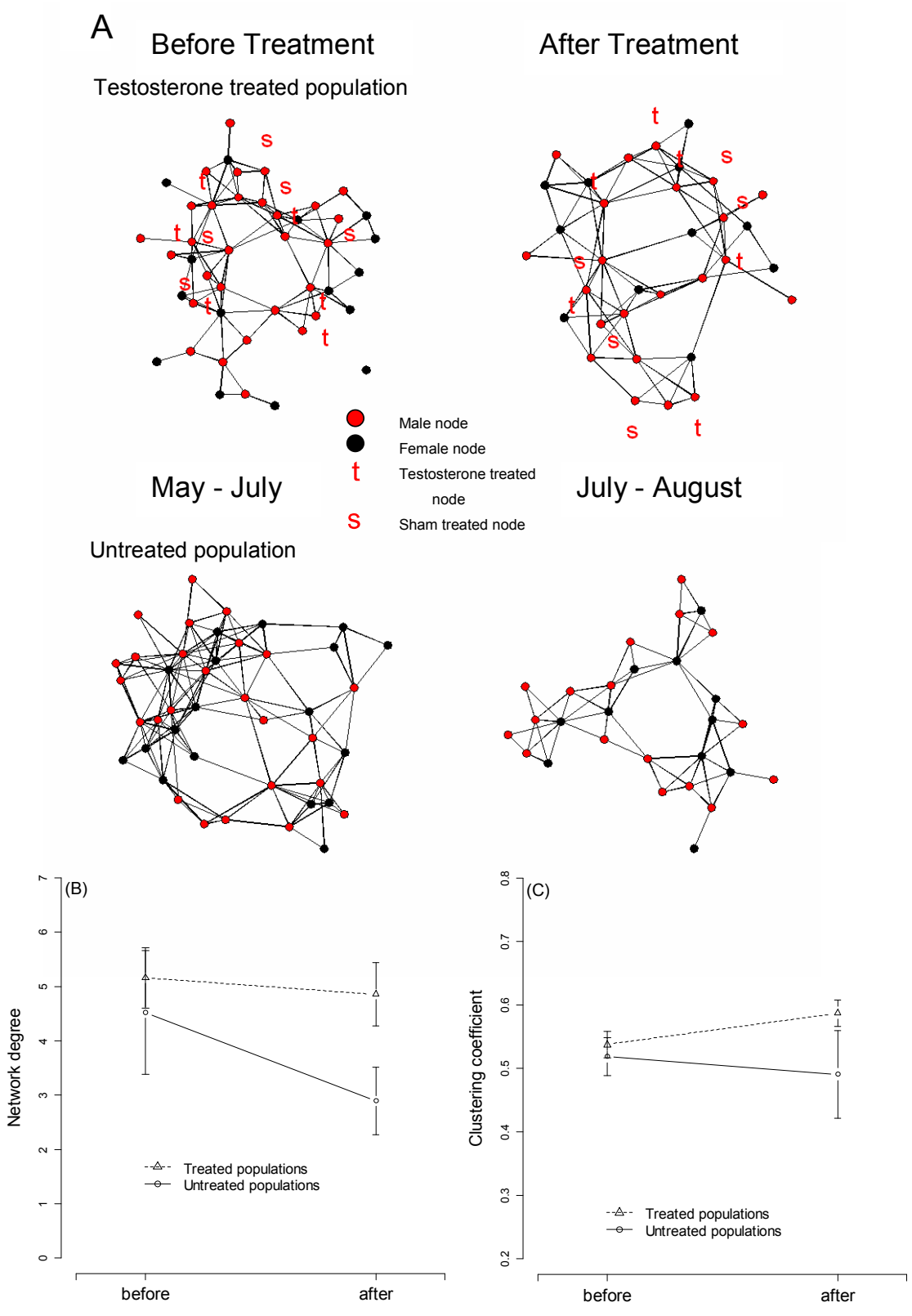
A
Before Treatment
After Treatment
Testosterone treated population
Male node
Female node
Testosterone treated node
Sham treated node
May - July
July - August
Untreated population
(B)
Network degree
Treated populations
Untreated populations
before
after
(C)
Clustering coefficient
Treated populations
Untreated populations
before
after

Figure 2.3

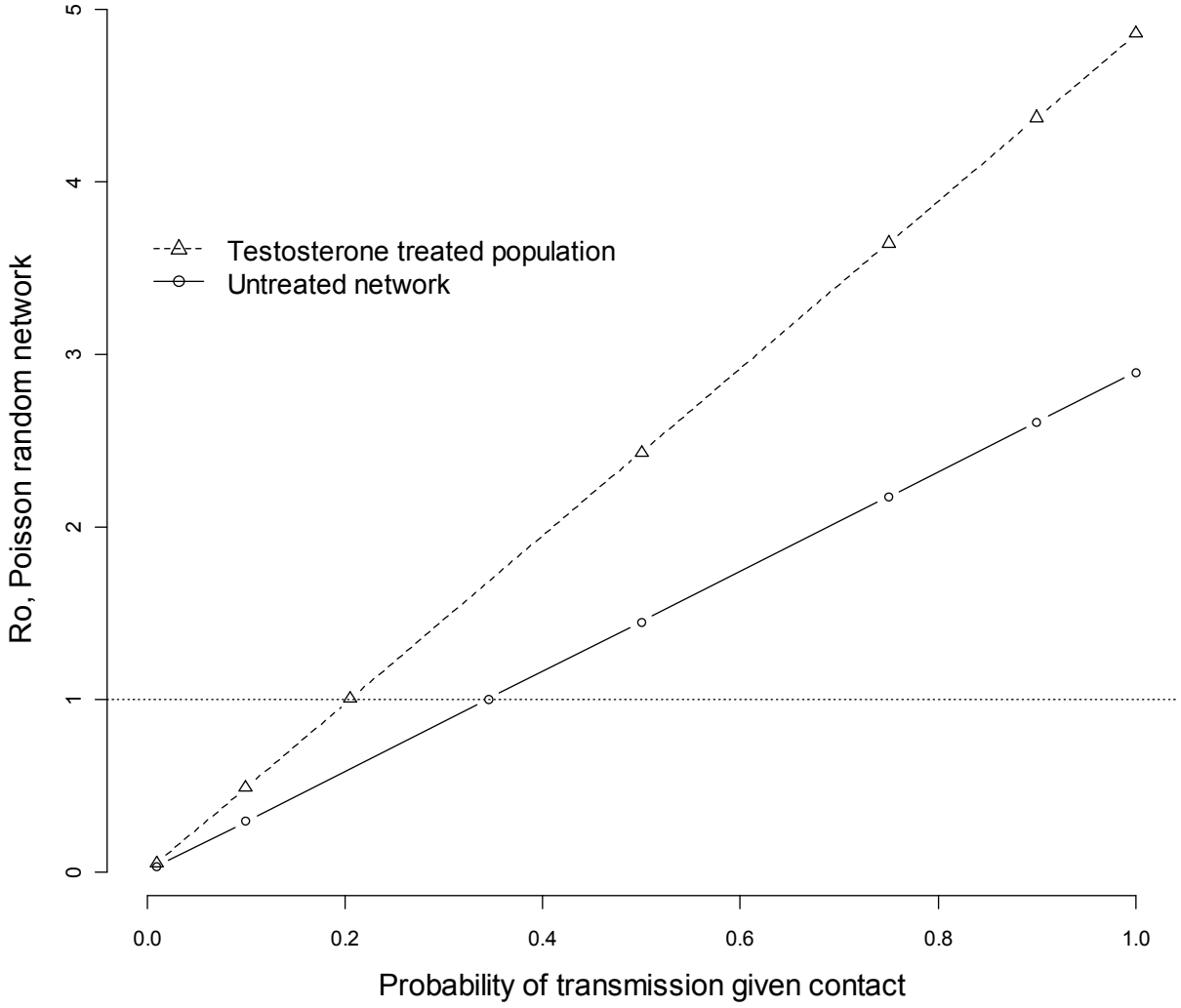
Testosterone treated population
Untreated network
Ro, Poisson random network
Probability of transmission given contact
0
1
2
3
4
5
0.0
0.2
0.4
0.6
0.8
1.0

Figure 2.1 Box and whisker plot of *Pterygodermatites peromysci* intensity from dissections of testosterone-treated (n = 6) or sham-treated (n = 8) male individuals recaptured at the end of the experiment. Boxes represent median with 1st and 3rd quartile of the range of intensity per infected mouse and whiskers represent an approximate 95% confidence interval around the median.

Figure 2.2 Visualization of *P leucopus* contact network graphs (A) from a testosterone treated population (top panels) and untreated population (bottom panels). Two networks were created for each replicate trapping site, one for the 6 week prior to testosterone treatment (left panels) and one for 6 weeks post treatment (right panels). (B) Average network degree of all individuals in populations with testosterone treated males (dashed lines) is maintained after treatment and populations with no experimental treatment (solid lines) decrease degree in the period after treatment. (C) Testosterone treatment increases the clustering coefficient, but that change is not statistically different from the non-treated populations. Error bars represent one standard error based on site level variation.

Figure 2.3 Basic reproductive number of a parasite, R_0, estimated based on the average degree (contact rate) of networks with random degree distribution observed for testosterone treated sites (4.86) and untreated sites (2.90). R_0 is 1.7 times higher on testosterone treated sites across a range of values for probability of transmission per contact. The epidemic threshold, $R_0 = 1$, is reached at a lower transmissibility on testosterone treated sites (0.21) compared to untreated sites (0.35).

Chapter 3. Sex-biased transmission of a complex life-cycle parasite: why males matter

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ABSTRACT

A central challenge in disease ecology is to identify and predict the individual hosts responsible for parasite transmission and the mechanisms that cause variation in infectiousness between hosts. The male-biased transmission hypothesis states that males produce more onward transmission events than females and proposes several competing mechanisms that generate male-biased transmission in mammals; including greater parasite intensity, higher prevalence, greater reproductive output per parasite, and greater behavioral-mediated spread of infective stages from males compared to females. The first goal of our study was to test for male-biased transmission in a system with no sex-biased infection: white-footed mice (*Peromyscus leucopus*) transmission of the trophically transmitted intestinal nematode parasite (*Pterygodermatites peromysci*). We performed a longitudinal field experiment and continuously removed intestinal nematode parasites of male or female white-footed mice with anthelmintic treatment and recorded the subsequent transmission to the non-treated sex of the mouse host. If males drive transmission, we predicted female mice would have lower infection rates on male-dewormed sites compared to controls. Our second goal was to determine the mechanisms generating this transmission pattern. To that end, we dissected a cross-sectional sample of hosts and compared the prevalence, intensity, and fecundity of intestinal parasites between host sexes to examine potential mechanisms for male-biased transmission. Removing parasites from male mice resulted in lower infection rates among female mice in male-treated sites compared to females in control sites. We detected no effect of female-deworming on transmission rates among male

mice. We found no difference in prevalence, intensity, or fecundity of *P. peromysci* parasites in the cross-sectional sample of mice. Our field experiment confirmed that male white-footed mice are responsible for driving transmission of a parasitic nematode, even when infection rates were not male-biased and transmission requires passage through an intermediate host. Without male-biased prevalence, intensity, or parasite fecundity, we concluded that male-biased transmission is unlikely to be created via physiological differences between male and female hosts and the parsimonious explanation is that male behavior spread infective particles in a more successful manner.

Keywords: sex-biased transmission; trophic interactions; gastrointestinal nematode; white-footed mouse; camel cricket;

INTRODUCTION

Males are often the most conspicuous portion of populations (Andersson 1982, Folstad and Karter 1992), yet, as Rankin and Kokko (2007) point out, they are usually ignored by population ecologists because females are the obvious source of population growth. Nevertheless, males can play an important role in shaping populations processes, that in turn, influence female vital rates (Rankin and Kokko 2007, Boukal et al. 2008, Heubal et al. 2009, Veran and Beissinger 2009). For example, when males are responsible for much of the transmission of infectious diseases this can have a profound impact on survival and fecundity of females and, consequently, shape population dynamics. (Skorping and Jensen 2004, Miller et al. 2007). In this study we addressed the role that males play in parasite dynamics and specifically identified male-biased transmission rates. Among mammal-parasite systems in particular, male hosts are implicated as the important host class for transmission because males tend to have higher infection rates and/or greater infection intensity than their female conspecifics (Zuk and

McKean 1996, Poulin 1996a, Skorping and Jensen 2004, Zuk 2009). However, a connection between infection rates and transmission rates has not been clearly established based on predictable host characteristics since there is the confounding effect of infection bias, males have more worms so this leads to more infection of both males and females and does not separate the relative infectiousness of the males. Many studies of vertebrate-parasite systems have successfully identified characteristics of the most infected host class (see reviews of Shaw and Dobson 1995, Poulin 1996a, Moore and Wilson 2002), and yet, only a few key experiments have identified that a minority of the population is responsible for the majority of transmission; and these individuals have been relatively large males (Perkins et al. 2003, Ferrari et al. 2004).

There are two non-exclusive sources of heterogeneity that may lead to male-biased transmission: first, males acquire infection at a greater rate than females and simply cause more infections because they have more parasites that generate proportionally more infective stages. Second, males acquire infection at an equal rate yet still produce more onward infection. In this latter case, male-biased transmission may still result from males shedding more infectious particles because parasites are able to extract resources more efficiently from males and are more fecund. Alternatively, the infectious particles shed by males may be more likely to successfully transmit, resulting in greater infectiousness per parasite infective stage.

Our study system focused on the white-footed mouse (*Peromyscus leucopus*), a generalist rodent of North America that harbors a diverse community of intestinal helminth parasites across its range (Grundman et al. 1976, Pedersen and Greives 2007, Vandegrift and Hudson 2009). In the forested regions of central Pennsylvania, the dominant intestinal parasite of the white-footed mouse is a nematode (*Pterygodermitites peromysci*, family: Rictularidae) with a complex life-cycle (Oswald 1958a, Oswald 1958b, Vandegrift and Hudson 2009). Adult *P. peromysci*

reproduce sexually in the small intestine lumen of white-footed mice and eggs are shed into the environment with host feces. The intermediate host of *P. peromysci* is a scavenger camel cricket (*Ceutophilus palipides*, and other *Ceutophilus spp.*). When a cricket consumes infectious mouse feces, the parasite eggs hatch in the cricket midgut, the larval nematode enters the hemoceol of the intermediate host and develops to an infectious stage within a cricket-derived cyst which becomes infective to mice after 1-2 weeks. The parasite life-cycle is completed when a mouse ingests a cricket with infectious cysts: the parasite larvae emerge from the cyst, develop into reproductive adults in the host intestinal-lumen, and begin to shed eggs after 4-6 weeks. *P. peromysci* intensity is not different between male or female white-footed mice and prevalence fluctuates between 10-60%: spatially, between years, and seasonally within years, but is also not significantly different between host sex (Vandegrift et al. 2008). Camel crickets produce 1-2 generations per season in our study area and abundance is highest in late summer and early autumn (Luong, unpublished data). Intensity of *P. peromysci* infective cysts in infective crickets ranges from 1-60 with prevalence highest in autumn (Luong et al 2009). Trophically transmitted parasites utilize the ecological link between predator and prey so that infectious particles are passed from one host to the next when consumed and heterogeneities in transmission may arise at several points in a trophic-transmission cycle. When the opportunity exists to sample parasites from all stages of a complex life-cycle, the sources of transmission heterogeneities that are difficult to measure in direct life-cycles, such as exposure to infectious stages and distribution of infectious stages, become tractable, particularly in parasites with trophic transmission where no reproduction takes place in the intermediate host (Keymer and Anderson 1979, Hansen et al. 2004, Luong et al. 2009).

We investigated the relative contribution of male and female white-footed mice to the transmission of *P. peromysci* by conducting a field experiment and selectively removing parasites from male or female mice. We asked the question, are males responsible for more transmission events when infection is similar in males and females? We postulated that male hosts contributed more transmission events compared to females and tested the prediction that females on male-dewormed sites will have lower infection rates compared to control females, while removal of parasites from female hosts will not affect transmission in male hosts.

We also asked, what is the mechanism of male-biased transmission when the prevalence and intensity of parasites does not differ between sexes? To address this question, we sampled hosts and compared the prevalence, intensity, and fecundity of intestinal parasites between host classes. Our objective was to evaluate what host characteristics were associated with variation in prevalence of infection, intensity of infection, and fecundity of parasites. Specifically, we tested the prediction that parasites from males hosts would be more fecund compared to parasites from female hosts.

METHODS

Mouse capture: field experiment

We live-captured white-footed mice on 9 open trapping grids, consisting of 64 traps in a 8 x 8 array at 10 meter intervals (Ugglan #2 multiple capture traps, Grahnab AB, Sweden). All grids were 20km south of State College, Pennsylvania, USA, in open forested habitat and separated by at least 500m. We recorded trap location, body length, body mass, sex, and breeding condition for each mouse capture. Feces were collected from each individual for parasite detection. We classified males in breeding condition if their testes were descended. We

classified females in breeding condition if they had a perforated vagina, were lactating, or were pregnant. We classified mice into two age classes: adults were defined as any mouse in breeding condition or any mouse with a mass greater than 15g and juveniles as less than 15g and not in breeding condition. We individually marked mice using a passive induced transponder tag (EIDAP, Sherwood Park, Alberta, Canada) and trapped every two weeks from 29-April to 10-September 2008 with 2 consecutive trap nights per trap session, for a total of 10 trap sessions over 20 weeks. We considered any individual captured during more than one trap session a resident and individuals captured during only one trap session as non-residents.

Experimental design and parasite identification

Beginning with the 3rd trapping session (3-June, 2008) and continuing to the end of the experiment, we selectively removed parasites from all male or female mice at each capture. We randomly selected three grids as male-anthelmintic treatments, three grids as female-anthelmintic treatments, and three grids as untreated controls. The treatment was an oral dose of anthelmintic (1 µl/gram of Levamisole Hydrochloride; dose: 36 mg/Kg, AgriLabs®, Missouri, USA), administered at every capture to individuals of the anthelmentic-treated sex (referred to hereafter as treated-sex). Individuals on experimental grids of the non-anthelmintic-treated sex (referred to hereafter as response-sex) and all individuals captured on control grids were given a sham treatment of 1 µl/g sterile water. All animal handling was approved by the Institutional Animal Care and Use Committee at Pennsylvania State University (IACUC #23268).

We collected fecal samples from trap contents and stored them at 4^{o}C in petri dishes lined with a damp towel (1ml water) overnight to standardize humidity. We determined parasite infection using a modified McMasters egg floatation for all fecal samples greater than 0.06g

(Sloss and Kemp 1978): 1g feces mixed with 10ml saturated-$MgSO_4$ solution, giving a minimum resolution of 37 eggs per gram feces (epg). We identified nematode eggs consistent with Rictularidae morphology as *P. peromysci*. *P. peromysci* is not found in any other mammal species on our study sites (Vandegrift, unpublished data) and we confirmed egg morphology via comparison with dissected nematodes. We identified other intestinal helminthes based on egg morphology and comparison with dissected specimens, but we limited our focus to *P. peromysci* because it was the numerically dominant helminth in our study.

Cross-sectional sampling for parasite fecundity

We obtained adult *P. peromysci* from necropsies of animals euthanized at the end of various unrelated field studies from August – October conducted between 2003 and 2007. We recorded standard morphometric data, as described above, for each mouse at the time of capture and before they were euthanized. Nematodes were dissected from hosts and transferred to a preservative of 90% ethanol and 10% glycerol. We photographed individual nematodes under a stereomicroscope (Leica® S6E) with a digital camera (Nikon® Coolpix 4500) and measured nematode length from the photographs with the ImageJ software package (Rasband 2007). We estimated parasite fecundity by counting eggs *in-utero*: we extracted the eggs from adult female *P. peromysci* by dissolving the nematode in household bleach (6% sodium hypochlorite) for 8-10 minutes; extra care was taken to not overexpose the eggs to bleach as this may cause the eggs to deteriorate. Once all the eggs were released, we rinsed individual samples with deionized water and centrifuged for 2 min. at 1600 RPM. We removed the supernatant and re-rinsed the pellet containing the eggs with deinonized water, and then re-suspended before analysis with a flow cytometer (Beckman-Coulter FC500, Miami Lakes, FL, USA). Just prior to running the sample,

we added fluorescent beads (Flourebrite™ 20 μm microsperes, Polyscience, Inc., Warrington, PA, USA) to calibrate the flow cytometer.

Statistical analysis: field experiment

We divided time into 4 periods corresponding to treatment and parasite life-cycle: before-treatment, 2-6 weeks post-treatment initiation, 8-10 weeks post-treatment, and 12-14 weeks post-treatment. We grouped the time up to 6 weeks post-treatment because the pre-patent period of *P. peromysci* is at least 6 weeks and, hence, we did not expect to detect any effect of removing parasite infective stages to the environment (i.e. manipulating exposure) until after one parasite infection cycle. We split the remaining period after treatment to correspond to the drastic increase in intermediate host abundance that begins in late summer (Luong et al. 2009). We only included adult mice because younger mice are rarely infected with *P. peromysci* (Vandegrift and Hudson 2009) and the long development time of *P. peromysci* suggests that detection via fecal egg output would produce increased likelihood of type II error compared to older mice because of the 6-8 week pre-patent period of the *P. peromysci*.

Anthelminthic effect on parasite transmission

A before-after-control-impact (BACI) study design was used to assess the effect of sex-specific anthelmintic treatment on transmission and was analyzed using generalized linear mixed-models (GLMM). Parasite infection status was considered a binomial response variable, with mass, site treatment, and time-post-treatment initiation (time-period) as fixed-effect independent variables and trapping grid and individual mouse identification as random-effect variables. The site treatment by time-period interaction explicitly tested for an experimental effect in this BACI analysis (McDonald, Erickson & McDonald 2000). The random effect terms

structured the error to allow for analysis of repeated samples of individuals in time and experimental units without committing psuedoreplication (Hurlbert 1984). Parasite intensity (eggs per gram feces) was not used as a response because subsequent analysis revealed no variation in parasite egg output among host classes and previous studies have shown that parasite intensity is randomly distributed among hosts with a low mean (Vandergrift & Hudson 2009b).

The effect of the male-anthelmintic treatment on the parasite infection status in females was tested separately from the effect of the female-anthelmintic treatement on the parasite infection status in males because the treatment effectively removed parasite egg shedding from the treated sex (details in results). In each analysis, the non-treated sex (response sex) was compared against that same sex from only the control sites because fitting statistical models for all individuals was problematic when there was no variation in response in some treatment levels; for example, there was no female infection in female-treated sites after treatment. Experimental effects were tested by comparing a series of nested GLMM models beginning with an additive model with site treatment, time-period, and mass compared to a model with a site treatment ^{x} time-period interaction and mass. A likelihood-ratio X^2 test was used to compare the overall model fit to data between the reduced model and each subsequent model with the additional term. A P-value ≤ 0.05 indicated the model with the additional parameter was a significantly better fit to the data compared to the reduced model and that term was kept in the model. If the anthelmintic-treatment ^{x} time-period interaction was included in the best-fit model we concluded that there was a significant experimental effect.

To further examine the effect size of the coefficients in the model selected in the process above, 95% Bayesian credible intervals (CIs) were constructed from 10,000 samples from the posterior distribution of fitted GLMM fixed-effect parameters using Markov-Chain-Monte-Carlo

methods. Coefficients with credible intervals that excluded zero were considered to have a significant effect on response-sex prevalence. The credible intervals for the time-period ^{x} site treatment coefficients were used to evaluate the magnitude of the experimental effect on transmission by indicating a difference in how parasite prevalence in the response-sex changed from the before time-period to the time periods after-treatment on control sites compared to the sites where the other sex was treated. GLMMS were fitted using package 'lme4' in R (Bates & Maechler 2010; R development core team 2009) and the package 'arm' was used to conduct samples of fitted models coefficients for Bayesian CI construction (Gelman *et al*. 2010).

Anthelminthic effect on breeding status

We evaluated population-level effects of anthelmintic treatment on probability of a mouse being in breeding condition using a similar GLMM framework with breeding condition as a binomial response. We began with a model including the additive effects of site treatment and time-period and sequentially added an anthelmintic-treatment ^{x} time-period interaction and mass to the reduced model. We kept the additional variables if a likelihood ratio X^2 test indicated a full model was a significantly ($P \leq 0.05$) better fit to data than a reduced model and evaluated the explanatory the significance of the explanatory variables in the best fit model by constructing 95% Bayesian credible intervals.

Statistical analysis: longitudinal parasite prevalence and egg shedding

Prevalence of infection on control sites

To investigate if male mice were producing more transmission events because of higher prevalence or parasite egg shedding, we analyzed the mice captured from our control sites during the longitudinal experiment to explore differences in both prevalence of infection and eggs shed

per gram feces of infected individuals based on sex, breeding condition, mass, and seasonality using a model selection approach with GLMMs. To describe prevalence patterns, we used infection status as a binomial response variable with mouse sex, mass, month, and breeding condition as fixed-effect explanatory variables, with trap grid and individual mouse identification as random-effect variables. We used a backward selection technique starting with a full model with sex, mass, month, breeding condition, interactions of sex x breeding interaction, sex x month interaction, breeding x month interaction, sex x mass interaction, and sex x breeding x month interaction. We sequentially tested explanatory variables starting with the most complicated interactions and proceeding to the simple additive variables if interactions were not significant. We kept a variable if a likelihood ratio X^2 test indicated a full model was a significantly ($P \leq 0.05$) better fit to data than a reduced model without the variable. We created 95% Bayesian CIs around the explanatory variable coefficients in our final model and considered a coefficient significant if the CI did not overlap zero.

Parasite egg shedding intensity on control sites

We used a similar approach to describe eggs shed per gram feces, using only infected individuals captured on control sites. We used the *log* transformed epg as a Gaussian response with the GLMM structure as above. We started with a simple intercept-only model and added one explanatory variable at a time, starting with the single variables and then added interactions; keeping a variable if a likelihood ratio X^2 test was significant ($P \leq 0.05$).

Statistical analysis: cross-sectional analysis of nematode fecundity and intensity

Parasite fecundity

We used GLMMs with gaussian error to describe patterns of *P. peromysci* fecundity (*log* transformed in-utero egg count) and length from our cross-sectional dissections with the following fixed-effect independent factors: host body mass, sex, breeding condition, and intensity of infection (number of nematodes per host) and with individual mouse as a random-effect variable. The minimal model was achieved by backwards deletion of variables using a X^2 likelihood ratio test as described previously. We began with a full model including sex ^{x} breeding condition interaction, along with each explanatory variable, and dropped terms that were not significant ($P < 0.05$) starting with the interactions and proceeding to the individual variables. We calculated 95% CIs for the terms in the final model to determine significance.

