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LARVAL DENSITY AND FEEDING SUCCESS OF *IXODES SCAPULARIS* ON TWO SPECIES OF *PEROMYSCUS*

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ABSTRACT: One potential mechanism for the regulation of tick populations is density-dependent feeding success on vertebrate hosts. In a series of laboratory experiments, we tested whether the density of larval *Ixodes scapularis* on the mice *Peromyscus maniculatus* and *Peromyscus leucopus* influenced tick feeding success. For both host species, the proportion of ticks feeding to repletion was constant (approximately 40–50%) over a range of infestation from 5 to 100 ticks per mouse. For *P. leucopus,* neither mass nor molting success of fed ticks was significantly related to tick density on the host. However, for *P. maniculatus,* we observed a statistically significant increase in molting success with increasing tick density on hosts, thus demonstrating facilitation rather than density-dependent regulation. Although results were not statistically significant, we observed a tendency for previously exposed *P. leucopus* to support lower tick feeding success than naive mice; however, even for previously exposed mice, tick feeding success was not density dependent. Our results do not support the notion that density-dependent feeding on hosts regulates density of *I. scapularis* populations at the numbers tested.

Lyme disease is a vector-borne zoonotic disease that is prevalent in North America, Europe, and Asia. It is caused by spirochetes in the genus *Borrelia,* usually *Borrelia burgdorferi* (Burgdorfer et al., 1982; Johnson et al., 1984), which are transmitted to humans by several tick vectors. In the northeastern United States, the principal vector of *B. burgdorferi* is the tick *Ixodes scapularis* (Fish, 1993; formerly *Ixodes dammini* [Oliver et al., 1993]). Although both adult and nymphal stages of *I. scapularis* may carry and transmit *B. burgdorferi,* nymphs are considered to be responsible for most human cases of Lyme disease (Piesman et al., 1987). For this reason, it is important to understand the mechanisms regulating population densities of nymphs in Lyme disease endemic areas.

The life cycle of the deer tick typically spans 2 yr, includes 4 developmental stages (egg, larva, nymph, adult), and requires 3 successful blood meals for completion (Fish, 1993). Host-seeking larvae feed on a wide variety of vertebrate hosts, including small mammals, birds, and reptiles. In the northeast, the white-footed mouse *Peromyscus leucopus* is recognized as the primary host of immature *I. scapularis* and has been shown to be a highly efficient reservoir of the Lyme disease spirochete (Levine et al., 1985; Spielman et al., 1985; Magnarelli et al., 1988; Mather et al., 1989). The closely related deer mouse *Peromyscus maniculatus* has received surprisingly little attention from Lyme disease researchers, despite its wide geographical range and ecological similarity with *P. leucopus.* However, its competency as a reservoir host has been convincingly demonstrated by Rand et al. (1993).

The abundance of nymphs in any given year is determined by 4 basic factors: (1) the abundance of larvae in the previous year; (2) the proportion of larvae able to find hosts; (3) the ability of larvae to successfully feed on these hosts; and (4) biotic and abiotic influences on survivorship and molting success of fed larvae. In this paper, we seek to determine whether feeding success of larval *I. scapularis* on a host is regulated in a density-dependent manner.

Numerous laboratory studies have demonstrated that host species differ in their ability to resist parasitism by ticks (Trager, 1939; Sonenshine and Atwood, 1967; Randolph, 1979; Davidar et al., 1989; James and Oliver, 1990; Galbe and Oliver, 1992). Differential feeding success may be a consequence of ticks evolving mechanisms to evade the specific immunological responses of the hosts with which they have been most commonly and continuously associated over time (Sonenshine, 1991, 1993). However, little research has been conducted to establish whether or not the density of ticks feeding on a host is a significant predictor of feeding success. Mortality of ticks (*Boophilus microplus*) feeding on cattle has been shown to be density-dependent on previously exposed hosts (Sutherst et al., 1973, 1979) but not on naive hosts (Sutherst et al., 1978). Sonenshine and Atwood (1967) experimentally infested meadow voles, white-footed mice, and Norway rats with densities ranging from 1 to 100 ticks (*Dermacentor variabilis*) per host and found no effect of infestation level on the proportion fed. Davidar et al. (1989) examined ticks removed from field-caught *P. leucopus* and found that the engorgement size of ticks increased with increasing feeding density, which ranged from 1 to 35 ticks per host. Thorough examinations of the possibility of density-dependent population regulation of *I. scapularis* at the larval feeding stage are lacking in our current understanding of the ecology of Lyme disease.

