Parasitol Res (2013) 112:1763–1770
DOI 10.1007/s00436-013-3335-1

ORIGINAL PAPER

Associations between innate immune function and ectoparasites in wild rodent hosts

Evelyn C. Rynkiewicz · Hadas Hawlena · Lance A. Durden · Michael W. Hastriter · Gregory E. Demas · Keith Clay

Received: 7 September 2012 / Accepted: 31 January 2013 / Published online: 17 February 2013

Abstract Immune function is an important component of host fitness, and high investment in immunity should occur when the benefits outweigh the costs, such as when risk of parasitism is high. We sampled two rodent hosts, white-footed mice (*Peromyscus leucopus*), and prairie voles (*Microtus ochrogaster*), and their tick, flea, and mite ectoparasites. A bacterial killing assay was used to measure the host's innate immune function. We hypothesized that classes of hosts (species, sexes, or age classes) with overall higher tick burdens would have a higher innate immune function as an evolutionary response to historically greater exposure. We hypothesized a weaker relationship between the fleas and mites and immune function because of high host specificity in fleas and the absence of known vector function in North American mites. Ectoparasites were significantly overdispersed on hosts. In accordance with our hypothesis, *Peromyscus* that had higher tick burdens also exhibited significantly higher bacterial killing ability compared to *Microtus*. There was no significant difference in total flea burden between rodent species and no relationship with bacterial killing ability. *Microtus* had higher burdens of mites in each order than *Peromyscus*, and female rodents had higher mite burdens than males. The benefits of maintaining high levels of innate immune factors appear to be greater than the energetic costs for *Peromyscus* compared to *Microtus*.

E. C. Rynkiewicz (✉) · G. E. Demas · K. Clay
Department of Biology, Indiana University, 1001 E 3rd St, Bloomington, IN 47405, USA
e-mail: ecrynkie@indiana.edu

H. Hawlena
Department of Life Sciences, Ben Gurion University of the Negev, Beer Sheva, Israel

L. A. Durden
Department of Biology, Georgia Southern University, Statesboro, GA, USA

M. W. Hastriter
Monte L. Bean Life Science Museum, Brigham Young University, Provo, UT, USA

Introduction

Immune function is an important component of host fitness, but it is energetically costly (Demas et al. 1997; Graham et al. 2010). High investment in immunity should occur when the benefits of high immune function outweigh the costs, such as in habitats dense with parasites or when host fitness is strongly affected by parasitism (Allen 1994; Baucom and de Roode 2011; Graham et al. 2011). Moreover, the vertebrate immune system is diverse with components adapted to respond to different kinds of challenges. The time scale and nature of host interactions with parasites should determine the evolutionary patterns of immune investment. Short-term interactions with parasites are expected to increase the benefit of investing in specific antibodies and cell-mediated responses. For example, kittiwake birds (*Rissa tridactyla*) exhibited a positive correlation between tick density and *Borrelia burgdorferi* antibodies in kittiwake eggs (Gasparini et al. 2001). In contrast, longer-term and more widespread interactions should select for higher investment in constitutive, innate responses. This is supported by observations of small ground finches (*Geospiza fuliginosa*) where birds from larger islands in the Galapagos with higher parasite diversity had higher levels of general immune measures and lower levels of cell-mediated immunity compared to birds from smaller islands (Lindstrom et al. 2004). Given that the host immune system interacts directly with parasites, a better understanding on the broader ecological consequences of the relationship between immune function and parasitism is needed (Bradley and Jackson 2008; Hawley and Altizer 2011; Pedersen and Fenton 2007).

There are several hypotheses predicting the relationship between immune function and parasitism in animal hosts. The immunocompetence handicap hypothesis (ICHH), proposed by Folstad and Karter (1992) in response to an analysis by Hamilton and Zuk (1982), states that there is a physiological trade-off between energetic investment in secondary sexual characteristics and immune function such that males with the showiest traits have the lowest immune function and higher susceptibility to parasites. This relationship, where hosts with low immunity have more parasites, has also been used to explain the formation of "superspreaders" (Shaw and Dobson 1995; Shaw et al. 1998), individuals that transmit far more infectious propagules than the majority of the population (Woolhouse et al. 1997). While there is evidence supporting the ICHH based on patterns found in nature and in laboratory experiments (Folstad et al. 1989; Muehlenbein 2006; Zuk et al. 1990a, b), many studies have found mixed results on the relationship between parasites and immune function (Bordes et al. 2012; Saino et al. 1995; Fuxjager et al. 2011; Ezenwa et al. 2011). These patterns suggest that the relationship between host immune function and parasites may be determined by long-term ecological interactions, the natural history of the host and parasites, and the type of immunity measured.

Another hypothesis derived from plant pathology predicts how host immune response can lead to resistance and/or tolerance to parasites (Fornoni et al. 2004; Puustinen et al. 2004; Bordes et al. 2012). It suggests that tolerant hosts with high parasite loads are able to reduce fitness costs of infection by confining parasite spread or investing in immune components that sequester parasites or protect host tissues. By contrast, highly resistant hosts may be as fit as tolerant hosts but have low parasite loads due to highly effective removal of parasites through immune clearance (Råberg et al. 2007; Baucom and de Roode 2011). Based on predictions from the resistance–tolerance trade-off hypothesis, observed superspreaders may not be the individuals with the "poorest" immune function, but rather individuals that invest in tolerance mechanisms and survive long enough to propagate many secondary infections.