Parasite intensity

We also looked for patterns of nematode intensity from the same sample by using a Poisson GLM with intensity as the response and host sex, mass, and breeding condition as explanatory variables. The minimal model was also achieved by backwards deletion of factors that were not significant (X^2 , $P < 0.05$) compared to a full model, beginning with sex ^{x} breeding condition interaction. We calculated 95% CIs for the terms in the final model to determine significance.

Parasite detection the spring following the experiment

We also trapped and performed fecal egg identification for 3 trap sessions in the spring of 2009 (1-April to 6-May) to examine if any treatment effects persisted over the winter. We did not treat any mice with anthelminthic. We analyzed the data in a GLMM with infection status of *P. peromysci* as a binomial response and treatment, sex, and mass as fixed effect explanatory variables and individual and site as random effects. We tested for a persistent treatment effect by

comparing a full additive model with a model containing a sex ^{x} treatment interaction with a likelihood ratio test and then calculating the 95% CIs for the better fitting model.

RESULTS

We recorded 1725 white-footed mouse captures of 453 individuals. We collected sufficient feces from 867 captures of 391 individuals to undertake parasite egg identification and counts. We identified 3 nematode species and 1 cestode from fecal egg counts. *P. peromysci* was the most common helminth with an overall prevalence of 14.5% on non-treated sites. We also detected uncommon infections of *Capillaria americana* (1.7%), *Syphacia peromysci* (5.8%), and a cestode in the genus *Hymenolepis* (0.3%). All subsequent parasite results only refer to *P. peromysci*.

Experimental effects of anthelminthic treatment

Anthelminthic effect on parasite transmission

The levamisole treatment successfully reduced parasite egg output from the target sex. Anthelmintic treatment of females resulted in no detection of parasite eggs in feces from resident individuals (Figure 1a), although a single infected non-resident female was captured during the last trapping session. Anthelmintic treatment of males resulted in reduced detection of parasites in the first session after treatment began (2 weeks) and parasites were only detected in one subsequent resident male, captured in the 2nd to last trapping session (Figure 1b). Three infected non-resident males were captured on male-treated grids after treatment initiation; 2 on the same male-treatment grid and one on a separate grid.

As predicted by the male-biased transmission hypothesis, prevalence of infection in females was significantly lower on male-treated sites 8-10 weeks after treatment began compared to female prevalence on control sites 8-10 weeks post-treatment (95% CI [-10.4 , -0.52]). The before-after-control-impact analysis indicated the term for an experimental effect improved the model fit to the data (time-post-treatment ^{x} grid treatment $X^2 = 14.9$, *d.f.* = 6, $P = 0.02$). Female mass also significantly improved the GLMM ($X^2 = 8.58$, *d.f.* = 1, $P < 0.01$) and had a positive effect on prevalence (95% CI [0.04 , 0.61]). The significant experimental effect on female response-sex prevalence was only detected 8-10 weeks post treatment and non-interaction coefficients did not have an effect on female prevalence; however, variation in female prevalence was difficult to estimate because very little female infection was detected while females were being treated (Figure 1a, Table 1).

Prevalence of infection in males as the response sex was not different compared to male prevalence on control-sites and there was no evidence for an experimental effect: interaction between time-post-treatment and treatment ($X^2 = 7.54$, *d.f.* = 6, $P = 0.27$). Mass improved the GLMM fit to the data but was not quite significant ($X^2 = 3.45$, *d.f.* = 1, $P = 0.06$) and had a weak positive effect on prevalence (95% CI [-0.02 , 0.34]). Non-interaction coefficients had no effect on male prevalence (Table 1).

Anthelmintic effects on breeding

There was no evidence that females were more likely to be in breeding condition in response to treatment: the treatment ^{x} time-post-treatment interaction did not improve the model fit to data ($X^2 = 11.01$, *d.f.* = 6, $P = 0.08$). Mass improved the model fit to the data ($X^2 = 104.5$, *d.f.* = 1, $P <0.001$) and had a significant positive effect on the probability of female being in

breeding condition (95% CI [0.28 , 0.45]). No other parameter had a significant effect of a female probability of being in breeding condition: 95% CI female treatment [-0.44 , 0.80], male treatment [-0.23 , 1.06], 2-6 weeks after treatment [-1.19 , 0.18], however, females captured later in the summer had a slightly lower probability of being in breeding condition: 8-10 weeks after [-1.50 , 0.01], 12-14 weeks after [-1.63 , 0.001].

Likewise, there was no evidence that males were more likely to be in breeding condition in response to treatment: the treatment ^{x} time-post-treatment interaction did not improve the model fit to data ($X^2 = 9.45$, *d.f.* = 6, $P = 0.15$). Mass improved the model fit to the data ($X^2 = 62.4$, *d.f.* = 1, $P < 0.001$) and had a significant positive effect on the probability of a male being in breeding condition (95% CI [0.19 , 0.34]). Males were more likely to be in breeding condition during the period 8-10 weeks post-treatment-initiation on all grids (95% CI [0.30 , 1.52]) and no other parameter had a significant effect of male probability of being in breeding condition: 95% CI female treatment [-1.16 , 0.01], male treatment [-0.92 , 0.21], 2-6 weeks after treatment [-0.24 , 0.79], 12-14 weeks after [-0.67 , 0.48].

Longitudinal parasite prevalence and egg shedding

Parasite prevalence on control sites

We examined prevalence of infection in our control sites during our longitudinal trapping and the month ^{x} breeding interaction was the only interaction that significantly improved the model fit to the data ($X^2 = 13.7$, *d.f.* = 5, $P = 0.017$). There was no evidence of a three-way interaction between sex, breeding, and month ($X^2 = 3.10$, *d.f.* = 4, $P = 0.54$). The mass ^{x} sex

interaction ($X^2 = 0.32$, *d.f.* = 1, $P = 0.57$), mass ($X^2 = 2.81$, *d.f.* = 1, $P = 0.09$) and sex ($X^2 = 0.85$, *d.f.* = 1, $P = 0.35$) were not significant variables. 95% CIs indicated that no coefficients for the month ^{x} breeding interaction were significant (Table 2), however, visual inspection of prevalence by breeding reveals that in June and August there were large differences in prevalence between breeding and non-breeding individuals (Figure 2). In June, we detected infection only in males and females that were in breeding condition. In August the pattern was the reverse and we detected infection in only non-breeding females and a higher prevalence of infection in non-breeding males (Figure 2).

Parasite egg shedding intensity on control sites

We detected no differences in egg shedding among time or host class of infected individuals. Neither sex ($X^2 = 0.41$, *d.f.* = 1, $P = 0.52$), mass ($X^2 = 2.34$, *d.f.* = 1, $P = 0.13$), breeding condition ($X^2 = 0.60$, *d.f.* = 1, $P = 0.44$), nor month ($X^2 = 0.85$, *d.f.* = 4, $P = 0.85$) provided a better fit to data compared to the mean of all individuals (mean *log epg* = 4.61 , 95% CI [4.44 , 4.77]).

Cross-sectional analysis of nematode fecundity and intensity

Parasite fecundity

We counted eggs *in utero* from 125 *P. peromysci*, obtained from a cross-sectional sample of 76 adult mice, yielding a mean of 59,772 ± 3775 SE eggs per nematode (Figure 3). The distribution of *in utero* egg counts was not well-described by negative binomial (likelihood ratio goodness-of-fit $P < 0.001$) or log-normal (Kolmogorov-Smirnov $P < 0.001$) distributions and we

analyzed *log* transformed egg count per worm to approximate a Gaussian distribution (Figure 3). There was a strong positive relationship between nematode eggs per female worm and length of worm ($X^2 = 70.7$, *d.f.* = 1, $P < 0.001$; 95% CI [0.10 , 0.15], Figure 4). Host mass also improved the fit of the model but was not significantly correlated with *in utero* egg count ($X^2 = 9.024$, *d.f.* = 1, $P < 0.002$; 95% CI [-0.03 , 0.10]). Host sex ($X^2 = 1.40$, *d.f.* = 1, $P = 0.24$), host breeding condition ($X^2 = 0.35$, *d.f.* = 1, $P = 0.56$), parasite intensity ($X^2 = 1.53$, *d.f.* = 1, $P = 0.22$), and a sex x breeding interaction ($X^2 = 0.59$, *d.f.* = 1, $P = 0.44$) were not important to explaining egg count.

Parasite size and intensity

We measured the body length 164 female *P. peromysci* from 92 adult mice resulting in a mean length of 18.46 ± 0.36 SE mm and a random frequency distribution. Host mass ($X^2 = 41.3$, *d.f.* = 1, $P < 0.001$) and breeding condition ($X^2 = 5.11$, *d.f.* = 1, $P = 0.02$) improved the GLMM fit, but 95% CIs indicated neither had a significant effect on worm length (mass: [-0.13 , 0.40]; breeding: [-0.86 , 2.34]. Host sex ($X^2 = 0.32$, *d.f.* = 1, $P = 0.57$), nematode intensity ($X^2 = 0.03$, *d.f.* = 1, $P = 0.87$), and sex x breeding interaction ($X^2 = 0.004$, *d.f.* = 1, $P = 0.95$) did not have an association with nematode length. Intensity of infection in the cross sectional dissection was not related to any host factor: sex x breeding interaction ($X^2 = 0.017$, *d.f.* = 1, $P = 0.90$), sex ($X^2 = 0.011$, *d.f.* = 1, $P = 0.918$), breeding ($X^2 = 0.020$, *d.f.* = 1, $P = 0.89$), mass ($X^2 = 0.014$, *d.f.* = 1, $P = 0.90$).

<u>Parasite detection the spring following the experiment</u>

There was no evidence for a persistent treatment effect in the spring following the termination of the deworming treatments ($X^2 = 0.44$, *d.f.* = 2, $P = 0.80$). We did not detect any host factors that influenced the probability of infection during this time (95% CIs): female treatment [-0.77, 2.91], male treatment [-0.68 , 2.84], sex [-0.87 , 1.75], mass [-0.09 , 0.28].

DISCUSSION

We experimentally demonstrated the disproportionate contribution of male hosts to the transmission of a trophically transmitted parasite in a system where there was no male bias in infection levels. The infection rates decreased in female mice when we removed parasites from male mice and, intriguingly, we observed no change in male infection rates when we removed parasites from females; thus, males hosts contribute more to parasite transmission compared to female hosts. Factors related to the seasonality of transmission, outside of the scale of our treatment, also appeared to be important to the parasite-dynamics in this system because we could no longer detect a male-biased treatment effect by the end of the season. Nonetheless, this finding is one of the few experiments in natural populations that addresses transmission and, along with previous experiments in a mouse-nematode system (Ferrari *et al*. 2004; Luong, Grear & Hudson 2009), supports the hypothesis that males are responsible for driving parasite dynamics (Zuk 2009). The implication of these results are that males maybe more important to driving infection than is generally suspected, even in systems with complex life cycles and no male bias in infection levels.

Hypotheses for the underlying mechanisms of male-biased transmission fall into two general categories: physiological and behavioral. These mechanisms are not mutually exclusive and may act independently or in tandem to result in male hosts producing more infectious stages

and/or shedding infective stages at times or places that result in greater probability of onward transmission compared to females. Physiological mechanisms of male-biased transmission often fall under the immuno-competence handicap hypothesis (ICH, Grossmann 1989; Folstad & Karter 1992); which predicts that male hosts acquire parasites at a faster rate and parasites can be more fecund because male hosts have a weaker immune response than females (Hughes & Randolph 2001; Muegot *et al*. 2005; Seivwright *et al*. 2005).

Our data did not provide any evidence to support physiologically-mediated mechanisms that could lead to male-biased transmission. We detected no difference in egg shedding rates between males and females. Indeed, there was no difference in worm fecundity or shedding rates among any host categories. We also observed similar intensities of *P. peromysci* infection among host sex and breeding condition from our cross-sectional study, as was the case in a more extensive parasite survey of white-footed mice in the same study area (Vandegrift & Hudson 2009b). Similarly, we found no overall difference between male and female prevalence, indicating that the male-biased transmission we detected was not driven by higher prevalence, higher intensity of infection, or greater parasite fecundity in male mice. Although differences among hosts, such as sex and body size can influence nematode size and fecundity in other vertebrate-nematode systems (Stear *et al*. 1995; Poulin 1996b; Stear *et al*. 1997; Tompkins & Hudson 1999; Wilkes *et al*. 2004), intra-specific parasite competition limits the intensity and variation in this system (Luong, Vigliotti & Hudson 2011). Patterns of infection reported from extensive samples over our study region across multiple years show that *P. peromysci* are randomly distributed around a mean of less than 2 worms per host (Vandegrift & Hudson 2009b).

Host behavior has the potential to compound physiological effects in generating male-biased transmission, or generate male-biased transmission even if susceptibility is equal. Sexually dimorphic body-size potentially increases infection rates when males are the larger sex because they present bigger habitats for parasites and there is an energetic trade-off between growth rate and immunity (Moore & Wilson 2002). Behavioral differences between sexes, such as greater space use and agonistic interactions in males, may also increase male exposure to parasites, resulting in more parasites and more infectious stages shed. Such behavioral differences may also result in greater probability of onward transmission from infectious particles shed by males, particularly in the case of greater space use or behaviors associated with testosterone (Seivwright *et al*. 2005; Grear, Perkins & Hudson 2009). Future studies should investigate whether sex-differences in exposure, timing and distribution of shedding of infective stages, could contribute to greater male transmission rates. Nonetheless, Luong *et al.* (2009) showed that parasite eggs shed from male white-footed mice result in higher intensities of infective stages in intermediate hosts, suggesting male-bias in transmission comes partly from the trophic interaction of the intermediate-host and feces from male mice.

While we can show that males play an important role in driving transmission dynamics via more efficient transmission of parasite eggs to intermediate hosts compared to females, the trophic interaction between mice and intermediate hosts, along with seasonality of the cricket-intermediate hosts appears to play an important role in the parasite dynamics. Interestingly, the experimental effect of male deworming was lost in late summer, despite constant male treatment (Figure 1a). Late summer infections are coincident with a large seasonal increase in the available cricket population and a functional response of mouse consumption of these crickets may have resulted in increased transmission despite our successful efforts to reduce exposure to the

intermediate host. Along with abundance, prevalence and intensity of *P. peromysci* infective stages in crickets increased dramatically during the late summer, even while both male and female treatment lowered the availability of infective stages (Luong, Grear & Hudson 2009). We hypothesize that white-footed mice have a type III functional response to crickets and greatly increase consumption of crickets as cricket density increases in the late summer (Holling 1959). Therefore, even with fewer infective stages in crickets when males were treated (Luong, Grear & Hudson 2009), this functional response to increased cricket abundance may be sufficient to ensure that mice consume enough parasite infective stages to noticeably increase transmission.

In conclusion, this experiment identified a male-bias in transmission that persisted through a complex parasite life-cycle. Importantly, we demonstrated a male-biased heterogeneity in transmission that would not have been predicted based on infection patterns. Male and female hosts carry a similar intensity and prevalence of parasites, but male hosts still generate more transmission events compared to female hosts. Unlike other systems where male-biased transmission has been reported (Ferrari *et al*. 2004), we could not identify any physiologically mediated mechanisms for this dynamic. Future studies should consider behavioral-mediated mechanisms that result in parasite infective stages being shed more efficiently from male. We recognize that physiological mechanisms related to increased male susceptibility are epidemiologically important and suggest that mechanisms related to differential behavior may add an additional layer of host heterogeneity that disease ecologists too often ignore. Finally, we demonstrated an emergent dynamic in support of a general pattern of male-driven parasite dynamics (Skorping & Jensen 2004; Zuk 2009) and in support of conclusions made by Rankin & Kokko (2007), whereby males matter to population ecology. Host population processes can be influenced by parasites (e.g. increased mortality, decreased fecundity; Anderson & May 1978;

May & Anderson 1978) and, thus, male-driven parasite transmission should be considered as a potential modifier of population dynamics.

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REFERENCES

Anderson, R. M. & May, R. M. (1978) Regulation and stability of host-parasite population interactions I, regulatory processes. *Journal of Animal Ecology*, 47, 219-247.

Andersson, M. (1982) Sexual selection, natural selection and quality advertisement. *Biological Journal of the Linnean Society*, 17, 375-393.

Bates, D., & Maechler, M. (2010) lme4: Linear mixed-effects models using S4 classes. R package version 0.999375-33. <http://CRAN.Rproject.prg/package=lme4>

Boukal, D. S., Berec, L. & Krivan, V. (2008) Does sex-selective predation stabilize or destabilize predator-prey dynamics? *PLoS One*, 3, e2687. doi:10.1371/journal.pone.0002687.

Ferrari, N., Cattadori, I. M., Nespereira, J, Rizzoli, A., & Hudson, P. J. (2004) The role of host sex in parasite dynamics: field experiments of the yellow-necked mouse *Apodemus flavicollis*. *Ecology Letters*, 7, 88-94.

Folstad, I. & A. J. Karter. 1992. Parasites, bright males, and the immunocompetence hypothesis. American Naturalist 139:603-622.

Gelman, A., Y., Su, S., Yajima, M/, Hill, J., Pittau, M. G., Kerman, J., & Zheng, T. (2010) arm: Data analysis using regression and multilevel/hierarchical models. R package version 1.3-02. <http://CRAN. R-project,org/package=arm>

Grear, D. A., Perkins, S. E. & Hudson, P. J. (2009) Does elevated testosterone result in increased exposure and transmission of parasites? *Ecology Letters*, 12, 528-537.

Grossman, C. (1989) Possible underlying mechanisms of sexual dimorphism in the immune response, fact and hypothesis. *Journal of Steroid Biochemistry*, 34, 241-251.

Grundman, A. W., Warnock, R. G., & Wasson, D. L. (1976) Some Mechanisms of natural regulation of parasitic helminth populations. *American Midland Naturalist*, 95, 347-360.

Hansen, F., Jeltsch, F., Tackmann, K., Staubach, C. & Thulke, H. H. (2004) Processes leading to a spatial aggregation of *Echinococcus multilocularis* in its natural intermediate host *Microtus arvalis*. *International Journal for Parasitology*, 34, 37-44.

Holling, C. S. (1959) The components of predation as revealed by a study of small-mammal predation of the European sawfly, *Neodiprion sertifer*. *Canadian Entomologist*, 91, 293-320.

Huebel, K. U., Rankin, D. J. & Kokko, H. (2009) How to go extinct by mating too much: population consequences of male mate choice and efficiency in a sexual-asexual species complex. *Oikos*, 118, 513-520.

Hughes, V. L. & Randolph, S. E. (2001) Testosterone increases the transmission potential of tick-borne parasites. *Parasitology*, 123, 365-371.

Hurlbert, S. H. (1984) Pseudoreplication and the design of ecological field experiments. *Ecological Monographs*, 54, 187-211.

Keymer, A. E. & Anderson, R M. (1979) Dynamics of infection of *Tribolium confusum* by *Hymenolepis diminuta*: influence of infective stage density and spatial distribution. *Parasitology*, 79, 195-207.

Luong, L. T., Grear, D. A. & Hudson, P. J. (2009) Male host are responsible for the transmission of a trophically transmitted parasite, Pterygodermitites peromysci, to the intermediate host in the absence of sex-biased infection. *International Journal for Parasitology*, 39, 1263-1268.

Luong, L. T., Vigliotti, B. A. & Hudson, P. J. (2011) Strong density-dependent competition and acquired immunity constrain parasite establishment: implications for parasite aggregation. *International Journal for Parasitology*, doi: 10.1016/j.ijpara.2010.12.003.

May, R. M. & Anderson, R. M. (1978) Regulation and stability of host-parasite population interactions II, destabilizing processes. *Journal of Animal Ecology*, 47, 249-267.

McDonald, T. L., Erickson, W. P, & McDonald, L. L. (2000) Analysis of count data from before-after control-impact studies. *Journal of Agricultural, Biological, and Environmental Statistics*, 5, 262-279.

Miller, M. R.,White, R. A. Wilson, K. & Boots, M. (2007) The population dynamical implications of male-biased parasitism in different mating systems. *PLoS One*, 2:e624. doi:10.1371/journal.pone.0000624.

Moore, S. L. & Wilson, K. (2002) Parasites as a viability cost of sexual selection in natural populations of mammals. *Science*, 297, 2015-2018.

Mougeot, F., Redpath, S. M., Piertney, S. B. & Hudson, P. J. (2005) Separating behavioral and physiological mechanisms in testosterone-mediated trade-offs. *American Naturalist*, 166, 158-168.

Oswald, V. H. (1958a) Studies on *Rictularia coloradensis* Hall, 1916 (Nematoda: Thelaziidae). II. Development in the definitive host. *Transactions of the American Microscopical Society*, 77, 413-422.

Oswald, V. H. (1958b) Studies on *Rictularia coloradensis* Hall, 1916 (Nematoda: Thelaziidae). I. Larval development in the intermediate host. *Transactions of the American Microscopical Society*, 77, 229-240.

Pedersen, A. B & Geives, T. J. (2007) The interaction of parasites and resources cause crashes in a wild mouse population. *Journal of Animal Ecology*, 77, 370-377.

Perkins, S. E., Cattadori, I. M., Tagliapietra, V. & Hudson, P. J. (2003) Empirical evidence for key hosts in persistence of a tick-borne disease. *International Journal for Parasitology*, 9, 900-917.

Poulin, R. (1996a) Sexual inequalities in helminth infections: a cost of being a male? *American Naturalist*, 147, 287-295.

Poulin, R. (1996b) Helminth growth in vertebrate hosts: Does host sex matter? *International Journal for Parasitology*, 26, 1311-1315.

R development core team. (2009) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. <http://www.R-project.org>

Rankin, D. J. & Kokko, H. (2007) Do males matter? The role of males in population dynamics. *Oikos*, 116, 335-348.

Rasband, W. (2007) ImageJ. U.S. National Institutes of Health, Bethesda, MD, USA.

Seivwright, L. J., Redpath, S. M., Mougeot, F., Lecki, F. & Hudson, P. J. (2005) Interactions between intrinsic and extrinsic mechanisms in a cyclic species: testosterone increases parasite infection in red grouse. *Proceedings of the Royal Society B,* 272, 2299-2304.

Shaw, D. J. & Dobson, A. P. (1995) Patterns of macroparasite abundance and aggregation in wildlife populations: a quantitative review. *Parasitology*, 111, (Suppl.), S111-S133.

Skorping A. & Jensen, K. H. (2004) Disease dynamics: all caused by males? *Trends in Ecology and Evolution*, 19, 219-220.

Sloss, M. W. & Kemp, R. L. (1978) *Veterinary Clinical Parasitology*. Iowa State University Press, Ames, Iowa, USA

Stear, M. J., Bairden, K., Duncan, J. L., Holmes, P. H., McKellar, Q. A., Park, M., Strain, S., Murray, M., Bishop, S. C. & Gettinby, G. (1997) How hosts control worms. *Nature,* 389, 27-27.

Stear, M. J., Bishop, S. C., Doligalska, M., Duncan, J. L., Holmes, P. H., Irvine, J., McCririe, L., McKellar, Q. A., Sinski, E. & Murray, M.. (1995) Regulation of egg production, worm burden, worm length and worm fecundity by host responses in sheep infected with *Ostertagia circumcincta*. *Parasite Immunology*, 17, 643-652.

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

Vandegrift, K. J, Raffel, T. R. & Hudson, P. J. (2008) Parasites prevent summer breeding in white-footed mice, *Peromyscus leucopus*. *Ecology*, 89, 2251-2258.

Vandegrift, K. J. & Hudson, P. J. (2009a) Response to enrichment, type and timing: small mammals vary in their response to a springtime cicada but not a carbohydrate pulse. *Journal of Animal Ecology*, 78, 202-209.

Vandegrift, K. J. & Hudson, P. J. (2009b) Could parasites destablilize mouse populations? The potential role of *Pterygodermatites peromysci* in the population dynamics of free-living mice, *Peromyscus leucopus*. *International Journal for Parasitology*, 39, 1253-1262.

Veran, S. & Beissinger, S.R. (2009) Demographic origins of skewed operational and adult sex ratios: perturbation analyses of two-sex models. *Ecology Letters,* 12, 129-143.

Wilkes, C. P., Thompson, F. J., Gardener, M. P., Paterson, S. & Viney, M. E. (2004) The effect of the host immune response on the parasitic nematode *Strongyloides ratti*. *Parasitology,* 128, 661-669.

Zuk, M. & McKean, K. A. (1996) Sex differences in parasite infections: patterns and processes. *International Journal for Parasitology,* 26, 1009-1024.

Zuk, M. (2009) The sicker sex. *PLoS Pathogens*, 5, e1000267.doi:10.1371/journal.ppat.1000267.

TABLE 3.1 – Generalized linear mixed model (GLMM) parameter coefficients for best-fitting statistical models of male or female white-footed mouse (*P. leucopus*) prevalence of infection with the nematode, *Pterygodermatites peromysci*, in a before-after-control-impact experimental design, where parasites were removed from all males (female response) or females (male response). Coefficient confidence intervals represent the change in response-sex prevalence in the coefficient category compared to control sites.

Response variable	Explanatory variable	Coefficients	Value[a]	95% CI[b]
Female prevalence		intercept[c]	-14.6	[-21.9 , -7.2]*
	Site treatment	male treatment	0.62	[-4.99 , 6.23]
		female treatment	2.61	[-3.61 , 8.82]
	Treatment time period	2-6 weeks post treatment	1.72	[-0.59 , 4.03]
		8-10 weeks post treatment	1.76	[-1.09 , 4.67]
		12-14 weeks post treatment	1.56	[-1.76 , 4.94]
		female mass	0.33	[0.04 , 0.62]*
	Site treatment × time post treatment initiation[d]	male treatment × 2-6 weeks post treatment	-3.16	[-6.90 , 0.42]
		male treatment × 8-10 weeks post treatment	-5.39	[-10.4 , -0.52]*
		male treatment × 12-14 weeks post treatment	0.78	[-4.41 , 5.94]
		female treatment × 2-6 weeks post treatment	-4.94	[-11.4 , 1.70]
		female treatment × 8-10 weeks post treatment	-17.01	NA[e]
		female treatment × 12-14 weeks post treatment	-6.07	[-14.3 , 2.14]
Male prevalence		intercept[c]	-5.35	[-9.08 , -1.63]*
	Site treatment	male treatment	-1.07	[-2.53 , 0.36]
		female treatment	0.16	[-1.06 , 1.27]
	Treatment time period	2-6 weeks post treatment	-0.19	[-1.34 , 0.81]
		8-10 weeks post treatment	-1.00	[-2.20 , 0.24]
		12-14 weeks post treatment	0.04	[-1.15 , 1.23]
		male mass	0.16	[-0.02 , 0.34]

[a]Untransformed coefficient values for the GLMM regression with individual mouse and trapping grid included as random-effect variables. Values indicate the change in response, relative to the intercept, or relative to the intercept + additive coefficients, in the case of interactions.