MATERIALS AND METHODS

We conducted a series of 3 experiments to determine whether the density of larval ticks feeding on 2 rodent hosts affects the feeding success of *I. scapularis.* We infested naive and previously exposed *P. leucopus* and naive *P. maniculatus* with varying numbers of larval ticks, and measured the feeding success of ticks in terms of (1) the proportion of infesting ticks feeding to repletion; (2) the average weight of engorged ticks; and (3) the proportion of fully engorged larvae molting to the nymphal stage.

Experimental subjects

Wild larval ticks (*I. scapularis*) were collected in mid-August 1994, using standard cloth drags in tick-infested maple woods on the property of the Institute of Ecosystem Studies (IES) in Millbrook, New York. Captive-reared larval ticks were obtained in early September 1994 from a colony maintained by Dr. Thomas Mather at the University of Rhode Island. Ticks were stored in glass vials capped with organdy cloth, which were held over a supersaturated solution of potassium phosphate in a plastic storage container to maintain high humidity (Winston and Bates,

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1960). The unfed ticks were kept in an incubator set to 21–23 C with a 16L:8D photoperiod.

Deer mice (*Peromyscus maniculatus bairdii*) and white-footed mice (*P. leucopus*) were captive-reared, adult males obtained from the *Peromyscus* Stock Center at the University of South Carolina. They had no prior exposure to ticks. Throughout the course of the series of experiments, mouse hosts were kept in a ventilated room at ~25 C, with a 14L:10D photoperiod. Except for 24 hr of confinement in PVC containers directly following infestation, mice were always supplied with laboratory chow and water, along with an occasional piece of apple or carrot. When not in use for experimentation, mice were held in groups of no more than 15 individuals in glass tanks lined with cedar shavings and containing cotton for bedding.

General procedures

In each of the 3 experiments, mouse hosts were assigned to 1 of 5 treatment groups, for a single infestation of 5, 10, 25, 50, or 100 larval ticks. Although individual *P. leucopus* may occasionally host > 100 ticks, this range in tick densities reflects natural infestation rates within Lyme disease endemic areas. To infest a mouse, 1 investigator held it by the scruff of the neck while another gently applied the prescribed number of ticks to the head and belly of the mouse using a soft camel hair brush (size 0001) or fine forceps. The mouse was then immediately placed in a piece of PVC pipe (10 cm long × 4 cm diameter) capped at both ends with 0.6 cm-mesh hardware cloth. The ends were covered with organdy cloth held in place with elastic bands in order to prevent ticks from escaping. The mouse remained in the PVC container for 24 hr, supplied with 3 squares of laboratory chow and a piece of apple or carrot.

After 24 hr, each infested mouse was released from its PVC container into a cage (29 cm × 10 cm × 9 cm) constructed of 0.6 cm-mesh hardware cloth. Each cage was suspended over a plastic dishpan containing water. Pans were checked daily for ticks dropping from hosts. All ticks from a pan were removed with a camel-hair brush, gently blotted dry on a Kim-wipe®, and placed together in a labeled glass vial containing a water-saturated, solid plaster-of-paris base. The water in the pan was then replaced. Pans were checked every day for a minimum of 3 days and a maximum of 8 days. If no ticks were recovered from a mouse during 2 successive checks, or if all infesting ticks were accounted for, the mouse was returned to a holding tank.

Ticks collected from pans were weighed to 0.0001 mg on an analytical balance, after which all fully engorged ticks were returned to glass vials. The vials were capped with organdy cloth, then placed in an incubator at 21–23 C with a 16L:8D photoperiod. Vials were checked at least once every 2 wk for molting ticks. After every check, water was added to the vial to saturate the plaster of paris base. Vial checks were continued until all ticks in a vial had died or molted, or until 8 wk had elapsed since their collection. Molting success was calculated as the percentage of fully engorged larvae that molted to the nymphal stage.

Experiment 1: Tick density and feeding success on naive *P. maniculatus bairdii*

A total of 21 deer mice were infested with larval ticks, all of which were collected from the field at IES. Five mice were infested with 25 ticks each; 4 mice were assigned to each of the other treatment groups. All mice were infested between 1700 hr and 2100 hr on 21 or 22 August 1994. Engorged ticks were collected from pans every morning between 0800 hr and 1000 hr. Ticks were weighed beginning at 1000 hr the same day. Whenever possible, all ticks collected from each mouse were weighed. However, it was necessary in some cases to take a random subsample of 15 ticks.

