

FACTORS INFLUENCING THE DISTRIBUTION OF LARVAL BLACKLEGGED TICKS ON RODENT HOSTS

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Abstract. Because of differences among hosts in reservoir competence for tick-borne diseases, the distribution of larval blacklegged ticks on hosts might determine tick infection prevalence and disease risk to humans. We conducted a three-part study to determine the factors responsible for greater burdens of larval blacklegged ticks on white-footed mice than on eastern chipmunks. A microhabitat study indicated that questing ticks have higher encounter rates with mice than with chipmunks. Laboratory experiments demonstrated that ticks oriented more strongly toward mice. However, larval ticks fed more successfully from chipmunks. Our results strongly suggest that mice are both more likely to use larval tick-infested microhabitats and to attract questing larvae than are chipmunks, leading to a dramatically higher initial infestation rate, which is then reduced by greater grooming activity by mice. The high mortality rate of larvae that were experimentally introduced onto mice suggests that grooming is a significant cause of mortality to larval blacklegged ticks.

INTRODUCTION

In recent decades, tick-borne infections have been emerging or expanding their geographic ranges and are increasingly recognized as a threat to human health worldwide. Many of the pathogens that cause tick-borne diseases undergo an enzootic cycle of transmission between ticks and their vertebrate hosts. For Lyme disease, human granulocytic ehrlichiosis, and babesiosis, which are transmitted by *Ixodes ricinus* complex ticks, transovarial transmission of the pathogens is highly inefficient, leading to negligible infection levels in larval cohorts.^{1–3} Therefore, each generation of ticks acquires infection via blood meals from infected vertebrate hosts. Larvae that acquire an infection during their single blood meal maintain the infection transstadially, allowing them to transmit pathogens during subsequent blood meals. Infection prevalence in post-larval stadia (particularly nymphs) is a key determinant of the risk of human exposure to these zoonoses.⁴ Interactions between tick populations and the community of vertebrate hosts are crucial in determining infection prevalence and therefore risk of exposure.

In eastern and central North America, Lyme disease (etiologic agent = *Borrelia burgdorferi*), which is transmitted predominantly by nymphal blacklegged ticks (*I. scapularis*), is the most common vector-borne disease of humans.^{5,6} Larval blacklegged ticks have been collected from dozens of species of mammalian, avian, and reptilian species, and therefore they are widely considered to be extreme host generalists.^{5,7} In areas endemic for Lyme disease, larval ticks that feed to repletion on field-caught white-footed mice (*Peromyscus leucopus*) have a high (up to 90%) probability of becoming infected, whereas those that feed on other rodents (e.g., eastern chipmunks [*Tamias striatus*] and tree squirrels [*Sciurus carolinensis*, *Tamiasciurus hudsonicus*, and *Glaucomys volans*]) have a lower (10–50%) probability of becoming infected, and those that feed on white-tailed deer (*Odocoileus virginianus*), mammalian carnivores, and some songbirds are unlikely ($\leq 10\%$ chance) to become infected (LoGiudice K and others, unpublished data). As a consequence, spatial and temporal variation in availability of different larval host species is thought to cause variation in infection prevalence in the tick population and therefore risk of human exposure to Lyme disease.^{8–10}

Such arguments typically assume that larval ticks feed non-selectively from all available hosts. Because larval ticks are weakly motile and therefore incapable of selecting hosts from a distance, successful host-finding requires that a host approaches within centimeters of the larva at its questing site, typically on the ground. If larval ticks fed entirely non-selectively, the distribution of larvae across the community of hosts would be a function of the relative encounter rates between larvae and the various host species. Encounter rates, in turn, would be functions of the relative abundance of each species of host, combined with its activity levels and patterns that might affect use of questing microhabitats. However, such a passive-encounter model of tick-host interactions has not been verified. In fact, despite the strong epidemiologic relevance of the distribution of larval meals across the community of potential vertebrate hosts, the factors responsible for determining tick burdens across the community of hosts are poorly understood.