[b]Bayesian credible intervals calculated from 10,000 MCMC samples from the posterior distribution of the model. CIs not overlapping zero were considered significant (*).

[c]Intercept value is the reference probability of infection over all site treatments during the time period before treatment.

[d]Treatment X time interaction tests for experimental effect, interaction did not significantly improve male prevalence model ($X^2 = 7.54$, $d.f. = 6$, $P = 0.27$)

[e]Confidence intervals could not be calculated because experimental treatment removed all infection (Fig. 1).

TABLE 3.2 - Generalized linear mixed model (GLMM) parameter coefficients for minimum models describing white-footed mouse (*P. leucopus*) prevalence of infection with the nematode, *Pterygodermatites peromysci*, on control sites.

Explanatory variable	Coefficient	Value[a]	95% CI[b]
	Intercept[c]	-2.45	[-9.01 , -1.16]*
Month	June	-16.19	NA[d]
	July	-0.17	[-2.48 , 2.14]
	August	0.43	[-1.91 , 2.77]
	September	1.53	[-0.55 , 3.62]
Breeding status	Breeding	-0.25	[-2.22 , 1.71]
Month x breeding status	June x breeding	17.54	NA[d]
	July x breeding	0.17	[-2.66 , 3.01]
	August x breeding	-0.87	[-3.79 , 2.06]
	September x breeding	-0.73	[-3.55 , 2.09]

[a]Untransformed coefficient values for the generalized linear mixed-model regression with individual mouse and trapping grid included as random-effect variables. Values indicate the change in response, relative to the intercept, or relative to the intercept + additive coefficients, in the case of interactions.

[b]Bayesian credible intervals calculated from 10,000 MCMC samples from the posterior distribution of the model. CIs not overlapping zero were considered significant (*).

[c]Intercept value is the reference probability of infection over all non-breeding control-site individuals during May.

[d]Confidence intervals could not be calculated because there was not enough variation in response (Fig. 2)

FIGURE 3.1- Effect of single-sex parasite removal on transmission of *Pterygodermatites peromysci* in white-footed mice. A. Prevalence of infection in female mice compared among females captured on control sites (shaded bars), male-treated sites (hatched bars), and female-treated sites (open squares). We detected a significant reduction in prevalence 8-10 weeks after treatment was initiated in females from sites where parasites were removed from males, compared to females on control sites. B. Prevalence of infection in male mice compared among males captured on control sites (shaded bars), male-treated sites (hatched bars), and female-treated sites (open diamonds). We detected no effect of removing parasites from females on prevalence in males. Infection was determined by presence of parasite eggs in host feces.

FIGURE 3.2 – Prevalence of *P. peromysci* in white-footed mice from non-treated control sites by month. A. Adult female mice in breeding condition (filled bars) and not in breeding condition (hatched bars). B. Adult male mice in breeding condition (filled bars) and not in breeding condition (hatched bars). The total number of mice sampled in each time and breeding class is indicated above each bar.

FIGURE 3.3 – *log* transformed egg counts per female *Pterygodermatites peromsyci*. Mean untransformed *in utero* egg count was 59,772 ± 3775 standard error.

FIGURE 3.4 – Positive relationship between *in utero* nematode fecundity and female nematode length (n = 125). Female nematode length was the strongest predictor of fecundity ($P < 0.001$) with no host characteristics influencing parasite fecundity.

Figure 3.1

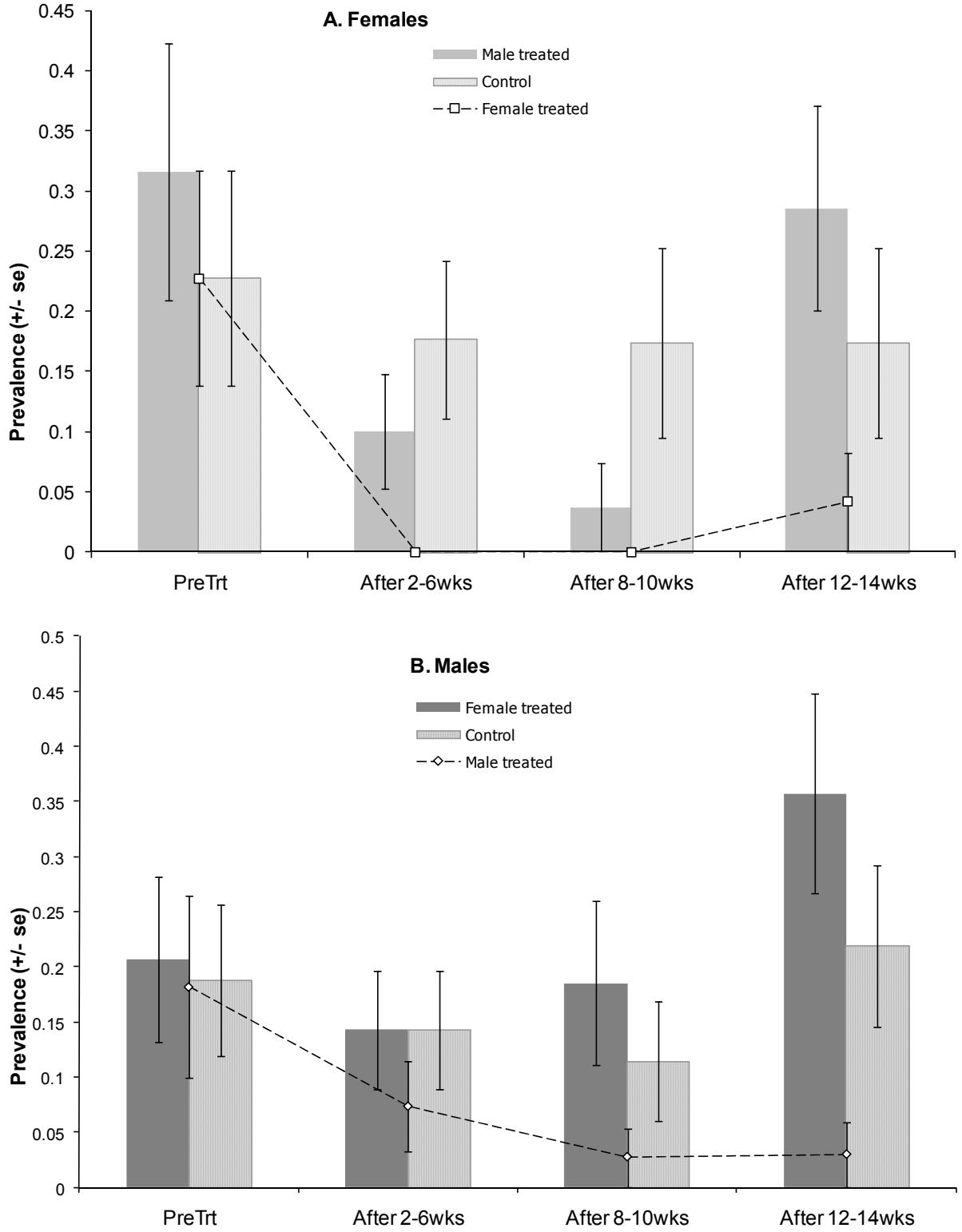

A. Females
Male treated
Control
Female treated
Prevalence (+/- se)
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
PreTrt
After 2-6wks
After 8-10wks
After 12-14wks
B. Males
Female treated
Control
Male treated
Prevalence (+/- se)
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
PreTrt
After 2-6wks
After 8-10wks
After 12-14wks

Figure 3.2

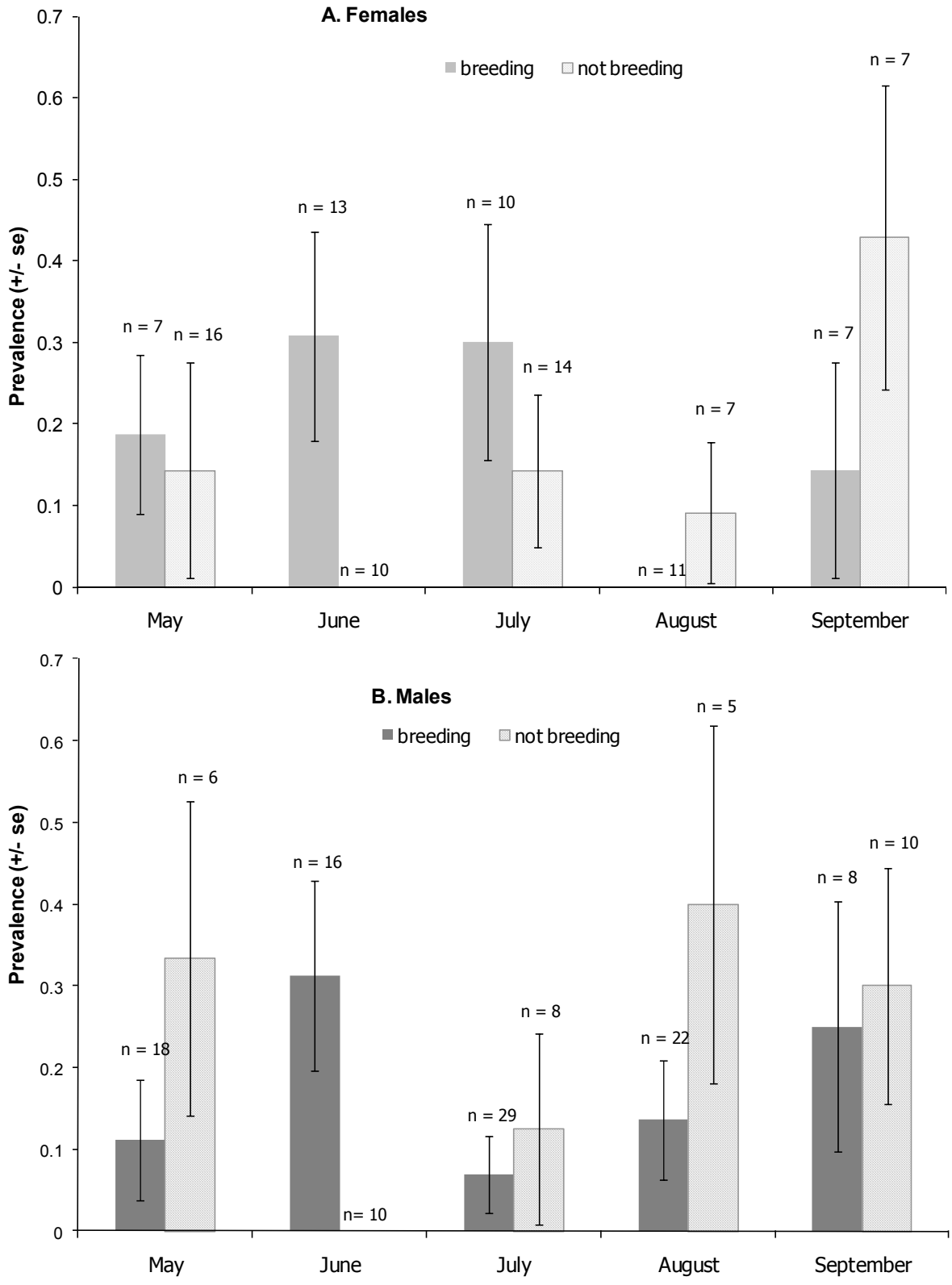

A. Females
breeding
not breeding
Prevalence (+/- se)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
n = 7
n = 16
n = 13
n = 10
n = 10
n = 14
n = 11
n = 7
n = 7
n = 7
May
June
July
August
September
B. Males
breeding
not breeding
Prevalence (+/- se)
n = 18
n = 6
n = 16
n= 10
n = 29
n = 8
n = 22
n = 5
n = 8
n = 10
May
June
July
August
September

Figure 3.3

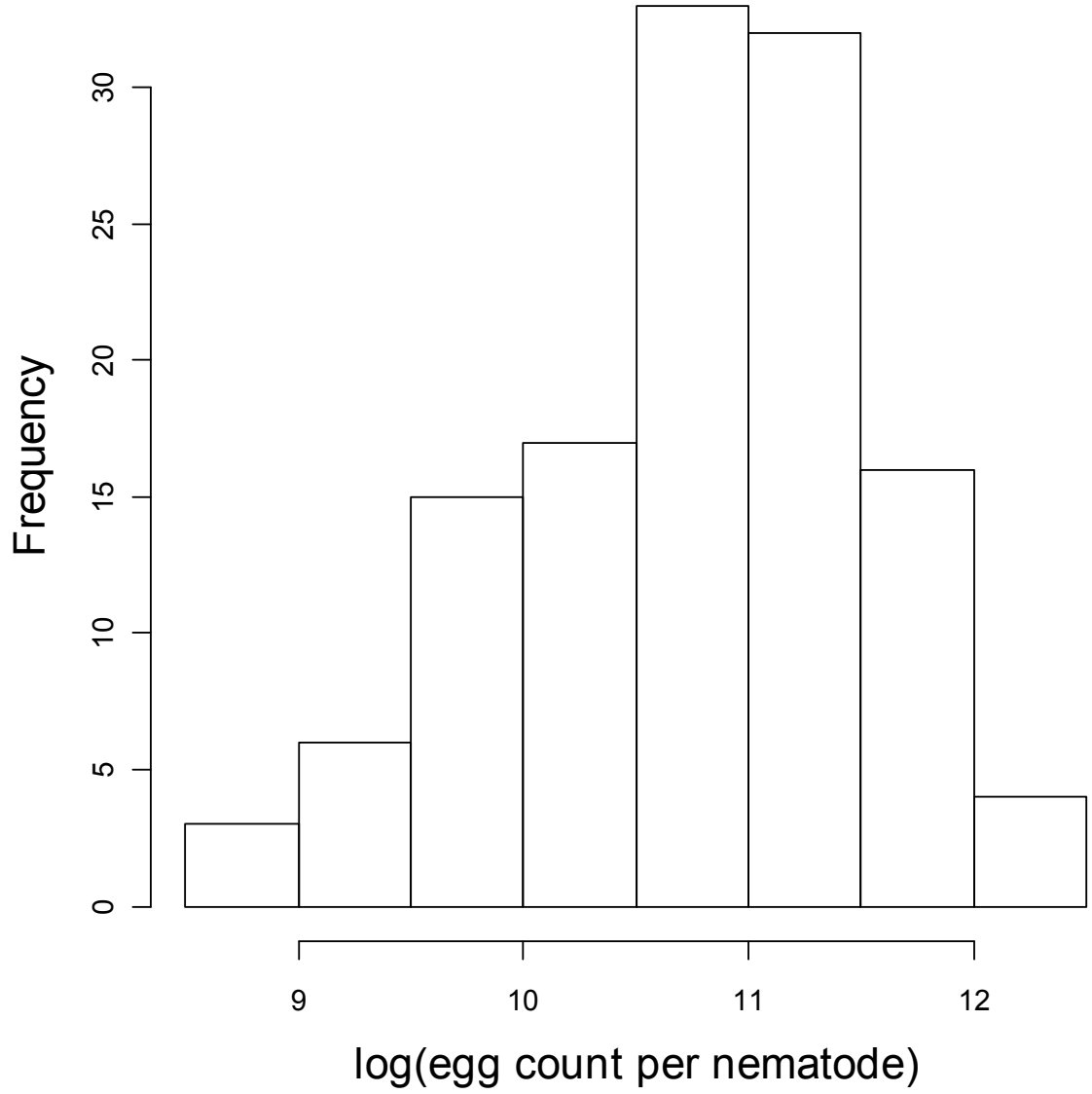
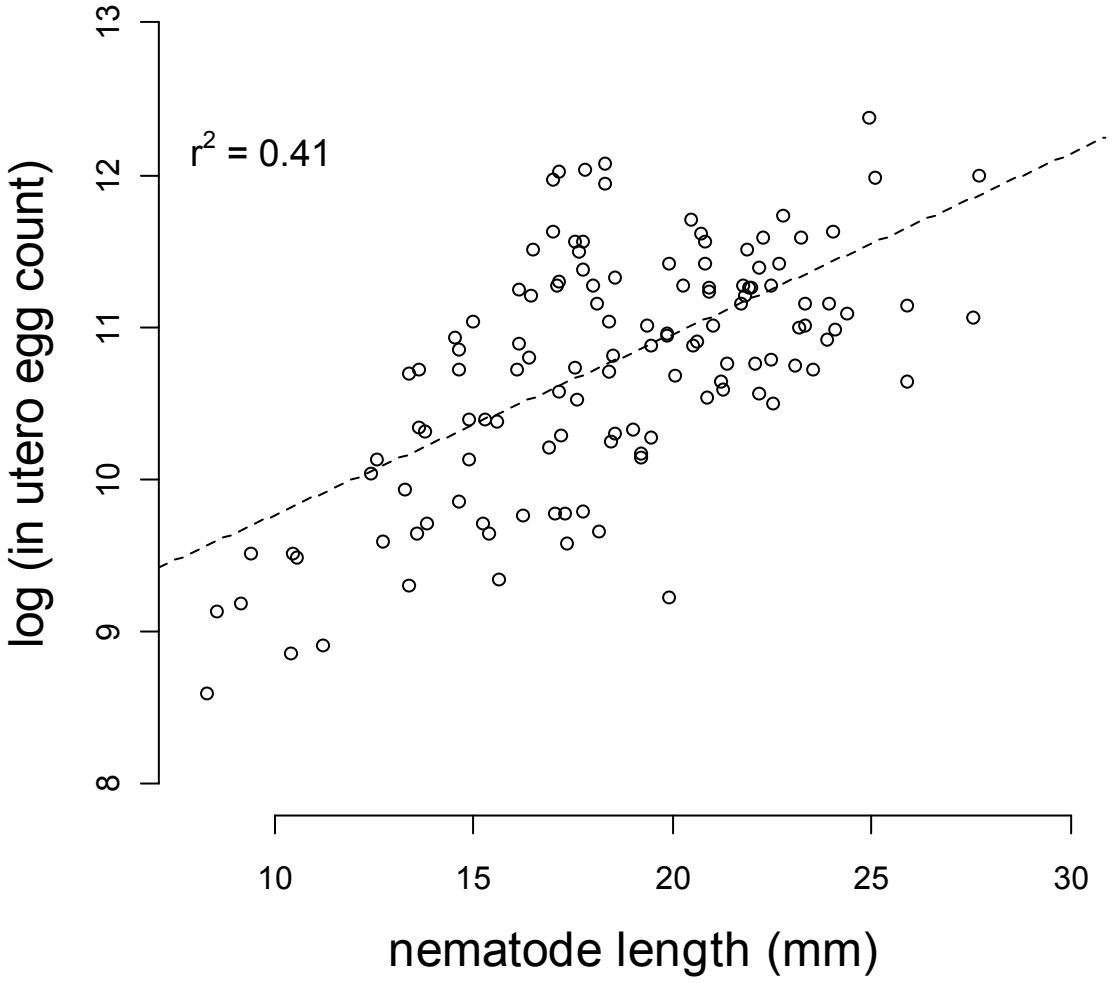

Frequency
0
5
10
15
20
25
30
9
10
11
12
log(egg count per nematode)

Figure 3.4


log (in utero egg count)
nematode length (mm)
r2 = 0.41
8
9
10
11
12
13
10
15
20
25
30

Chapter 4. Feeding and foraging behaviors interact to influence the parasite community of the eastern chipmunk (*Tamias striatus*)

ABSTRACT

The process of disease transmission is determined by the interactions of susceptibility and exposure to parasite infectious stages. Host behavior is an important determinant of the spread of, and exposure to, infectious stages, but is difficult to measure and often ignored by disease ecologists. We examined two aspects of host foraging behavior that influence parasite transmission: the availability and spatial distribution of food in relation to parasites with fecal-oral transmission routes. We hypothesized that supplemental food in an aggregated distribution would alter foraging space-use, lead to increased contact rates, and, hence, increase transmission of directly transmitted parasites. We also hypothesized that supplemental food would generate a diet switch away from invertebrates that serve as intermediate hosts for trophically transmitted parasites and decrease their transmission. We employed an experimental approach and added supplemental food, in uniform or aggregated spatial distributions, to natural populations of the eastern chipmunk and monitored the response of chipmunk foraging behavior and infection levels of their gastro-intestinal helminth community using replicated longitudinal capture-mark-recapture techniques. Using network metrics, we predicted that network degree and prevalence of directly transmitted parasites would increase in response to supplemental food provided in an aggregated distribution. Providing a supplemental food source, regardless of spatial distribution, decreased the transmission of a trophically-transmitted parasite, i.e. transmission that requires ingestion of invertebrates. The contact networks appeared to become sparser in response to either spatial distribution of supplemental food compared to controls, but was confounded by

biased trapability in response to treatment. The prevalence of two directly transmitted gastro-intestinal nematode parasites was strongly correlated to network in-degree, while the prevalence of trophically transmitted parasites was not. We concluded that the relative availability of different food sources is an important influence on the transmission of parasites and the specific transmission route, relative to the type of available food, is a key factor in determining transmission and community composition of parasites.

Keywords: macroparasite, transmission dynamics, contact networks, foraging behavior, eastern chipmunk, food supplementation, trophic transmission

INTRODUCTION

Hosts and parasites are embedded in heterogeneous environments and host behaviors, such as space-use, inter-host interactions, and diet, are crucial to determining the exposure of susceptible hosts and distribution of infectious stages by infected hosts. Parasite-mediated host behavior receives much attention because of the fascinating phenomena it can produce (Moore 2002; Poulin 2010a), but host behavior, as it relates to population level transmission dynamics, receives less attention and is difficult to study (Altizer *et al.* 2003; Kjaer *et al.* 2008). Empirically measuring and evaluating the importance of individual host exposure heterogeneity, in a manner that is meaningful when the transmission of parasites is considered, is an emerging challenge.

Recently, the important role of individual heterogeneity in the probability of contact between an infectious and susceptible individual has been highlighted using network theory incorporated with traditional models of transmission (Myers *et al.* 2005; Aparicio & Pascual 2007; Bansal *et al.* 2007; Bansal *et al.* 2010). Conventional methods for evaluating contact

heterogeneities in natural animal populations have been limited to estimating contact probability from home range overlap or observing individual behaviors (Revilla & Palomares 2002; Weihong *et al*. 2005; Schauber *et al.* 2007). The former is usually measured at a spatial and temporal scale that is too coarse to accurately estimate potential transmission and the latter is too time and labor intensive to collect the amount of data to make general conclusions. Graph theory and network analysis have been applied successfully to several human and animal disease systems in an effort to quantify heterogeneous infectious contacts (see reviews of May 2006; Poulin 2010b). Network approximations of transmission risk derived from host locations represent a significant improvement over techniques such as home-range analysis because these networks can incorporate all location information, in time and space, to specifically estimate transmission risk according to interactions defined by the parasite(s) in question.

Thus far, network ecology has inferred empirical contact or social networks for a handful of species in relation to potential parasite transmission (Cross *et al.* 2004; Hamede *et al.* 2009; Perkins *et al.* 2008; Grear *et al.* 2009; Perkins *et al.* 2009, Craft *et al.* 2010, Drewe 2010; Salathe *et al.* 2010), but very few transmission networks have been fully characterized with both empirical host contact information and explicit incorporation of a parasite (but see, Godfrey *et al.* 2009). Our goal was to explicitly address the role of behaviorally mediated exposure in the transmission of multiple parasite species infecting the same host. Predictions about how host behaviors will influence parasite transmission must be considered from the perspective of parasite. In the case of directly transmitted parasites, social behavior can generate heterogeneities in exposure to parasites transmitted by intimate contact. These exposure heterogeneities may translate into heterogeneous infection patterns and heterogeneous transmission (Loehle *et al.* 1995; Delahay *et al.* 2000; Altizer *et al.* 2003; Grear *et al.* 2010).

Alternatively, host foraging behaviors that influence contact with contaminated food sources are key factors disease transmission for parasites that ingested by their hosts (Hall et al 2007).

In this study, we examined the role of foraging behavior on transmission using network techniques to infer how an ecological contact network influenced parasite infection, as well as tested how food availability influenced the transmission of parasites with direct (fecal-oral) and trophic (fecal-intermediate host-oral) transmission modes among a wildlife host in an experimental framework. We studied the role of foraging behavior and diet composition on the exposure and subsequent transmission of parasites in natural populations of eastern chipmunks (*Tamias striatus*) and their community of gastrointestinal parasites. Eastern chipmunks are central place foragers with their home-range space-use tied closely to foraging behavior (Yahner 1978; Kramer & Nowell 1980). Chipmunks maintain home-ranges that change quickly in response to resource availability (Mares *et al.* 1982; Lacher & Mares 1996). They are highly territorial of a central burrow where they maintain long-term food caches, but do not defend exclusive fixed home-ranges outside of their burrows (Elliot 1978; Yahner 1978).

Eastern chipmunks harbor a community of gastro-intestinal helminthes with fecal-oral transmission modes including direct host-environment-host and trophic host-environment-invertebrate-host life-cycles (Rausch & Tiner 1948; Snyder 1982; Ehman 2001). By using these two life cycles we can examine how foraging space-use and feeding behavior influences the transmission of parasites according to transmission mode. Based on previous parasite surveys of eastern chipmunks in the northeast United States, we expected to find at least 5 species of parasitic gastrointestinal helminthes infecting the chipmunks (Ehman 2000; Geibel, unpublished data). Two of these nematode species, *Capillaria tamiasstriati* and *Citellinema bifurcatum*, have direct fecal-oral transmission routes. Parasite eggs are shed into the environment with host feces

and larvae require 1-2 weeks (Anderson 2000) of development before they are infectious upon ingestion by the same or another chipmunk. *C. bifurcatum* larvae emerge from the egg and develop as free-living larvae and *C. tamisstriati* larvae remain ensheathed in the egg as they develop (Anderson 2000). A third nematode species, identified based on morphology to the genus, *Syphacia*, also has a direct fecal-oral transmission mode, but does not require a full 1-2 weeks to become infectious. Other congeneric *Syphacia* species infecting rodents are able to generate host-self-reinfection by depositing eggs on the host peri-anal skin to be transmitted through grooming behaviors (Adamson 1994); although the extent to which the chipmunk *Syphacia* species employs host-self-reinfection versus transmission via the environment is not known. The remaining two species of GI helminthes are transmitted trophically when scavenger invertebrates consume chipmunk feces containing parasite eggs and the parasites larvae develop to the infectious stage within the invertebrate intermediate host. Transmission is completed when a chipmunk ingests an invertebrate with infective parasite larvae. *Ricularia halli* is a nematode species that has a camel cricket in the genus *Ceutaphilus* as its putative intermediate host. The other trophically transmitted helminth is a cestode, *Hymenopelis sensu latu*, with an unknown intermediate host species range.