Experiment 2: Tick density and feeding success on naive *P. leucopus*

Twenty-five naive white-footed mice were assigned to the same 5 experimental treatment groups. We infested mice in blocks of 5, each block containing 1 mouse from each treatment group. All mice in a block were infested between 1500 hr and 1700 hr on the same day. Blocks were infested between 10 and 26 September 1994. Blocks 1–3 were infested with colony-reared larvae, whereas Blocks 4–5 were infested with field-caught larvae. Engorged ticks were collected from pans between 0700 hr and 0900 hr each morning, and ticks were weighed between 0900 hr and 1300 hr the same day.

Experiment 3: Tick density and feeding success on naive and previously exposed *P. leucopus*

Fifteen white-footed mice, previously infested with 10–50 larvae during the course of Experiment 2, were randomly reassigned to new treatment groups. As before, we infested mice in blocks of 5. We first preexposed each mouse in a block with an infestation of 25 larvae. Eight days later, each mouse in the block was infested with 5, 10, 25, 50, or 100 larvae, and ticks were collected as in the previous experiment. These postexposure infestations were between 3 November and 6 December 1994. We infested 1 control block of 5 naive mice (1 mouse in each density treatment) on 12 November. All infestations took place between 1500 hr and 1700 hr. Ticks were collected from pans daily between 1400 hr and 1600 hr and were immediately weighed and stored as in previous experiments.

We compared the feeding success of ticks fed on three distinct groups of white-footed mice. We refer to the 25 naive mice initially infested in Experiment 2 as "initial naive mice." We refer to the 15 mice reinfested in this third experiment as "pre-exposed mice." We refer to the 5 naive mice infested during the same time frame as the pre-exposed mice as "control naive mice."

Statistical analyses

Our basic statistical approach was to employ stepwise polynomial regression analysis to find optimum regression models describing the relationships between tick densities and indices of feeding success. Unless otherwise indicated, a first-order regression provided the optimum fit and reported statistics refer to a linear model. We regressed (1) the proportion of infesting ticks that fed to full engorgement against the number of infesting ticks; (2) the average weight of engorged ticks against the total number engorged on a host; (3) the molting success of engorged ticks against the average engorged weight; and (4) the molting success of engorged ticks against the total number engorged on a host.

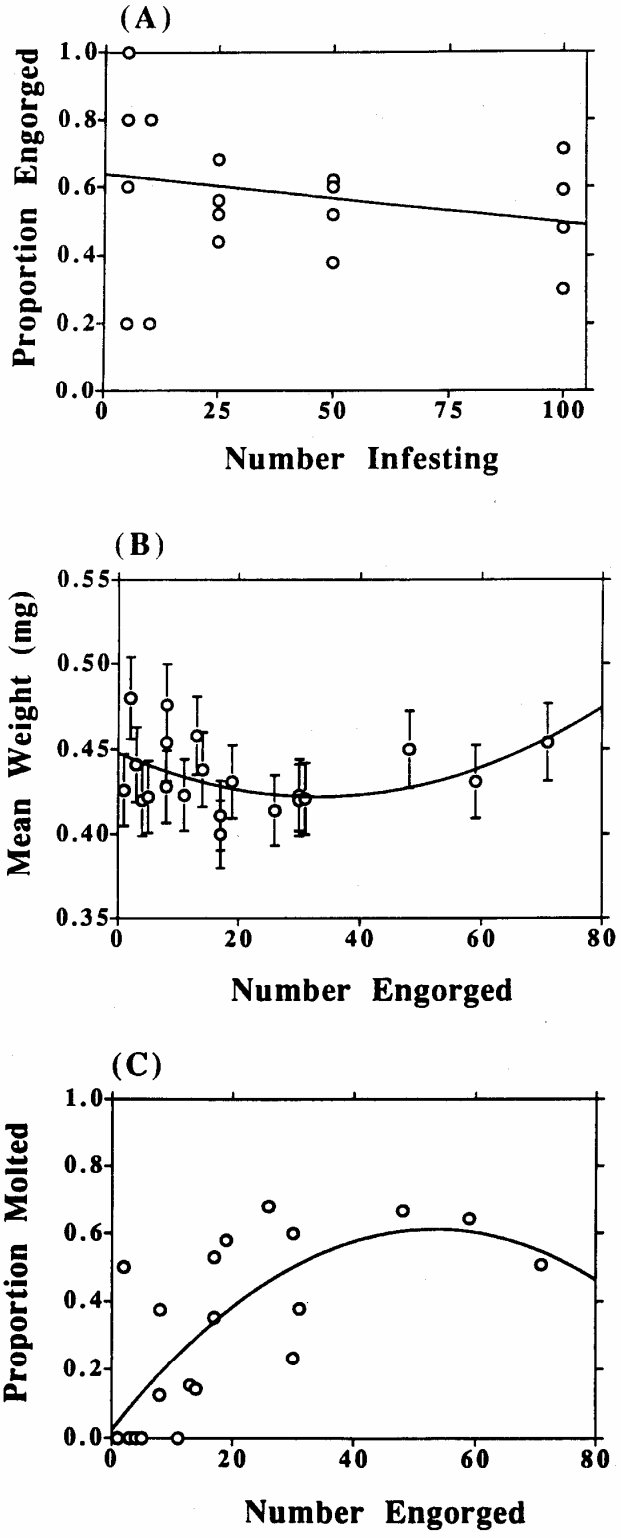
We employed analysis of covariance (ANCOVA) to (1) determine whether the origin of ticks (field-caught or laboratory-reared) that fed on *P. leucopus* was relevant to feeding success; and (2) compare feeding success of ticks fed on naive and pre-exposed *P. leucopus*. All proportions were transformed [$\arcsin(x^{1/2})$] before proceeding with analyses. We report means (±standard error) of untransformed values.

