

INFESTATION OF *PEROMYSCUS LEUCOPUS* AND *TAMIAS STRIATUS* BY *IXODES SCAPULARIS* (ACARI: IXODIDAE) AS A RESULT OF TICK HOST PREFERENCE, HOST GROOMING EFFICIENCY, AND HABITAT UTILIZATION

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INTRODUCTION

Lyme disease is the most prevalent vector-borne zoonosis in the United States and Europe (Steere 1989; Gray 1998). In the United States alone over 125,000 cases of Lyme disease have been reported since 1982 (CDC Website). The disease was first discovered in Europe in the early 1900's under the name of "erythema migrans syndrome" (Ostfeld 1997). Not until 1975 in Lyme, Connecticut was the disease recognized in the U.S. following a cluster of cases of rheumatoid arthritis in children (Reed 1993, White et al. 1991) The causative agent was found to be a spirochete bacterium *Borrelia burgdorferi*, which was discovered in 1981 in the midgut diverticula of *Ixodes scapularis* ticks (Gray 1998). Since then several species of *ixodid* ticks have been found naturally infected with *B. burgdorferi*, including *I. pacificus* in the western United States, and *I. ricinus* and *I. persulcatus* in Eurasia (Lane et al. 1991; Gray 1998; Reed 1993). The primary tick vector harboring Lyme borreliosis in the eastern and upper- midwestern United States, *I. scapularis* is most commonly referred to as the "black-legged tick" (Formerly *Ixodes dammini* Oliver et al. 1993; Levin and Fish 1997; Lane et al. 1991).

Black-legged ticks undergo four life stages (egg, larvae, nymph, adult) over the course of a two-year life span. Eggs hatch into six-legged larva in the midsummer, which actively seek small hosts (i.e. rodent, bird, lizard) and feed for their first blood meal lasting from 3-7 days (pers. observation). These fed larvae then detach from the host after feeding to repletion and molt in to the next life stage within a month. The subsequent nymphal tick has eight legs and is larger than its larval predecessor. This tick searches for a small-medium size host (i.e. chipmunk, dog, raccoon) usually in the following summer after a seasonal diapause and undergoes its second blood meal. Once engorged, the tick drops off the host into the leaf litter and molts into the adult three months later. The considerably larger adult will feed on a large animal host (i.e. white-tailed deer). The adult males will mate and then expire and the females will oviposit a large egg mass the following spring composed of a few hundred to a few thousand eggs and then expire (Ostfeld 1997). The larval ticks that hatch in midsummer emerge free of the Lyme bacterium (Gray 1998; Lane 1991).

Lyme disease is a zoonosis in which the etiological agent, maintained through transmission from a black-legged tick to a vertebrate host (Reed 1993) and back. Trans-stadial transmission (stage-to-stage i.e. from larvae to nymph) rather than transovarial transmission (from infected mother to her eggs) has been found to be the primary means for Lyme disease dissemination. Meaning that because larvae hatch out of eggs uninfected, ticks can only pick up the infection by feeding on an infected host during their larval or nymphal meals. Once the tick becomes infected it maintains the bacterium through all life stages. The subsequent infected nymphal or adult ticks are then only capable of transmitting the infection during their blood meal on another host (Gray 1998; Ostfeld 1996). As a consequence, only the nymphal and adult life stages are able to transmit the bacterium to an uninfected host, including humans. Nymphal ticks have been found to cause the majority of human cases of Lyme disease in the northeastern United States (Levin and Fish 1998). Because nymphal ticks acquire the spirochetes during their larval blood meal, the host the larval tick feeds on during the blood meal is critical in determining the density of

infected nymphs capable of spreading the Lyme bacterium. Most human Lyme disease cases occur in mid-summer months (June- August) with a peak in July, which corresponds with nymphal tick activity (Lane et al 1991). For this reason the population density of questing infected nymphal ticks has been determined to be the greatest factor influencing the risk of human exposure to Lyme disease (Ostfeld et al. 1996; Schmidt et al., 1999).

Juvenile black-legged ticks have been reported to parasitize nearly 60 vertebrate host species encompassing the classes Mammalia, Aves and Reptilia (Bosler et al. 1984; Gray 1998; Lane et al. 1991; Oliver 1989; Schmidt 1999). These hosts vary in their ability to transmit the *B. burgdorferi* pathogen to feeding ticks. The efficiency of disease transmission from host to tick is defined as reservoir competence, which refers to the probability of a tick becoming infected with the bacterium during the course of a blood meal on an infected host. In other words, ticks feeding on some hosts, such as the white-footed mouse, are highly likely to acquire the Lyme bacterium. These hosts are called competent reservoirs. In contrast, ticks feeding on some other host species, such as deer, are extremely unlikely to become infected with the Lyme bacterium.

Because of the wide variety of hosts that have been found to be parasitized by *I. scapularis* this tick species has been considered to be a generalist in host selection (Gray J.S. 1998; James A.J. and Oliver J.H. 1990; Lane et al. 1991; Oliver J.H. 1989; Ostfeld & Keesing 2000; Reed G.H. 1993). It has not been empirically determined whether *I. scapularis* are in fact generalists in host selection or not. If *I. scapularis* are indiscriminate feeders then the chances of their feeding on a competent reservoir host for Lyme disease is solely dependent on the abundance of particularly infective hosts and the frequency for which ticks encounter them. If *Ixodes* ticks are preferentially feeding on a particular host then the maintenance of Lyme disease becomes dependent on the ability of the preferred host to transmit the disease to a feeding tick. Because the abundance of reservoir hosts able to harbor and transmit the spirochetes within a habitat is crucial to the establishment of infected tick populations, determining whether *I.scapularis* are generalists or specialist feeder is extremely important in understanding the ecology and maintenance of Lyme disease.