Our objective was to examine two aspects of exposure to parasites with trophic and direct fecal-oral transmission routes: 1) the availability of different food sources and 2) the foraging behaviors that determine where food is being consumed. We performed an experimental manipulation of the food supply of the eastern chipmunk using two treatment levels: uniform and aggregated distribution of a supplemental food. We hypothesized that a cacheable supplemental food source in an aggregated distribution would increase transmission of directly transmitted parasites by altering chipmunk foraging behavior and causing greater overlapping foraging. We

predicted that we would observe an increase in the density of network connections (degree) in response to the aggregated treatment of supplemental food compared to a uniform distribution of supplemental food or unfed controls. We also predicted that there would be an associated increase of the prevalence and intensity of infection of directly transmitted parasites resulting from the increased contacts.

Our second hypothesis was that an abundant supplemental food source would decrease the transmission of trophically transmitted parasites by switching host diet away from invertebrates that serve as intermediate hosts for transmission. We predicted that the prevalence of trophically transmitted parasites would decrease in response to food supplementation in both spatial distributions compared to the unsupplemented controls.

We also explored the relationship between factors influencing host infection and parasite community composition in relation to contact network properties. Specifically, we addressed whether the network degree of hosts was correlated to infection with individual and multiple parasite species. By defining our connections based on the transmission mode of directly transmitted parasites, we tested the prediction that, after accounting for intrinsic host factors, the network degree would positively correlate with infection by directly transmitted nematodes and have no correlation with infection by trophically transmitted nematodes.

METHODS

Animal capture and field experimental design

We live-captured eastern chipmunks on 12 open trapping grids, consisting of 64 traps in a 8 x 8 array at 12.5 meter intervals (100 ha, Ugglan #2 multiple capture traps, Grahnab AB, Sweden). All grids were 20km south of State College, Pennsylvania, USA, in open forested

habitat and separated by at least 800m. We recorded trap location, body length, body mass, sex, and breeding condition for each chipmunk capture. Feces were collected from each capture for parasite detection. We individually marked chipmunks using a passive induced transponder tag (EIDAP, Sherwood Park, Alberta, Canada) and trapped every week from 1-June to 29-August 2009 with 2 consecutive trap days per trap session, for a total of 12 trap sessions and 1536 trap days. A trap day was an 8-10 hour session, with traps set at 0600-0700 and checked and opened at 1200-1600 the first trap day; and traps set at 0900-1000 and checked at 1600-1900 the second consecutive trap day.

Experimental design

Beginning with the 7th trapping session (13-July, 2009) and continuing to the end of the experiment, we applied a 2-level supplemental feeding treatment. Four randomly selected grids received 9 kg/week sunflower seeds in a uniform distribution across the trapping grid: the seeds were divided into 8 equal parts by weight, and hand broadcast continuously along 8 regular transects through the trapping grid to achieve as uniform of distribution as possible. Four other randomly selected trapping grids received 9 kg/wk sunflower seeds in three-3 kg piles (aggregated distribution). The locations of the piles were chosen randomly from interior 36 trap locations. Each 3 kg of sunflower seeds were placed inside a 0.5h x 0.5w x 0.5l wooden box with four-4 inch round access holes at the base on each side. These boxes had a secure top and were fastened to the ground with 8 inch metal stakes in order to prevent easy access by birds and larger mammals. Three similar boxes were placed at random locations at each uniform-treatment and control site, but were never filled with sunflower seeds. Four trapping grids remained untreated control sites. The supplemental food was distributed after the 2nd trapping day each week (i.e. trapping was completed 6-7 days after the supplemental food was provided). All

animal handling was approved by the Institutional Animal Care and Use Committee at Pennsylvania State University (IACUC #23268).

Parasite identification

We collected fecal samples from trap contents at each capture and stored them at 4^{o}C in petri dishes lined with a damp towel (1ml water) overnight to standardize humidity. We determined parasite infection using a modified McMasters egg floatation for all fecal samples greater than 0.06g (Sloss and Kemp 1978): 1g feces mixed with 10ml saturated-$MgSO_4$ solution, giving a minimum resolution of 37 eggs per gram feces (epg). We identified helminth eggs based on morphology and comparison with dissected specimens.

Empirical contact networks

Node and edge definitions

We defined our network edges based on the transmission of directly transmitted GI nematodes, such as *C. bifurcatum* and *C. tamiasstriati*, and each individual chipmunk was defined as a node with attributes defined for sex (male or female), average mass, and infection status (presence or absence during any capture) based on fecal egg identification of the five helminthes described above. Directed edges approximated a potential infectious interaction based on proximity of capture in time and space according to the transmission mode of a direct fecal-oral transmission mode with an obligate environment development period. An edge between node *i* and node *j* was defined if *j* was captured in the same or nearest nine traps (area with radius 17.67m) to *i* after a 1-2 week time lag. In the directed network visualization framework, an arrow represents an edge with its origin (out-degree) at node *i* and its termination (in-degree) at node *j*. An edge between two nodes can be unidirectional or have a termination

(in-degree) at both nodes, indicating the two individuals each fulfilled the out-degree and in-degree definition in relation to each other. An edge between two nodes was not created if the two individuals were captured in spatial proximity within the same trapping week.

Two cumulative networks were created for each of the trapping grids: one representing all trapping sessions up to and including the week treatments were applied (weeks 2-6: before-treatment period) and the second for all subsequent trapping sessions (7-12: after-treatment period). We included candidate out-degree captures from weeks 5 and 6 in our after-treatment networks because we were most interested in how the altered foraging behavior influences in-degrees and contact with infectious parasites already in the environment.

Network characterization and analysis

We focused on the in-degree statistics for the analysis of the networks. The in-degree of a node is the number of edges that terminate at that node and represent the time and space interactions that are necessary for potential parasite contact from other nodes. We also considered subsets of in-degrees that originated from infected nodes, by each parasite species, as an additional metric. The network statistics were calculated using the package 'Statnet' in R (Handcock *et al.* 2003; R Development Core Team 2008). We characterized the degree distributions of each of our networks by fitting the observed degrees per node to a negative binomial and a Poisson distribution using direct maximum likelihood estimation and evaluated the goodness-of-fit using the deviance of the maximum likelihood estimate according to the methods of Shaw *et al.* (1998). To choose the best-fitting distribution to each of our networks we compared the difference in Akaike's information criterion corrected for small sample size (AIC_C) between the Poisson and the negative binomial fit and considered one distribution a superior fit if

the absolute value of the difference was greater than 4 and the goodness-of-fit statistic indicated that distribution adequately described the data (Burnham & Anderson 2002).

Statistical analyses: field experiment

Network response to treatments

A before-after-control-impact (BACI) study design was used to assess the effect of the supplemental food treatments on network in-degree and transmission of the parasite species and was analyzed using generalized linear mixed-models (GLMM). We first examined the network response to treatment by using in-degree as a response variable with Poisson error structure with mean mass, host sex, site-treatment (uniform, aggregated, control), and treatment-period (before, after) as fixed-effect independent variables and trapping grid and individual chipmunk identification as random-effect variables. The interaction of site treatment and time-period explicitly tested for an experimental effect in this BACI analysis (McDonald, Erickson & McDonald 2000). We began with an additive model with all fixed effects and compared it to a model with a site-treatment ^{x} time-period interaction. A likelihood-ratio X^2 test was used to compare the overall model fit to data between the reduced model and each subsequent model with the additional term. A *P*-value ≤ 0.05 indicated the model with the additional parameter was a significantly better fit to the data compared to the reduced model and we kept the additional term in the model. If the site-treatment ^{x} time-period interaction was included in the best-fit model we concluded that there was a significant experimental effect.

To further examine the effect size of the coefficients in the model selected from the process above, 95% Bayesian credible intervals (CIs) were constructed from 10,000 samples from the posterior distribution of fitted GLMM fixed-effect parameters using Markov-Chain-

Monte-Carlo methods. Coefficients with credible intervals that excluded zero were considered to have a significant effect on response parasite prevalence. The CIs for the time-period ^{x} site treatment coefficients were used to evaluate the magnitude of the experimental effect on network in-degree by indicating a difference in how in-degree on the treatment sites changed from the before treatment time-period to after treatment compared to the change on control sites. GLMMS were fitted using package 'lme4' in R (Bates & Maechler 2010; R development core team 2009) and the package 'arm' was used to conduct samples of fitted model coefficients for CI construction (Gelman *et al*. 2010).

Capture and trapability response to treatment

We evaluated the effect of our treatment on the capture of the chipmunks by comparing three metrics in the same BACI framework: minimum days known alive (*DKA*) per individual, number of captures per individual and trapability index. The trapability index was the residuals of the regression between the number of captures per individual and *DKA*.

Parasite prevalence response to treatment

Parasite infection status was considered a binomial response variable where an individual was considered infected if we detected parasites eggs in feces at any capture, with separate infection data for the before and after time periods. Mean mass, host sex, site-treatment (uniform, aggregated, control), treatment-period (before, after), and in-degree were fixed-effect independent variables and trapping grid and individual chipmunk identification as random-effect variables in the same GLMM framework as described above, separately for each parasite species. The interaction of site treatment, time-period, and in-degree explicitly tested for an experimental effect in this BACI analysis. We began with an additive model with all fixed effects terms and sequentially added comparisons to models with a time-period ^{x} site-treatment

interaction (hypothesis: supplemental food influences transmission), time-period ^{x} in-degree interaction (hypothesis: network changes influence transmission), and time-period ^{x} in-degree ^{x} site-treatment interaction (hypothesis: supplemental food treatments interacted with networks and influenced transmission). If the likelihood-ratio X^2 test indicated an interaction model was a better fit to the data compared to the reduced model ($P \leq 0.05$), we concluded that there was evidence for an experimental effect on parasite transmission and calculated 95% CIs for the fixed-effect coefficients in the most complex model. If either the time-period ^{x} site-treatment or the time-period ^{x} in-degree interaction did not provide a better fit to the data, we did not proceed to test the full 3-way interaction.

Parasite egg-shedding intensity response to treatment

Parasite intensity (*ln* eggs per gram feces, epg) was used as a response with Gaussian errors in a separate set of GLMMs for each parasite species, with the same methods as above to examine the experimental effect on the intensity of parasite egg shedding of infected individuals. The epg response was analyzed at an individual capture level to assess the variation in epg across the time-periods. As such, network in-degree was not included in this analysis because our measure of network in-degree was a cumulative statistic calculated across all before or after treatment trapping sessions. In addition, analysis of the network response to treatment indicated that the network response could not be measured reliably because the treatment biased capture rates. Thus, we began with an additive model with mass, sex, site-treatment, and time-period and compared it to a model with a site-treatment ^{x} time-period interaction to test for an experimental effect on the intensity of parasite egg shedding. If the site-treatment ^{x} time-period interaction provided a better fit to the data compared to an additive model (X^2 $P \leq 0.05$), we

evaluated the experimental effect by calculating 95% CIs for the fixed-effect variable coefficients.

Statistical Analysis – longitudinal sampling of parasites on contact networks

We evaluated correlations of host characteristics and network in-degree with parasite infection using the networks calculated from sites before treatment was applied.

Network correlations to individual parasite species prevalence in the period before treatment

We first evaluated the probability of infection with individual parasite species using GLMMs where infection at any capture was a binomial response with sex, mean mass, total in-degree, and the proportion of in-degrees generated from nodes infected with the focal parasite species (infected in-degree) as fixed-effect explanatory variables and trapping grid as a random-effect variable. We used a forward selection framework: testing models with each fixed-effect variable separately against a null model and retaining the variable if the likelihood-ratio X^2 test between the full and nested reduced model indicated the full model was a better fit to the data ($P \leq 0.05$). For each parasite, we tested the additive effect of the proportion of infected in-degree only in addition to total in-degree. We only tested fixed-effect interactions if multiple fixed-effects provided a better fit to the data additively. We evaluated the magnitude of correlations between the fixed-effects in the best fit model by calculating 95% CIs for the fixed-effect coefficients.

Parasite community correlations

Finally, we explored a parasite community correlation to host characteristics and network in-degree in the same forward-selection framework using species richness (count of parasite species per individual) as a response with Poisson error structure. We also calculated the C-

score index of species co-occurrence (Stone & Roberts 1990) and V-ratio of variability in species richness per animal (Schluter 1984) to evaluate if the patterns of parasite species richness were non-random. We calculated these indices and evaluated the significance with 5000 randomization simulations in the program EcoSim 7.0 (Gotelli & Entsminger 2001). We explicitly tested for evidence of a more structured co-occurrence pattern than random, where the C-score *P*-value was the proportion of simulations with a larger C-score than observed and the V-score *P*-value was the proportion of simulations with a smaller V-score than observed.

RESULTS

Animal captures

We recorded 1321 captures of 250 unique individual chipmunks across the 12 sites. The number of unique individuals per trapping grid ranged from 3-31 (mean = 15.75, SD = 7.9) in the period before treatment and from 7-20 (mean = 15.25, SD = 4.8) in the period after treatment. One trapping grid from the control treatment and one from the aggregated food treatment were excluded from the analysis due to the low abundances. For the analysis, the abundances over the10 sites ranged from 8-31 (mean=17.9, SD = 6.6) and 9-20 (mean = 16.7, SD = 3.7) before and after treatment, respectively (Table 1).

Parasite identification

We collected 834 fecal samples from 238 individuals for parasite egg identification and counts. Four nematode species and 1 cestode were identified from fecal egg counts. Overall prevalence of the five species were: *C. tamiasstriati* (39.0%), *C. bifurcatum* (12.6%), *R. halli* (8.0%), *Hymenolepis s. l.* (2.6%), and *Syphacis spp* (1.8%). All subsequent individual parasite

results focus on *C. tamiasstriati*, *C. bifurcatum*, and *R. halli* because they were numerically most abundant.

Network characterization

The mean in-degree per site ranged from 1.8-5.5 for the networks before treatment was applied. There was not strong evidence to suggest that the degree distributions were overdispersed (negative binomial) (Table 1); hence, we used Poisson error distribution to examine network in-degree responses in the statistical analyses.

Statistical analyses: field experiment

Network properties in response to treatment

There was a significant experimental effect of treatment on in-degree ($X^2 = 60.8$, $df = 2$, $P < 0.001$). Coefficient estimates and CIs indicated that in-degree was significantly reduced in both treatments compared to the control sites in response to treatment: aggregated ^{x} after-treatment = -0.61 (95%CI [-0.96, -0.31]) and uniform ^{x} after-treatment = -1.21 (95% CI [-1.53, -0.92]) (Figure 1a). Mean mass of an individual (coef = 0.002, 95% CI[-0.01, 0.31]) and sex (coef = 0.12, 95% CI [-0.07, 0.31]) did not have a significant effect on the in-degree.

Capture and trapability response to treatment

Analysis of the days known alive and trapability metrics indicated that the experimental treatments had a significant influence on *DKA* ($X^2 = 214.69$, $df = 2$, $P < 0.001$) with the *DKA* decreasing significantly on the aggregated (95% CI [-0.80, -0.46]) and uniform treatment sites (95% CI [-1.36, -1.03]) compared to control sites after treatment (Figure 1b). The *DKA* increased significantly on control sites after treatment compared to the before treatment period

(95% CI [0.18, 0.40]) and neither sex (95% CI [-0.22, 0.36]) nor mass (95% CI [-0.03, 0.002]) had an effect on the minimum days known alive. There was a significant effect of treatment on the number of captures per individual ($X^2 = 43.7$, $df = 2$, $P < 0.01$) with the number of capture per individual decreasing on aggregated (95% CI [-0.71, -0.18]) and uniform (95% CI [-1.10, -0.57]) treatment sites in response to treatment (Figure 1c). Neither sex (95% CI [-0.06, 0.30]) nor mass (95% CI [-0.02, 0.01]) had an effect of the number of captures per individual. There was not evidence for an experimental effect on the trapability index ($X^2 = 3.75$, $df = 2$, $P = 0.15$), although there was a weak trend for the trapability to decrease on aggregated (95% CI [-0.53, 0.07]) and uniform (95% CI [-0.58, 0.01]) treatment sites in the period after treatment compared to trapability on control sites (Figure 1d). There was a weak trend for males to have a higher trapability index compared to females (95% CI [-0.001, 0.25]) and mass did not influence the trapability index (95% CI [-0.01, 0.01]).

We did not include the network in-degree as an explanatory factor for the analysis of the parasite response to treatment because the treatments had a significant effect of the capture of individuals and made our estimates of the networks incomparable in response to the treatments.

Parasite prevalence: response to treatment

The response of each of the three most abundant parasite species were analyzed separately at the individual capture level to assess the effect of feeding treatment on the probability of infection. There was no evidence for an experimental treatment effect on the prevalence of *C. tamiasstriati* (site-treatment ^{x} time-period; $X^2 = 1.57$, $df = 2$, $P = 0.45$; Figure 2a). Coefficient estimates indicated that males were significantly less likely to be infected compared to females, and no other factors had a significant effect (Table 2).

Likewise, there was no evidence for an experimental treatment effect on the prevalence of *C. bifurcatum* (site-treatment ^{x} time-period; $X^2 = 5.19$, $df = 2$, $P = 0.07$; Figure 2b). Chipmunks on sites that received the aggregated food treatment were more likely to be infected with *C. bifurcatum* before treatment and there was no effect of mass or sex on probability of infection (Table 2).

There was evidence for an experimental treatment effect on the prevalence of *R. halli* (site-treatment ^{x} time-period; $X^2 = 6.77$, $df = 2$, $P = 0.03$; Figure 2c). Coefficient CIs did not indicate that any explanatory variable had a significant effect on probability of infection with *R. halli* (Table 2), but visual inspection of the data showed a that *R. halli* prevalence increased on control sites in the period after treatments were applied, while the prevalence declined in both treatments (Figure 2c).

Parasite egg-shedding intensity: response to treatment

The response of each of the three most abundant parasite species were analyzed separately at the individual capture level to assess the effect of feeding treatment on the intensity of eggs being shed (*epg*). There was no evidence for an experimental treatment effect on the *epg* of *C. tamiasstriati* (site-treatment ^{x} time-period; $X^2 = 2.95$, $df = 2$, $P = 0.23$; Figure 3a). Coefficient estimates indicated that males shed significantly fewer *C. tamiastriati* eggs per gram feces compared to females (Table 3), and no other factors had a significant effect.

There was evidence for an experimental treatment effect on the prevalence of *C. bifurcatum* (site-treatment ^{x} time-period; $X^2 = 5.94$, $df = 2$, $P = 0.05$; Figure 3b), and chipmunks on sites that received the aggregated food treatment shed more *C. bifurcatum* eggs per gram feces during the before treatment period (Table 3). There was a weak trend for a greater increase

in *epg* on the uniform treatment sites in response to treatment compared to the change on the other treatments (Figure 3b) and no effect of mass or sex on *epg* (Table 3).

There was no evidence for an experimental treatment effect on the *epg* of *R. halli* (site-treatment ^{x} time-period; $X^2 = 2.83$, $df = 2$, $P = 0.24$) (Figure 2c). Coefficient CIs did not indicate that any explanatory variable had a significant effect on *epg* of *R. halli* (Table 3).

Statistical Analysis – longitudinal sampling of parasites on contact networks

Network correlations to individual parasite species prevalence in the period before treatment

There was a strong positive correlation between infection with *C. bifurcatum* and in-degree, as well as with the in-degrees sourced from individuals infected with *C. bifurcatum* (Figure 4a-b). The in-degree of an individual ($X^2 = 14.7$, $df = 1$, $P < 0.01$) and the additive effect of the proportion of infected in-degrees significantly improved the model fit to the data ($X^2 = 16.2$, $df = 1$, $P < 0.01$). The in-degree was the only explanatory factor that was significantly correlated to *C. bifurcatum* infection (95% CI [0.08, 0.41]). The additive coefficient for the proportion of infected in-degrees did not have a significant effect (95% CI [-2.89, 0.34]) and neither mass ($X^2 = 0.32$, $df = 1$, $P = 0.57$) nor sex ($X^2 = 0.09$, $df = 1$, $P = 0.75$), improved the model fit.

*C. tamia*sstriati infection was not correlated to any single explanatory variable, but the additive effect of in-degree and proportion of infected in-degree had a strong correlation ($X^2 = 38.1$, $df = 1$, $P < 0.01$). The in-degree did not have an effect on the probability of infection with *C. tamiasstriati* (95% CI [-0.11, 0.19]), but the proportion of those in-degrees that were generated from nodes infected with *C. tamiasstriati* had a strong positive effect on infection

(95% CI [2.43, 4.55]; Figure 4c-d). Host mass ($X^2 = 0.53$, $df = 1$, $P = 0.47$) and sex ($X^2 = 2.44$, $df = 1$, $P = 0.12$) did not improved the model fit to data.

R. halli infection was not correlated to any single explanatory variable, but the additive effect of in-degree and proportion of infected in-degree significantly improved the model fit to the data ($X^2 = 19.6$, $df = 1$, $P < 0.01$). However, neither in-degree (95% CI [-0.13, 0.19]) nor the proportion of those in-degrees that were generated from nodes infected with *R. halli* had a strong positive effect on infection (95% CI [-1.09, 2.75]; Figure 4e-f). Host mass ($X^2 = 0.38$, $df = 1$, $P = 0.54$) and sex ($X^2 = 0.01$, $df = 1$, $P = 0.9$) did not improved the model fit to data.

Parasite community correlations

The species richness of parasites infecting the same host was weakly correlated to in-degree ($X^2 = 2.94$, $df = 1$, $P = 0.08$; Figure 5) and neither mass ($X^2 = 0.69$, $df = 1$, $P = 0.4$) nor host sex ($X^2 = 0.12$, $df = 1$, $P = 0.73$) had any correlation with parasite species richness per host. There was no evidence for non-random co-occurrence of species infecting the same host based on the C-score ($P = 0.20$) or V-score ($P = 0.94$).

DISCUSSION

What hosts eats and where they eat it has important consequences for parasite transmission when foraging is the mode of transmission. We were able to show that what food is available influences the transmission of parasites that are trophically transmitted. Further, the between-host interactions that result from where hosts forage, in relation to other hosts, has a strong correlation to infection with parasites that are directly transmitted. We were able to precisely define infectious interactions in a system where exposure could be examined through

foraging and feeding behaviors that reflect the transmission of an endemic community of parasites. Individual heterogeneities in between-host interactions have recently been recognized as key parameters in epidemiological models using a social network framework (Myers *et al.* 2005; Aparicio & Pascual 2007; Bansal *et al.* 2007). While these theoretical examinations of heterogeneities in host interactions have illustrated the potential importance of heterogeneous host contacts to parasite dynamics, few studies have incorporated empirical host and parasite information (Godfrey *et al.* 2009) and this study represents the first to do so in an experimental setting with a natural host-parasite system.

What you eat is important for transmission dynamics

We experimentally documented a decrease in prevalence of a trophically transmitted parasite that requires an invertebrate intermediate host in response to supplemental food (Figure 2c). We believe this reflects a decrease in transmission and hypothesize that the mechanism was a reduction in exposure because of a diet switch away from invertebrate intermediate hosts that transmit the trophically transmitted nematode *R. halli*. We have indirect evidence that the food addition did not alter nutrition status that could have impacted parasite establishment or survival, based on host and parasite responses to treatment. First, chipmunk mass did not change in response to either supplemental food treatment compared to control sites (data not shown). Additionally, there was no evidence that the intensity of parasite egg shedding changed in either treatment compared to controls for any of the parasites (Table 3). Nutritional status of hosts can impact the establishment and survival of intestinal parasites (Koop & Kyriazakis 1999). Food supplementation experiments in wildlife tend to increase the body mass of target species (Boutin 1990), as well as increase measures of immunocompetance (Bachman 2003, De Neve *et al.* 2007). However, our supplemental sunflower seed food source represents a carbohydrate dense

food source that is also easily cacheable. Vandegrift & Hudson (2009) measured a greater density response of rodents to a cicada emergence event (protein-rich, non-cacheable food pulse) compared to a supplemental sunflower seed addition. Potentially, the chipmunks switched foraging attention to the supplemental food source but did not increase their consumption, by energy content, compared to what was available on control sites because they were caching the supplemental seeds. Previous research indicates that chipmunks in eastern mast-tree dominated forests are not food-limited during the summer season, but rather devote most of their time to foraging to build food caches that are necessary for winter survival (Yahner 1978, Ostfeldt *et al.* 1996).

Alternative mechanisms for the decrease in prevalence of *R. halli* in response to treatment are less likely, but we do not have evidence to support or refute other mechanisms. The treatment could have influenced cricket feeding away from chipmunk feces and reduced the exposure of crickets to infection, as opposed to reducing the exposure of chipmunks to crickets. We did not sample crickets to assess a change in the abundance of parasite infectious stages, although, this would be unlikely to yield meaningful results because *R. halli* is hosted by other co-occuring Sciurids (Rausch & Timer 1948) and a *Peromyscus leucopus* (white-footed mouse) specific parasite *Pterygadermatites peroymsci* (in the same family, Rictularidae) is also abundant in the study areas, has the same putative intermediate host range, but we are unable to differentiate the parasite species within the intermediate hosts (Luong, unpublished).

Where you eat is important for transmission dynamics

While we were unable to document the predicted changes in foraging exposure in response to the spatial arrangement of supplemental resources, we were able to provide documentation of a strong empirical correlation between host network structure and parasite

infection. There was a positive correlation between network in-degree and the probability of infection with directly transmitted parasites. This correlation was only present when the definition of a network edge corresponded to the transmission route (Figure 4a-d). Further, there was an even stronger correlation with infection among the subset of in-degrees that were generated from infected individuals (Figure 4b,d).