Several factors could undermine the passive-encounter model of tick distributions among hosts. First, ticks could actively orient towards some hosts, and weakly or not at all towards others, which approach within detection distance.¹¹ Second, once on a host, ticks could more readily reject and drop off some host species than others. Third, species-specific grooming behavior by hosts might result in larval ticks having a poorer success rate in feeding from some host species than others, despite equivalent encounter rates.¹¹ Fourth, ticks could orient towards particular microhabitat features, introducing a bias in their probability of encountering particular hosts.

In this study, we assessed the potential for each of these factors to influence the distribution of larval blacklegged ticks on hosts. We focused our attention on two of the most commonly parasitized, widespread, and epidemiologically important tick hosts: white-footed mice and eastern chipmunks. These two rodent species are sympatric throughout much of North America and overlap extensively in microhabitat and macrohabitat preferences, diet, and behavior. Both are repeatedly noted as among the most heavily parasitized tick hosts within areas endemic for Lyme disease.^{12–15} Prior studies at our site demonstrate that populations of both species fluctuate in parallel, largely as a consequence of the autumnal acorn (genus *Quercus*) crop, with white-footed mice main-

taining population densities about twice that of chipmunks.¹⁶ Averaging among years, larval burdens on mice are approximately three times higher than those on chipmunks.¹⁶ We assessed whether heavier larval burdens on mice than on chipmunks were caused by 1) microhabitat segregation between mice and chipmunks that results in greater exposure by mice to questing larvae; 2) preferential orientation by larvae toward mice; and/or 3) more efficient grooming by chipmunks than by mice. Because reservoir competence of mice is approximately double that of chipmunks at our site (LoGiudice K and others, unpublished data), the determinants of larval burdens on these two species should be important influences on the risk of Lyme disease to humans.

MATERIALS AND METHODS

General approach. We conducted a three-part study to assess the mechanism(s) that underlie the greater larval tick burdens on white-footed mice than on eastern chipmunks at our sites. The initial component, patterned after analyses by Schmidt and others,¹⁶ assessed whether microhabitat segregation by mice and chipmunks is correlated with the degree of divergence in tick burdens on these two host species. If so, a passive encounter model (i.e., more ticks on mice than on chipmunks because of higher encounter rates with mice) would be supported. The second component assessed the degree to which larval ticks given a choice between a mouse and a chipmunk oriented toward one host species. In the third component, we determined the fates of larval ticks that we introduced onto captive hosts of both species. Integration of the three sets of results would allow us to infer the key processes that cause differences in larval burdens on these hosts. All experiments were conducted on the property of the Institute of Ecosystem Studies (IES) in Dutchess County in southeastern New York (41°50'N, 73°45'W). The maintenance and care of experimental animals complied with the National Institutes of Health guidelines for the humane use of laboratory animals.

Microhabitat segregation assessment. This experiment used data from a small-mammal monitoring program conducted on three 2.25-hectare trapping grids at IES from 1997 to 1999, during which rodent density was moderate to high. Trapping grids consisted of an 11 × 11 point array of trap stations, with two Sherman live traps (Sherman Traps, Inc., Tallahassee, FL) per station (242 traps per grid), and 15 meters between trap stations. Traps were baited with whole oats, provided with cotton batting for insulation during cold weather, and covered with a plywood board for protection against rain and sun. They were set between 4:00 PM and 6:00 PM, checked between 8:00 AM and 11:00 AM the following morning, and closed during the middle of the day. Each year, live-trapping was conducted for 2–3 consecutive days at least once per month from May through November. All captured rodents were transferred from the trap to a plastic bag, removed from the bag by hand, and given a uniquely numbered metal eartag for identification at first capture. No anesthesia was used. At all captures, we recorded species identity, tag number, gender, reproductive condition, body mass, and trap station, and then released animals at the point of capture. On the first capture of each individual during each trapping session, we counted the number of larval ticks attached to the ears, head, and neck of the host, which are the preferred sites of attach-

ment.¹⁶ Previous research at the same sites demonstrated that *I. scapularis* is the only species of larval tick found on these hosts at this time of year (Ostfeld R, unpublished data). Direct counts of larval ticks attached to live-trapped rodents underestimate true larval burdens by 10–30% (Ostfeld R, unpublished data).¹⁶ However, because our approach was to compare the difference in tick burdens between mice and chipmunks that overlapped in microhabitat use versus those that did not overlap, this underestimate does not introduce a bias into our analyses. We restricted our analyses to trap sessions from June to September each year, a period that corresponds to the questing activity period of larval black-legged ticks at our sites.¹⁷