In the northeastern United States the white-footed mouse (*Peromyscus leucopus*) is the most abundant host, as well as the most frequently parasitized, and the most infective to uninfected ticks making it the primary reservoir host for Lyme disease (Apperson 1993; Bolsler et al. 1984; Levin and Fish 1998; Ostfeld et al. unpublished data; Schmidt et al. 1999). Because of the role of the white-footed mouse in infecting larval ticks, factors influencing the abundance of larval ticks infestation on white-footed mice are crucial determinants of the abundance of subsequent populations of infected nymphs. Schmidt et al. (1999) determined larval tick burdens to be 2-3 times higher on white-footed mice than on chipmunks (*Tamias striatus*), despite the fact that both white-footed mice and chipmunks are abundant within the same eastern deciduous forest habitat and are of relatively similar size. These two hosts are the most commonly parasitized vertebrate host by larval black-legged ticks, yet eastern chipmunks are not as efficient as white-footed mice at transmitting Lyme disease to uninfected ticks making it a less competent reservoir in Lyme disease transmission (Lane et al. 1991).

In this study, I examined the causes of why larval infestation rates are higher on white-footed mice than on eastern chipmunks. I tested three mechanisms, each of which could account for the higher larval burdens on mice. First, I tested whether black-legged ticks preferentially orient towards white-footed mice rather than chipmunks. Second, I examined whether habitat utilization by mice cause them to encounter more ticks than chipmunks, and finally, I tested whether chipmunks were more efficient at grooming than mice.

METHODS

Study Sites

All experiments were conducted on the property of the Institute of Ecosystem Studies (IES) in Dutchess County, southeastern NY (41°50'N, 73°45'W). Experiments were conducted in the Animal Rearing Facility at IES from

late June-July when immature ticks are actively seeking hosts. The IES Institutional Animal Care and Use Committee approved all protocols for this study.

White-footed mice (*Peromyscus leucopus*) and eastern chipmunks (*Tamias striatus*) used in the laboratory experiments were trapped from forest areas within IES property using Sherman live traps. Only adult and sub-adult males of both species were used for the experiment to prevent use of pregnant or lactating females with dependent young. All animals were released at the exact location where they were trapped. Ticks were collected from IES property using a 1-m² white corduroy drag cloth (Falco and Fish 1992). All ticks were maintained in glass vials (~ 20 mL) with nylon mesh and polypropylene tops in a cooling unit at ~2.5-4.0⁰ and ~ 85 % relative humidity (RH) when not being used to conduct experiments. The photophase was 14:10 (light: dark) for hosts and ticks during all experiments.

Host Preference Experiment

Because juvenile ticks require high relative humidity, the experiment was conducted within an environmentally controlled enclosure (91.4 cm x 33 cm x 30.5 cm) constructed of .64 cm plexi-glass (Figure 1). A saturated salt solution (B) along with aluminum pans (C) of water within the chamber were used to maintain an average of 85% RH within the apparatus throughout all experiments. Secured on the farthest ends within the enclosure, were two 750mL tubs filled with a saturated salt solution consisting of 330 g of K₂SO₄ and 100 mL of H₂O to generate humidity within the chamber (Winston et al. 1960). Air vents were established in the chamber to allow air circulation to maintain the temperature within the apparatus and sustain the animals comfortably. Four air vents (D) each consisting of clusters of five .64 cm holes located on the lid and two farthest walls of the chamber ventilated the enclosure. Two of the vents were located on each of the farthest walls of the chamber and two vents were located at the far ends of the chamber's lid. The interior of the chamber was maintained at ~ 85% RH and at 22 ± 2°C during all experiments.

The apparatus for holding animals and conducting the experiment was nested within the humidified chamber. The inner experimental apparatus consisted of two plexi-glass cages (E) (21.5 cm x 21.5 cm x 15.0 cm) with 5, 0.64 cm holes (F) in the lid and farthest walls of the two cages, which provided further air circulation between the inner cages and the humidified chamber for the animals. The two cages were connected by a 2.54 cm diameter, 16 cm long plastic tube (G), in which larval ticks were placed at the beginning of each orientation experiment. The connecting tube had a .64 cm diameter hole placed equidistant from both cages that was used for the addition of ticks into the center of the tube. The base (H) of the inner apparatus, in which the plexi-glass cages were nested, was made of pressure-treated wood covered with a polyurethane sealant (add specific one and vendor). The base under the two plexi-glass cages was constructed of hardware cloth (I), which permitted the cages to be suspended over water basins, allowing for waste and tick collection. At opposite ends of both cages, two humidity gauges (J) were placed within the apparatus to monitor humidity during the experiments. A water bottle (K) was centrally located 5 cm vertically from the cage base on the wall farthest from the connection tube in each cage.

Mouse and chipmunk pairs were added in the inner plexi-glass cages, a chipmunk in one cage and a mouse in the other cage. Animals were supplied with equal amounts of *ad libitum* food (Rat Nutri-plus; add name of manufacturer). In order to saturate the chamber with host odor, the animals were held for one hour in the apparatus before the experiment began. A hardware cloth nest box covered in aluminum foil (L), which was replaced at the beginning of every experiment, was placed over the opening of the connecting tube in both plexi-glass cages to further concentrate host odor near the opening of the connecting tube. (Figure 1, picture of apparatus)

At the start of each experiment, twenty-five larval black-legged ticks were collected in 2 mL of water and placed on the tip of a fine bristle paintbrush (000 mm) and then added to the central connecting tube between the two plexi-glass cages. To facilitate measurements of tick movements, the tube was marked every 2 cm from the central point where the ticks were added. To ensure sterility of conditions to prevent scent contamination from

one trial to the next, an acetate lining was placed within the central tubing before every experiment. To prevent ticks from actually accessing the host animals, the ends of the acetate were covered with a fixed volume (~ 2 cc?) of Tanglefoot® vendor applied with a plastic syringe.

Each trial lasted 3 hours, during which ticks could move in the direction of either the mouse or the chipmunk, away from the center of the tube where they were added, or could remain in the center of the tube. After three hours, the inner acetate lining was removed and the distance that all 25 individual ticks had traveled was recorded.

The entire apparatus was rotated 180 degrees after every experiment to prevent a directional bias. In order to equalize the placement of the hosts for each trial the cages were marked with an X and an O and the animal placement was recorder each time to alternate the position of the animals for each experiment. After each trial, the entire chamber and inner apparatus were washed with 50% ethanol and allowed to stand for a minimum of 2 hours.