RESULTS

Experiment 1: Tick density and feeding success on naive *P. maniculatus bairdii*

The proportion of ticks that fed to repletion ranged from 0.2 to 1.0, but was unrelated to infestation level (Fig. 1A). The mean proportion of larvae feeding to repletion across all infestation levels was 0.53 (±0.05). The number of ticks engorging on a host did not affect the average weight of engorged ticks recovered from the host (Fig. 1B). Furthermore, the average weight of engorged ticks was not a predictor of molting success ($r^2 = 0.013$, df = 19, $P = 0.62$).

The molting success of ticks was positively related to the number of ticks that engorged on a host (Fig. 1C). To elucidate further the relationship between tick feeding density and molting success, we regressed the total number of molting ticks against the total number fed to repletion on a host. We did not transform values prior to this analysis because we were interested in the shape of the regression curve, which would have been altered by transformation. We found a highly significant curvilinear relationship (Fig. 2). Ticks feeding at very low densities had poor molting success. As feeding density increased, molting success improved sharply but began to level off at the highest feeding densities.



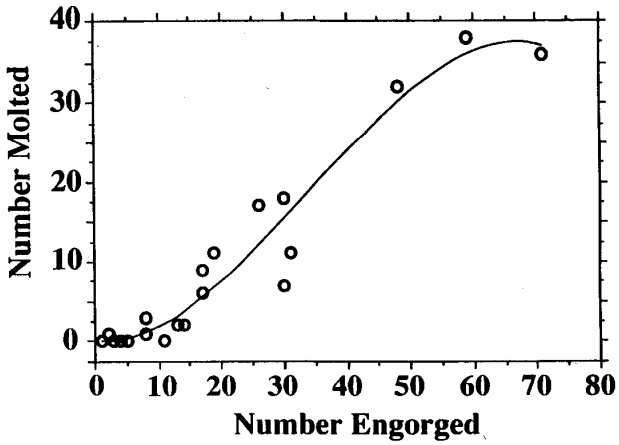


FIGURE 2. The number of molted *I. scapularis* ticks as a function of the number of engorged larvae fed on naive *P. maniculatus bairdii* hosts. Cubic regression, no-intercept model: $y = -0.00034x^3 + 0.034x^2 + 0.094x$, $r^2 = 0.543$, df = 18, $P < 0.001$.

Experiment 2: Tick density and feeding success on naive *P. leucopus*

The origin of ticks (colony-bred versus field-caught) infesting naive white-footed mice did not influence engorgement rates or molting success but had a significant impact on the average weight of engorged ticks (Fig. 3). In subsequent analyses, therefore, we lumped all engorgement and molting success data, irrespective of tick origin, but we did not include the weights of field-caught larvae.

The engorgement rate of ticks was independent of the number of ticks infesting a naive white-footed mouse (Fig. 3A). Overall, 0.37 (±0.04) of ticks fed to repletion at all levels of infestation. The average weight of ticks was not affected by the number of ticks engorged on a host (Fig. 3B) and was not a predictor of molting success ($r^2 = 0.113$, df = 12, $P = 0.24$). On average, 0.62 (±0.06) of engorged larvae molted, and molting success was independent of the number of larvae fed to repletion on a host (Fig. 3C).