To assess the effects of microhabitat use on host tick burdens, we first calculated the average larval burden on each host species at each of the three trapping grids in each of the three years. This provided population-level means for all mice and chipmunks irrespective of the locations where they were captured. We then calculated these same values, but restricted the analysis to individual mice and chipmunks (heterospecific pairs) that were captured at the same trap station during the same week. (When >1 individual of either species was captured, we used only the first individual caught.) This criterion restricted the comparison of host-specific parasite loads to individuals of each species that overlapped with an individual of the other species in space and time, i.e., that used the same microhabitats. Finally, we determined tick burdens on mice and chipmunks that did not overlap with a heterospecific (i.e., non-paired animals). If the observed heavier larval burdens on mice are caused by greater overlap between mice and questing larvae than between chipmunks and questing larvae, we expected the ratio of larvae on mice to larvae on chipmunks to be lower when comparing only mice and chipmunks that co-occurred at trap stations to those that did not co-occur. To test this hypothesis, we generated for each grid and year, ratios of larvae on mice to larvae on chipmunks for both the subset of mice and chipmunks that were captured at the same location and the remaining unpaired mice and chipmunks. Because of a small sample size of grids (three) and years (three), we could not verify normality of the data; therefore, we subjected these ratios to a nonparametric Mann-Whitney U test.

Host preference experiment. Both the host preference and host grooming experiments were conducted in the Animal Rearing Facility at IES in the summer of 2001. White-footed mice and eastern chipmunks used in both 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 to prevent separation of pregnant or lactating females from their dependent young. After the experiments, all animals were released at point of capture. Individual rodents were used only once in any given experiment. Ticks were collected from IES property using a 1-meter² white corduroy drag cloth.¹⁸ All ticks were maintained in glass vials (~20 mL) with nylon mesh and polypropylene tops in a cooling unit at ~2.5–4.0°C and a relative humidity of ~85% before use. The photophase was 14:10 (light:dark) for hosts and ticks during all experiments.

Because juvenile ticks require high relative humidity, the experiment was conducted within an environmentally-controlled enclosure (91.4 cm × 33 cm × 30.5 cm) constructed of 0.64 cm thick Plexiglas (Figure 1A). A saturated salt solu-