We were not able to evaluate the experimental influence on the network structure because there was a large difference in the apparent capture rate of chipmunks between the supplemental feeding treatments and the control treatment (Figure 2). We postulate that the presence of supplemental food decreased the trapability of chipmunks, resulting in a capture bias and an inability to detect an accurate measure of the contact networks in response to treatment. The contact networks appeared to become sparser in response to treatment (Figure 1a), but we doubt this reflects the actual contact structure because of the trapping bias caused by the treatment. The number of individual chipmunks captured did not change in a manner that appeared to be biased by treatment (Table 1), but the number of captures per individual, the minimum days known alive, number of captures per chipmunk, and index of trapability decreased on treatment sites compared to control sites (Table 1, Figure 1b-d). Previous food supplementation experiments have not documented a bias in trapability in response to food supplementation, although these studies were lacking replication or controls (Lacki *et al.* 1984a,b; Mares *et al.* 1982). Eastern chipmunks have been observed to engage in a late summer activity lull that appears unrelated to available food resources (Wrazen 1980) but the trapping rate of chipmunks only declined in response to our feeding treatments and appeared to increase on control sites (Figure 1b-d). We speculate that the chipmunks found the traps (baited with food) less enticing when there was an abundance of seeds on the ground. We cannot rule out other mechanisms that

reduced the per individual capture rates in response to treatment, such as decreased survival due to a predator response, parasite infection, or an interaction between parasite infection and host activity because the capture data per individual were insufficient. Nonetheless, we feel confident that we sampled enough individuals to accurately detect the change in prevalence with the trophically transmitted *R. halli* and evaluate the hypothesis that what food is available determines the exposure to trophically transmitted parasites.

Our data from before the treatment indicated the network of between-host contacts is a key factor in the parasite dynamics of our chipmunk-helminthes system. Notably, the network framework for quantifying the host interactions that are correlated to parasite infection is only relevant when the interactions were properly defined to reflect parasite transmission mode. We detected a positive correlation between the network in-degree and probability of infection with the two most common directly transmitted parasites, *C. bifurcatum* and *C. tamiasstriati* (Figure 4a-b). The correlation between total in-degree and infection was only statistically significant for *C. bifurcatum* (Figure 4a). However, when we incorporated information about the proportion of in-degrees generated by infected individuals, correlations with both directly transmitted parasite species became stronger (Figure 4b,d). The importance of defining the edges with biologically meaningful interaction criteria is highlighted by the lack of correlation with our most common trophically transmitted parasite, *R. halli* (Figure 4e-f).

An alternative explanation for the network-parasite correlations is that the network position resulted from the parasite infection, rather than the parasite infection patterns resulting from network position. Studies of other rodents, have shown that experimental infections with macroparasites does not change feeding rates of deer mice (*Peromyscus maniculatus,* Schwanz 2006), but reduces activity (Poirier *et al*. 1995). Yet, studies in wild wood mice (*Apodemus*

sylvaticus) observed that males infected with the nematode parasite *Heligmosomoides polygyrus* had larger territories than uninfected males (Brown *et al*. 1994). The influence of parasites on the foraging area and activity of eastern chipmunks is unknown, but it is possible that an increased energetic demand caused by parasite infection could necessitate an increase in foraging time or area and result in parasitized chipmunks accumulating more transmission contacts. Yet, the lack of a correlation between network in-degree and infection with the trophically transmitted *R. halli* suggests that the interpretation of networks influencing infection via exposure may be robust (Figure 4 e-f). If infection patterns result from network structures, we do not expect a trophically transmitted parasite to correlate to networks defined by host-host interactions. Conversely, there is no *a priori* reason to expect the influence of network position by parasites should be different based on infection by the different nematode species.

Surprisingly, the infra-host community of GI parasites had a similar positive correlation with network in-degree (Figure 5). Analyses for species co-occurrence patterns provided no evidence for any pairwise structuring of parasite species infecting an individual host. Given the correlations between network structure and in-degree for the directly transmitted, *C. bifurcatum* and *C. tamiasstriati*, and the lack of correlation with the trophically transmitted *R. halli*, we hypothesize that the parasite species richness is being driven by increased exposure to the directly transmitted parasites when individuals are more connected. Empirical analysis of gidgee skink (*Egernia stokesii*) contact networks found that skinks infected with multiple blood parasites and infested with ectoparasites were twice as connected as individuals infected with fewer parasite species (Godfrey *et al.* 2009). Further, we speculate that the network measures of connectedness may reflect an underlying process whereby more observed contacts represents an accumulation curve of infectious contacts. This process may be strongly influenced by the

prevalence of parasite species: as the network connectedness increases, an infectious contact and subsequent infection with rare parasite species are only achieved by the most connected individuals.

Exposure is important

Exposure is a key process generating host-parasite dynamics. Relevant measures of exposure are difficult to empirically measure, despite the theoretical recognition of the central role that individual heterogeneity plays in disease dynamics (Keeling 1999; Lloyd-Smith *et al.* 2005; Myers *et al.* 2005; Aparicio & Pascual 2007; Bansal *et al.* 2007). Defining the appropriate measure for exposure is strongly dependent on an understanding of the transmission mode of the parasite(s) of interest. We have examined the relevant foraging behaviors (food type availability and individual connections during foraging) that are necessary for the transmission of parasites with two different modes of fecal-oral transmission. This examination of foraging behavior is analogous to studying the number and identity of sexual partners in regards to HIV transmission (Jones & Handcock 2003) or social grouping behaviors in the transmission of parasites transmitted by intimate contact (Altizer *et al.* 2003; Craft *et al.* 2010). Within-host processes, at the interaction of hosts and parasites, will always remain a key half of the infection and transmission process. We strongly believe that further integration of host-host and host-environment interactions that determine exposure to, and spread of, parasite infectious stages will be necessary for fuller understanding of host-parasite dynamics.

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REFERENCES

Adamson, M. (1994) Evolutionary patterns in the life histories of Oxyruida. *International Journal for Parasitology*, **24,** 1167-1177.

Altizer, S., Nunn, C.L., Thrall, P.H., Gittleman, J.L., Antonovics, J., Cunningham, A.A., Dobson, A.P., Ezenwa, V., Jones, K.E., Pederson, A.B., Poss, M. & Pulliam, J.R.C. (2003) Social organization and parasite risk in mammals: integrating theory and empirical studies. *Annual Review of Ecology, Evolution, and Systematics*, 34, 517-547.

Anderson, R. C. (2000) Nematode parasites of vertebrates: their development and transmission. 2nd edition. CABI Publishing, New York, NY, USA.

Aparicio, J.P. & Pascual, M. (2007) Building epidemiological models from R0, an implicit treatment of transmission networks. *Proc. R Soc. B Biol. Sci.,* 274, 505-512.

Bachman, G. C. (2003) Food supplements modulate changes in leucocyte numbers in breeding male ground squirrels. *The Journal of Experimental Biology*, 206, 2373-2380.

Bansal, S., Grenfell, B.T., & Myers, L.A. (2007) When individual behaviour matters: homogeneous and network models in epidemiology. *J. R. Soc. Interface*, 4, 879-891.

Bansal, S., Read, J., Pourbohloul, B., & Myers, L. A. (2010) The dynamic nature of contact networks in infectious disease epidemiology. Journal *of Biological Dynamics*, 4, 478-489. doi: 10.1080/17513758.2010.503376

Bates, D., & Maechler, M. (2010) lme4: Linear mixed-effects models using S4 classes. R package version 0.999375-33. <http://CRAN.Rproject.prg/package=lme4>

Brown, E.D., Macdonald, D.W., Tew, T.E., & Todd, I.A. (1994) *Apodemus sylvaticus* infected with *Heligmosomoides polygyrus* (Nematoda) in arable ecosystems: epidemiology and effects of infection on the movement of male mice. *J. Zool., Lond.*, 234, 623-640.

Craft, M. E., Volz, E., Packer, C., & Myers, L. A. (2009) Disease transmission in territorial populations: the small-world network of Serengeti lions. *J. R. Soc. Interface*, 28, doi: 10.1098/rsif.2010.0511.

Cross, P.C., Lloyd-Smith, J.O., Bowers, J.A., Hay, C.T., Hofmeyr, M., & Getz, W.M. (2004) Integrating association data and disease dynamics in a social ungulate: bovine tuberculosis in African buffalo in the Kruger National Park. *Ann. Zool. Fenn.,* 41, 879-892.

De Neve, L., Soler, J. J., Ruiz-Rodriguez, M., Martin-Galvez, D., Perez-Contreras, T., & Soler, M. (2007) Habitat-specific effects of a food supplementation experiment on immunocompetence in Eurasian Magpie *Pica pica* nestlings. *Ibis*, 149, 763-773.

Delahay, R.J., Langton, S., Smith, G.C., Clifton-Hadley, R.S. & Cheeseman, C.L. (2000) The spatio-temporal distribution of Mycobacterium bovis (bovine tuberculosis) infection in a high-density badger population. *Journal of Animal Ecology*, 69, 428-441.

Drewe, J. A. (2010) Who infects whom? Social networks and tuberculosis transmission in wild meerkats. *Proc. R. Soc. B.*, 277, 633-642. doi: 10.1098/rspb.2009.1775

Ehman, K. D. (2001) Nematode parasites of the eastern chipmunk from Armstrong county, Pennsylvania. *Journal of the Pennsylvania Academy of Science*, 75, 62-65.

Elliot, L. (1978) Social behavior and foraging ecology of the eastern chipmunk (*Tamias striatus*) in the Adirondack Mountains. *Smithsonian Contributions to Zoology*, 265, 1-107.

Gelman, A., Y., Su, S., Yajima, M/, Hill, J., Pittau, M. G., Kerman, J., & Zheng, T. (2010) arm: Data analysis using regression and multilevel/hierarchical models. R package version 1.3-02. <http://CRAN. R-project,org/package=arm>

Godfrey, S. S., Bull, C. M., James, R., & Murray, K. (2009) Network structure and parasite transmission in a group living lizard, the gidgee skink, *Egernia stokesii*. *Behavioral Ecology and Sociobiology*, 63, 1045-1056.

Gotelli, N.J. & Entsminger, G. L. (2001) EcoSim: Null models software for ecology. Version 7.0. Acquired Intelligence Inc. & Kesey-Bear, http://homepages.together.net/~gentsmin/ecosim.htm.

Grear, D.A., Perkins, S.E. & Hudson, P.J. (2009) Does elevated testosterone result in increased exposure and transmission of parasites? *Ecology Letters*, 12, 528-537.

Grear D.A., Samuel, M.D.,Scribner, K.,Weckworth, B.V., & Langenberg, J. A. (2010) Influence of genetic relatedness and spatial proximity on CWD transmission among female white-tailed deer. *Journal of Applied Ecology***,** 47, 532-540.

Hall, S. R., Sivars-Becker, L., Becker, C., Duffy, M. A., Tessier, A. J., and Caceres, C. E. (2007) Eating yourself sick: transmission of disease as a function of foraging ecology. *Ecology Letters*, 10, 207-218. doi: 10.1111/j.1461-0248.2006.01011.x

Hamede, R. K., Bashford, J., McCallum, H., & Jones, M. (2009) Contact networks in a wild Tasmanian devil (*Sarcophilus harrisii*) populations: using social network analysis to reveal seasonal variability on social behaviour and its implications for transmission of devil facial tumor. *Ecology Letters*, 12, 1147-1157. doi: 10.1111/j.1461-0248.2009.01370.x

Handcock, M.S., Hunter, D.S., Butts, C.T., Goodreau, S.M., & Morris, M. (2003) Statnet: A suite of R packages for the Statistical Modeling of Social Networks. Version 2.0, URL http://www.csde.washington.edu/statnet.

Jones, J. H. & Handcock, M. S. (2003) Social networks (communication arising): sexual contacts and epidemic models. *Nature*, 423, 605-606.

Keeling, M.J. (1999) The effects of local spatial structure of epidemiological invasions. *Proc. R Soc. B Biol. Sci.,* 266, 859-867.

Kjaer, L.J., Schauber, E.M., & Nielsen, C.K. (2008) Spatial and temporal analysis of contact rates in female white-tailed deer. 72, 1819-1825. doi: 10.2193/2007-489

Kramer, D. L. & Nowell, W. (1980) Central place foraging in the Eastern chipmunk, *Tamias striatus*. *Animal Behavior*, 28, 772-778.

Koop, R. L., & Kyriazakis, I. (1999) Nutrition-parasite interaction. *Veterinary Parasitology*, 84, 187-204.

Lacher, T. E. & Mares, M. A. (1996) Availbility of resources and use of space in eastern chipmunks, *Tamias striatus*. *Journal of Mammalogy*, 77, 833-849.

Lacki, M. J., Gregory, M. J., & Williams, P. K. (1984a) Summer activity of *Tamias striatus* in response to supplemental food. *Journal of Mammalogy*, 65, 521-524.

Lacki, M. J., Gregory, M. J., & Williams, P. K. (1984b) Spatial response of an eastern chipmunk population to supplemented food. *American Midlands Naturalist*, 111, 414-416.

Loehle, C. (1995) Social barriers to pathogen transmission in wild animal populations. *Ecology*, 76, 326-335.

Lloyd-Smith, J. O., Schreiber, S. J., Kopp, P. E., & Getz, W. M. (2005) Superspreading and the effect of individual variation on disease emergence. *Nature*, 438, 355-359.

Mares, M.A, Lacher, T.E., Willig, M.R., Bitar, N.A., Adams, R., Klinger, A., & Tazik, D. (1982) An experimental analysis of social spacing in *Tamias striatus*. *Ecology*, 63, 267-273.

May, R.M. (2006) Network structure and the biology of populations. *Trends in Ecology and Evolution*, 7, 394-399.

McDonald, T. L., Erickson, W. P, & McDonald, L. L. (2000) Analysis of count data from before-after control-impact studies. *Journal of Agricultural, Biological, and Environmental Statistics*, 5, 262-279.

Moore, J. (2002) Parasites and the behavior of animals. Oxford University Press, Oxford, UK.

Myers, L.A., Pourbohloul, B., Newman, M.E.J., Skowronski, D.M., & Brunham, R.C. (2005) Network theory and SARS: predicting outbreak diversity. *Journal of Theoretical Biology*, 232, 71-81.

Ostfeldt, R. S., Jones, C. G., & Wolff, J. O. (1996) Of mice and mast. *Bioscience*, 46, 323-330.

Perkins, S.E., Cagnacci, F., Stradiotto, A., Arnoldi, D., & Hudson, P.J. (2009). A comparison of social networks derived from ecological data: implications for inferring infectious disease dynamics. *Journal of Animal Ecology*, 78, 1015-1022. doi: 10.1111/j.1365-2656.2009.01557.x

Perkins, S.E., Ferrari, M.F., & Hudson, P.J. (2008) The effect of social structure and sex-biased transmission on macroparasite infection. *Parasitology*, 135, 1561-1569.

Poirier, S.R., Rau, M.E., & Wang, X. (1995) Diel locomotory activity of deer mice (*Peromyscus maniculatus*) infected with *Trichinella nativa* or *Trichinella pseudospiralis*. *Canadian Journal of Zoo*logy, 73, 1323-1334.

Poulin, R. (2010a) Parasite manipulation of host behavior: an update and frequently asked questions. *Advances in the Study of Behavior*, 41, 151-186.

Poulin, R. (2010b) Network analysis shining light n parasite ecology and diversity. *Trends in Parasitology*, 26, 492-498. doi:10.1016/j.pt.2010.05.008

R development core team. (2009) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. <http://www.R-project.org>

Rausch, R. & Tiner, J. D. (1948) Studies on the parasitic helminthes of the north central states. I. Helminths of Sciuridae. *American Midland Naturalist*, 39, 728-747.

Revilla, E. & Palomares, F. (2002) Spatial organization, group living and ecological correlates in low-density populations of Eurasian badgers, *Meles meles*. *Journal of Animal Ecology*, 71, 497-512.

Salathé, M., Kazandjieva, M., Lee, J. W., Levis, P., Feldman, M. W., & Jones, J. H. (2010) A High-Resolution Human Contact Network for Infectious Disease Transmission. *Proceedings of the National Academy of Science USA*, 107, 22020-22025.

Schauber, E.M., Storm, D.J., & Nielsen, C.K. (2007) Effects of joint space use and group membership on contact rates among white-tailed deer. *Journal of Wildlife Management*, 71, 155-163.

Schluter, D. (1984) A variance test for detecting species associations, with some example applications. *Ecology* 65, 998-1005.

Schwanz, L.E. (2006) Schistosome infection in deer mice (*Peromyscus maniculatus*): impacts on host physiology, behavior and energetics. *Journal of Experimental Biology*, 209, 5029-5030.

Shaw, D. J., Grenfell, B. J., & Dobson, A. P. (1998) Patterns of macroparasite aggregation in wildlife host populations. *Parasitology*, 117, 597-610.

Sloss, M. W. & Kemp, R. L. (1978) *Veterinary Clinical Parasitology*. Iowa State University Press, Ames, Iowa, USA

Snyder, D. P. (1982) Tamias striatus. *Mammalian Species*, 168, 1-8.

Stone, L., & Roberts, A. (1990) The checkerboard score and species distributions. *Oecologia*, 85, 74-79.

Vandegrift, K. J. & Hudson, P. J. (2009) Response to enrichment, type and timing: small mammals vary their response to a springtime cicada but not a carbohydrate pulse. *Journal of Animal Ecology*, 78, 202-209. doi: 10.1111/j.1365-2656.2008.01456.x

Weihong, J.I, White, P.C.L., & Clout, M.N. (2005) Contact rates between possums revealed by proximity data loggers. *J. Appl. Ecol.*, 42, 595-604.

Wrazen, J. A. (1980) Late summer activity changes in populations of eastern chipmunks (*Tamias striatus*). *Canadian Field Naturalist*, 94, 305-310.

Yahner, R. H. (1978) Burrow system and home range use by eastern chipmunks, *Tamias striatus*: ecological and behavioral considerations. Journal of Mammalogy, 59, 324-329.

TABLE 4.1. Summary of the network size, in-degree distribution, and capture metrics of eastern chipmunks (*Tamias striatus*) for each replicate trapping site before (May-June) and after (July-August) the supplemental feeding experiment treatment.

	Site	Nodes	Time Period	Average In-Degree	Standard Error[a]	dAICc[b] (Poisson – neg. binom.)	Poisson GOF[c] *P*-value	Neg. Binomial GOF *P*-value	Mean number of captures per individual	Mean days known alive	Trapability index[d]
Untreated sites	II	17	Before Treatment	1.8	0.64	-2.31	**0.35**	**0.34**	3.5	10.9	0.089
		17	After Treatment	4.9	0.75	**12.9**	<0.01	0.01	7.7	24.7	0.522
	V	19	Before	2.5	0.64	-1.94	**0.09**	**0.10**	3.1	11.9	-0.062
		18	After	5	0.75	2.00	0.01	0.02	6.1	19.9	0.075
	VIII	31	Before	5.5	0.64	**33.1**	<0.01	0.04	5.4	20.0	-0.124
		20	After	4.5	0.75	**13.0**	<0.01	**0.25**	6.0	18.4	-0.103
Aggregated treatment sites	IV	24	Before	4.7	0.54	1.46	**0.07**	**0.21**	4.4	16.5	0.035
		18	After	2.4	0.36	-2.30	0.03	0.03	4.2	12.5	-0.131
	VII	8	Before	0.9	0.54	-2.94	<0.01	<0.01	2.9	8.9	-0.049
		14	After	0.9	0.36	2.66	0.04	**0.36**	2.4	6.2	0.001
	X	18	Before	2.9	0.54	-1.72	**0.08**	**0.10**	3.3	12.0	-0.003
		17	After	2.7	0.36	**7.22**	0.04	**0.69**	3.5	9.3	0.037
Uniform treatment sites	VI	22	Before	4.2	0.56	1.74	0.02	**0.08**	5.1	19.9	-0.126
		21	After	1.6	0.22	-1.23	**0.30**	**0.44**	2.6	3.1	-0.062
	IX	12	Before	2.8	0.56	-0.16	**0.06**	**0.13**	3.3	14.0	-0.293
		9	After	0.3	0.22	**-35.2**	<0.01	<0.01	2.0	5.7	-0.591
	XII	15	Before	4.3	0.56	**3.60**	0.01	**0.11**	4.5	15.1	0.105
		13	After	1.5	0.22	2.55	0.01	**0.07**	3.9	9.5	-0.168
	XIV	13	Before	3.0	0.56	-2.45	**0.72**	**0.71**	5.3	22.5	-0.170
		20	After	1.6	0.22	-2.25	**0.33**	**0.33**	2.9	8.1	-0.204

[a]Standard error calculated from fitted generalized linear mixed-models including individual and trapping site as random effects.
[b]Akaike's information criteria corrected for small sample size. Negative values indicate the degree distribution was better described by a negative binomial distribution and positive values indicate a better fit to a Poisson distribution. A superior fit was assigned (bold) if the absolute difference was >4.
[c]Goodness-of-fit was evaluated according to Shaw et al. 1998. *P*-values > 0.05 (bold) indicate an adequate fit to the distribution.
[d]Trapability index is the residuals of a regression between number of captures and minimum days known alive.

TABLE 4.2. Generalized linear mixed model (GLMM) parameter coefficients for best fitting models describing eastern chipmunk (*Tamias striatus*) prevalence of infection with three nematode species in response to supplemental food treatments.

Parasite response	Explanatory variable	Coefficients	Value[a]	95% CI[b]
Capillaria tamiasstriati		intercept[c]	-0.14	[-6.91 , 5.68]
prevalence	Site treatment (relative to	Aggregated treatment	1.04	[-4.80 , 7.33]
(direct fecal-oral	control sites)	Uniform treatment	1.53	[-4.35 , 7.30]
transmission)	Treatment time period			
	(relative to before treatment)	After treatment	0.19	[-0.55 , 0.90]
		Mass	-0.02	[-0.08 , 0.04]
		Sex: male (relative to females)	-1.77	[-2.90 , -0.62]*
Citellinema bifurcatum		intercept[c]	-1.37	[-3.93 , 1.05]
prevalence	Site treatment (relative to	Aggregated treatment	0.66	[0.05 , 1.26]*
(direct fecal-oral	control sites)	Uniform treatment	-0.17	[-0.84 , 0.46]
transmission)	Treatment time period			
	(relative to before treatment)	After treatment	0.04	[-0.41 , 0.47]
		Mass	-0.01	[-0.04 , 0.02]
		Sex: male (relative to females)	-0.05	[-0.53 , 0.39]
Rictularia halli		intercept[c]	-9.73	[-17.4 , -0.84]*
prevalence	Site treatment (relative to	Aggregated treatment	1.05	[-3.23 , 5.03]
(trophic fecal-oral	control sites)	Uniform treatment	1.85	[-1.67 , 5.16]
transmission)	Treatment time period			
	(relative to before treatment)	After treatment	1.08	[-0.31 , 2.34]
		Mass	0.02	[-0.09 , 0.11]
		Sex: male (relative to females)	-0.35	[-3.02 , 2.40]
	Site treatment x time	Aggregated treatment, after treatment	0.25	[-1.89 , 2.70]
		Uniform treatment, after treatment	-0.81	[-2.81 , 1.52]

*Significant coefficients

[a]Untransformed coefficient values for the generalized linear mixed-model regression with individual chipmunk and trapping grid included as random-effect variables. Values indicate the change in response, relative to the intercept, or relative to the intercept + additive coefficients, in the case of interactions.

[b]Bayesian credible intervals calculated from 10,000 MCMC samples from the posterior distribution of the model. CIs not overlapping zero were considered significant

[c]Intercept value is the reference probability of infection over all site treatments during the time period before treatment.

TABLE 4.3. Generalized linear mixed model (GLMM) parameter coefficients for best fitting models describing eastern chipmunk (*Tamias striatus*) intensity of parasite egg shedding with three nematode species in response to supplemental food treatments.

Response parasite	Explanatory variable	Coefficients	Value[a]	95% CI[b]
Capillaria tamiasstriati		intercept[c]	1.44	[0.39 , 2.58]*
egg shed per gram	Site treatment (relative to	Aggregated treatment	-0.001	[-1.39 , 1.16]
feces (*epg*)	control sites)	Uniform treatment	0.16	[-1.04 , 1.22]
(direct fecal-oral	Treatment time period			
transmission)	(relative to before treatment)	After treatment	0.002	[-0.10 , 0.10]
		Mass	-0.01	[-0.01 , 0.004]
		Sex: male (relative to females)	-0.27	[-0.50 , -0.09]*
Citellinema bifurcatum		intercept[c]	0.37	[-0.18 , 0.84]
epg	Site treatment (relative to	Aggregated treatment	0.19	[0.04 , 0.35]*
(direct fecal-oral	control sites)	Uniform treatment	-0.07	[-0.22 , 0.08]
transmission)	Treatment time period			
	(relative to before treatment)	After treatment	-0.02	[-0.15 , 0.11]
		Mass	-0.002	[-0.01 , 0.004]
		Sex: male (relative to females)	-0.01	[-0.08 , 0.07]
	Site treatment x time	Aggregated treatment, after treatment	-0.13	[-0.32 , 0.08]
		Uniform treatment, after treatment	0.15	[-0.06 , 0.35]
Rictularia halli		intercept[c]	0.05	[-0.50 , 0.55]
epg	Site treatment (relative to	Aggregated treatment	0.09	[-0.10 , 0.28]
(trophic fecal-oral	control sites)	Uniform treatment	0.13	[-0.05 , 0.30]
transmission)	Treatment time period			
	(relative to before treatment)	After treatment	0.05	[-0.03 , 0.11]
		Mass	0.0003	[-0.01 , 0.01]
		Sex: male (relative to females)	-0.005	[-0.13 , 0.11]

*Significant coefficients

[a]Untransformed coefficient values for the generalized linear mixed-model regression with individual chipmunk and trapping grid included as random-effect variables. Values indicate the change in response, relative to the intercept, or relative to the intercept + additive coefficients, in the case of interactions.

[b]Bayesian credible intervals calculated from 10,000 MCMC samples from the posterior distribution of the model. CIs not overlapping zero were considered significant

[c]Intercept value is the reference probability of infection over all site treatments during the time period before treatment.