Experiment 3: Tick density and feeding success on naive vs. previously exposed *P. leucopus*

Although indices of the feeding success of ticks on the 2 groups of naive mice did not differ significantly, a biological trend was evident (Fig. 4; see Materials and Methods for explanation of

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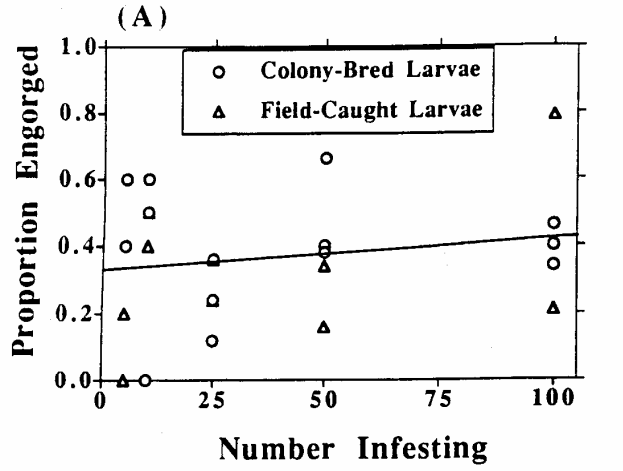
FIGURE 1. Indices of feeding success of *Ixodes scapularis* larvae as a function of tick density on naive *Peromyscus maniculatus bairdii*. Mean tick weights in (B) are shown with standard error bars. Proportions in (A) and (C) were transformed [$\arcsin(x^{1/2})$] prior to statistical analysis. Optimum-fit regression curves for untransformed data are shown. Results of stepwise polynomial regression analyses: (A) linear, $r^2 = 0.065$, df = 19, $P = 0.27$; (B) quadratic, $r^2 = 0.196$, df = 18, $P = 0.14$; (C) quadratic: $y = -0.00027x^2 + 0.027x - 0.061$, $r^2 = 0.543$, df = 18, $P < 0.001$.



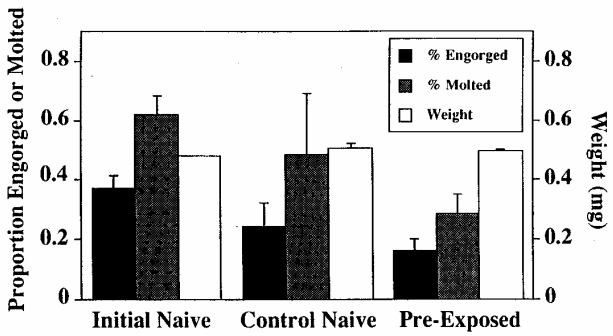


FIGURE 4. Feeding success of *I. scapularis* larvae on initial naive, control naive, and pre-exposed groups of *Peromyscus leucopus* (see Materials and Methods: Experiment 3 for explanation of groups). Bars show means ± standard errors of untransformed values. Results of ANCOVA of group effect on engorgement rates: $F_{1,39} = 2.12$, $P = 0.13$; on molting rates: $F_{1,33} = 3.96$, $P = 0.03$ (SNK pairwise comparisons not significant at $P = 0.05$); on mean engorged weight of colony-bred larvae: $F_{1,24} = 1.29$, $P = 0.29$.

groups). Of the ticks infesting the control naive mice, a smaller proportion fed to engorgement, and a smaller proportion of the engorged ticks molted to the nymphal stage than was the case for ticks feeding on the initial naive mice. In addition, ticks took longer to feed on the control group. Weights of engorged ticks, however, were comparable for both groups. We attributed the general decline in feeding success to the aging of larvae in storage, because the control naive mice were infested approximately 2 mo later than the initial naive mice, and we felt it was inappropriate to pool the results from the control naive mice with those of the initial naive mice. Comparing engorgement rates, weights, and molting success of ticks fed on control naive versus pre-exposed mice, we obtained no statistically significant results but again observed a biological trend: ticks fed less successfully on pre-exposed mice than on control naive mice, in terms of both engorgement rates and molting success (Fig. 4).

As with the naive mice infested in Experiment 2, there was no sign of density-dependent effects on ticks feeding on pre-exposed *P. leucopus*. The proportion of ticks engorging was unrelated to infestation level ($r^2 = 0.039$, df = 13, $P = 0.48$). Tick weights and molting success were independent of the number of ticks fed to repletion (weights: $r^2 = 0.002$, df = 10, $P = 0.90$; molting success: $r^2 = 0.136$, df = 10, $P = 0.24$).