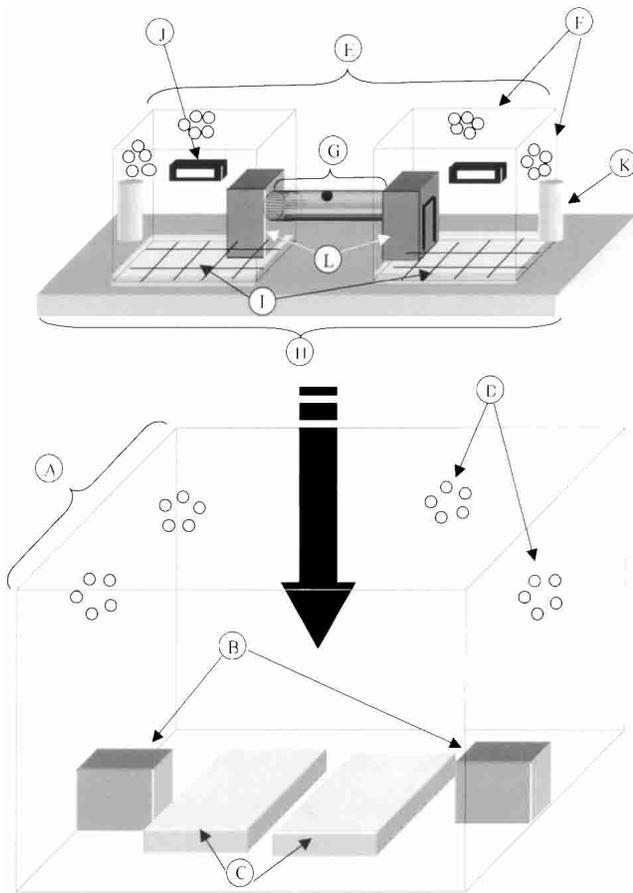


FIGURE 1. Diagram of the orientation experiment apparatus showing the humidity- and temperature-regulated chamber with the following features: **A**, Outer plexiglas chamber; **B**, Saturated salt solution ($K_2SO_4 + H_2O$) to regulate humidity; **C**, Pans of water for humidity and waste collection; **D**, Air vents; **E**, Plexiglas cages for holding animals; **F**, Inner vents; **G**, Tube for tick addition connected to both cages; **H**, Base of inner apparatus made of pressure treated wood; **I**, Hardware cloth base for underneath inside cages; **J**, Humidity gauges; **K**, Water bottles; **L**, Hardware cloth and aluminum foil nest boxes.

tion (Figure 1B) along with aluminum pans (Figure 1C) of water within the chamber were used to maintain an average relative humidity of 85% within the apparatus throughout all experiments. Secured on the farthest ends of the enclosure were two 750-mL tubs (Figure 1B) filled with a saturated salt solution consisting of 330 g of K_2SO_4 and 100 mL of water to generate constant, high humidity within the chamber.¹⁹ Air vents were established to allow air circulation and even temperature within the apparatus and sustain the animals comfortably. Four air vents (Figure 1D), each consisting of clusters of five 0.64 cm-diameter holes located on the lid and two farthest walls of the chamber ventilated the enclosure. The interior of the chamber was maintained at $22 \pm 2^\circ C$ at all times.

The apparatus that housed hosts and ticks was nested within the humidified chamber. The inner experimental apparatus consisted of two Plexiglas cages (Figure 1E) (21.5 cm \times 21.5 cm \times 15.0 cm) with five 0.64 cm-diameter holes (Figure 1F) in the lid and farthest walls of the two cages, which provided additional air circulation between the inner cages and the humidified chamber. The two cages were connected by a

2.54 cm-diameter, 16 cm-long plastic tube (Figure 1G), in which larval ticks were placed at the beginning of each orientation experiment. The connecting tube had a 0.64 cm-diameter hole placed equidistant from both cages that was used for the addition of ticks into the center of the tube. The base under the two Plexiglas cages was constructed of hardware cloth (Figure 1I), which permitted the cages to be suspended over water basins, allowing for waste and tick collection. Two humidity gauges (Figure 1J) were placed at opposite ends of both cages. A water bottle (Figure 1K) 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 Plexiglas cages and were supplied with equal amounts of *ad libitum* food. To saturate the chamber with host odor, the animals were held for one hour in the apparatus before adding ticks. A hardware cloth nest box covered in aluminum foil (Figure 1L), which was replaced at the beginning of every experiment, was placed over the opening of the connecting tube in both Plexiglas cages to further concentrate host odor near the opening of the connecting tube.

At the start of each experiment, 25 larval blacklegged ticks were collected in 2 mL of water, placed on the tip of a fine bristle paintbrush (size 000), and added to the midpoint of the connecting tube between the two Plexiglas cages. To facilitate measurements of tick movements, the tube was marked every 2 cm from the central point. To prevent scent contamination from one trial to the next, a new 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 lining were covered with a sticky paste (Tanglefoot®; The Tanglefoot Company, Grand Rapids, MI) applied with a plastic syringe. After the placement of the ticks in the tube, the apparatus was undisturbed for three hours, during which ticks were free to move within the central tube, and after which the direction and 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. After each trial, the entire chamber and inner apparatus were washed with 50% ethanol and allowed to stand for a minimum of two hours.