In this study, we measured ectoparasite burdens (ticks, fleas, and mites) on two common rodent hosts in order to investigate how ectoparasite exposure relates to innate immune function of the host. We employed a bacterial killing assay as a measure of general, innate immunity that is independent of previous exposure and thus does not measure acquired immunity to specific agents (Millet et al. 2007; Tieleman et al. 2005). We hypothesized that classes of hosts (e.g., species, sexes, or age classes) with consistently higher tick burdens will have a higher innate immune response. While individual hosts may vary greatly in tick and other ectoparasite burden in the short term (Calabrese et al. 2011; Krasnov et al. 2006), over the longer term, certain classes of hosts are more likely to be parasitized than others (Perkins et al. 2008; Ostfeld et al. 1995) and are more likely to be exposed to vector-borne pathogens (Ginsberg 2008). By contrast, because the flea species, *Ctenophthalmus pseudagyrtes* and *Orchopeas leucopus*, are specific to the rodent hosts examined here (Traub 1985), we expected that they would be poor predictors for immunocompetency. The mites found in our study are facultative hematophages, not feeding exclusively on blood (Whitaker 1982; Radovsky 1985), and mite-borne pathogens in North America are very rare (Zavala-Castro et al. 2009; Whitaker et al. 2007; Iwasa et al. 1990), suggesting that mites would also be weak predictors of immunocompetency.

Methods

Study system

The most common rodent species in our study sites in southern Indiana (USA) are *Peromyscus leucopus* (white-footed mouse) and *Microtus ochrogaster* (prairie voles). Both hosts support a diversity of ectoparasites (Whitaker 1982; Durden 1992). *P. leucopus* is found mostly in forested habitats, while *M. ochrogaster* is found in open, grassy habitats and prefers shorter and varied vegetation (Krebs 1970; Mumford and Whitaker 1982). The most common rodent ectoparasites are the ticks, *Ixodes scapularis* (black-legged deer tick) and *Dermacentor variabilis* (American dog tick); the fleas, *C. pseudagyrtes* and *O. leucopus*; and mites from the orders Mesostigmata and Trombidiformes (Mumford and Whitaker 1982; Durden 1992). Lice may also infest these rodents, but their quantification would have required sacrificing animals, which was not done here (Whitaker 1982). The two tick species found in this study are generalists that use a wide variety of hosts during their lifetime (Klompen et al. 1996; Whitaker 1982). In contrast, the two flea species are highly host specific, with *O. leucopus* parasitizing *Peromyscus* spp. and *C. pseudagyrtes* parasitizing *Microtus* spp. almost exclusively. Mites within a family or order can vary greatly in their host specificity and their feeding ecology (Whitaker 1982; Radovsky 1985). *Peromyscus* and *Microtus* species are reservoir hosts for human and wildlife vector-borne pathogens such as *B. burgdorferi* (LoGiudice et al. 2003; Ostfeld and LoGiudice 2003), *Babesia microti* (Anderson et al. 1986; Burkot et al. 2000), and *Bartonella* spp. (Hofmeister et al. 1998).

Field collections

Eight sites in southern Indiana were sampled for small mammals and their ectoparasites from May to July, 2009. Three 40×50-m grids were established in forested and open, grassy habitats at each site. Forty Sherman live traps (3×

3.5×9 in; H.B. Sherman Traps, Tallahassee FL, USA) were placed in each grid for a total of 120 traps per site, and each site was surveyed for three consecutive days for a total of 2,880 trap nights (8 sites×3 nights×120 traps). Traps were baited each evening before dusk with millet seeds, puffed corn peanut butter snack, and cotton batting and were checked early the next morning. Each rodent was censused for ectoparasites only at its first capture in order to have equal sampling effort for each individual. The skin and fur of each rodent was thoroughly searched with forceps. All ticks and fleas observed were collected, and the majority (68 %) of observed mites were collected for identification. Mites were numerous on certain individual rodents and, unlike ticks, were highly mobile, making it difficult to collect 100 % of the observed mites. Ticks, fleas, and mites collected from rodents were placed in 70 % ethanol and stored in the laboratory at −20 °C until they were identified to species.

After sampling rodents for ectoparasites, a 100-μl blood sample was taken under anesthesia once per sampling period using retro-orbital sinus bleed. Non-heparinized blood samples were kept on ice until they were centrifuged (12,000 RPM for 15 min) to separate red blood cells from serum. Serum was aliquoted, and both blood components were stored separately at −80 °C. Serum samples were then used for immune assays. After sampling, all rodents were marked with a numbered, steel ear tag and were released at the exact site of their capture. All rodent handling procedures were approved by the Bloomington Institutional Animal Care and Use Committee (protocol no. 11-001).

Immune assay

A bacterial killing assay (BKA) was used to measure each host's innate immune function; it does not measure current pathogen infection in the host (modified from Chester et al. 2010; Millet et al. 2007). Because serum samples were stored frozen, bacterial killing here was primarily due to complement proteins in the rodent serum which create pores on the surface of bacterial cells causing them to lyse (Boughton et al. 2011; Chester et al. 2010). Individual variation in background levels of immune factors is expected and reflected as measurable variation in bacterial killing ability. To perform the BKA, a pellet of a known number of *Escherichia coli* cells [Epower™ Microorganisms no. 0483E7, MicroBioLogics, St. Cloud, MN; 1 pellet=10^7 cells or colony forming units (CFU)] was prepared in glutamine-enriched, CO_2-independent growth media (Gibco no. 18045, Carlsbad, CA). The pellet was activated by incubating in 40 ml sterile 1 M phosphate-buffered saline (PBS) at 37 °C for 30 min, resulting in a 500,000-CFU/ml stock. A 10 % working stock of *E. coli* was made using 1 M PBS, resulting in a 50,000-CFU/ml solution.