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FIGURE 3. Indices of feeding success of *I. scapularis* larvae as a function of tick density on naive *Peromyscus leucopus*. (A, C) Tick origin (colony-bred versus field-caught) was not significant, so data were lumped; proportions were transformed [arcsin($x^{1/2}$)] prior to statistical analysis. (B) Mean weights are shown with standard errors; only data from colony-bred ticks were included in subsequent analyses because tick origin had a significant effect on weight (ANCOVA, $F_{1,19} = 29.57$, $P < 0.001$). Optimum-fit regression curves for untransformed data are shown. Results of stepwise polynomial regression analyses: (A) linear, $r^2 = 0.21$, df = 23, $P = 0.32$; (B) linear, $r^2 = 0.01$, df = 12, $P = 0.77$; (C) linear, $r^2 = 0.01$, df = 21, $P = 0.67$.

DISCUSSION

In this study, we showed that the molting success of larval *I. scapularis* was positively related to tick density on naive *P. maniculatus bairdii* hosts. We are not the first to have found evidence of ticks benefitting by feeding at high densities. Davidar et al. (1989) found that the engorgement index (=body length/scutum length) of larval *I. scapularis* increased with increasing numbers engorging on *P. leucopus*. Schorderet and Brossard (1993) showed that fecundity of female ticks was influenced by the magnitude of previous, recent infestations of their rabbit hosts. "High infestation" rabbits were given 2 successive infestations of 25 adult *Ixodes ricinus* pairs, whereas "low infestation" rabbits were infested twice with 5 pairs. Subsequently, both groups of rabbits were infested with 15 *I. ricinus* pairs. At this third infestation, low infestation rabbits became more resistant to ticks than high infestation rabbits, and the feeding and fecundity of ticks fed on the high infestation group were improved.

In contrast, other researchers have found an opposite pattern of decreasing feeding success with increasing tick density. Survival to maturity of larval *B. microplus* fed on cattle with acquired tick resistance decreased with increasing levels of infestation (Sutherst et al., 1973). Randolph (1994) showed that molting success of larval *Ixodes trianguliceps* decreased with increasing numbers of ticks attached to naive bank voles (*Clethrionomys glareolus*). In addition, engorgement rates and molting success decreased with increasing cumulative totals of ticks attached during previous infestations.

How can feeding success of ticks be positively influenced by tick density in some tick–host systems and negatively influenced in others? The outcome of a tick–host interaction is determined by 2 basic factors: (1) the vigor of the host's immune response to tick antigens, and (2) the ability of the tick to evade the host's immune response. Ixodid ticks do not feed continuously while attached to a host; fluid uptake is periodically interrupted by periods of salivation (Sonenshine, 1993). The roles of tick saliva and host immune response in the feeding process have frequently been reviewed and discussed (Kemp et al., 1982; Wikel and Allen, 1982; Ribeiro, 1987, 1989; Brown, 1988; Sonenshine, 1991). As a source of antigenic material, tick saliva can trigger immune responses by hosts, thereby diminishing feeding success and possibly resulting in outright rejection of the parasite. High densities of feeding ticks result in high levels of tick antigen being introduced into the host, which is likely to result in a heightened immune response by the host and lowered feeding success of ticks. On the other hand, tick feeding success may be promoted by salivary compounds that suppress host inflammatory responses and prevent hemostasis, so that density may have a positive effect on feeding success. In fact, the saliva of *I. scapularis* has been shown to contain anti-inflammatory and immunosuppressive agents (Ribeiro et al., 1985). At low feeding density, these salivary agents may be too dilute or localized to overcome the host's defenses; increased levels of immunosuppressants introduced into the host may facilitate feeding at higher tick densities (Davidar et al., 1989).

The feeding success of larval *I. scapularis* on naive *P. leucopus*, a natural host, was not regulated in a density-dependent manner. Engorgement rates of ticks were similar at all infestation levels, and tick weights and molting success were independent of the number of ticks engorging on a host. Likewise, Sutherst et al. (1978) found no effect of density on the survivorship of larval *B. microplus* feeding on naive cattle. Sonenshine and Atwood (1967) found no relationship between infestation levels and the proportion of larval and nymphal *D. variabilis* feeding on meadow voles, white-footed mice, or laboratory rats.