Figure 4.1

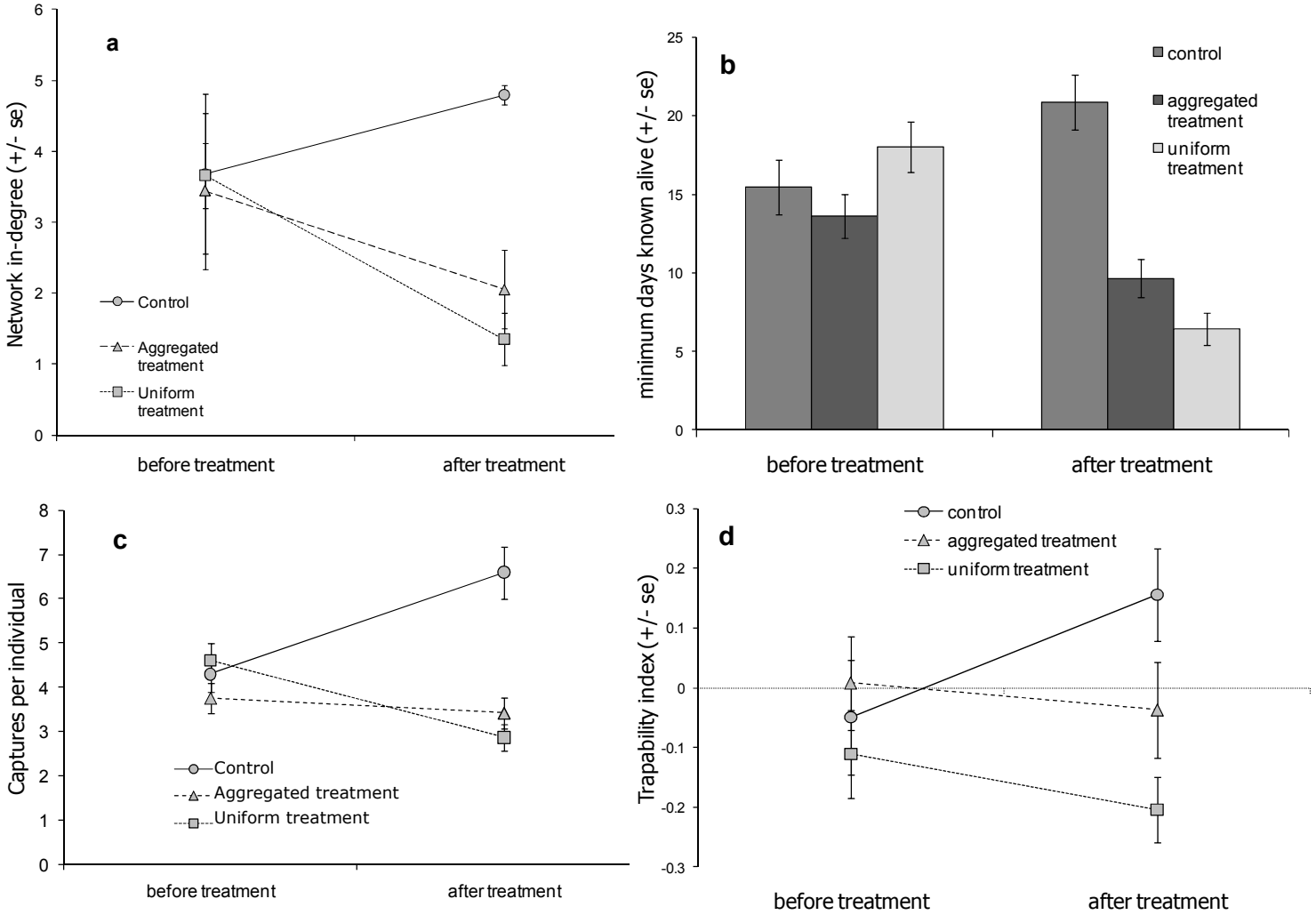
a
Network in-degree (+/- se)
Control
Aggregated treatment
Uniform treatment
before treatment
after treatment
b
minimum days known alive (+/- se)
control
aggregated treatment
uniform treatment
before treatment
after treatment
c
Captures per individual
Control
Aggregated treatment
Uniform treatment
before treatment
after treatment
d
Trapability index (+/- se)
control
aggregated treatment
uniform treatment
before treatment
after treatment

FIGURE 4.1: Network in-degree and trapping metrics of eastern chipmunks (*Tamias striatus*) in response to two supplemental feeding treatments compared to unfed controls: 9kg/week sunflower seeds provided in an aggregated distribution and a uniform distribution. Network in-degree decreased in both treatments (a), however, the reliability of capture data cannot be compared because treatment also reduced the minimum days known alive (b), number of captures per individual (c) and trapability index (d) compared to control sites. Trapability index was the residuals from the regression between the number of captures and days known alive.

Figure 4.2

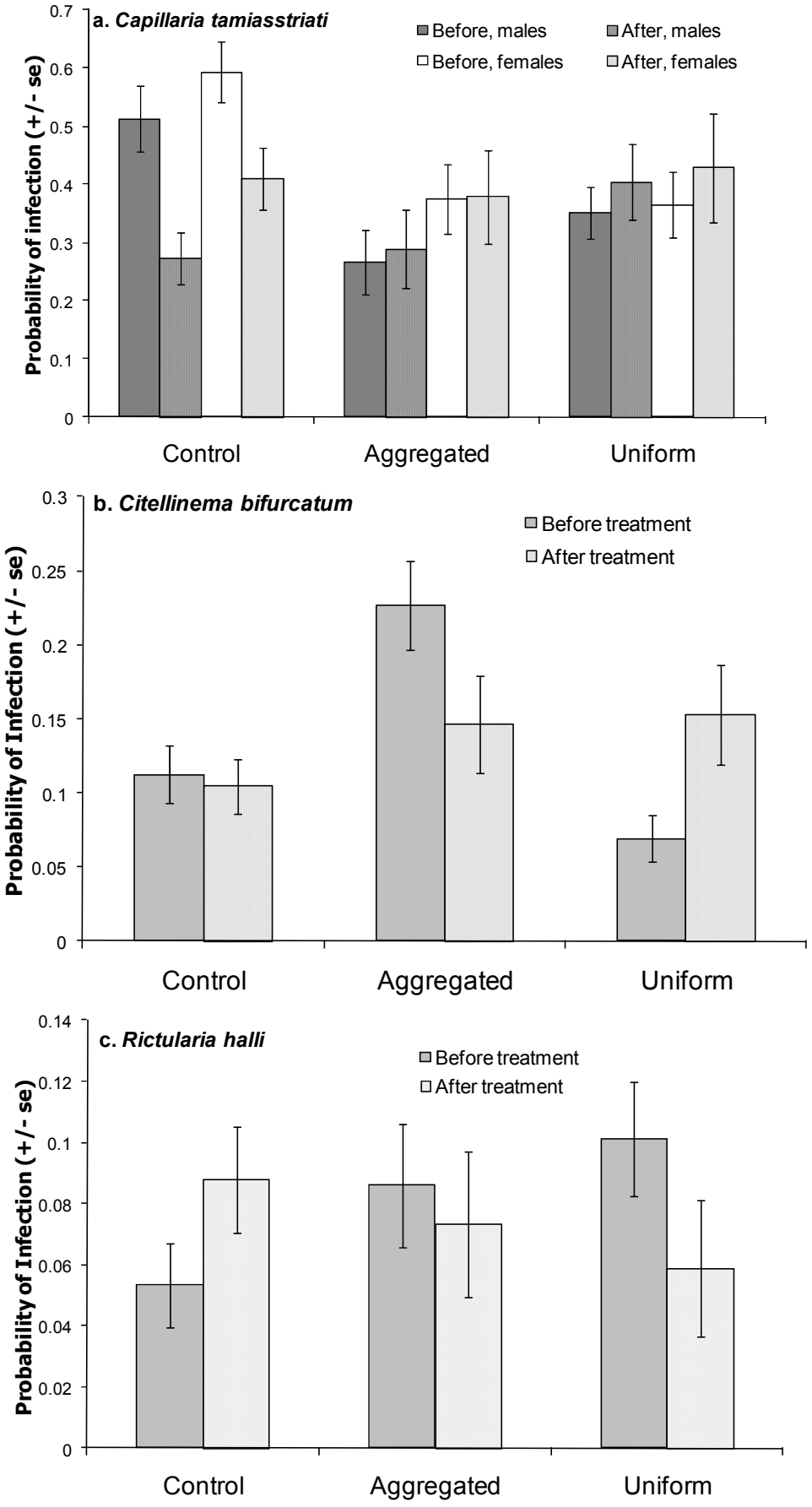
a. Capillaria tamiasstriati
Before, males
After, males
Before, females
After, females
Probability of infection (+/- se)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Control
Aggregated
Uniform
b. Citellinema bifurcatum
Before treatment
After treatment
Probability of Infection (+/- se)
0.3
0.25
0.2
0.15
0.1
0.05
0
Control
Aggregated
Uniform
c. Rictularia halli
Before treatment
After treatment
Probability of Infection (+/- se)
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Control
Aggregated
Uniform

FIGURE 4.2: Response of the prevalence of infection of the three most common eastern chipmunk (*Tamias striatus*) gastrointestinal helminth parasites to two supplemental feeding treatments compared to unfed controls: 9kg/week sunflower seeds provided in an aggregated distribution and a uniform distribution. We did not detect a different change in the prevalence of two directly transmitted parasites, *C. tamiasstriatus* (a) and C. *bifurcatum* (b), in response to treatment. Male chipmunks were less likely to be infected with *C. tamiasstriati* (a) compared to females (95% CI [-2.90 , -0.62]). There was a significant effect of treatment on the prevalence of the trophically transmitted *R. halli* (c) in response to treatment ($X^2 = 6.77$, $df = 2$, $P = 0.03$) with prevalence decreasing after treatment was applied compared to an increase on control sites.

Figure 4.3

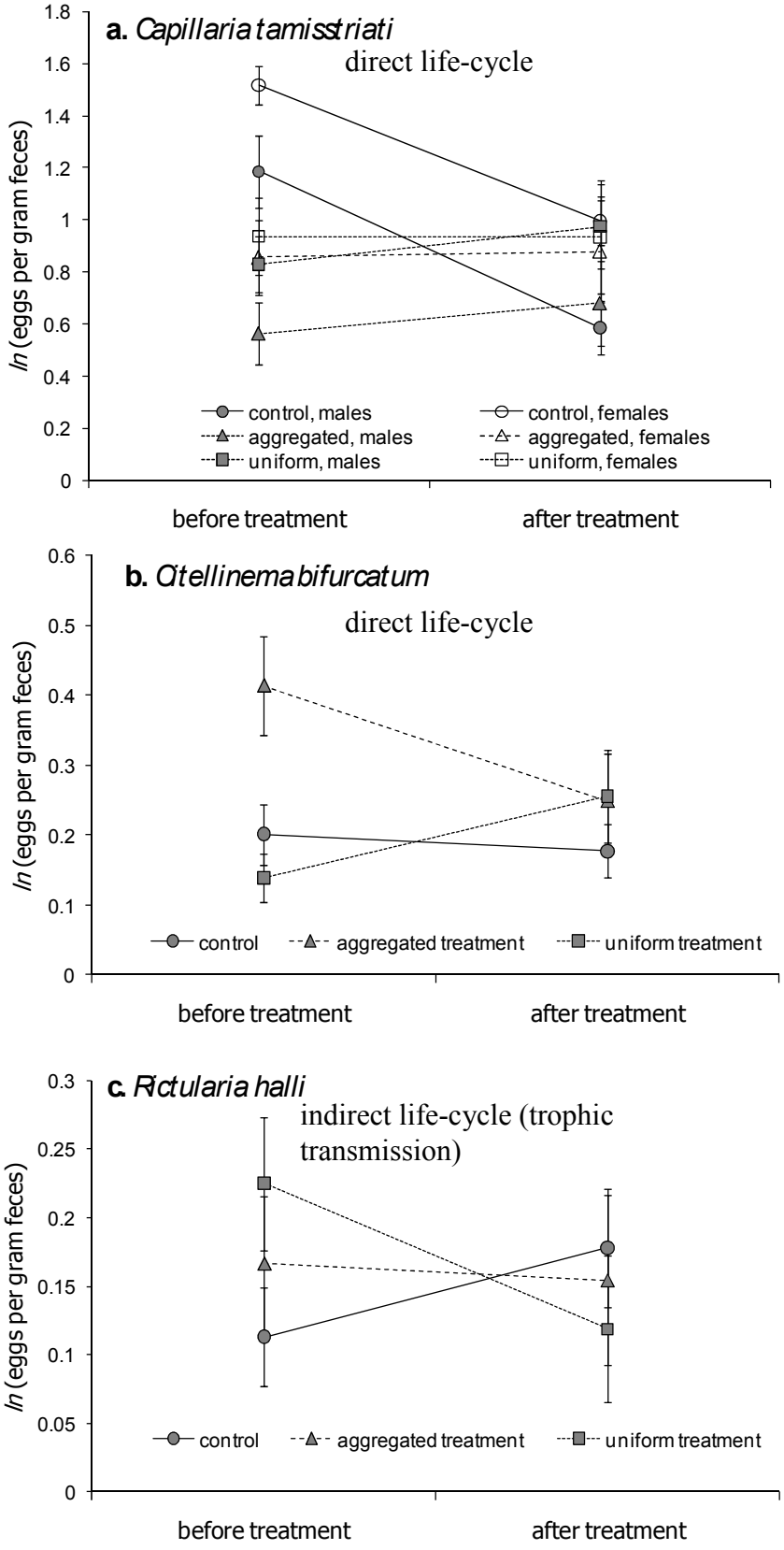
a. Capillaria tamisstriati
direct life-cycle
ln (eggs per gram feces)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
control, males
control, females
aggregated, males
aggregated, females
uniform, males
uniform, females
before treatment
after treatment
b. Citellinema bifurcatum
direct life-cycle
ln (eggs per gram feces)
0.6
0.5
0.4
0.3
0.2
0.1
0
control
aggregated treatment
uniform treatment
before treatment
after treatment
c. Rictularia halli
indirect life-cycle (trophic transmission)
ln (eggs per gram feces)
0.3
0.25
0.2
0.15
0.1
0.05
0
control
aggregated treatment
uniform treatment
before treatment
after treatment

FIGURE 4.3: Response of the intensity of parasite eggs shed of the three most common eastern chipmunk (*Tamias striatus*) gastrointestinal helminth parasites to two supplemental feeding treatments compared to unfed controls: 9kg/week sunflower seeds provided in an aggregated distribution and a uniform distribution. We did not detect a difference in the change of the egg shedding intensity of *C. tamiasstriati* (a) and male chipmunks shed fewer parasite eggs compared to females (95% CI [-0.50 , -0.09]). We detected an experimental response of C. *bifurcatum* (b), with a weak trend for a greater increase in parasite egg shedding rates on uniform treated sites after treatment compared to the other treatments ($X^2 = 5.94$, $df = 2$, $P = 0.05$). There was no effect of treatment on the trophically transmitted *R. halli* (c).

Figure 4.4

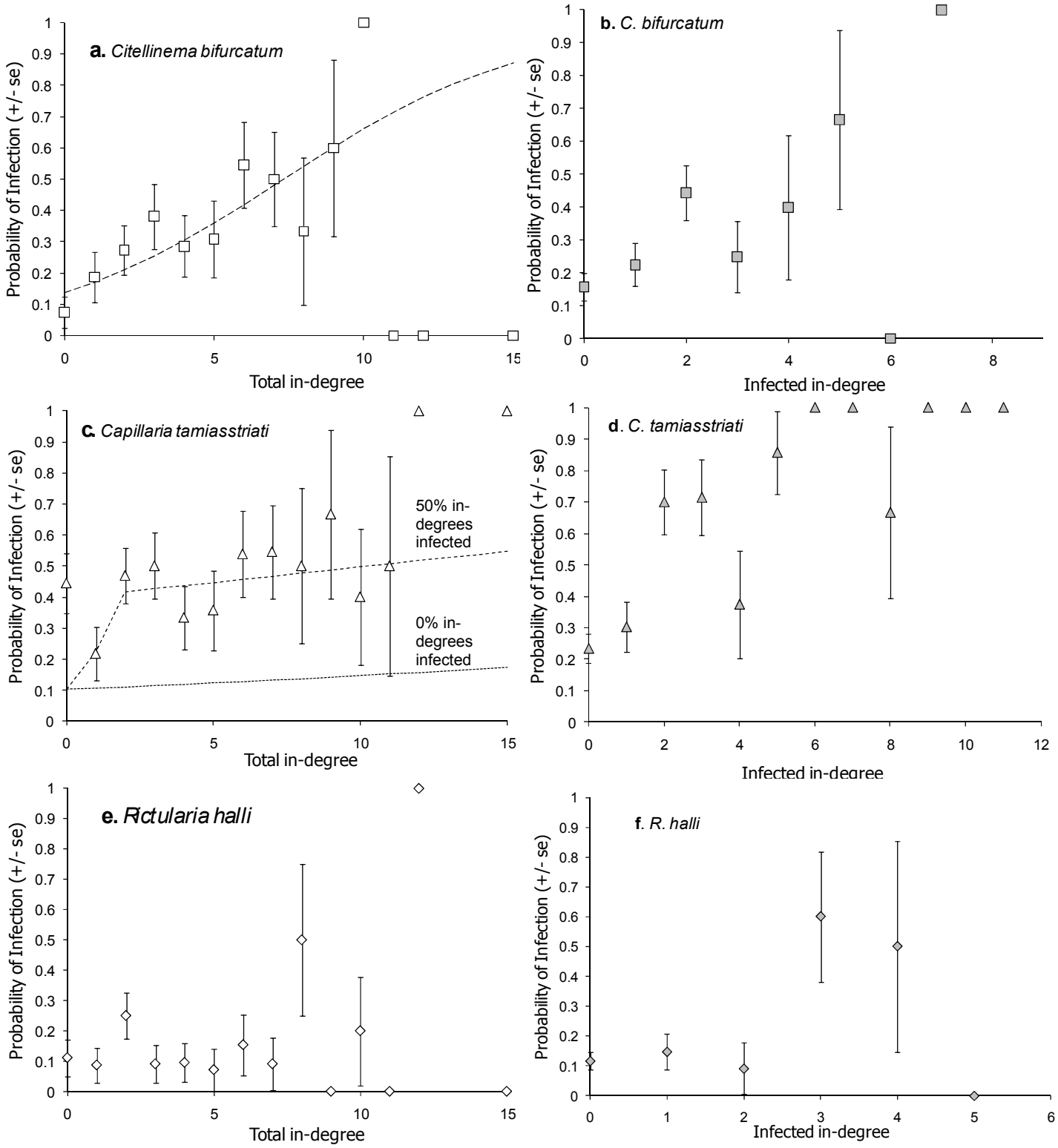
a. Citellinema bifurcatum
b. C. bifurcatum
c. Capillaria tamiasstriati
50% in-degrees infected
0% in-degrees infected
d. C. tamiasstriati
e. Rictularia halli
f. R. halli
Probability of Infection (+/- se)
Total in-degree
Infected in-degree

FIGURE 4.4: Relationship between network in-degree (a, c, e), the subset of in-degrees generated from infected nodes (b, d, f), and the probability of parasite infection of the three most common eastern chipmunk (*Tamias striatus*) gastrointestinal helminth parasites. There were significant correlations between network in-degree and infection with the two directly transmitted parasites (a-d) and no relationship with the trophically transmitted parasite, *R. halli* (e-f). There was a significant relationship between total network in-degree and probability of infection with *C. bifurcatum* (a, 95% slope CI [0.08, 0.41]). There was a similar correlation between *C. bifurcatum* and the subset of in-degree generated by nodes infected with *C. bifurcatum*, but that did not improve the statistical correlation beyond total in-degree (b). There was no correlation between total in-degree and probability of infection with *C. tamisstriati* (c, dotted line, 95% slope CI [-0.11, 0.19]). The proportion of total in-degrees generated from a node infected with *C. tamiasstriati* was strongly correlated with probability of infection (c-d, dashed line, 95% slope CI [2.43, 4.55]).

Figure 4.5

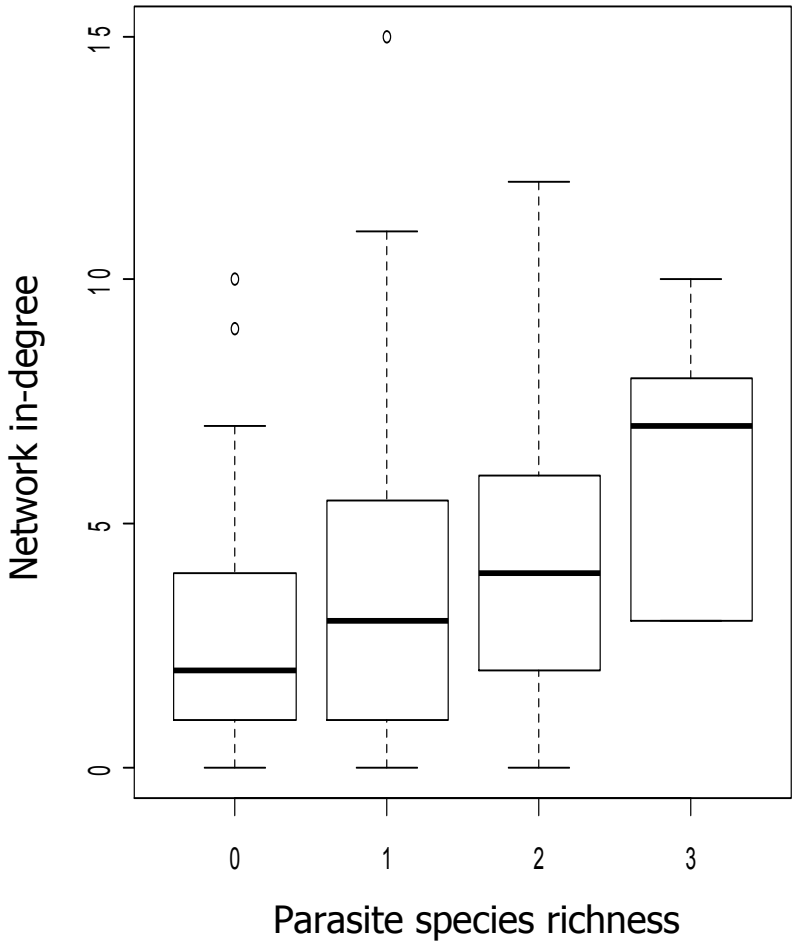

FIGURE 4.5: Positive relationship between network in-degree and parasite species richness per host (number of helminth parasite species infecting a single chipmunk, *Tamias striatus*) (X^2 = 2.94, *df* = 1, *P* = 0.08). Bold lines are the median network in-degree, boxes represent the 25th and 75th percentile, and whiskers the 5th and 95th.

Chapter 5. The dynamics of macroparasite host-self-infection: a study of the patterns and processes of pinworm (Oxyuridae) aggregation

Daniel A. Grear, Peter Hudson

ABSTRACT

Among parasites, Taylor's power law identifies a tight relationship in aggregation of macroparasite infection intensity with few exceptions; notably, the nematode family Oxyuridae tends to have higher than expected aggregation. Oxyuridae infect a wide range of mammalian hosts and have a unique reproductive strategy that involves conventional horizontal transmission, as well as re-infection of an already infected host. We asked the question, does the unique aspects of pinworm life-history can explain an exception to the widely observed patterns of aggregation of parasite populations? We empirically examined the differences among Oxyuridae (genus: *Syphacia*) compared with other helminth (genus: *Heligmosomoides*) parasite aggregation in two rodent hosts with similar ecology: the yellow-necked mouse (*Apodemus flavicollis*) from Trento, Italy and the white-footed mouse (*Peromyscus leucopus*) from Pennsylvania, USA. To investigate the effects of pinworm life-history characteristics on generating aggregation, we present a stochastic model that explores aggregation under a range of host-self-infection, parasite death, and transmission scenarios. Oxyuridae parasites had consistently greater aggregation compared to other nematodes regardless of host or parasite species identity and pinworm aggregation exceeded the range of macroparasite aggregation described previously. Our simulations demonstrated that host-self-infection, on its own, is sufficient to generate greater than predicted aggregation values.

Keywords: aggregation, Taylor's power law, Oxyuridae, macroparasite dynamics, *Apodemus*, *Peromyscus*

INTRODUCTION

The aggregation of macroparasites among hosts is common in natural host-parasite systems, such that a few hosts harbor heavy infections while most hosts have few or no parasites (Shaw and Dobson, 1995; Wilson *et al*. 2002). The variation in parasite abundance in a population of hosts is consistently greater than the mean and follows Taylor's power law (Taylor, 1961), which states that the variance of the abundance of individuals per habitat (parasite per host, intensity) scales with mean abundance of individuals as an exponent of abundance, ***b***: the degree of aggregation. The intriguing feature of Taylor's power law is that the degree of aggregation is remarkably consistent among taxa, in both parasitic and free-living species, suggesting that similar constraints drive the degree of aggregation regardless of life-history traits (Taylor and Taylor, 1977; Taylor and Woiwod, 1980; Shaw and Dobson, 1995; Morand and Guegan, 2000). Parasitic infections exhibit a similarly consistent variance to mean relationship, within and between parasite species, such that most species exhibit a degree of aggregation: $\boldsymbol{b} = 1.55 \pm 0.04$ SD (Shaw and Dobson, 1995). The causes of aggregation within host-parasite relationships have been attributed primarily to variation in host exposure, host susceptibility and environmental conditions (Anderson and Gordon, 1982), although, in a more general sense, the degree of aggregation can be generated via stochasticity in the vital population rates (birth, death, immigration, emigration) within heterogeneous habitats (Anderson *et al*. 1982).

Nematode parasites in the family Oxyuridae (referred to hereafter as pinworms) are gastrointenstinal parasites with a direct life cycle wherein females deposit eggs in the intestinal tract and eggs pass into the environment with host feces. In addition, females of many pinworm species infecting mammalian hosts can exit the intestine and deposit eggs on the perianal region

of the host, allowing for host-self-infection during grooming behavior (Prince, 1950; Schad, 1957; Adamson, 1989; Adamson, 1994; Anderson, 2000). In general, pinworms are considered benign relative to other parasitic nematodes, as they have no extra-intestinal migration and don't feed on tissue or host food but consume bacteria in the posterior gut of their hosts (Adamson, 1994); as opposed to nearly all other parasitic nematodes that have at least one life-stage involving migration through or consumption of host tissue.

The pinworm reproductive strategy of host-self-infection suggests that the intensity of infection in hosts already infected would increase faster than the rate of infection in uninfected hosts, resulting in a highly aggregated intensity distribution. Whereas, conventional nematode parasites are subject to the population processes of death (natural senescence and host mediated) and infection (immigration), pinworms have the additional host-self-infection process, which equates to within-host birth. Pinworm host-self-infection rates also vary with the level of infection, further suggesting the potential for high aggregation: host harboring higher pinworm intensities are subject to higher self-infection rates. The consequence of pinworm life-history for aggregation within a population of hosts is that the variance in intensity per host may be greater compared to conventional nematodes because of the addition of a birth process.