Serum samples were diluted to 1:40 with CO_2-independent media (5 μl serum to 195 μl media; dilution determined by pilot optimization). Next, 20 μl of working stock bacteria was added to the 200 μl serum dilution. The positive control consisted of 20 μl working stock bacteria (but no serum) added to 195 μl media and 5 μl PBS. The negative control was made using 25 μl PBS and 195 μl growth media. All samples were incubated at 37 °C for 30 min. Then, 50 μl of each sample was plated onto a Trypticase Soy Nutrient Agar plate. Each sample was plated in triplicate. All plates were incubated at 37 °C for 8 h, after which colonies were counted. The mean number of colonies was calculated from the three plates for each sample, and the number of colonies on sample plates was compared to positive control plates to obtain "proportion alive" for each rodent host. Bacterial killing ability, or "proportion killed", was calculated as 1—proportion alive.

Statistical analysis

A generalized linear model analysis with a negative binomial distribution was used to analyze differences in ectoparasite burdens between hosts. A general linear mixed model (GLMM) was used to analyze differences in bacterial killing ability between host classes. A square-root transformation was used to improve the normality of the bacterial killing data. Host sex, species, age, body mass, reproductive condition, and habitat were fixed variables in both analyses, and collection site was included as a random variable in the GLMM. Measures of ectoparasite burden (number of ticks, fleas, or mites collected from each host) were added as fixed variables in GLMM analysis of bacterial killing ability.

Results

Ectoparasite distributions on hosts

A total of 192 rodents were trapped during this study [126 *P. leucopus*, 62 *M. ochrogaster*, 3 *Microtus pinetorum* (pine vole), and 1 *Microtus pennsylvanicus* (meadow vole); Table 1]. Further analyses were conducted only on *P. leucopus* (hereafter, *Peromyscus*) and *M. ochrogaster* (hereafter, *Microtus*), due to the small numbers of *M. pinetorum* and *M. pennsylvanicus* captured. From *Peromyscus* and *Microtus*, a total of 273 ticks (157 *D. variabilis* and 116 *I. scapularis*), 80 fleas (26 *C. pseudagyrtes* and 54 *O. leucopus*), and 201 mites were collected. Eight mite species in the order Mesostigmata were identified (153 mites total), with *Androlaelaps fahrenholzi* and *Laelaps kochi* being the most abundant, while one species in the order Trombidiformes was collected (*Eutrombicula alfreddugesi*, 48 mites total; Table 1). One individual *Listrophorus mexicana*, a hair mite (order Sarcoptiformes), was also collected.

Table 1 Summary of ectoparasites (ticks, fleas, and mites) collected from rodent hosts divided by species and sex

	Peromyscus (126) Sum (Mean±SE)	Female (51)	Male (75)	*Microtus* (62)	Female (28)	Male (34)
	306 (2.43±0.22)	90 (1.76±0.24)	216 (2.88±0.31)	351 (5.75±0.32)	143 (5.11±0.56)	208 (6.03±0.38)
Class Acari						
Ticks						
Dermacentor variabilis	**141 (1.05±0.18)**	34 (0.67±0.28)	107 (1.43±0.23)	**16 (0.26±0.26)**	7 (0.25±0.38)	9 (0.27±0.35)
Ixodes scapularis	**52 (0.38±0.24)**	11 (0.22±0.37)	41 (0.55±0.31)	**64 (1.03±0.34)**	22 (0.79±0.50)	42 (1.27±0.46)
Mites						
Androlaelaps fahrenholzi	**34 (0.272±0.14)**	10 (0.196±0.18)	24 (0.324±0.20)	**45 (0.726±0.199)**	17 (0.607±0.26)	28 (0.823±0.29)
Eulaelaps stabularis	**1 (0.008±0.09)**	1 (0.019±0.14)	0 (0)	**0 (0)**	0 (0)	0 (0)
Eutrombicula alfreddugesi	**21 (0.168±0.26)**	18 (0.353±0.43)	3 (0.04±0.15)	**27 (0.435±0.54)**	27 (0.964±0.80)	0 (0)
Haemogamasus liponyssoides	**2 (0.016±0.09)**	0 (0)	2 (0.027±0.12)	**3 (0.048±0.12)**	3 (0.107±0.18)	0 (0)
Haemogamasus longitarsus	**0 (0)**	0 (0)	0 (0)	**1 (0.016±0.13)**	0 (0)	1 (0.029±0.171)
Laelaps alaskensis	**0 (0)**	0 (0)	0 (0)	**7 (0.113±0.18)**	3 (0.107±0.24)	4 (0.117±0.27)
Laelaps kochi	**0 (0)**	0 (0)	0 (0)	**46 (0.741±0.17)**	11 (0.393±0.22)	35 (1.029±0.22)
Listrophorus mexicana	**0 (0)**	0 (0)	0 (0)	**1 (0.016±0.13)**	0 (0)	1 (0.029±0.171)
Ornithonyssus bacoti	**5 (0.04±0.16)**	5 (0.098±0.26)	0 (0)	**8 (0.129±0.12)**	3 (0.107±0.18)	5 (0.147±0.16)
Class Insecta						
Fleas						
Ctenophthalmus pseudagyrtes	**3 (0.02±0.05)**	1 (0.02±0.08)	2 (0.027±0.06)	**23 (0.39±0.07)**	17 (0.61±0.11)	6 (0.182±0.10)
Orchopeas leucopus	**54 (0.42±0.07)**	19 (0.37±0.11)	35 (0.47±0.09)	**0**	0	0

All values are presented as total (mean±1 SE)

Data in bold highlight the host species sums and means for each ectoparasite

We found an overdispersed (aggregated) pattern of ectoparasites on rodent hosts. Most hosts had zero or a few ectoparasites, while a few hosts had many ectoparasites (variance/mean ratio=7.081, skewness=3.756±0.178; Fig. 1). High variance/mean ratio and skewness greater than 1.0 indicate that the distributions of ectoparasites on hosts are best described by a negative binomial distribution (Petney et al. 1990), which accurately describes the distribution of many macroparasites (Fenton et al. 2010; McAloon and Durden 2000; Shaw and Dobson 1995).