Although not statistically significant, our data suggest that larval *I. scapularis* fed less successfully on pre-exposed *P. leucopus* than on the control naive mice, in terms of both the proportion engorged and molting success. Davidar et al. (1989) found no significant differences in engorgement rates and molting success between *I. scapularis* fed on naive and pre-exposed white-footed mice. However, ticks fed on pre-exposed mice weighed significantly less than those fed on naive mice. After repeatedly infesting white-footed mice with *I. scapularis* larvae, Allan and Appel (1993) found that the percentage of engorged larvae decreased significantly from the initial to the third and fourth exposures but found no effect on weights of engorged ticks. It seems that white-footed mice may in fact develop some degree of resistance against *I. scapularis*. Other studies have indicated that acquired resistance in a pre-exposed host is associated with a density-dependent reduction in tick feeding success (Sutherst et al., 1973; Randolph, 1979, 1994). In the current study, we found no evidence that feeding success of ticks on pre-exposed mice is regulated in a density-dependent manner. However, because of the reduced vigor of ticks used to infest the pre-exposed mice in this study, we believe that further investigations are required to establish firmly whether or not acquired resistance, density dependence, or both, are important factors in the association between *I. scapularis* and *P. leucopus* and tick feeding success.

For both *P. maniculatus* and *P. leucopus*, considerable variation existed among individuals in tick feeding success (Figs. 1, 3). The causes of this variation are not clear but appear worthy of study because such variability affects both experimental design and, potentially, the dynamics of Lyme disease risk.

A thorough understanding of the interaction between a disease vector and its principal host is crucial to designing and implementing control strategies for the disease. The dynamics of the disease will be greatly affected by the nature of the tick-host interaction. If tick feeding success is negatively influenced by tick density on a host, then the tick population will be regulated in a density-dependent manner. If there is no such density-dependent mechanism, or if high densities actually facilitate tick feeding, tick populations will have potential for explosive population growth. In this study, we have shown evidence for facilitation of larval *I. scapularis* feeding at high densities on naive *P. maniculatus bairdii*. Whether this type of interaction occurs in forest subspecies of *P. maniculatus*, which may be significant Lyme disease reservoirs, is uncertain. We found no support for density-dependent regulation of *I. scapularis* populations by their principal host, *P. leucopus*.

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LITERATURE CITED

ALLAN, S. A., AND M. J. APPEL. 1993. Aquired resistance to *Ixodes dammini*: Comparison of hosts. *In* Host regulated developmental mechanisms in vector arthropods: Proceedings of the third symposium, D. Borovsky and A. Spielman (eds.). University of Florida–IFAS, Vero Beach, Florida, p. 255–262.

BROWN, S. J. 1988. Highlights of contemporary research on host immune responses to ticks. Veterinary Parasitology **28:** 321–334.

BURGDORFER, W., A. G. BARBOUR, S. F. HAYES, J. L. BENACH, E. GRUNWALDT, AND J. P. DAVIS. 1982. Lyme disease—A tick-borne spirochetosis? Science **216:** 1317–1319.

DAVIDAR, P., M. WILSON, AND J. M. C. RIBEIRO. 1989. Differential distribution of immature *Ixodes dammini* (Acari: Ixodidae) on rodent hosts. Journal of Parasitology **75:** 898–904.

FISH, D. 1993. Population ecology of *Ixodes dammini*. *In* Ecology and environmental management of Lyme disease, H. S. Ginsberg (ed.). Rutgers University Press, New Brunswick, New Jersey, p. 25–42.

GALBE, J., AND J. H. OLIVER. 1992. Immune response of lizards and rodents to larval *Ixodes scapularis* (Acari: Ixodidae). Journal of Medical Entomology **29:** 774–783.

JAMES, A. M., AND J. H. OLIVER. 1990. Feeding and host preference of immature *Ixodes dammini, I. scapularis,* and *I. pacificus* (Acari: Ixodidae). Journal of Medical Entomology **27:** 324–330.

JOHNSON, R. C., G. P. SCHMID, F. W. HYDE, A. G. STEIGERWALT, AND D. J. BRENNER. 1984. *Borrelia burgdorferi* sp. nov.: Etiologic agent of Lyme disease. International Journal of Systematic Bacteriology **34:** 496–497.

KEMP, D. H., B. F. STONE, AND K. C. BINNINGTON. 1982. Tick attachment and feeding: Role of the mouthparts, feeding apparatus, salivary gland secretions and the host response. *In* Physiology of ticks, F. D. Obenchain and R. Galun (eds.). Pergamon Press, New York, New York, p. 119–168.

LEVINE, J. F., M. L. WILSON, AND A. SPIELMAN. 1985. Mice as reservoirs of the Lyme disease spirochete. American Journal of Tropical Medicine and Hygiene **34:** 355–360.