Two methods of analysis were used differing in the parameters to be met in order to consider that a choice had been made for one animal over another. The first considers each tick an independent data point. For the movement of an individual tick to be considered a choice it had to travel >2cm from the point of addition. For this analysis of individual preference all ticks that moved farther than the set distance criterion of 2cm towards a host was considered to have oriented towards that particular host. To analyze the data a test of the sampling distribution of sample proportion was used. This distribution revealed the tendency for ticks to orient towards either host.

The second test considered each group of 25 ticks in each of the 20 trials to be an individual data point. This analysis does not assume each tick to be an individual unit working independently of the other 24 ticks. For each trial the proportion of ticks within that population that oriented towards a mouse, a chipmunk and neither was determined. To conclude whether each trial of 25 ticks oriented towards a particular host, at least half of the total number of ticks added had to move past the set 2cm mark in either direction away from the central point of addition. In order for a trial to be considered a choice made by the majority of the population at least twice the number of ticks moving past the 2 cm mark had to be on one side over the other. A chi-squared analysis was used to examine the number of trials that were considered a choice for either host.

Grooming Efficiency Experiment

This experiment took place in conjunction with the host preference experiment. Ticks were maintained in the rearing facility laboratory at IES at 22+2⁰C and ~ 75-80% RH throughout the course of this experiment. The mice and chipmunks used for this test were those that had been previously used for the host selection experiment. Before being used in this test the animals had been held in hardware cloth cages over water for 72 hours to eliminate field tick burdens. All hosts had at least 24 hours during which no ticks were collected from the water basins, signifying the end of field burdens (pers. communication R. Ostfeld). After being used in the host preference experiment, mice and chipmunks were placed in wire cages of .64 cm mesh (30.5 cm x 10.2 cm x 10.2 cm) and suspended 10 cm above a water basin (Figure 4). The mesh cages did not restrict grooming behaviors of either host. The animals were supplied with water and food *ad libitum* (Rat Nutri-plus VENDOR) throughout the experiment.

After ticks acquired in the field had been allowed to fall from the host, experimental ticks were added to each host. These ticks were collected in 2mL of water and concentrated to the tip of a fine bristle brush (000 mm) and then added manually to each host's back and neck while the hosts were held within the mesh cages. Larval ticks were added to each host in clusters of 10 ticks; clusters were added every 10 minutes for a total of 50 ticks per animal. Over a period of 120 hours all ticks were allowed to either feed to repletion, drop off without feeding, or be groomed off. The water below the animals was checked 1 hour after the addition of all ticks and every 12 hours after that for a total of 120 hours. Ticks were counted and their condition (chewed/unchewed) was noted as they

were recovered; the number of ticks ingested by the host was calculated by subtracting the total recovered from the total of 50 added at the start of the trial.

The total number of ticks that fed to repletion and the number of ticks that died in the course of grooming, either by being ingested or chewed was compared between the two hosts. The number of ticks that fed and the number that were assumed dead were analyzed using a standard t-test.

Habitat Utilization Experiment

This experiment used data collected by R.S. Ostfeld on three 2.25-ha trapping grids at IES from 1997-1999. Data from 1996 and 2000 were not analyzed due to very low mammal density during those years. Each year animals were live-trapped at least once per month from May-November and the number of larval ticks visible on the ears and head of each host were counted. Detailed descriptions of small mammal trapping and larval burden estimates are available in Schmidt et al. (1999).

To assess the effects of habitat use on host tick burdens, I compared larval tick burdens on mice and chipmunks that were captured, analyzing only data from June-September, when larval ticks are active at these sites. First, I calculated the average larval burden on each host at each of the three trapping locations in each of the three years. I then calculated these same values, but restricted the analysis to mice and chipmunks that were captured at the same trap station during the same week, to compare burdens on hosts that utilized the same areas of the grid, restricting habitat use. The larval burdens on mice and chipmunks that were found in the same trap station paired with another host of the other species, were then used to find the mean larval burden. Then the larval burdens on these animals were compared to the larval burdens found at the population level over all three plots. The analysis compared larval burdens on mice and chipmunks with no restriction on habitat use and mice and chipmunks that were considered to be utilizing the same habitat in time.

Statistical analysis was done using a paired t- test to compare the ratio of larvae found on mice and chipmunks over the three plots for three years for the both paired and total population of mice and chipmunks.

RESULTS

Orientation Experiment

In all experiments ticks were found to significantly preferentially orient towards mice over chipmunks. When tick movement past 2cm in the direction of either host was analyzed treating each individual tick (n=289) in each group of 25 across the 20 replicates as an independent data point, there was a significant preference for the ticks to orient towards mice ($p > .58$; $P < 0.025$)(Figure 2). When considering the number of trials (n=20) where a choice was made for one animal over another, which was determined by the proportion of each group of 25 ticks that oriented towards one host, ticks significantly oriented towards mice ($\chi^2 = 8.333$; $df = 1.000$; $P = 0.004$) (Table 1). Of the 20 trials, 10 were considered an orientation towards mice and none were considered as having made a choice for chipmunks.

Grooming Efficiency Experiment

Mice were found to be more effective at grooming away larval ticks than chipmunks. The number of ticks that were not removed by grooming and were able to feed to repletion was significantly higher on chipmunks (n=10) than mice (n=10) ($t = 3.768$; $df = 18$; $P = 0.001$)(Figure 3). The number of ticks that were groomed away and either ingested or chewed resulting in death during grooming was not significantly different between the two hosts ($P > 0.05$)(Figure 4).

Habitat Utilization Experiment

When examining mice captured and examined for larval ticks the average burden of ticks per host for mice was 7.37. The average burden of larval ticks per chipmunk was found 3.02. An analysis of mice and chipmunks that were paired because they were trapped at the same station during the same session, found the mean mouse burden per host to be $6.32 \pm$ and the mean chipmunk burden to be $2.61 \pm$ (Figure 7). If habitat use was the primary factor affecting the difference in tick burdens, one would expect the ratio of the larval burden on mice to the larval burden on chipmunks in the paired population to be close to 1. When mice and chipmunks were paired they were still found to have an average of 3 times as many larval ticks as chipmunks (Table 2). This discrepancy suggests that habitat use is a weaker factor influencing the larval burdens on mice and chipmunks. If habitat were a partial explanation of the higher burden on mice then the ratio of larvae on paired animals should approach 1. No significant difference was found in the ratios of larval burdens between the total population and the paired population ($t=0.98$; $df=8$; $P=0.36$), suggesting that habitat does not account for the difference in tick burdens between mice and chipmunks (Figure 6).