Dermacentor ticks were found at all eight sites and on both host species, whereas *Ixodes* ticks were found only at two sites. Therefore, further analyses on tick distributions were conducted only for *Dermacentor*. There was a significant difference in tick burden between rodent species with *Peromyscus* carrying significantly more *Dermacentor* ticks than *Microtus* (likelihood ratio=26.27, degrees of freedom (*df*)=1, $p<0.0001$; Fig. 2). When considering all hosts, male rodents had higher *Dermacentor* burdens than females

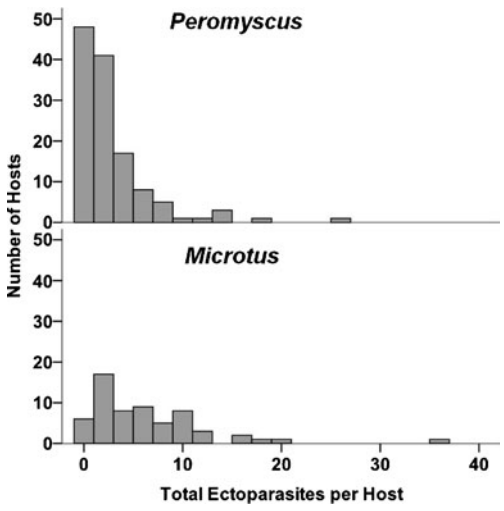

Fig. 1 Distribution of all ticks, fleas, and mites on the two host species. A majority of individuals carry no or few ticks and a minority carry many ticks, leading to the observed skewed distribution (variance/mean ratio=7.081, skewness=3.756±0.178)

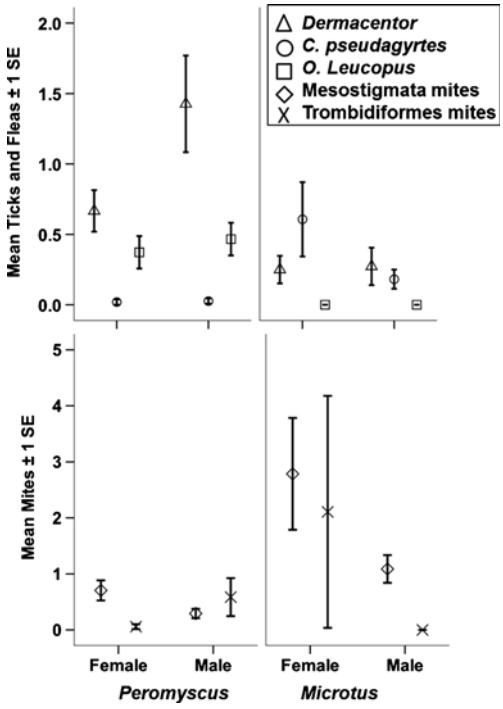

Fig. 2 Mean [±1 standard error (SE)] *D. variabilis* tick (*triangles*), *C. pseudagyrtes* flea (*circles*), *O. leucopus* flea (*squares*, *top panel*), Mesostigmata (*diamonds*), and Trombidiformes (*X*s, *bottom panel*) burdens on *Peromyscus* and *Microtus* hosts

(likelihood ratio=9.91, p=0.002). There was not a significant species×sex interaction. However, when host species were analyzed separately, the sex difference was driven by male *Peromyscus*, which had significantly higher tick burdens than female *Peromyscus* (Fig. 2; likelihood ratio=8.24, p=0.004). No sex differences in tick burden were found between male and female *Microtus*. Habitat, date, body size, age, reproductive condition, and host density did not affect tick burden (p>0.20 for all listed variables).

C. pseudagyrtes fleas were found nearly exclusively on *Microtus* (likelihood ratio=30.56, df=1, p<0.0001) and *O. leucopus* occurred only on *Peromyscus* (Table 1). This is consistent with previous results reporting high host specificity in these flea species (Durden 1992; Mumford and Whitaker 1982). There were no significant species differences in total flea burden between hosts, but there was a significant species×sex interaction (likelihood ratio=5.71, p=0.017), with *Peromyscus* males and *Microtus* females having higher flea burdens. Habitat, date, body size, age, reproductive condition, and host density did not affect flea burden (p>0.20 for all listed variables).

Mites were binned by order for statistical analysis (excluding Sarcoptiformes because only a single *L. mexicana* mite was found; Table 1). *Microtus* had significantly more Mesostigmata (likelihood ratio=15.87, p<0.0001) and Trombidiformes mites (likelihood ratio=39.91, df=1, p<0.0001) than *Peromyscus*. Female rodents also had higher mite burdens than males (Mesostigmata, likelihood ratio=16.012, p<0.0001; Trombidiformes, likelihood ratio=3.424, p=0.064). There were no significant host species×sex effects for either mite order.