MAGNARELLI, L. A., J. F. ANDERSON, K. E. HYLAND, D. FISH, AND J. B. MCANINCH. 1988. Serologic analyses of *Peromyscus leucopus,* a rodent reservoir for *Borrelia burgdorferi,* in northeastern United States. Journal of Clinical Microbiology **26:** 1138–1140.

MATHER, T. N., M. L. WILSON, S. I. MOORE, J. M. C. RIBEIRO, AND A. SPIELMAN. 1989. Comparing the relative potential of rodents as reservoirs of the Lyme disease spirochete (*Borrelia burgdorferi*). American Journal of Epidemiology **130:** 143–150.

OLIVER, J. H., M. R. OWSLEY, H. J. HUTCHESON, A. M. JAMES, C. CHEN, W. S. IRBY, E. M. DOTSON, AND D. K. MCLAIN. 1993. Conspecificity of the ticks *Ixodes scapularis* and *I. dammini* (Acari: Ixodidae). Journal of Medical Entomology **30:** 54–63.

PIESMAN, J., T. N. MATHER, G. J. DAMMIN, S. R. TELFORD III, C. C. LASTAVICA, AND A. SPIELMAN. 1987. Seasonal variation of transmission risk of Lyme disease and human babesiosis. American Journal of Epidemiology **126:** 1187–1189.

RAND, P. W., E. H. LACOMBE, R. P. SMITH, S. M. RICH, C. W. KILPATRICK, C. A. DRAGONI, AND D. CAPORALE. 1993. Competence of *Peromyscus maniculatus* (Rodentia, Cricetidae) as a reservoir host for *Borrelia burgdorferi* (Spirochaetares, Spirochaetaceae) in the wild. Journal of Medical Entomology **30:** 614–618.

RANDOLPH, S. E. 1979. Population regulation in ticks: The role of acquired resistance in natural and unnatural hosts. Parasitology **79:** 141–156.

———. 1994. Density-dependent acquired resistance to ticks in natural hosts, independent of concurrent infection with *Babesia microti.* Parasitology **108:** 413–419.

RIBEIRO, J. M. C. 1987. Role of saliva in blood-feeding by arthropods. Annual Review of Entomology **32:** 463–468.

———. 1989. Role of saliva in tick/host interactions. Experimental and Applied Acarology **7:** 15–20.

———, G. T. MAKOUL, J. LEVINE, D. R. ROBINSON, AND A. SPIELMAN. 1985. Antihemostatic, antiinflammatory, and immunosuppressive properties of the saliva of a tick, *Ixodes dammini.* Journal of Experimental Medicine **161:** 332–344.

SCHORDERET, S., AND M. BROSSARD. 1993. Changes in immunity to *Ixodes ricinus* by rabbits infested at different levels. Medical and Veterinary Entomology **7:** 186–192.

SONENSHINE, D. E. 1991. Biology of ticks, Vol. 1. Oxford University Press, New York, New York, 447 p.

———. 1993. Biology of ticks, Vol. 2. Oxford University Press, New York, New York, 465 p.

———, AND E. L. ATWOOD. 1967. Dynamics of feeding of the American dog tick, *Dermacentor variabilis* (Acarina: Ixodidae). Annals of the Entomological Society of America **60:** 362–373.

SPIELMAN, A., M. L. WILSON, J. E. LEVINE, AND J. PIESMAN. 1985. Ecology of *Ixodes dammini*-borne human babesiosis and Lyme disease. Annual Review of Entomology **30:** 439–460.

SUTHERST, R. W., K. B. W. UTECH, M. J. DALLWITZ, AND J. D. KERR. 1973. Intraspecific competition of *Boophilus microplus* (Canestrini) on cattle. Journal of Applied Ecology **10:** 855–862.

———, ———, J. D. KERR, AND R. H. WHARTON. 1979. Density dependent mortality of the tick, *Boophilus microplus,* on cattle—Further observations. Journal of Applied Ecology **16:** 397–403.

———, B. M. WAGLAND, AND J. A. ROBERTS. 1978. The effect of density on the survival of *Boophilus microplus* on previously unexposed cattle. International Journal for Parasitology **8:** 321–324.

TRAGER, W. 1939. Acquired immunity to ticks. Journal of Parasitology **25:** 57–81.

WIKEL, S. K., AND J. R. ALLEN. 1982. Immunological basis of host resistance to ticks. *In* Physiology of ticks, F. D. Obenchain and R. Galun (eds.). Pergamon Press, New York, New York, p. 169–196.

WINSTON, P. W., AND D. H. BATES. 1960. Saturated solutions for the control of humidity in biological research. Ecology **41:** 232–237.