DISCUSSION

Orientation Experiment

Black-legged ticks appear to be indiscriminate in host selection based on the range of hosts that they are collected from in nature (Gray 1998; James and Oliver 1990; Lane et al. 1991; Oliver 1989; Ostfeld & Keesing 2000; Reed 1993;). From this experiment however, *I. scapularis* are clearly capable of exerting preference for one particular host over another. In the experiments previously described black-legged ticks preferentially oriented toward white-footed mice over eastern chipmunks. Every test in which a choice was made the ticks consistently chose mice.

Though they are apparently capable of feeding on many hosts, *Ixodes* ticks also seem capable of making a choice when alternative hosts are available. So long as potential hosts are abundant and the probability of encountering more than one host is high, then host selectivity would be advantageous to the ticks. When the probability of encountering a host is low, however, refusing one host in hopes of finding a better one would be counter-productive, leaving the tick unable to obtain a necessary blood meal.

Grooming Experiment

Because larval burdens are 2-3 times higher on white-footed mice than chipmunks it was hypothesized that chipmunks were more effective at grooming off larval ticks than mice. However, the results from this study run counter to this prediction. White-footed mice were significantly more efficient at grooming off larval ticks than chipmunks. Of the 50 ticks added to each host an average of 2.8 larval ticks were able to successfully feed on mice. Chipmunks averaged nearly 10.5 larval ticks able to successfully feed per host. It might be surmised that the proficiency of mice at grooming off larval ticks in fact might be because they are the preferred host. A high larval burden regularly infesting white-footed mice may induce more regular grooming behavior. Yet the higher grooming efficiency in mice still does not counteract the larger effect of white-footed mice being preferred by ticks.

Habitat Use Experiment

Based on analysis of trapping data, differences in habitat use did not account for the discrepancy between mouse and chipmunk larval burdens. Mouse and chipmunk pairs that were trapped in the same trap station in the same week had vastly different larval burdens. Mice had nearly four times as many larval ticks as did chipmunks. This ratio was not significantly different from that obtained for all animals regardless of where they were trapped. This implies that differences in habitat utilization do not account for the discrepancy between larval burdens on these

two hosts. If it did, it would be expected that the ratio of larvae on mice versus larvae on chipmunks would approach 1 when habitat was restricted. In this experiment, I assumed that animals that were trapped in the same trap week at the same trap station were using the same habitat area. The animals that were used in this analysis were captured at the same space and time. However, this does not signify that the space they used overlapped and might therefore underestimate the effect of habitat use on larval burdens.

An alternate approach to test the effect of habitat use on larval burdens would be to equalize habitat use experimentally and then examine subsequent larval burdens on hosts under the set conditions. For example, hosts could be caged and placed in close proximity in the field over a period of days and examined for consequent larval burdens. This would restrict differential habitat use while still allowing larvae to access the hosts equally. This test would more accurately control for habitat utilized between the two animals. If habitat use is a strong signal affecting larval burdens, hosts confined in this way would be expected to have equal larval burdens. Any discrepancy in larval burdens could be assumed to be due to factors other than habitat use such as tick orientation and host grooming. However, this experiment would have to be conducted in the mid-summer months and would last for many days at a time. Because of this, animals might suffer from the effects of heat, rendering this experimental approach unacceptable.

Net Effect of Experiments on Larval Burdens

The combined result of the three experiments elucidates the natural processes affecting larval burdens on mice and chipmunks. Based on my results, mice and chipmunks encounter equivalent numbers of larval ticks. However, larvae preferentially orient towards white-footed mice over chipmunks. Mice, being more efficient at grooming, will remove a larger number of ticks than chipmunks, but because of the higher burden resulting from host preference, the overall grooming of mice is not effective enough to cause the net effect of lower larval burdens than that found on chipmunks. The end result finds mice more frequently parasitized by larval ticks than are chipmunks.

Implications

In this experiment, the most competent reservoir for the transmission of the bacterium that causes Lyme disease was found to be the preferred host for immature *Ixodes* ticks. The transmission of the Lyme pathogen by a vector to a competent reservoir host, that is then capable of returning the pathogen to a feeding vector is the only means of maintaining this disease cycle. It has been suggested by Ostfeld and Keesing 2000, that the Lyme pathogens may selectively specialize on the most “numerically dominant” member of the community in order to promote survival. The pathogen, by being exposed to the most abundant host more frequently, is then capable of evolving within that host community to be highly infective and persistent in that particular host. In the Northeastern United States white-footed mice have been reported as the most competent reservoir for Lyme bacterium transmission and they are the most abundant vertebrate host for ticks. A positive correlation has been found between higher burdens on hosts and increased reservoir competence (Ostfeld and Keesing 2000). This suggests that the Lyme bacterium may be very opportunistic allowing it to adapt within a vertebrate species thereby increasing that hosts’ competence as a reservoir. As a result, an active choice for white-footed mice by *Ixodes* ticks results in the most efficient transmission and maintenance of the Lyme bacterium.

Ixodes ticks that can feed on a range of species within a community may also evolve in that same ecological community on the most dominant vertebrate host species to specialize on the most proficient host for successful feeding. In communities where the dominant vertebrate species is disproportionately abundant relative to the other hosts, specialization on the most abundant host would lead to increased tick survival. The majority of blood meals received by larval black-legged ticks in the Northeastern United States are on the most dominant vertebrate species, the white-footed mouse. Larval ticks have been found to molt more successfully when having fed on a white-footed mouse compared to other vertebrate hosts (R.S. Ostfeld pers. com). The specialization in host selection of *I. scapularis* to white-footed mice further ensures increased survival of nymphal tick populations.