Because we could not be certain that individual ticks were orienting in the tube independently of all other ticks in each trial, we considered each group of 25 ticks in each of the 20 trials to be our sampling units. We established two criteria *a priori* to assess whether the ticks in each trial had made a choice. To meet the first criterion (population orientation), at least half of the 25 ticks per trial had to travel ≥ 2 cm from the central point of the connecting tube. Trials not meeting this criterion were eliminated from analysis. To meet the second criterion (population choice for a host), at least twice the number of ticks moving past the 2-cm mark had to orient toward one of the two hosts. A chi-square analysis with Yates' correction 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 at $22 \pm 2^\circ C$ and a relative humidity of 75–80% throughout the experiment. 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. To be considered free of ticks, ≥ 24 hours had to elapse during which no ticks were collected from the water basins. After being used in the host preference experiment, mice and chipmunks were placed in wire mesh cages (30.5 cm \times 10.2 cm \times 10.2 cm) with 0.64-cm mesh and suspended 10 cm above a water basin. Grooming by captive animals was not restricted. The animals were supplied with water and food *ad libitum* throughout the experiment.

Ticks to be added to each host were collected in 2 mL of water and then added manually to each host's back and neck with a fine bristle brush (000 mm) and 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. The water below the cages was checked one 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 was noted as they were recovered. Dead larvae with broken exoskeletons or that were otherwise not intact were considered to have been chewed by the host. 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 1) fed to repletion; 2) were killed and damaged (chewed); and 3) were never retrieved and assumed to have been consumed, was compared between host species using a multivariate analysis of variance (MANOVA).

RESULTS

Microhabitat segregation assessment. During the three years of the field study, the total mean \pm SE burden of larval ticks per host was 11.31 ± 1.34 for mice and 3.67 ± 1.18 for chipmunks. For mice and chipmunks that were paired because they were trapped at the same station during the same session, the mean \pm SE larval burden was 7.66 ± 1.59 for mice and 5.02 ± 2.03 for chipmunks (Figure 2). Larval tick burdens

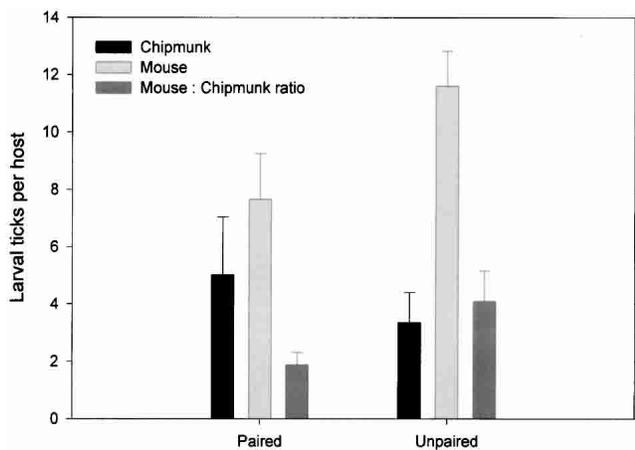


FIGURE 2. Mean \pm SE larval burdens for white-footed mice and eastern chipmunks on six forested plots in southeastern New York. Paired bars represent mice and chipmunks that were captured at the same trapping station during the same 2- or 3-day trapping session, and therefore overlapped in microhabitat use. Unpaired bars represent all other mice and chipmunks on the trapping grid. Also shown is the ratio of larvae on mice to larvae on chipmunks for the two categories.

for mice and chipmunks, respectively, that were not paired with a heterospecific were 11.60 ± 1.22 and 3.36 ± 1.04 . For individual trapping grids and years, ratios of larval burdens on mice to those on chipmunks varied from 1.07 to 6.18. The ratio of tick burdens on mice versus burdens on chipmunks for unpaired captures (mean \pm SE ratio = 4.09 ± 1.07) was more than twice as high as the ratio of tick burdens on heterospecific pairs that occupied the same trap station (mean \pm SE ratio = 1.88 ± 0.43 ; Figure 2). The difference in ratios between paired and unpaired animals was statistically significant (chi-square approximation of Mann-Whitney U statistic = 3.86, degrees of freedom [df] = 1, $P = 0.05$).

Orientation experiment. Of the 268 individual larval ticks that moved beyond the 2-cm criterion distance from the midpoint, 181 (62%) oriented toward a mouse. In 10 of the 20 trials a choice was made by the population of 25 ticks, as indicated by satisfying both criteria, namely that at least half the ticks moved beyond the 2-cm distance, and that more than twice as many oriented toward one of the two hosts available. In all 10 of these trials, orientation was toward mice ($\chi^2 = 8.333$, df = 1, $P = 0.004$).