Immune response of hosts and ectoparasites

Blood samples were collected from a total of 114 rodents, with 56 *Peromyscus* and 14 *Microtus* samples providing sufficient serum for immune analysis. There was no significant linear relationship between individual bacterial killing ability and tick (R^2=0.001, F_1=0.094, p=0.76) or flea burden (R^2=0.003, F_1=0.217, p=0.64). There was also no significant linear relationship between mite burden for either order and bacterial killing ability (Mesostigmata, R^2=−0.012, F_1=0.16, p=0.69; Trombidiformes, R^2=0.006, F_1=1.40, p=0.24).

There was, however, a significant difference in bacterial killing ability between host species, with *Peromyscus* serum samples killing more bacteria (proportion killed=0.76±0.03) than *Microtus* serum samples (0.33±0.06, $F_{1/63.56}$=48.31, p<0.0001). There was also a nonsignificant trend for *Peromyscus* males (0.78±0.037) to kill more bacteria than females (0.74±0.05, $F_{1/53.07}$=2.35, p=0.13; Fig. 3), but there was no difference in bacterial killing between male and female *Microtus*. Habitat, body size, age, reproductive condition, and host density did not affect bacterial killing ability (p>0.20 for all listed variables). While there can be changes in rodent physiology due to day length and other seasonal changes (Nelson and Demas 1996; Zysling et al. 2009), date was also not a significant factor in our analyses.

Discussion

The results of our field sampling and laboratory assays provide partial support for our hypothesis that classes of

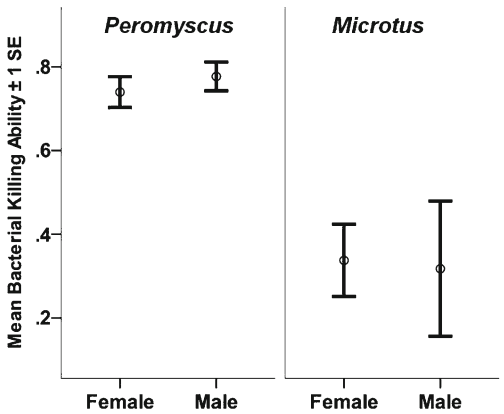

Fig. 3 Mean bacterial killing ability (±1 SE) of both host species and both host sexes. Bacterial killing ability was square root transformed to improve normality

hosts with higher tick burdens will have higher innate immune function. Here, *Peromyscus*, the host species with the higher tick burden, had significantly higher bacterial killing ability than *Microtus*. As predicted, there was no relationship between flea burden and bacterial killing. There was a significant negative correlation between mite burden and bacterial killing, reflecting that *Microtus* had the highest mite burden and the lowest bacterial killing ability. In addition, male *Peromyscus* had higher tick burdens than female *Peromyscus* and a higher, but nonsignificant, bacterial killing ability, which is partially consistent with the ICHH (Folstad and Karter 1992; Roberts et al. 2004). The differences in tick burden and innate immune function between rodent species, however, were more consistent with the hypothesis that classes of hosts with high parasite loads are more tolerant and exhibit high innate immune function (Baucom and de Roode 2011). The immunocompetence of an individual may represent a combination of both resistance and tolerance mechanisms (Keil et al. 2001).

We found significant differences in innate immune function between two host species, with *Peromyscus* showing higher bacterial killing ability than *Microtus*. The observed host species differences could result from longer-lived *Peromyscus* investing more in immune function than shorter-lived *Microtus* (Martin et al. 2007). However, habitat differences and parasite exposure between the two host species could also provide different selective environments for investment in innate immune function. At our study sites, *Peromyscus* was found primarily in forests, where tick densities on vegetation were higher, while *Microtus* inhabited open, grassy areas with lower tick densities (Durden 1992; Rynkiewicz et al., unpublished data). The benefits of maintaining high innate immune function may outweigh the costs for *Peromyscus* if they are more likely to encounter ticks and vector-borne pathogens, while the same energetic expenditure may be less beneficial for *Microtus*. Ticks and fleas vector a number of microbial pathogens, and hosts with more ectoparasites will be more frequently exposed to vector-borne pathogens (Ginsberg 2008). Because the innate immune system responds nonspecifically to challenges and may be more responsive to long-term, predictable patterns of host–parasite interactions, variation in ectoparasite burden among classes of hosts may provide a stronger selective force than short-term variation among individuals.

There was no relationship between flea burden and bacterial killing ability. *Microtus* and *Peromyscus* had similar flea burdens, yet *Peromyscus* had higher bacterial killing ability. The host species-specific fleas sampled in this study can spend their entire adult life on the same individual (Mumford and Whitaker 1982; Whitaker 1982). Given this high level of specificity between host and ectoparasite, we would expect higher host investment in adaptive, as opposed to innate, immunity. While these fleas can vector pathogenic bacteria (Hawlena et al. 2013; Abbot et al. 2007; Erickson et al. 2009), they are quite host specific, unlike the ticks sampled here; so, a host is less likely to be exposed to novel flea-borne pathogens over their lifetime (Traub 1985). However, this pattern may not be universal for all flea and host species (Brinkerhoff et al. 2011; Gomez-Diaz et al. 2007; Jones and Britten 2010).

Mesostigmata and Trombidiformes mites were more abundant on *Microtus*, and female rodents had more mites of both orders than males. While *Microtus* had higher mite burdens than *Peromyscus* and lower bacterial killing ability, the mites found on the rodents in this study are not known to be vectors of pathogens. Some biting mites may cause small wounds on the host, which could lead indirectly to infection. However, most of the mite species found in this study are facultative hematophages and often feed on skin secretions or debris or on body fluids from other arthropods on the host or in the host nest (Radovsky 1985). Therefore, the mites sampled here are unlikely sources of infection and probably do not impose a fitness cost on their host and so may provide a weak selective pressure on host innate immune function.