However, there are costs associated with being too aggregated: as parasite numbers per host increase, forces such as host immune response to increased virulence and intraspecific competition for energy or space will act to reduce intensity per host via increased parasite death rate. At the extreme, parasite induced host mortality removes an entire patch of parasites at parasite intensity dependent rates. Such forces constrain the upper limit of the variance to mean ratio by reducing intensity within, or removing, the few hosts that harbor the most parasites, as well as decreasing the potential for transmission and creating an evolutionary compromise

between parasite intensity (transmission, immigration/emigration) and host fitness (Read, 1994; Gandon *et al* 2002).

The unique aspects of pinworm life-history provide an interesting case to study the trade-offs between direct-reproduction within hosts (birth rates), transmission (immigration & emigration), and death (parasite death rate and host death rate) on generating the widely observed patterns of aggregation of parasite populations. We asked the question: does pinworm life-history lead to greater aggregation across host species compared to other orders of nematode parasites? Specifically, we empirically examined the differences among pinworm (genus: *Syphacia*) aggregation within and between two wild rodent hosts with similar ecology, *Apodemus flavicollis* from Trento, Italy and *Peromyscus leucopus* from Pennsylvania, USA. We also examined aggregation patterns in a non-pinworm nematode parasite, *Heligmosomoides polygyrus*, in *A. flavicollis* hosts. In addition, we combine our data with other published reports of abundance and variance of pinworms and compare the pinworm variance-mean relationship to the previously reported variance-mean relationship across a wide range of nematode parasites (Shaw and Dobson, 1995). We predicted that the self-infecting characteristic of pinworms leads to a greater variance *vs* mean relationship than nematode parasites with a direct life-cycle that do not host self-infect. Finally, to further investigate the effects of pinworm life-history characteristics on generating the observed variance-mean relationship, we present a stochastic simulation model that compares simulated variance *vs* mean relationships under a range of host-self-infection, parasite death, and transmission rates.

METHODS

Rodent – macroparasite data

Yellow-necked mice were collected from mixed broadleaf woodland of the Italian Alps (Magla Campo, Trentino) at 6 sites separated by at least 500m. Trapping was carried out for three consecutive nights at each site, during July 2002. White-footed mice were collected from open-forested habitat in central Pennsylvania, USA across 11 sampling sites: sampled from 2005 - 2007. All sites were separated by at least 1km and sampled once. All parasites were identified and counted by dissection of the host intestinal tract. All host collections were carried out under the approval of the appropriate animal care and use oversight (Pennsylvania State University Institutional Animal Care and Use Committee approval numbers 16061 and 23268).

Additional reports of mean and variance were assembled from reports of dissections from the literature for *Apodemus sylvaticus* (the wood-mouse) – *H. polygyrus* and *A. sylvaticus* – *Syphacia stroma* (Behnke *et al*. 1999; Muller-Graf *et* al. 1999; Abu Madi *et al*. 2000; Fuentes, 2004), as well as for *P leucopus – S peromysci* (Grundman *et al.* 1976, Vandegrift and Hudson, 2009).

Analysis of parasite intensity and variance

We calculated mean intensity per host, variance around the mean intensity, and prevalence at each trapping site for *S. frederici* and *H. polygyrus* in *A. flavicollis* from the Trentino sites and for *S. peromysci* in *P. leucopus* from the Pennsylvania sites. We fitted the number of parasites per host to the negative binomial and Poisson distribution using direct maximum likelihood estimation and evaluated the goodness-of-fit using the deviance of the maximum likelihood estimate according to the methods of Shaw *et al.* (1998). We then tested for differences in mean parasite abundance (negative-binomial mean, μ) and aggregation (negative-binomial, k) as described in the analysis of dispersion test in Shaw *et al.* (1998) between host sex in *A. flavicollis – S. frederici*, *A. flavicollis – H. polygyrus*, and *P. leucopus – S.*

peromysci, as well as between *H. polygyrus – S. frederici* in *A. flavicollis* and between *S. frederici –S. peromysci* in their respective hosts. We tested for differences in prevalence among each host-parasite relationship using odds ratios and 95% confidence intervals around the odds ratios between each pair of host and parasite and considered prevalence different if the odds ratio CI did not overlap one.

We evaluated the relationship between mean intensity per host and variance around that mean for the parasite samples along with our additional literature values from other host species according to Taylor's power law (Taylor, 1961):

$$s^2 = e^a \mu^b,$$

where, μ, is the mean abundance of parasites per individual, s^2 is the variance, and ***a*** and ***b*** are parameters that describe the power relationship. We used each spatially independent sample of *A. flavicollis – S. frederici*, *A. flavicollis – H. polygyrus*, and *P. leucopus – S. peromysci* as separate data points and included each additional host-parasite report from the literature as a single data point. We fit linear regressions to the natural *log*-transformed variance as the dependent variable and the natural *log*-transformed mean of each population sample as the independent variable. We compared 95% confidence intervals around the slope parameter, ***b,*** between the regression lines fitted with pinworm parasites and non-pinworm parasites, as well as to the previously reported ***b*** for host-macroparasite interactions, 1.55 +/- 0.037 SD (Shaw and Dobson, 1995). We considered the slopes significantly different if the confidence intervals around (*log* ***b***) did not overlap.

Simulation model

To explore the population processes that generate aggregation, we created a stochastic model to simulate the number of parasites per host based on infection (immigration), death, and

birth (host-self-infection) processes. Our aim was to simulate the processes that may lead to aggregated patterns of macroparasite intensity in a population of hosts as a result of a conventional transmission mode of macroparasites and contrast these aggregation patterns with a transmission model where:

- A birth process is present (i.e. direct reproduction within a host, host-self-infection)
- Predisposition to infection is increased (i.e. no migration through host tissue)
- Parasite death is decreased and intensity-dependent death rate is weakened (i.e. no consumption of tissue or other intimate host interactions that invoke strong immune responses)

These three aspects of the transmission process correspond to the aspects of pinworm life history that differ from most other families of vertebrate parasitic nematodes. Our approach was to contrast general tradeoffs in population processes that may generate high levels of aggregation without invoking any mechanisms specific to nematode life-history (e.g. nothing specific about the genus *Heligmosomoides* was reflected in our baseline models), except for host-self-infection.

The model for the conventional nematode infection process described the number of parasites, P, in a host, i, based on stochastic force of infection, ρ, the probability that a host is exposed to a parasite infective stage and that infective stage establishes (predisposition to infection), φ, and parasite death rate d (equation 1a). A parasite-intensity dependence, ψ, was included as a modifier to the death rate ($d_{max}\psi$, equation 1a) based on the current intensity of parasites and a carrying capacity, k; such that death rate approached zero when there was very low intensity of infection ($P \rightarrow 0$, $\psi \rightarrow 0$), and increased linearly to the maximum death rate, d_{max}, as the intensity of infection approached k ($P \rightarrow k$, $\psi \rightarrow 1$). The models for simulated pinworm transmission processes included infection-death processes without intensity-dependent

death (equation 1b) and infection-death processes with host-self–infection, with (equation 2a) and without intensity-dependent death rates (equation 2b). Host-self-infection was parameterized by the proportion of total fecundity a female deposits for host-self-infection, τ, the sex ratio of parasites, π, and the current number of parasites per host P (equation 2a,b).

$$P_{(i,t+1)} = \rho\ \varphi - d_{\max}\ \psi_{k,\,P\,(i,t)}\, P_{(i,t)} \quad (1a)$$

$$P_{(i,t+1)} = \rho\ \varphi - dP_{(i,t)} \quad (1b)$$

$$P_{(i,t+1)}) = \rho\ \varphi - d_{\max}\psi_{k,\,P(i,t)}\, P_{(i,t)} + \tau\ \pi P_{(i,t)} \quad (2a)$$

$$P_{(i,t+1)}) = \rho\ \varphi - dP_{(i,t)} + \tau\ \pi P_{(i,t)} \quad (2b)$$

Force of infection, ρ, was modeled as a stochastic process with the number of potential infective stages that a host could be exposed to drawn from a Poisson distribution with mean = ρ, representing an environmental pool of infection. We assumed that mean force of infection was an endemic number of infective stages for each model realization and ran series of simulations at ρ = 3, 5, 10, 20, 100. The predisposition to infection, φ, was modeled as a host characteristic: the probability that an infective stage is encountered and subsequently established in a host, such that most hosts had a low predisposition to infection and few hosts had high predisposition to infection. This predisposition distribution was chosen to represent heterogeneities in exposure and susceptibility that have been proposed to generate aggregated distributions of parasites in host populations, but are not explicitly modeled because they are beyond the scope of this simulation (Anderson and Gordon, 1982; Shaw *et al*. 1998; Wilson *et al*. 2002). This distribution was a represented by a gamma distribution (shape = 3, rate =1) adjusted to range from 0 to 1, and fixed for each host at the beginning of a simulation. Based on initial simulation results, we assumed that, in the absence of intensity-dependent death, the parasite death rate was fixed at d = 0.2 and in the presence of intensity-dependent death, the maximum death rate was,

$d_{max} = 0.5$. The strength of the intensity dependence (the intensity where $d\psi = d_{max}$) on the death rate was determined by varying the carrying capacity, k, relative to the maximum mean exposure, ρ_{max}. The strongest intensity-dependence was set at $k = \rho = 100$ and weaker intensity-dependence was explored over several orders of magnitude ($k = 2\rho_{max}$, $5\rho_{max}$, $10\rho_{max}$, $50\rho_{max}$, $100\rho_{max}$, $500\rho_{max}$, $1000\rho_{max}$).

For each simulation, we simulated 50 hosts with birth rate equal to death rate. We assumed that host death is independent of parasite intensity and hosts that die are replaced by an uninfected host at a fixed rate of 0.02. At the beginning of each simulation, all hosts were uninfected and at each time step they were subject to a force of infection, drawn from a Poisson distribution with mean, ρ. We assumed that there was no time delay for parasite maturation and any parasite that established became an adult at the next time step. Parasite sex ratio was, $\pi = 0.5$. We measured our model output using the degree of aggregation parameter from Taylor's power law, $\boldsymbol{b}$, based on a set of simulations run with mean force of infection, $\rho = 3, 5, 10, 20, 100$. We report the middle 95% of $\boldsymbol{b}$ values from 10000 realizations at the 5 mean force of infection values.

The effect of intensity dependent death on parasite aggregation was examined by contrasting model realizations based on equation (1a) and equation (1b) with the strength of parasite intensity-dependent death rates varying with $k = 2\rho_{max}$, $5\rho_{max}$, $10\rho_{max}$, $50\rho_{max}$, $100\rho_{max}$, $500\rho_{max}$, $1000\rho_{max}$. We also explored the effect of the maximum death rate in model realizations of equation (1a, $k = 50\rho_{max}$) with $d_{max} = 0.1, 0.2, 0.3, 0.4\ 0.5, 0.6, 0.7, 0.8, 0.9$. We further explored the effect of decreased parasite death rate on aggregation by contrasting 2 sets of realizations, one based on equation (1a) with parasite death rate decreasing proportionally at 0.9 – 0.1 of $d_{max} = 0.5$, at 0.1 intervals and the strength of intensity dependence set with $k = 50\rho_{max}$.

The second set contrasted model realizations based on equation (1b, no intensity-dependent death rate) as described above from a reference death rate, $d = 0.2$.

The effect of increased predisposition to infection was examined by contrasting 2 sets of realizations, one based on equation (1a) with predisposition values proportionally increased 1.1 – 3 times at 0.1 intervals from a reference φ distribution, as described above, and the strength of intensity dependence set with $k = 50\rho_{max}$. The second set contrasted model realizations based on equation (1b, no intensity-dependent death rate, $d = 0.2$) as described above.

The effect of direct reproduction via host-self-infection was examined by contrasting two sets of model realizations based on equation (2a, $d_{max} = 0.5$, $k = 500\rho_{max}$) and equation (2b, $d = 0.2$) with host-self-infection rate, $\tau = 0.001, 0.002, 0.003, 0.003, 0.004, 0.005, 0.006, 0.007, 0.008, 0.009, 0.010, 0.011, 0.0125, 0.015, 0.0175, 0.02, 0.03, 0.04, 0.05$. We further explored a potential trade-off between host-self-infection and the force of horizontal transmission by comparing model realizations based on equation (2a, $d_{max} = 0.5$, $k = 50\rho_{max}$) and equation (2b, $d = 0.2$) across the range of host-self-infection rates described above, with mean force-of-infection, ρ, decreasing proportionally from 0.9 - 0.1 of ρ, at 0.2 intervals.

RESULTS

Empirical parasite abundance and variance

The abundance distribution of each host-parasite combination was an adequate fit to a negative binomial distribution and was not described well by a Poisson distribution (Table 1). We tested for differences in mean, μ, and aggregation, k, between host sexes within each parasite-host relationships and found no evidence for differential mean parasite burden between males and females in any host-parasite relationship (Table 2). There was a trend for parasites to be more aggregated in female hosts in the *A. flavicollis – H. polygyrus* relationship (analysis of

dispersion $P = 0.02$) and a weak trend for parasites to be more aggregated in female hosts in the *P. leucopus – S. peromysci* relationship (analysis of dispersion $P = 0.06$). There was no evidence for a difference in aggregation of *S. frederici* in male and female *A. flavicollis* hosts (analysis of dispersion $P > 0.1$). Prevalence was not different between the pinworm infections in *P. leucopus* and *A. flavicollis* (odds ratio = 1.32, 95% CI [0.74 , 2.36]). Prevalence was greater in *H. polygyrus* infection of *A. flavicollis* compared to *S. frederici – A. flavicollis* (odds ratio = 2.72, 95% CI [1.89 , 3.91]) and compared to *S. peromysci – P. leucopus* (odds ratio = 3.59, 95% CI [2.23 , 5.78]).

Analysis of the mean and variance of intensity using Taylor's power law revealed that the *log* variance *vs log* mean slope was significantly greater for host-pinworm relationships (***b*** = 2.82, 95% CI [2.4 4, 3.22]) compared to host-*H. polygyrus* relationships (***b*** = 1.82, 95% CI[1.41 , 2.23]) and the previously reported ***b*** for c. 260 host-macroparasite relationships (***b*** = 1.55 +/- 0.037 SD; Shaw and Dobson, 1995) (Fig. 1).

Simulation model

Simulation output of the *log* variance *vs log* mean slope, ***b***, demonstrated that host-self-infection is necessary to increase the degree of aggregation, ***b***, to levels that exceed the range reported for macroparasites (Shaw and Dobson, 1995), as well as many other taxa (Taylor 1961). Simulations that included a parasite intensity-dependent death rate showed that aggregation is constrained to levels that reflect a random distribution (***b*** = 1) of parasites per host when the intensity-dependence was very strong (Fig. 2A). The values of ***b*** rose significantly when the strength of intensity-independence were weakened within an order of magnitude and reached ***b*** values matching the empirically range (***b*** = 1.5-1.6) when the intensity dependence was weakened by 1 or more orders of magnitude. Adjusting the maximum parasite death rate did not

influence ***b*** (Fig. 2B) and all subsequent simulations including intensity-dependent death rates were run with the strength of intensity-dependence fixed at $k = 50\rho_{max}$ and $d_{max} = 0.5$. Decreased parasite death rate (Fig. 2C, D) did not cause a significant change in ***b***. Increasing predisposition to infection without intensity-dependent parasite death (Fig. 2E) resulted in a relatively slow increase in aggregation, with ***b*** becoming significantly increased over the reference simulations when predisposition to infection was 2 times greater. The increase in aggregation reached an asymptote as the relative predisposition to infection approached a 3-fold increase from the reference simulations, but at a lower value of ***b*** than simulations including host-self-infection. Increasing predisposition to infection with intensity-dependent parasite death did not significantly increase ***b*** (Fig. 2F).

Simulations that included host-self-infection yielded the highest aggregation levels; provided host-self-infection rate exceeded 0.01 (Fig. 3A) or 0.006 in the absence of intensity-dependent death (Fig. 3B). In simulations with intensity-dependent death rates, low-levels of self-infection decreased aggregation slightly, but as host-self-infection increased, ***b*** increased and significantly exceeded the level of aggregation of simulations without host-self-infection (Fig. 3A). As relative force-of-infection decreased, the initial decrease in ***b*** with self-infection was dampened and, at the lowest relative force-of-infection, ***b*** increased significantly over reference simulations at host-self-infection rates approximately one order of magnitude lower compared to simulations with equal force-of-infection (Fig 3A). Without intensity-dependent death rates, host-self-infection generated high levels of aggregation at host-self-infection levels that were an order of magnitude weaker than simulations with intensity-dependent death (Fig. 3B). The rate of host-self-infection necessary to increase ***b*** over reference simulations without intensity-

dependent death increased as relative force-of-infection decreased, but did not change the shape of host-self-infection *vs* ***b*** relationship (Fig. 3B).

DISCUSSION

The empirical data for mouse gastrointenstinal parasites falls within the degree of aggregation reported by Shaw and Dobson (1995), although the degree of aggregation was consistently greater in the pinworm-rodent relationships; both within host samples and between two ecologically similar rodent hosts (Fig. 1). The consistently greater aggregation in pinworm species is an exception to the general constraints on aggregation in parasites and provides an opportunity to explore the mechanisms that drive aggregation patterns through examination of life history characteristics. We proposed three possible mechanisms related to pinworm life-history that could drive larger increases in variance with increasing mean (Taylor's ***b***) : 1. Host self-infection, 2. increased host predisposition to infection, and 3. decreased strength of parasite intensity dependence and decreased absolute parasite death rate. We used stochastic simulations to evaluate what life-history characteristic or combination of characteristics could lead pinworm aggregation to be an exception to Taylor's power law and also mimic empirical pinworm aggregation. In line with our prediction, host self-infection had the strongest effect on generating high aggregation and was the only characteristic that resulted in the slope of the *log*-variance *vs* *log*-mean, ***b***, in our simulations increasing at a magnitude that was similar to the relative empirical difference between aggregation of pinworm and non-pinworm nematodes (Fig. 3). Strong intensity-dependence parasite death decreased aggregation and decreased parasite death rate had no significant effect**.** Increasing predisposition to infection had a small effect at the greatest relative predisposition levels we simulated, but that ***b*** value still did not approach the levels of aggregation empirically measured from pinworms. Host-self-infection is dependent on

the current parasite population size and, thus, creates heterogeneous rates of within-host population growth over a collection of hosts. The other mechanisms that we tested, decreased death rate and increased predisposition to infection, did not increase the variance to mean slope, even when combined with intensity-dependent parasite death rates; which creates heterogeneous rates of population decline. In using pinworm life-history as a template for our simulation, we believe that our assumption is reasonable given that pinworm parasites do not have the type of interaction with host-tissues that evoke strong immune responses (Parker *et al*. 2009).

We were also able to obtain insights into the degree of host-self-infection that is needed to increase aggregation to the high levels we observed. Shortly after Talyor *et al*. (1978) established the constancy of the variance to mean relationship, others determined that stochasticity in population vital rates could lead to the observed aggregation patterns, with the exact level of aggregation being a balance between the variability in these population processes (Anderson *et al.* 1982). Our simulation revealed that only a small proportion (1-2%) of fecundity devoted towards host self-infection is required to increase ***b*** above the expected aggregation levels and then aggregation quickly asymptotes with additional host self-infection rates (Fig 2B). When the relative force of infection (immigration) of self-infecting parasites was reduced (Fig 3A), ***b*** decreased at the smallest values of host-self-infection rates, but the degree of aggregation still increased over the expectations at higher rates of host-self-infection. Regardless of the relative decrease in force of infection, the level of host-self-infection required to increase ***b***, was small, indicating that even a small additional source of within-host population growth can increase aggregation of individuals.

The characterization of an aggregated macroparasite distribution is no surprise given the vast number of epidemiological reports indicating such similar degree of aggregation using

statistical distributions and Taylor's power law (Shaw and Dobson, 1995; Shaw *et al*. 1998). Indeed, the expectation that any distribution of organisms is aggregated, within a relatively narrow degree, is hardly surprising according to Taylor's power law (Taylor, 1961). The aggregated distribution of individuals within populations is usually attributed to a balance of population processes that have evolved within a species. The generally narrow range of aggregation among macroparasites, suggests that the underlying evolutionary processes that constrain the degree of aggregation are similar across many different host-parasite relationships (Shaw and Dobson, 1995). Generally, the constraint on the upper limit of aggregation is attributed to parasite virulence or host response to parasites, where the tail of the aggregated distribution is removed when parasites at high intensities kill their host or evoke an immune response that increases parasite mortality. Indeed, our simulations revealed that aggregation can be greatly reduced with strong intensity-dependent parasite death rate. One of the presumed benefits of virulence is increased body size and fecundity, primarily attributed to tissue migration in macroparasites (Read and Skorping, 1995). Pinworms do not require migration through host tissues and are relatively small-bodied compared to other parasitic nematodes infecting the same host, and hence less fecund. Reports of female pinworm parasites (genus, *Syphacia*) of other mouse species are c. 2-4 mm (Chan, 1952, Hussey, 1957), while *H. polygyrus* females are c. 20-30 mm. in similar sized hosts (Luong *et al*. 2010). Pinworm parasites appear to have been able to escape the cost of high aggregation via life-history traits that reduce their impact on hosts and their evolutionary independence from other parasitic nematodes suggests that pinworms' life-history and resulting aggregation is indeed an exception among macroparasites (Adamson, 1994).

In examining the patterns and processes of pinworm aggregation, we present one mechanism that can generate higher than expected aggregation. The addition of a within-host birth process, even at a small magnitude, to a conventionally immigration-death population dynamic appears sufficient to increase aggregation. Previous attempts to elucidate a general mechanism that generates the narrow range of aggregation in animal populations have evoked complex explanations such as spacing behavior (Taylor and Taylor, 1977), dispersal (Hanski, 1980), and inter-specific community interactions (Kilpatrick and Ives, 2003). While such complex explanations are able to characterize the general constancy of empirical variance-mean relationships, we offer an explanation based on basic life-history characteristics that can explain the general observed aggregation patterns, as well as an exception to the rule.

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REFERENCES

Abu-Madi, M. A., Behnke, J. M., Lewis, J. W., and Gilbert, F. S. (2000). Seasonal and site specific variation in the component community structure of intestinal helminthes in *Apodemus sylvaticus* from three contrasting habitats in south-east England. *Jounral of Helminthology*, 74, 7-15.

Adamson, M. (1989), Evolutionary biology of the Oxyurida (Nematoda): biofacies of a haplodiploid taxon. *Advances in Parasitology*, 28, 175-228.

Adamson, M. (1994). Evolutionary patterns in the life histories of Oxyruida. *International Journal for Parasitology*, 24, 1167-1177.

Anderson R. C. (2000). *Nematode parasites of vertebrates: their development and transmission*. 2nd edition. CABI Publishing, New York.

Anderson, R. M., and Gordon, D. M. (1982). Processes influencing the distribution of parasite numbers within host populations with special emphasis on parasite-induced host mortalities. *Parasitology*, 85, 373-398.

Anderson, R. M., Gordon, D. M., Crawley, M. J., and Hassell, M. P. (1982).Variability in the abundance of animal and plant species. *Nature*, 296, 245-248.

Behnke, J. M., Lewis, J. W., Mohd Zain, S. N., and Gilbert, F. S. (1999). Helminth infections in Apodemus sylvaticus in southern England: interactive effects of host age, sex and year on the prevalence and abundance of infections. *Jounral of Helminthology*, 73, 31-44.

Chan, K. F. (1952). Life-cycle studies on the nematode *Syphacia obveleta*. *American Journal of Hygiene*, 56, 14-21.

Fuentes, M. V., Saez, A., Trelis, M., Galan-Puchades, M.T., and Esteban, J. G. (2004). The helminth community if the wood mouse, *Apodemus sylvaticus*, in the Sierra Espuna, Murcia, Spain. *Journal of Helminthology*, 78, 219-223.

Gandon, S, van Baalen, M., and Jansen, V. A. A. (2002). The evolution of parasite virulence, superinfection, and host resistence. *American Naturalist*, 159, 658-669.

Grundman, A. W., Warnock, R. G., and Wasson, D. L. (1976). Some Mechanisms of natural regulation of parasitic helminth populations. *American Midland Naturalist*, 95, 347-360.

Hanski, I. (1980). Spatial patterns and movements in coprophagous beetles. *Oikos*, 34, 293-310.

Hussey, K. L. (1957). *Syphacia muris* vs. *S. obvelata* in laboratory rats and mice. *The Journal of Parasitology*, 43, 555-559.

Kilpatrick, A. M. and Ives, A .R. (2003). Species interactions can explain Taylor's power law for ecological time series. *Nature,* 422,65-68.

Luong, L. T., Perkins, S. E., Grear, D. A., Rizzoli, A., and Hudson, P. J. (2010). The relative importance of host characteristics and co-infection in generatin varitation in *Heligmosomoides polygyrus* fecundity. *Parasitology*, 137, 1003-1012. doi:10.1017/S0031182009991892.

Morand, S., and Guegan, J. F. (2000). Distribution and abundance of parasitic nematodes: ecological specialization, phylogenetic constraint or simply epidemiology? *Oikos*, 88, 563-573.

Muller-Graf, C. D. M., Durand, P., Feliu, C., Hugot, J. P., O'Callaghan, C. J. O., Renaud, F., Santalla, F., and Morand, S. (1999) Epidemiology and genetic variability of two species of nematodes (*Heligmosomoides polygyrus* and *Syphacia stroma*) of *Apodemus* spp. *Parasitology*, 118, 425-432.

Parker, G. A.., Ball, M. A., and Chubb, J. C. (2009). Why do larval helminthes avoid the gut of intermediate hosts? *Journal of Theoretical Biology*, 260, 460-473.

Prince, M. J. (1950). Studies on the life cycle of *Syphacia obveleta*, a common nematode parasite of rats. *Science*, 111, 66-67.

Read, A. F. (1994). The evolution of virulence. *Trends in Microbiology*, 2, 73-76.

Read, A. F., and Skorping, A. (1995). The evolution of tissue migration by parasitic nematode larvae. *Parasitology*, 111, 359-371.

Schad, G. A. (1957). Preliminary observations of the life history of the sheep pinworm, *Skrjabinema ovis*. *Journal of Parasitology*, 43, 13.

Shaw, D. J. and Dobson, A. P. (1995). Patterns of macroparasite abundance and aggregation in wildlife populations: a quantitative review. *Parasitology*, 111 (Suppl.) S111-S133.

Shaw, D. J., Grenfell, B. J., and Dobson, A. P. (1998). Patterns of macroparasite aggregation in wildlife host populations. *Parasitology*, 117, 597-610.

Taylor, L.R. (1961). Aggregation, variance and the mean. *Nature*, 189, 732-735.