The high reservoir competence of white-footed mice results in greater than 90% of ticks that feed on this particular host becoming infected with the Lyme bacterium (Schmidt and Ostfeld 2000). Greater feeding success of larval ticks on white-footed mice ensures that larvae molt into infected nymphs capable of spreading Lyme disease. The disproportionate effect of white-footed mice on nymphal infection prevalence is the result of human destruction of natural habitat and the creation of fragmented forest that cannot sustain a higher diversity of natural competitors and predators of white-footed mice. An increase in biodiversity of hosts for tick vectors in the Lyme disease system has been hypothesized to reduce Lyme disease risk by diluting the impact of the most competent reservoir hosts (white-footed mice) on the prevalence of infected ticks (Ostfeld and Keesing 2000). By increasing the abundance of vertebrate species that are less competent reservoirs for the disease but are capable of successfully feeding ticks, the relative abundance of the infective species is decreased (via competition and predation), which then decreases the probability of a larval tick receiving a blood meal from an infective host, resulting in a decreased number of infected ticks (Ostfeld and Keesing 2000).

Increased biodiversity has only been suggested for disease systems where the vector transmitting the pathogen is indiscriminate in host selection (Ostfeld and Keesing 2000). *Ixodes* ticks though previously considered indiscriminate in host selection, are capable of exercising a selective choice for white-footed mice. Increased biodiversity can be effective on a specialized vector so long as the addition of other vertebrate hosts significantly decreases the total abundance of the most competent reservoir species, while still permitting proficient hosts for larval feeding. Host preference may then change in tick communities in response to changes in the density and composition of the available host community. This would then mitigate the necessity for specialization on white-footed mice by providing other increasingly abundant candidates for successful blood meals by larval ticks. The necessity for a comprehensive analysis of the degree of vector specialization in the ecology of vector-borne zoonosis has been largely underestimated. Understanding the affect of vector as well as pathogen specialization on disease proliferation is essential for combating these disease systems in nature.

The distribution of *Ixodes* ticks on white-footed mice versus other hosts can dramatically affect the proliferation of the Lyme disease epidemic. The results from this study suggest that host preference may be a major factor influencing the total abundance of ticks on white-footed mice being higher than that found on other available hosts. Further studies of host preference are necessary in order to understand the nature of ecological interactions between *Ixodes* ticks harboring Lyme disease and their hosts.

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LITERATURE CITED

- Anderson, J. et al. 1987. Prevalence of *Borrelia burgdorferi* in White-Footed Mice and *Ixodes dammini* at Fort McCoy, Wis. *Journal of Clinical Microbiology* **25** (8): 1495- 1497.
- Apperson, C. et al. 1993. Relative utilization of reptiles and rodents as hosts by Immature *Ixodes Scapularis* (Acari: Ixodidae) in the Coastal Plain of North Carolina, USA. *Experimental and Applied Acarology* **17**: 719-731.
- Bosler, E et al. 1984. Prevalence of the Lyme disease spirochete in populations of white-tailed deer and white-footed mice. *The Yale Journal of Biology and Medicine* **57**: 651-659.
- Giardina, A. et al. 2000. Modeling the role of songbirds and rodents in the ecology of Lyme disease. *Canadian*

- Journal of Zoology **78**: 2184-2197.
- Gray, J.S. et al. 1992. Studies on the ecology of Lyme disease in a deer forest in County Galway, Ireland. Journal of Medical Entomology **29** (6): 915-920.
- Gray, J.S. 1998. The ecology of ticks transmitting Lyme Borreliosis. Experimental and Applied Acarology **22**: 249-258.
- James, A., and J. Oliver. 1990. Feeding and host preference of Immature *Ixodes dammini*, *I. scapularis*, and *I. pacificus* (Acari: Ixodidae). Journal of Medical Entomology **27** (3): 324-329.
- Lane, R.S., J. Piesman, and W. Burgdorfer. 1991. Lyme Borreliosis: Relation of its causative agent to its vectors and hosts in North America and Europe. Annual Review of Entomology **36**: 587-609.
- Levin, M.L. and Fish, D. 1998. Density-dependent factors regulating feeding success of *Ixodes scapularis* Larvae (Acari: Ixodidae). Journal of Parasitology **84** (7): 000-000.
- Markowski, D. et al. 1998. Reservoir competence of the meadow vole (Rodentia: Cricetidae) for the Lyme disease spirochete *Borrelia burgdorferi*. Journal of Medical Entomology **35** (5): 804-808.
- Mather, N., S. R. Telford III., and G.H. Adler. 1991. Absence of transplacental transmission of Lyme disease spirochetes from reservoir mice (*Peromyscus leucopus*) to their offspring. The Journal of Infectious Diseases **164**: 564-567.
- Mills, J., and J.E. Childs. 1998. Ecological studies of rodent reservoirs: Their relevance for human health. Emerging Infectious Diseases: Perspectives **4** (4): 529-537.
- Peisman, J. 1993. Standard system for infecting ticks (Acari: Ixodidae) with the Lyme disease spirochete, *Borrelia burgdorferi*. Journal of Medical Entomology **30** (1): 199-203.
- Oliver, J. 1989. Biology and Systematics of Ticks (Acari: Ixodidae). Annual Review of Ecological Systems **20**: 397-430.
- Oliver, J. 1990. Feeding and host preference of Immature *Ixodes dammini*, *I. scapularis*, and *I. pacificus* (Acari: Ixodidae). Journal of Medical Entomology **27** (3): 324-330.
- Oliver, J.H., Owsley, M.R., Hutcheson, H.J., James, A.M., Chen, C., Irby, W.S., Dotson, E.M. and McLain, D.K. 1993. Conspecificity of the ticks *Ixodes scapularis*, and *Ixodes dammini* (Acari: Ixodidae). Journal of Medical Entomology. **30**: 54-63.
- Ostfeld, R., M.C. Miller, and K.R. Hazler. 1996. Causes and consequences of tick (*Ixodes scapularis*) burdens on white-footed mice (*Peromyscus leucopus*). Journal of Mammology **77** (1): 266-273.
- Ostfeld, R. 1997. The ecology of Lyme disease risk. American Scientist. 85: 338-346.
- Ostfeld, R., and F. Keesing. 2000. The function of biodiversity in the ecology of vector-borne zoonotic diseases. Canadian Journal of Zoology **78**: 2061-2078.
- Reed, G. 1993. Lyme disease and other tick-borne diseases: A review. Journal of Environmental Health **55** (8): 6-10.
- Schmidt, K.A., R.S. Ostfeld, and E.M. Schaubert. 1999. Infestation of *Peromyscus leucopus* and *Tamias striatus* by *Ixodes scapularis* (Acari: Ixodidae) in relation to the abundance of hosts and parasites. Journal of Medical Entomology **36** (6): 749-757.
- Telford, S. et al. 1987. Incompetence of deer as reservoirs of the Lyme disease spirochete. American Journal of Tropical Medical Hyg. **39** (1): 105-109.
- Winston, P.W., and D.H. Bates. 1960. Saturated solutions for the control of humidity in biological research. Ecology **41**: 232-23
- White, D.J et al. 1991. The geographic spread and temporal increase of the Lyme Disease epidemic. JAMA. 266 (9): 1230-1236.