Our results suggest that there are species-specific differences in innate immune function. Mammals are known to develop antibodies both to components of tick saliva as well as to tick-borne pathogens (Brown 1988; Schwanz et al. 2011; Wikel 1999). However, the innate immune factors active in the bacterial killing assay we utilized are first responders to infection (Janeway et al. 2007). Complement proteins are always circulating in the blood, but contact with bacterial surface proteins and lipopolysaccarides, such as those on the surface of many gram-negative, vector-borne bacteria, can locally activate proliferation of complement proteins (Tilly et al. 2008; Amerault et al. 1981; Francis et al. 1980). A metagenomic survey of ticks and fleas from the same sampling sites reported here revealed high levels of bacterial diversity (Hawlena et al. 2013). The higher innate immune function in highly parasitized classes of hosts may therefore also reflect defense against ectoparasite-borne bacteria. Detailed analyses of vector-borne infections within hosts, immune function, and bacterial communities of ectoparasites would bring further clarity to issues of tolerance and resistance in these rodent hosts.

Our analyses of natural communities of ectoparasites and rodent hosts indicate that *P. leucopus* have higher tick burdens and higher bacterial killing ability than *M. ochrogaster*. Higher tick burdens in *Peromyscus* may have selected for high innate immune function to enhance their tolerance to high tick burdens and potential vector-borne pathogen exposure throughout their lifetime. Thus, these results indicate that potential superspreaders may have higher, rather than lower, immune function than the majority of the population

(Baucom and de Roode 2011; Bordes et al. 2012). Hawley and Altizer (2011) recently highlighted the need for field data linking immune function, host life history characteristics, and aggregated distribution of parasites to determine how they lead to patterns of host–parasite interactions observed in nature. Our results on rodent innate immune function, variation among life history and taxonomic classes, and ectoparasite aggregation help address this knowledge gap.

Acknowledgments The authors thank J. Kagemann, A. Avalos, and W. Snydermann for their help with rodent trapping and ectoparasite collection, and Dr. J. Whitaker and Dr. R. Pinger for ectoparasite identification. We thank Dr. E. Chester and Dr. M. Ruíz for their guidance in optimizing the bacterial killing assay and for helpful discussions of the results. Funding was provided through an Indiana Academy of Science Senior Research Grant and an NSF Doctoral Dissertation Improvement Grant (award no. 1110514) to ER, NSF-NIH grant DEB-03268742 to KC, an Indiana Metabolomics and Cytomics Initiative of Indiana University (IU) grant awarded to KC, and an IU Center for Research in Environmental Sciences grant awarded to KC.

References

Abbot P, Aviles AE, Eller L, Durden LA (2007) Mixed infections, cryptic diversity, and vector-borne pathogens: evidence from *Polygenis* fleas and *Bartonella* species. Appl Environ Microbiol 73:6045–6052

Allen JR (1994) Host resistance to ectoparasites. Rev Sci Tech Off Int Épiz 13:1287–1303

Amerault TE, Rose JE, Kuttler KL (1981) Comparative titration of *Anaplasma marginale* antibodies by card agglutination and complement-fixation tests. Am J Vet Res 42:1055–1056

Anderson JF, Johnson RC, Magnarelli LA, Hyde FW, Myers JE (1986) *Peromyscus leucopus* and *Microtus pennsylvanicus* simultaneously infected with *Borrelia burgdorferi* and *Babesia microti*. J Clin Microbiol 23:135–137

Baucom RS, de Roode JC (2011) Ecological immunology and tolerance in plants and animals. Funct Ecol 25:18–28

Bordes F, Ponlet N, de Bellocq JG, Ribas A, Krasnov BR, Morand S (2012) Is there sex-biased resistance and tolerance in Mediterranean wood mouse (*Apodemus sylvaticus*) populations facing multiple helminth infections? Oecologia 170:123–135

Boughton RK, Joop G, Armitage SAO (2011) Outdoor immunology: methodological considerations for ecologists. Funct Ecol 25:81–100

Bradley JE, Jackson JA (2008) Measuring immune system variation to help understand host-pathogen community dynamics. Parasitology 135:807–823

Brinkerhoff RJ, Martin AP, Jones RT, Collinge SK (2011) Population genetic structure of the prairie dog flea and plague vector, *Oropsylla hirsuta*. Parasitology 138:71–79

Brown SJ (1988) Highlights of contemporary research on host immune-responses to ticks. Vet Parasitol 28:321–334

Burkot TR, Schneider BS, Pieniazek NJ, Happ CM, Rutherford JS, Slemenda SB, Hoffmeister E, Maupin GO, Zeidner NS (2000) *Babesia microti* and *Borrelia bissetii* transmission by *Ixodes spinipalpis* ticks among prairie voles, *Microtus ochrogaster*, in Colorado. Parasitology 121:595–599

Calabrese JM, Brunner JL, Ostfeld RS (2011) Partitioning the aggregation of parasites on hosts into intrinsic and extrinsic components via an extended poisson-gamma mixture model. PLoS One 6:e29215

Chester EM, Bonu T, Demas GE (2010) Social defeat differentially affects immune responses in Siberian hamsters (*Phodopus sungorus*). Physiol Behav 101:53–58

Demas GE, Chefer V, Talan MI, Nelson RJ (1997) Metabolic costs of mounting an antigen-stimulated immune response in adult and aged C57BL/6 J mice. Am J Physiol Regul Integr Comp Physiol 273:R1631–R1637