Taylor, L .R., and Taylor, R. A. J. (1977). Aggregation, migration, and population mechanics. *Nature*, 265, 415-420.

Taylor, L. R., Woiwod, I. P., and Perry, J. N. (1978). The density-dependence of spatial behaviour and the rarity of randomness. *Journal of Animal Ecology*, 47, 383-406.

Taylor, L. R., and Woiwod, I. P. (1980). Temporal stability as a density-dependent species characteristic. *Journal of Animal Ecology*, 49, 209-224.

Vandegrift, K. J., and Hudson, P. J. (2009). Could parasites destabilize mouse populations? The potential role of *Pterygodermitites peromysci* in the population dynamics of free-living mice, *Peromyscus leucopus*. *International Journal for Parasitology*, 39, 1253-1262.

Wilson, K., Bjornstad, O. N., Dobson, A. P., Merler, S., Poglayen, G., Randolph, S. E., Read, A. F., and Skorping, A. (2002). Heterogeneities in macroparasite infections: patterns and processes. In *The Ecology of Wildlife Diseases*. (ed. Hudson, P. J., Rizzoli, A., Grenfell, B. T., Heesterbeek, H., and Dobson, A. P.). Oxford University Press, Oxford.

TABLE 5.1. Parameter estimates and goodness of fit (GOF) statistics for Poisson and negative binomial (NB) distributions fitted to parasite abundance distribution of *Syphacia-Apodemus* and *Heligmosomoides-Apodemus* from northen Italy and *Syphacia-Peromyscus* from eastern United States. N is the number of host individuals, $\hat{k}$ and $\hat{u}$ are fitted parameters of the NB distribution, $\bar{u}$ is the observed mean parasite abundance, and GOF p-value refers to the significance of the deviance goodness-of-fit test.

Host Species	Parasite Species	n	Prevalence	Poisson GOF p-value	NB GOF p-value	$\hat{k}$	$\hat{u}$	$\bar{u}$
Apodemus flavicollis	*Syphacia frederici*	232	0.12[a]	<0.001	0.079	0.091	2.297	2.297
Apodemus flavicollis	*Heligmosomoides polygyrus*	232	0.58[b]	<0.001	0.989	0.273	5.547	5.547
Peromycus leucopus	*Syphacia peromysci*	249	0.07[a]	<0.001	>0.999	0.013	3.360	3.360

[a] Prevalence of pinworms did not differ between hosts
[b] Prevalence of *H. polygyrus* was greater than prevalence of either pinworm

TABLE 5.2. Summary comparing mean, $\hat{u}$, and aggregation, $\hat{k}$, parameters from the negative binomial distribution for various host subsets fit to parasite abundance data from *Syphacia-Apodemus* and *Heligmosomoides-Apodemus* from northen Italy and *Syphacia-Peromyscus* from eastern United States. $\hat{u}_c$ *p*-value is the significance of the analysis of dispersion test for a common mean assuming a common k, $\hat{k}_c$ -value is the significance of the analysis of dispersion test for a common *k* and is only performed if there is no evidence that *u* is different for the subsets.

Host species	Parasite species	Subset	$\hat{u}$	$\hat{u}_c$ *p*-value	$\hat{k}$	$\hat{k}_c$ *p*-value
Apodemus	*Syphacia*	host-male	2.487	0.702	0.110	0.193
		host-female	2.097		0.072	
Apodemus	*Heligmosomoides*	host-male	5.555	0.992	0.350	0.018
		host-female	5.540		0.206	
Peromyscus	*Syphacia*	host-male	2.468	0.316	0.019	0.065
		host-female	5.000		0.007	
Apodemus	*Syphacia - Heligmosomoides*	*Syphacia*	2.297	<0.001	0.091	NA
		Heligmosomoides	5.547		0.273	
Peromyscus - Apodemus	*Syphacia*	*Peromyscus*	2.297	0.409	0.013	<0.001
		Apodemus	3.360		0.091	

FIGURE 5.1. Taylor's power law relationship indicated that the slope, ***b*** [95% CI], of the *log* variance to *log* mean abundance of parasites per host was consistently greater for pinworm parasites (open points, dashed line) compared to Heligmosomoid parasites (filled points, solid line). The dotted line indicates the *log* variance *vs log* mean slope of a wide range of host-macroparasite distributions, ***b*** = 1.55 (from Shaw and Dobson 1995).

Figure 5.1

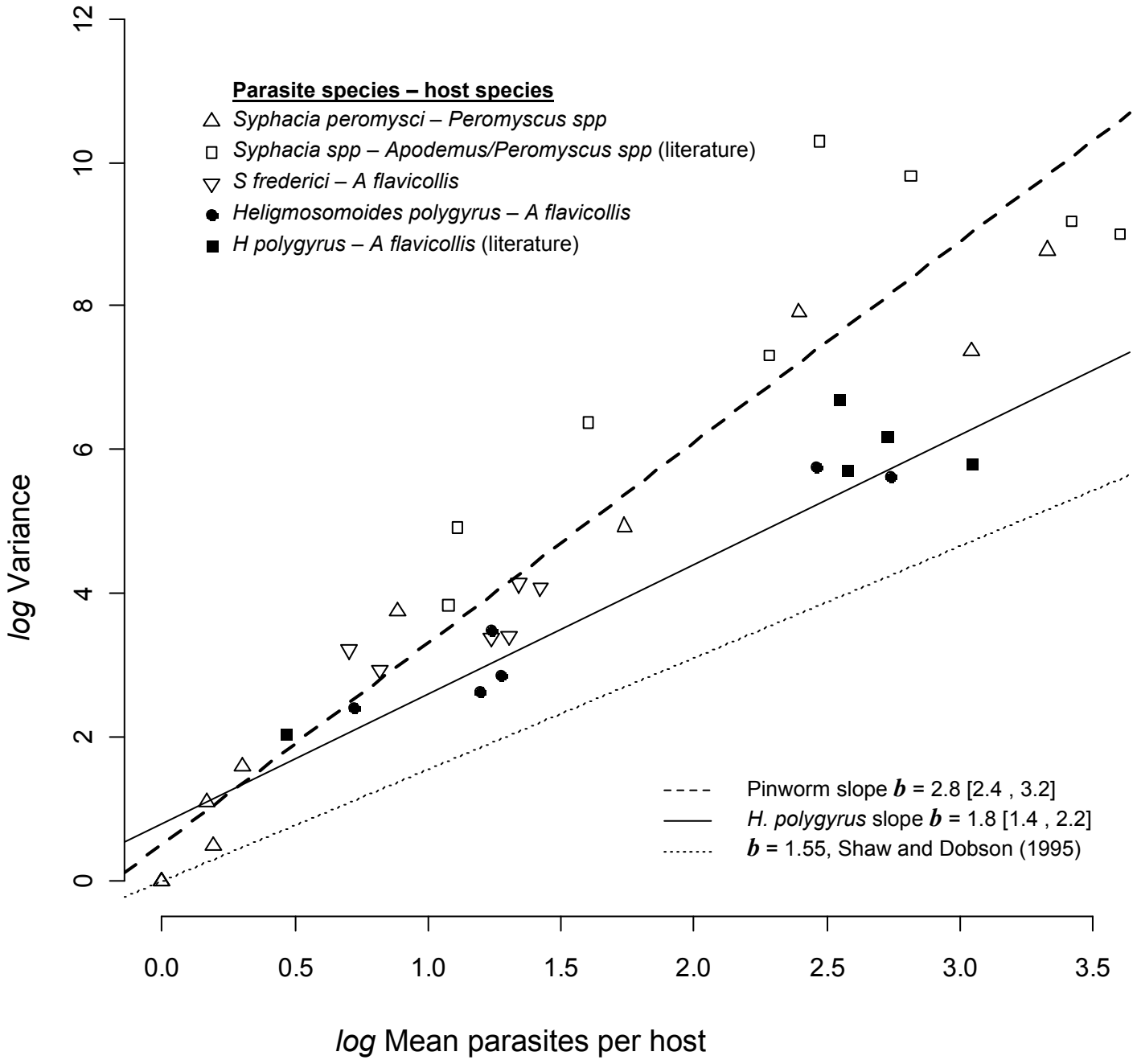

Parasite species – host species
Syphacia peromysci – Peromyscus spp
Syphacia spp – Apodemus/Peromyscus spp (literature)
S frederici – A flavicollis
Heligmosomoides polygyrus – A flavicollis
H polygyrus – A flavicollis (literature)
Pinworm slope b = 2.8 [2.4 , 3.2]
H. polygyrus slope b = 1.8 [1.4 , 2.2]
b = 1.55, Shaw and Dobson (1995)
log Variance
log Mean parasites per host
0
2
4
6
8
10
12
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5

FIGURE 5.2. Simulation realizations of the *log* variance vs *log* mean slope (***b***, y axis) across a range of intensity-dependence strength, $\psi_{k,P}$, parasite death rate, d, and predisposition to infection, φ, for a simulated infection (immigration) – death model of macroparasite intensity per host. The model outputs are median (lines) and 95% confidence values (shaded regions) for of the aggregation parameter, Taylor's power law ***b*,** from simulations where the parameter of interest (dark shading, dashed lines) is adjusted in relation to a fixed value (light shading, dotted lines). (A) Strong intensity-dependent parasite death rates reduce aggregation and decreasing strength of intensity dependence (increasing carrying capacity, k) increases aggregation. The strength of intensity dependence (x-axis) is represented by the carrying capacity of worms per host relative to the maximum simulated force of infection, ρ_{max}; with the strongest intensity dependence at $k = 1 = \rho_{max}$. (B) The maximum death rate in simulations with intensity-dependent death has no effect on aggregation ($\psi_{k = 50\rho max}$). (C) Decreasing the relative death rate has no effect on aggregation without intensity dependent dependence (dashed line relative to d = 0.2). (D) Decreasing the relative death rate has no effect on aggregation with intensity dependent death (dashed line relative to $d_{max} = 0.5$, $\psi_{k = 50\rho max}$). (E)-(F) Increasing predisposition to infection, φ, increased aggregation, ***b***, in simulations without intensity-dependent parasite death (E, $d = 0.2$), but not in simulations with intensity-dependent death rates (F, $d_{max} = 0.5$, $\psi_{k = 50\rho max}$).

Figure 5.2

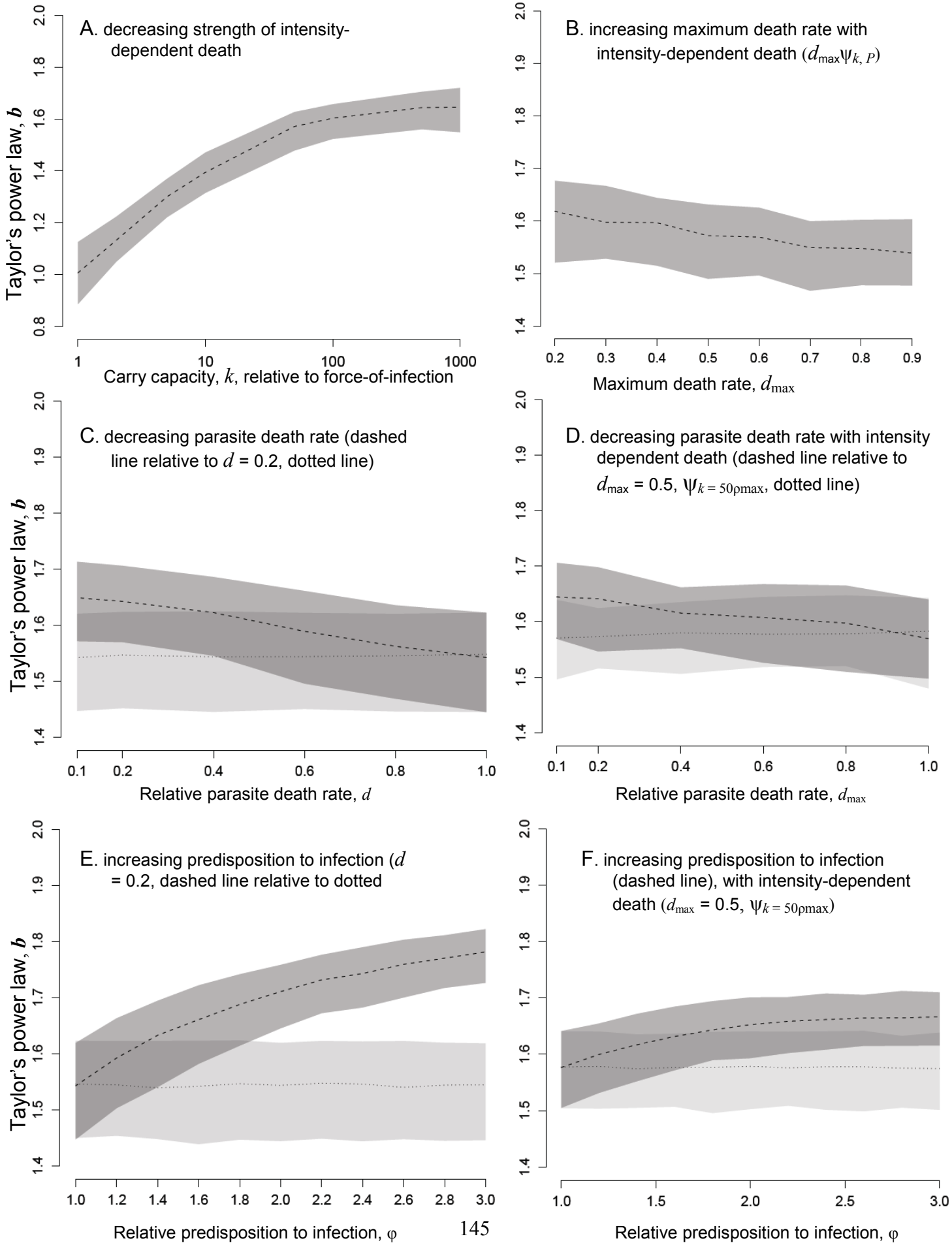
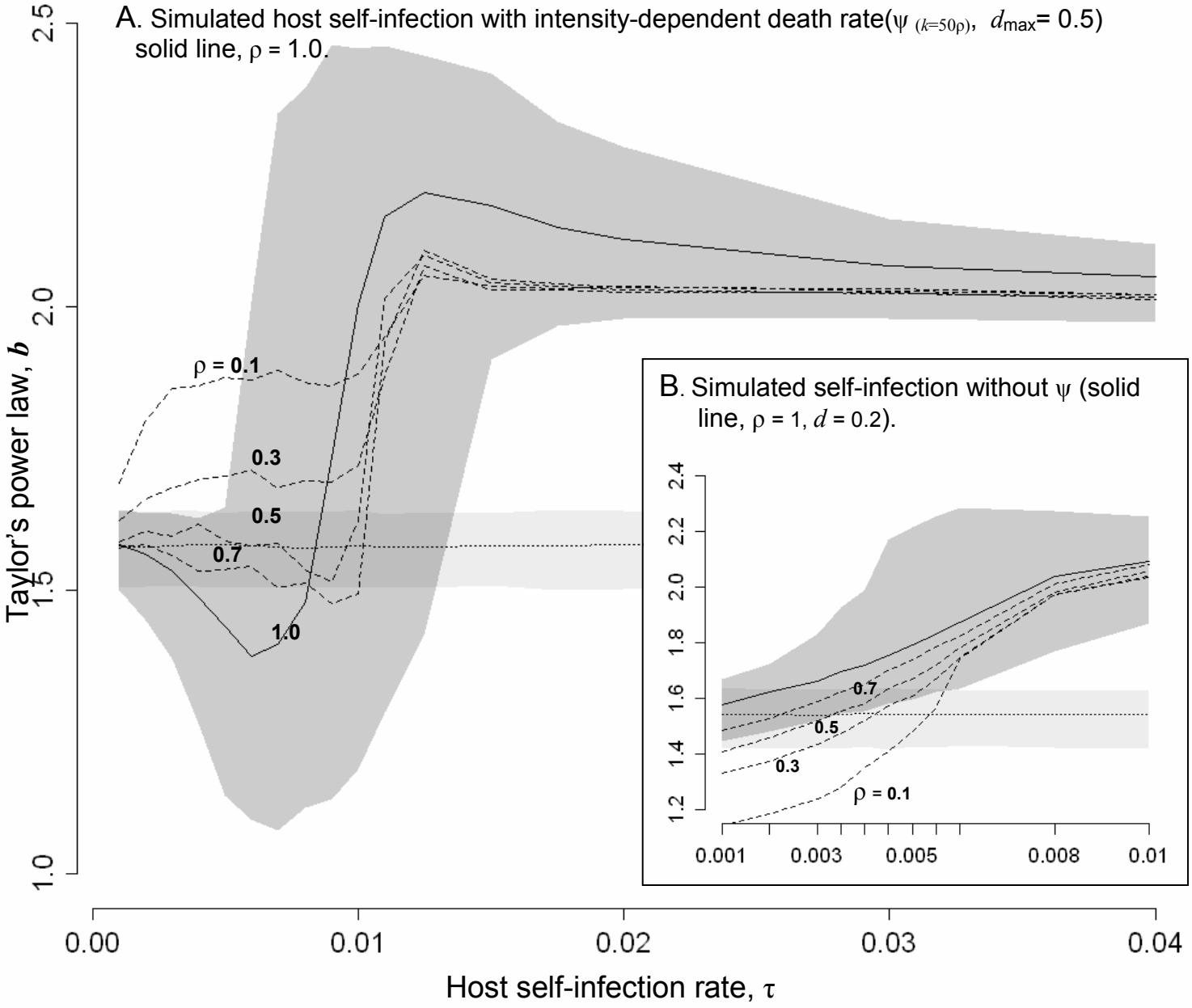

A. decreasing strength of intensity-dependent death
Taylor's power law, b
Carry capacity, k, relative to force-of-infection
B. increasing maximum death rate with intensity-dependent death ($d_{max}\psi_{k,P}$)
Maximum death rate, d_{max}
C. decreasing parasite death rate (dashed line relative to d = 0.2, dotted line)
Relative parasite death rate, d
D. decreasing parasite death rate with intensity dependent death (dashed line relative to d_{max} = 0.5, $\psi_{k=50\rho max}$, dotted line)
Relative parasite death rate, d_{max}
E. increasing predisposition to infection (d = 0.2, dashed line relative to dotted
Relative predisposition to infection, φ
F. increasing predisposition to infection (dashed line), with intensity-dependent death (d_{max} = 0.5, $\psi_{k=50\rho max}$)
Relative predisposition to infection, φ

FIGURE 5.3. Simulation realizations of the *log* variance *vs log* mean slope (***b***, y axis) across a range of host-self-infection rates, τ. The model outputs are median (lines) and 95% confidence values (shaded regions) for of the aggregation parameter, ***b*,** from simulations that include a host-self-infection process (dark shading, solid lines) compared to the 95% confidence values around ***b*** for a reference simulation with no host-self-infection (dotted line, light shading). (A) Model simulations that included parasite-intensity-dependent death ($k = 50\rho_{max}$, $d_{max} = 0.5$) with median ***b*** outputs from simulations with decreasing force of infection (dashed lines, $\rho = 0.1, 0.3, 0.5, 0.7$) and 95% confidence values shaded for $\rho = 1$. (B) Model simulations without parasite-intensity-dependent death ($d = 0.2$) with median ***b*** outputs from simulations with decreasing force of infection (dashed lines, $\rho = 0.1, 0.3, 0.5, 0.7$).

Figure 5.3


A. Simulated host self-infection with intensity-dependent death rate(ψ (k=50ρ), dmax= 0.5) solid line, ρ = 1.0.
ρ = 0.1
0.3
0.5
0.7
1.0
B. Simulated self-infection without ψ (solid line, ρ = 1, d = 0.2).
0.7
0.5
0.3
ρ = 0.1
Taylor's power law, b
Host self-infection rate, τ

Chapter 6. Summary and conclusions: Patterns and process of parasite infection and transmission

Summary

The emergent dynamics in any host-parasite system are the result of between-host and within-host processes that influence the transmission process. The purpose of the research in this dissertation was to explicitly test hypotheses about the transmission process and the mechanisms that drive transmission dynamics. In the framework of the hypotheses I set out in the introduction, the major findings of my dissertation research follow.

4. **Male hosts have higher transmission rates because they have a greater infection rate and produce infectious stages in proportion to their parasite intensity** (chapters 2, 3, 5).

Surprisingly, there was no male-bias in infection among the rodent-macroparasite systems I examined in central Pennsylvania. Therefore, I could not properly evaluate this hypothesis. Even the testosterone experiment, which explicitly tested a mechanism that predicted an increase in male infection rate, was not successful because of the peculiarities of white-footed mouse parasite community. More generally I showed in chapter 5 that, not only do host characteristics influence heterogeneous infection patterns, parasite life-history can have profound impacts on aggregated infection patterns. The unique life-history character of host-self-reinfection in pinworms generates an extra layer of transmission heterogeneity in addition to the between-host processes that were central to my main hypotheses.

5. **Male hosts have higher transmission rates because the parasites they harbor are more fecund, thus producing more infectious stages per parasite** (chapters 2, 3)

An advantage of the lack of male-bias in white-footed mouse infection rates in my experiments was that between host infection levels were already controlled for in a natural system. However, I found no evidence that parasites were bigger, more fecund, or generated greater fecal-egg-

shedding in male mice (naturally or in response to testosterone treatment), even though I was able to document the potential for greater male transmission due to testosterone and that males actually drove transmission of the intestinal parasite *P. peromysci*.

6. **Male hosts have higher transmission rates because their behaviors cause the infectious stages their parasites produce to more efficiently infect susceptible hosts** (chapters 2, 4)

Behavioral contributions to parasite transmission are difficult to study in natural systems, but by using an experimental framework and new techniques for quantifying host-host contacts, I was able to demonstrate evidence in support of this hypothesis. The testosterone manipulation tested a male characteristic that had population level consequences for behavioral spread of parasites. Finally, the manipulation of food resources of chipmunks provided evidence that foraging behavior plays an important role in exposure and transmission of parasite, even if the mechanisms are not directly connected to male characteristics.

Conclusions

Apparent heterogeneities in exposure and susceptibility that lead to heterogeneities in infection levels can determine transmission dynamics (Perkins *et al*. 2003; Ferrari *et al.* 2004). However, I was able to demonstrate that heterogeneities in transmission dynamics can arise even when there are no apparent heterogeneities in susceptibility and infection rates (Grear *et al.* 2011; chapter 3).

In a more general sense, these hypotheses can be summarized as influencing exposure and susceptibility to parasites. The contributions of exposure and susceptibility to transmission are often very difficult to empirically measure and test, especially when these two processes interact and covary. Aspects of susceptibility to infection receive much attention because the intimate relationship between parasite and host produces disease symptoms of interest to the

medical and veterinary fields. However, the disease symptoms that result from the host-parasite interactions can only occur after a host contacts parasite infective stages.

Often, transmission is treated as a black box and much research tries to reduce the complexity of transmission by focusing on independent parameters that influence only a small part of susceptibility or exposure. My approach to deconstruct the transmission process in an ecologically relevant scope has yielded insights into how males are key hosts for parasite transmission, even if they do not appear to be disproportionally infected. In particular, the epidemiologically important behaviors related to testosterone are potentially crucial because of the simultaneous effect on susceptibility (*Meugot et al.* 2005). The contributions of host behaviors to the transmission process can be difficult to study in natural systems. Considering specific host behaviors, which are defined by transmission routes, provides an ecologically relevant framework to approach behavioral aspects of transmission. The utility of this framework is illustrated by the influence that chipmunk foraging behaviors have on forage-transmitted parasites. Foraging behavior was only important for the parasites whose transmission matched the specific behavior. Understanding the integration of the processes that drive exposure and susceptibility is the key to better understand host-parasite dynamics and the aims and products of my dissertation provided mechanistic insights into these dynamics.

References

Ferrari, N., Cattadori, I.C., Nespereira, J., Rizzoli, A., & Hudson, P.J. (2004). The role of host sex in parasite dynamics: field experiments on the yellow-necked mouse *Apodemus flavicollis*. *Ecol. Lett.*, 7, 88-94.

Grear, D.A., Luong, L.T., & Hudson, P. J. (2011) Sex-biased transmission of complex life-cycle parasite: why males matter. *J. Anim. Ecol.,* in review.

Mougeot, F., Redpath, S.M., Piertney, S.B., & Hudson, P.J. (2005). Separating behavioral and physiological mechanisms in testosterone-mediated trade-offs. *Am. Nat.,*166, 158-168.

Perkins, S.E., Cattadori, I.C., Tagliapietra, V., Rizzoli, A.P., & Hudson, P.J. (2003). Empirical evidence for key hosts in persistence of a tick-borne disease. *Parasitology*, 33, 909-917.

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Grear DA, MD Samuel, K Scribner, BV Weckworth, and JA Langenberg. 2010. Influence of genetic relatedness and spatial proximity on CWD transmission among female white-tailed deer. **Journal of Applied Ecology,** 47, 532-540.

Luong, LT, SE Perkins, *DA Grear*, A Rizzoli, and PJ Hudson. 2010. The relative importance of host characteristics and co-infectionin generating variation in *Heligmosomoides polygyrus* fecundity. **Parasitology,** 137, 1003-1012.

Grear, DA, SE Perkins, and PJ Hudson. 2009. Does elevated testosterone result in increased exposure and transmission of parasites? **Ecology Letters**, 12, 528-537. (This paper has been designated a Faculty of 1000 "***Must Read***" factor 8)

Luong, LT, *DA Grear*, and PJ Hudson. 2009. Male hosts are responsible for the transmission of a trophically transmitted parasite, *Pterygodermatites peromysci* to the intermediate host in the absence of sex-biased infection. **International Journal for Parasitology**, 39, 1263-1268.

Grear, DA, MD Samuel, JA Langenberg, and D Keane. 2006. Demographic patterns of CWD prevalence and harvest vulnerability of CWD infected white-tailed deer in Wisconsin. **Journal of Wildlife Management**. 70, 546-553.

Recent Service, Professional Affiliations, and Awards

J. Brain Horton Award, outstanding achievement and service to the Penn State graduate program in ecology, 2011

Penn State Ecology Program **Student Assistantship**: responsible for coordinating seminar series and being a liaison for student and program activities, 2009-2010

Student Chair, Ecology Program Andersen Travel Award, 2010

Vice-President, Penn State Ecology Graduate Student Organization, 2008-2009

Member, Ecological Society of America

Braddock Research Award, outstanding research achievement by graduate student in Biology, 2010

University Graduate Fellowship, Penn State University, 2006-2007

Graham Endowed Fellowship, Penn State University, 2006-2008