APPENDIX

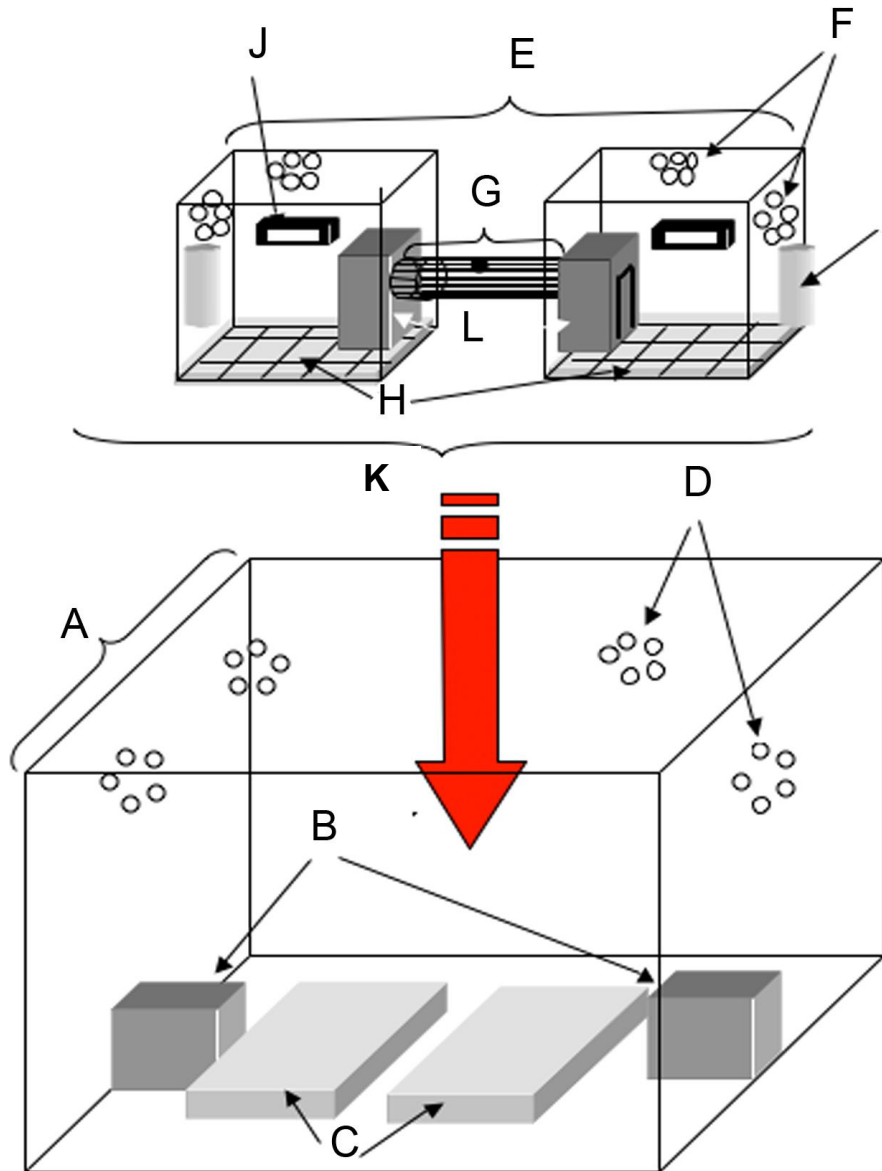


FIGURE 1. Orientation apparatus: Humidity and temperature regulated chamber (A) Saturated salt solution [K₂SO₄ + H₂O] to regulate humidity (B) Pans of water for humidity and waste collection (C) Air vents (D) Plexiglass cages for holding animals (E) Inner vents (F) Tube for tick addition connected to both cages (G) Hardware cloth base for underneath inside cages (H) Water bottles (I) Humidity gauges (J) Base of inner apparatus made of pressure treated wood (K) Hardware cloth and aluminum foil nest boxes (L)