Grooming efficiency experiment. In general, larval ticks fed more successfully on chipmunks than on mice. The mean \pm SE number of ticks that fed to repletion was higher on chipmunks (10.2 ± 1.7) than mice (2.8 ± 1.1). The number of ticks groomed off and either ingested or chewed, resulting in death during grooming, was similar between the two hosts (mean \pm SE = 25.3 ± 2.0 for mice, mean \pm SE = 23.5 ± 2.1 for chipmunks; Figure 3). On average, 21.9 ticks dropped off mouse hosts unfed but alive, versus 16.3 unfed ticks dropping off chipmunks (Figure 3). A MANOVA revealed a highly significant difference between mice and chipmunks in the fate of larval ticks (multivariate test: $F_{3,16} = 10.09$, $P = 0.001$). Univariate F-tests revealed that chipmunks fed more larvae than mice did ($F_{1,18} = 14.20$, $P = 0.001$), mice chewed more larvae than chipmunks did ($F_{1,18} = 20.930$, $P = 0.001$), but the hosts did not differ in the number of larvae ingested ($F_{1,18} = 0.513$, $P = 0.48$).

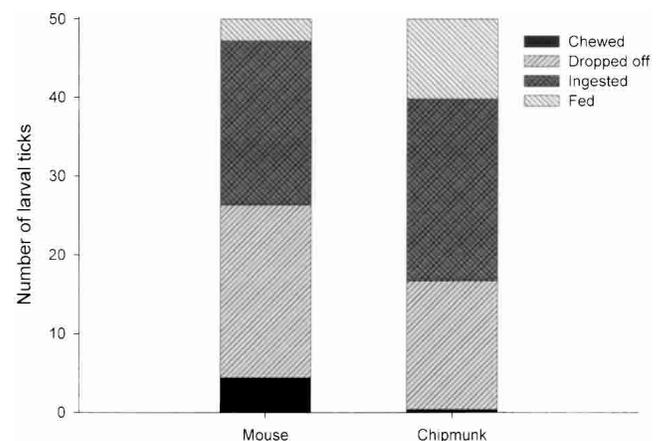


FIGURE 3. Disposition of field-collected larval ticks placed on captive white-footed mice or eastern chipmunks in the laboratory and later collected from water pans placed below the hosts. Chewed ticks had broken exoskeletons; dropped off were neither damaged nor replete; fed were replete; ingested comprised the remainder of the ticks.

DISCUSSION

White-footed mice and eastern chipmunks are both ubiquitous inhabitants of forests within Lyme-disease endemic areas, and the two species overlap extensively in both habitat and trophic niches.^{20,21} Population density of both species tends to fluctuate, and often densities of mice are approximately twice that of chipmunks.^{16,17,22} Despite the ecologic similarities between these two hosts, burdens of larval black-legged ticks typically are considerably higher on mice.^{12–16} Although it is tempting to conclude that differences among hosts in tick burdens represent host preferences by the ticks,^{23,24} factors other than preferences by the ticks may be important.

The number of ticks attached to hosts is a function of 1) entry by the potential host to within the perceptual range of questing ticks, which we term encounter rate; 2) orientation by ticks toward the approaching host; and 3) interactions between tick and host while in contact, for example, acceptance or rejection of the host by the tick and grooming by host. We assessed all three phenomena, directly or indirectly, to determine the mechanisms that underlie the observed greater larval burdens on mice than chipmunks.

Accurate assessment of encounter rates between hosts and ticks is extremely difficult. Our approach was to divide mouse and chipmunk populations into individuals that we knew overlapped with heterospecifics and those that did not, and to test the hypothesis that overlapping pairs would show greater similarity in larval burdens than non-overlapping pairs. In fact, we found that unpaired mice had the highest larval burdens and unpaired chipmunks had the lowest larval burdens, suggesting that microhabitat segregation by these species promotes greater tick burdens on mice. However, larval burdens converged when analyses were limited to those individuals that overlapped with a heterospecific. Because the ratio of ticks on mice versus ticks on chipmunks was significantly lower for overlapping pairs than for non-overlapping individuals, our hypothesis was supported. The observation that mouse-chipmunk pairs that overlapped in time and space had larval burden ratios less than half those of the remaining non-paired mice and chipmunks suggests that different encounter rates play an important role in explaining species differences in larval burdens.