Durden LA (1992) Ectoparasites of sympatric meadow voles and white footed mice at Fort Detrick, Maryland. J Med Entomol 29:761–766

Erickson DL, Anderson NE, Cromar LM, Jolley A (2009) Bacterial communities associated with flea vectors of plague. J Med Entomol 46:1532–1536

Ezenwa VO, Ekernas LS, Creel S (2011) Unravelling complex associations between testosterone and parasite infection in the wild. Funct Ecol 26:123–133

Fenton A, Viney ME, Lello J (2010) Detecting interspecific macroparasite interactions from ecological data: patterns and process. Ecol Lett 13:606–615

Folstad I, Karter AJ (1992) Parasites, bright males, and the immunocompetence handicap. Am Nat 139:603–622

Folstad I, Nilssen AC, Halvorsen O, Andersen J (1989) Why do male reindeer (*Rangifer-T tarandus*) have higher abundance of 2nd and 3rd instar larvae of *Hypoderma-tarandus tarandi* Than Females. Oikos 55:87–92

Fornoni J, Nunez-Farfan J, Valverde PL, Rausher MD (2004) Evolution of mixed strategies of plant defense allocation against natural enemies. Evolution 58:1685–1695

Francis DH, Buening GM, Amerault TE (1980) Characterization of immune-responses of cattle to erythrocyte stroma, *Anaplasma* antigen, and dodecanoic acid-conjugated *Anaplasma* antigen: humoral immunity. Am J Vet Res 41:362–367

Fuxjager MJ, Foufopoulos J, Diaz-Uriarte R, Marler CA (2011) Functionally opposing effects of testosterone on two different types of parasite: implications for the immunocompetence handicap hypothesis. Funct Ecol 25:132–138

Gasparini J, McCoy KD, Haussy C, Tveraa T, Boulinier T (2001) Induced maternal response to the Lyme disease spirochaete *Borrelia burgdorferi* sensu lato in a colonial seabird, the kittiwake *Rissa tridactyla*. Proc R Soc Lond B Biol Sci 268:647–650

Ginsberg HS (2008) Potential effects of mixed infections in ticks on transmission dynamics of pathogens: comparative analysis of published records. Exp Appl Acarol 46:29–41

Gomez-Diaz E, Gonzalez-Solis J, Peinado MA, Page RDM (2007) Lack of host-dependent genetic structure in ectoparasites of *Calonectris* shearwaters. Mol Ecol 16:5204–5215

Graham AL, Hayward AD, Watt KA, Pilkington JG, Pemberton JM, Nussey DH (2010) Fitness correlates of heritable variation in antibody responsiveness in a wild mammal. Science 330:662–665

Graham AL, Shuker DM, Pollitt LC, Auld SKJR, Wilson AJ, Little TJ (2011) Fitness consequences of immune responses: strengthening the empirical framework for ecoimmunology. Funct Ecol 25:5–17

Hamilton WD, Zuk M (1982) Heritable true fitness and bright birds—a role for parasites. Science 218:384–387

Hawlena H, Rynkiewicz E, Toh E, Alfred A, Durden LA, Hastriter MW, Nelson DE, Rong R, Munro D, Dong Q, Fuqua C, Clay K (2013) The arthropod, but not the vertebrate host or its environment, dictates bacterial community composition of fleas and ticks. ISME J 7:221–223

Hawley DM, Altizer SM (2011) Disease ecology meets ecological immunology: understanding the links between organismal immunity and infection dynamics in natural populations. Funct Ecol 25:48–60

Hofmeister EK, Kolbert CP, Abdulkarim AS, Magera JMH, Hopkins MK, Uhl JR, Ambyaye A, Telford SR III, Cockerill FR III, Persing DH (1998) Cosegregation of a novel *Bartonella* species with *Borrelia burgdorferi* and *Babesia microti* in *Peromyscus leucopus*. J Infect Dis 177:409–416

Iwasa M, Kasuya S, Noda N, Hioki A, Ito A, Ohtomo H (1990) Trombiculid mites (Acari: Trombiculidae) and *Rickettsia tsutsugamushi* isolated from wild rodents in a new endemic area of Japan. J Med Entomol 27:501–508

Janeway CA, Travers P, Walport M, Shlomchik M (2007) Immunobiology, 7th edn. Garland Publishing, New York

Jones PH, Britten HB (2010) The absence of concordant population genetic structure in the black-tailed prairie dog and the flea, *Oropsylla hirsuta*, with implications for the spread of *Yersinia pestis*. Mol Ecol 19:2038–2049

Keil D, Luebke RW, Pruett SB (2001) Quantifying the relationship between multiple immunological parameters and host resistance: probing the limits of reductionism. J Immunol 167:4543–4552

Klompen JSH, Black WCV, Keirans JE, Oliver JH Jr (1996) Evolution of ticks. Annu Rev Entomol 41:141–161

Krasnov BR, Shenbrot GI, Khokhlova IS, Hawlena H, Degen AA (2006) Temporal variation in parasite infestation of a host individual: does a parasite-free host remain uninfested permanently? Parasitol Res 99:541–545

Krebs CJ (1970) *Microtus* population biology—behavioral changes associated with population cycle in *M. ochrogaster* and *M. pennsylvanicus*. Ecology 51:34–52

Lindstrom KM, Foufopoulos J, Parn H, Wikelski M (2004) Immunological investments reflect parasite abundance in island populations of Darwin's finches. Proc R Soc Lond B Biol Sci 271:1513–1519

LoGiudice K, Ostfeld RS, Schmidt KA, Keesing F (2003) The ecology of infectious disease: effects of host diversity and community composition on Lyme disease risk. PNAS 100:567–571