**Orientation of Individual Ticks (n=289)
That Made a Choice**

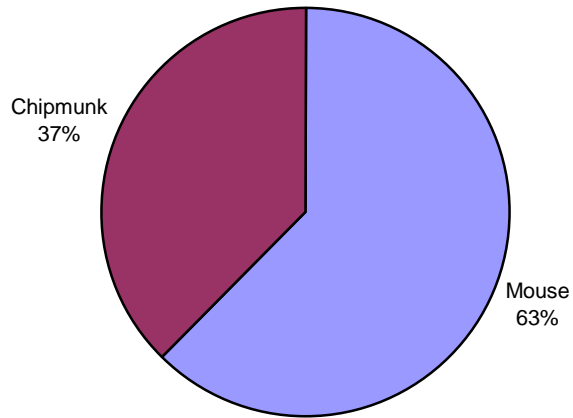


FIGURE 2. Proportion of ticks (n=289) orienting towards a mouse or a chipmunk

Larval Tick Feeding Success

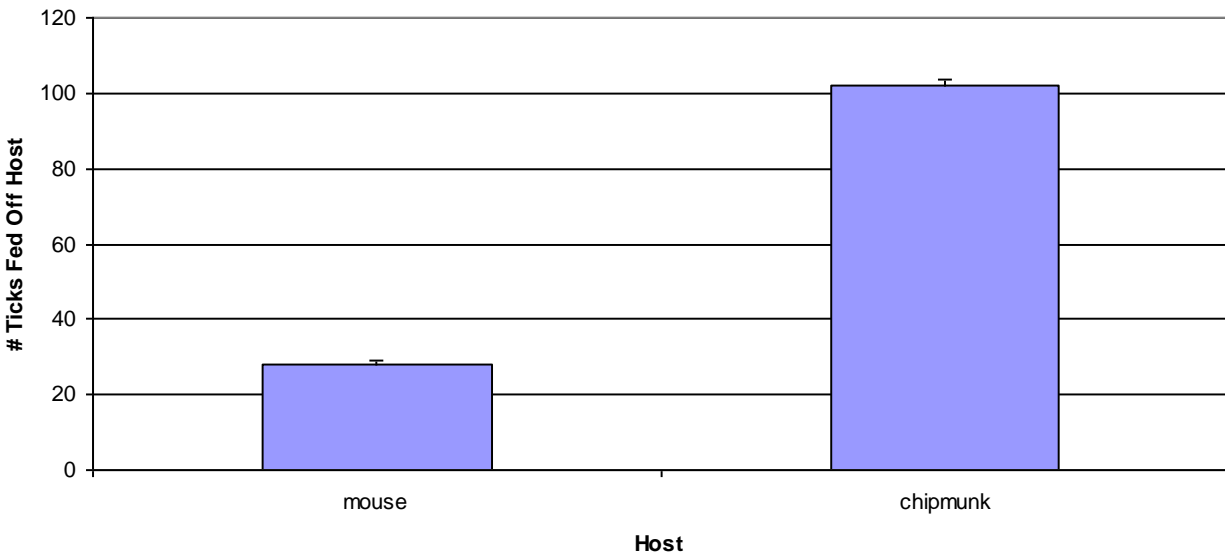


FIGURE 3. Total number of larval ticks able to feed to repletion from mice (n=10) and chipmunks (n=10)

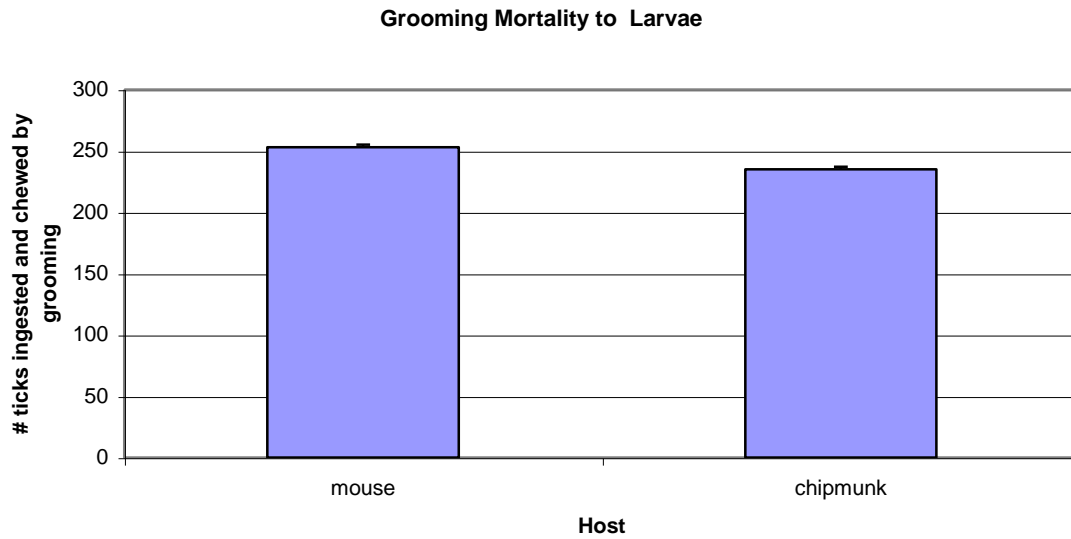


FIGURE 4. Total number of tick mortality suffered from grooming of mice (n=10) and chipmunks (n=10)

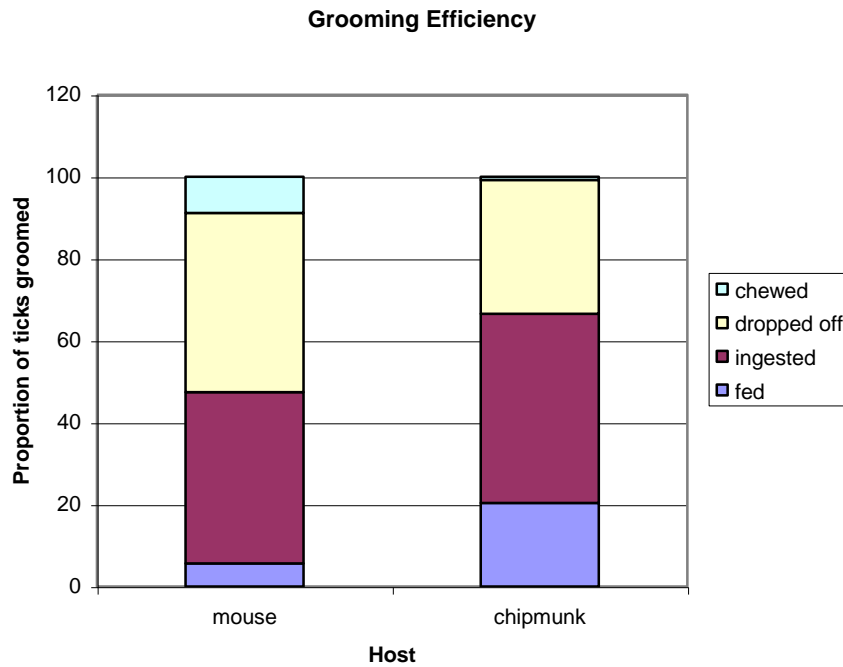


FIGURE 5. Grooming mechanisms and larval tick feeding success of mice (n=10) and chipmunks (n=10)