This approach clearly has limitations. For example, mice and chipmunks use space in different ways, and this could affect rates of encounter with larval ticks. Mice are nocturnal whereas chipmunks are diurnal; any diel patterns of host-seeking activity by larval ticks could cause a bias in host-specific encounter rate. Due to their smaller size and home ranges,²⁵ mice probably use space in a more fine-grained way, resulting in more thorough coverage of smaller areas, and this could bias encounter rates with ticks in unpredictable ways. In addition, trap station overlap indicates that members of the two host species overlapped at least at one point within a 2–3-day period, but no information is available concerning the amount of home range and microhabitat overlap that actually occurs over finer spatial and temporal scales.

Larval ticks clearly oriented towards mice when presented with a choice. Our host-choice apparatus was designed to maximize the ability of ticks to perceive distinct cues from two opposite directions. Orientation toward mice probably was not due simply to greater quantity of stimulus (e.g., CO₂,

or infrared radiation), because chipmunks are approximately four times the mass of mice and have similar mass-specific metabolic rates.²⁵ Therefore, we presume chipmunks were emitting more CO₂ and heat than were mice. Similar results were obtained by James and Oliver;¹¹ larval *Ixodes* ticks oriented preferentially toward laboratory mice when given a choice between a mouse and a chicken or between a mouse and a lizard, but not necessarily toward the biggest host.

Once on a host, however, larval ticks clearly fed to repletion more successfully on chipmunks than on mice. This surprised us since mice are often thought to be among the highest-quality host for larval blacklegged ticks.³ Moreover, we would expect ticks to orient toward a host on which they have a high probability of feeding successfully. Our results contrast with those of James and Oliver,¹¹ who found concordance between host orientation and feeding success by larval *I. scapularis* that were exposed to laboratory mice, chickens, and lizards. It remains possible that grooming behavior by hosts was affected by the laboratory situation, and that the captive situation affected chipmunks more strongly than mice, reducing the ability of the former to remove ticks. Clearly, time-energy budgets of the rodents could be affected strongly by captivity such that time spent grooming is enhanced or grooming efficiency is reduced.

Poorer feeding success of larvae on mice than on chipmunks could have been caused by a stronger degree of anti-tick host immunity in the former.²⁶ Because all hosts of both species were adults, and all were known to have been infested previously with larval *I. scapularis*, the host species had similar prior experience with tick salivary antigens. However, it remains possible that mice develop a stronger or faster anti-tick immunity than do chipmunks, owing to their greater encounter rates with and stronger attractiveness to ticks. This potentially greater anti-tick immunity in mice would be expected to stimulate grooming, thereby contributing to the reduced feeding success by larvae on mice.

Taken together, the results of our three-part study suggest that 1) microhabitat segregation by mice and chipmunks results in greater encounter rates between larvae and mice than between larvae and chipmunks; 2) larvae orient much more strongly to approaching mice than to chipmunks, resulting in a strong difference in initial tick burden; but 3) greater grooming efficiency by mice reduces this bias somewhat. Our results strongly suggest that mice are both more likely to use larval tick-infested microhabitats and to attract questing larvae than are chipmunks, leading to a dramatically higher initial infestation rate, which is then reduced by greater grooming activity by mice. The high mortality rate of larvae that were experimentally introduced onto mice suggests that grooming is a significant cause of mortality to larval black-legged ticks. Further studies will be necessary for quantitative assessment of the net effects of the species-specific bias in encounter, orientation, and grooming in determining ectoparasite burdens across hosts. Because larval ticks that feed to repletion from mice are approximately twice as likely to become infected with *B. burgdorferi*,²⁷ the distribution of ticks on these two common hosts is an important determinant of Lyme disease risk to humans.

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