Martin LB, Weil ZM, Nelson RJ (2007) Immune defense and reproductive pace of life in *Peromyscus* mice. Ecology 88:2516–2528

McAloon FM, Durden LA (2000) Attachment sites and frequency distribution of erythraeid mites, *Leptus indianensis* (Acari: Prostigmata), ectoparasitic on harvestmen, *Leiobunum formosum* (Opiliones). Exp Appl Acarol 24:561–567

Millet S, Bennett J, Lee KA, Hau M, Klasing KC (2007) Quantifying and comparing constitutive immunity across avian species. Dev Comp Immunol 31:188–201

Muehlenbein MP (2006) Intestinal parasite infections and fecal steroid levels in wild chimpanzees. Am J Phys Anthropol 130:546–550

Mumford RE, Whitaker JOJ (1982) Mammals of Indiana. Indiana University Press, Bloomington

Nelson RJ, Demas GE (1996) Seasonal changes in immune function. Q Rev Biol 71:511–548

Ostfeld RS, LoGiudice K (2003) Community disassembly, biodiversity loss, and the erosion of an ecosystem service. Ecology 84:1421–1427

Ostfeld RS, Cepeda OM, Hazler KR, Miller MC (1995) Ecology of Lyme disease: habitat associations of ticks (*Ixodes scapularis*) in a rural landscape. Ecol Appl 5:353–361

Pedersen AB, Fenton A (2007) Emphasizing the ecology in parasite community ecology. Trends Ecol Evol 22:133–139

Perkins SE, Ferrari MF, Hudson PJ (2008) The effects of social structure and sex-biased transmission on macroparasite infection. Parasitology 135:1561–1569

Petney TN, Vanark H, Spickett AM (1990) On sampling tick populations: the problem of overdispersion. Onderstepoort J Vet Res 57:123–127

Puustinen S, Koskela T, Mutikainen P (2004) Direct and ecological costs of resistance and tolerance in the stinging nettle. Oecologia 139:76–82

Råberg L, Sim D, Read AF (2007) Disentangling genetic variation for resistance and tolerance to infectious diseases in animals. Science 318:812–814

Radovsky FJ (1985) Evolution of mammalian mesostigmate mites. In: Kim KC (ed) Coevolution of parasitic arthropods and mammals. Wiley, New York

Roberts ML, Buchanan KL, Evans MR (2004) Testing the immunocompetence handicap hypothesis: a review of the evidence. Anim Behav 68:227–239

Saino N, Moller AP, Bolzern AM (1995) Testosterone effects on the immune system and parasite infestations in the barn swallow (*Hirundo rustica*): an experimental test of the immunocompetence hypothesis. Behav Ecol 6:397–404

Schwanz LE, Voordouw MJ, Brisson D, Ostfeld RS (2011) *Borrelia burgdorferi* has minimal impact on the Lyme disease reservoir host *Peromyscus leucopus*. Vector-Borne Zoon Dis 11:117–124

Shaw DJ, Dobson AP (1995) Patterns of macroparasite abundance and aggregation in wildlife populations: a quantitative review. Parasitology 111:S111–S133

Shaw DJ, Grenfell BT, Dobson AP (1998) Patterns of macroparasite aggregation in wildlife host populations. Parasitology 117:597–610

Tieleman BI, Williams JB, Ricklefs RE, Klasing KC (2005) Constitutive innate immunity is a component of the pace-of-life syndrome in tropical birds. Proc Royal Soc B Biol Sci 272:1715–1720

Tilly K, Rosa PA, Stewart PE (2008) Biology of infection with *Borrelia burgdorferi*. Infect Dis Clin N Am 22:217–234

Traub R (1985) Coevolution of fleas and mammals. In: Kim KC (ed) Coevolution of parasitic arthropods and mammals. Wiley, New York, pp 295–437

Whitaker JO (1982) Ectoparasites of mammals of Indiana. Indiana Academy of Science, Indianapolis

Whitaker JO Jr., Walters BL, Castor LK, Ritzi CM, Wilson N (2007) Host and distribution lists of mites (Acari), parasitic and phoretic, in the hair or on the skin of North American wild mammals north of Mexico: records since 1974. Faculty Publications from the Harold W Manter Laboratory of Parasitology, University of Nebraska, Lincoln 1:1–173

Wikel SK (1999) Tick modulation of host immunity: an important factor in pathogen transmission. Int J Parasitol 29:851–859

Woolhouse MEJ, Dye C, Etard JF, Smith T, Charlwood JD, Garnett GP, Hagan P, Hii JLK, Ndhlovu PD, Quinnell RJ, Watts CH, Chandiwana SK, Anderson RM (1997) Heterogeneities in the transmission of infectious agents: implications for the design of control programs. PNAS 94:338–342

Zavala-Castro JE, Zavala-Velazquez JE, Peniche-Lara GF, Sulu Uicab JE (2009) Human rickettsialpox, Southeastern Mexico. Emerg Infect Dis 15:1665–1667

Zuk M, Johnson K, Thornhill R, Ligon JD (1990a) Parasites and male ornaments in free-ranging and captive red jungle fowl. Behaviour 114:232–248

Zuk M, Thornhill R, Ligon JD, Johnson K (1990b) Parasites and mate choice in red jungle fowl. Am Zool 30:235–244

Zysling DA, Garst AD, Demas GE (2009) Photoperiod and food restriction differentially affect reproductive and immune responses in Siberian hamsters *Phodopus sungorus*. Funct Ecol 23:1–10