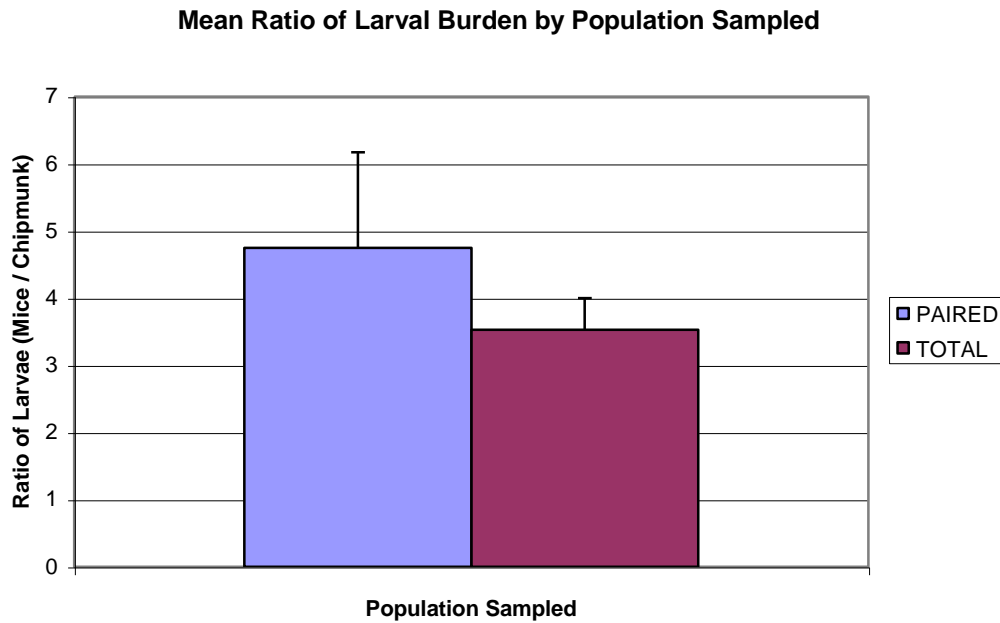


FIGURE 6. Ratio of larval ticks found by comparing mice and chipmunks from 1997-1999 on all 3 control grids.

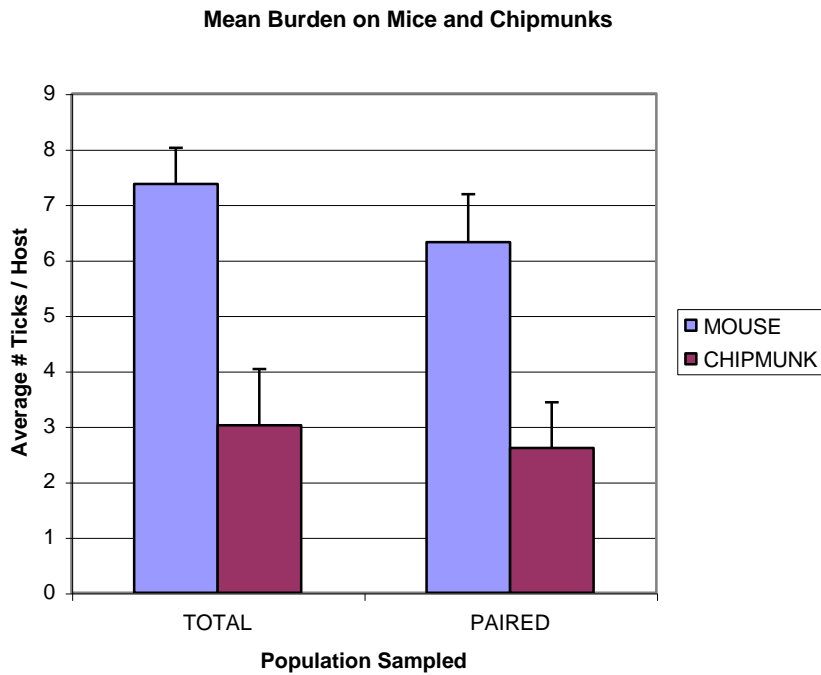


FIGURE 7. Mean burden of larval ticks on white-footed mice and eastern chipmunks collected from 1997-1999 over 3 control grids.

TABLE 1. Result from each individual orientation trial result. The first column represents the trial number followed by the host chosen by the individual ticks (mouse, chipmunk, no choice; did not travel farther than 2cm) and the end result of that trial, whether a choice for mouse, chipmunk, or no choice made.

2-8 cm Distance				
Trial	Mouse	Chipmunk	No Choice	Host Chosen or Neither
1	8	4	13	Neither
2	10	8	7	Neither
3	9	6	10	Neither
4	10	8	7	Neither
5	6	11	8	Neither
6	7	8	10	Neither
7	13	7	5	Neither
8	8	9	8	Neither
9	5	3	17	Neither
10	3	6	16	Neither
11	10	3	12	Mouse
12	11	2	12	Mouse
13	10	5	10	Mouse
14	9	4	12	Mouse
15	11	4	10	Mouse
16	9	4	12	Mouse
17	9	4	12	Mouse
18	12	3	10	Mouse
19	12	5	8	Mouse
20	9	4	12	Mouse

TABLE 2. Data used for the analysis of habitat use and its effect on larval burdens. The first column represents the site sampled, next the year, and finally the ratio of larval burdens on mice divided by chipmunk larval burdens. The average ratio of mouse larval burdens divided by chipmunk larval burdens is at the end of each table along with the calculated standard deviation.

TOTAL POPULATION		
Site	Year	Ratio (Mice/ Chipmunks)
GCG	1997	4.629542
GCG	1998	4.727964
GCG	1999	4.299037
RCG	1997	4.564375
RCG	1998	4.178791
RCG	1999	4.087443
YCG	1997	2.661706
YCG	1998	1.028413
YCG	1999	1.602701

PAIRED POPULATION		
Site	Year	Ratio (Mice/ Chipmunks)
GCG	1997	2.084898
GCG	1998	4.071481
GCG	1999	2.458694
RCG	1997	10.2
RCG	1998	13.5
RCG	1999	1.469636
YCG	1997	3.452055
YCG	1998	4.51458
YCG	1999	1.002673