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DENSITY-DEPENDENT PREPRANDIAL MATING BY DEER TICKS

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ABSTRACT. To determine whether the prevalence of preprandial insemination in deer ticks reflects their local abundance, we sampled adult ticks by flagging vegetation at selected sites in eastern Massachusetts in a standardized manner. Resulting female ticks were dissected to determine whether they contained endospermatophores, and the frequency of insemination was compared to the number of questing ticks flagged at each site. The prevalence of insemination correlated closely with density of ticks. The frequency of insemination increased linearly during the 1st 2 months during the fall. The mean daily probability of insemination during this period was about 1% when, on average, about 4 ticks were flagged per minute. A predictive equation was derived via multiple regression expressing deer tick abundance as a function of collection date and insemination prevalence ($P < 0.05$). The frequency of preprandial mating, thereby, was correlated with the abundance of questing deer ticks. Insemination prevalence increased predictably as the season of adult activity progressed. We conclude that a season-specific analysis of the frequency of preprandial insemination provides a robust indicator of the abundance of deer ticks that is unaffected by short-term fluctuations in the weather.

KEY WORDS Insemination, *Ixodes*, mating, ticks

INTRODUCTION

Various *Ixodes* ticks mate preprandially, before they have attained host-contact, as well as perprandially, after they have begun to feed. Males complete spermatogenesis and females become receptive to insemination within a few days after the nymphal–adult molt (Balashov 1956). Thereafter, they seem to mate as soon as appropriate contact is attained. Such preprandial mating may be so frequent that more than 50% of the female *Ixodes dammini* Spielman, Clifford, Piesman and Corwin (*Ixodes scapularis* Say of some authors) sampled by flagging in coastal Massachusetts (Yuval al. 1990), 68% of European *Ixodes ricinus* L. (Gray 1987), and 94% of *Ixodes rubicundus* Neumann (Fourie and Kok 1995) bear spermatophores. On the other hand, *Ixodes muris* Bishopp and Smith, in the same region, invariably mate preprandially (Smith 1941). Although a frequent occurrence, the relevance of preprandial mating to the biology of prostriate ticks remains unknown.

Deer ticks mate preprandially in eastern Massachusetts beginning when adults become evident in late September and continuing until adult females cease questing for hosts in the spring (Yuval and Spielman 1990). The frequency of insemination in infested sites presumably would increase throughout this hibernal period of adult activity and would vary with the likelihood of male–female contact. Indeed, female *I. ricinus* in Europe seem to bear endospermatophores more frequently where these ticks are numerous than where they are scarce (Gray 1987). However, the relationship between insemination frequency and tick density in Europe is obscured because sequential generations overlap and because adults seem to develop throughout the permissive season (Matuschka and Spielman 1986). In contrast, the developmental cycle of deer ticks in the northeastern United States is seasonally punctuated and sequential generations are distinct. The relationship between density of Nearctic deer ticks and frequency of preprandial insemination remains ill defined.

If the density of questing *I. dammini* influences the frequency of preprandial insemination, a season-specific measure of insemination frequency might provide a robust indicator of the density of these vector ticks. Accordingly, we determined whether endospermatophore prevalence in eastern Massachusetts correlates with season and density in these ticks. In particular, we visited diverse sites in eastern Massachusetts to follow the changes in insemination prevalence over the course of a hibernal season and recorded the relationship between insemination prevalence and tick density.

MATERIALS AND METHODS

Study sites: Ticks were sampled by flagging weekly from collection sites distributed over 2 locations in Ipswich, MA, between September 25 and November 20, 1992, with an additional collection on May 21, 1993. Samples were also taken from the same sites on November 15, 1994, and between October 4, 1996, and May 28, 1997, and also at Great Island, MA, on November 17, 1994. Locales were divided into distinct sampling sites ranging from 1 to 5 ha in size and demarcated by obvious features of terrain and vegetation. Each site contained extensive areas of forest ecotone delineated by meadows, dirt roads, pond shores, and sand dunes. Ticks could be flagged consistently from 8 of the 12 Ipswich sites. For certain analyses data from sites that were contiguous and similar in character were pooled.

Sampling technique: Questing ticks were sampled by means of a 0.6 × 1.0-m flag fashioned from upholstery cloth and mounted on a 1-m broomstick. The flag was swept through vegetation while walk-

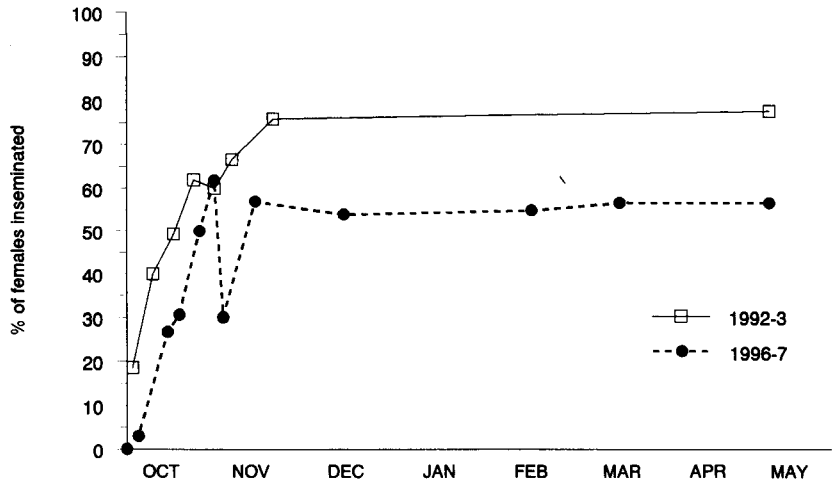

Fig. 1. Mean insemination prevalence by week among female deer ticks pooled from 7 collection sites in Ipswich, MA, over an 8-month period in 1992–93 and 1996–97.

ing at a slow, steady pace. Vegetation types included waist-high weeds, shrubs, and brush piles. Flagging episodes were timed by means of a stopwatch. Ticks were removed from flags after each 15- to 20-sec sweep. The time required to remove ticks from flags was not included as part of the overall flagging time. Sweeps were repeated until at least 20 female ticks were collected or until 30 min of flagging was completed. Sweeping routes were varied within each site each week such that no route was sampled twice. A single collector minimized variance due to differences in flagging technique. Ticks were separated by sex, stored in moist, plaster-bottomed 3-dram polystyrene vials, transported to the laboratory, and stored at 4–5°C.

Endospermatophore detection: Female ticks were dissected in Shen's saline by means of sharpened Dumont #5 forceps and a 27-gauge hypodermic needle. Their reproductive tracts were removed, cover-slipped, and examined via phase-contrast microscopy to determine the number of spermatophores present and whether the common oviduct or cervical vagina contained sperm. When possible, the entire sample collected from each site was dissected.

Statistical analysis: We expressed tick density at each site sampled on a given date in terms of the number of ticks collected per minute of flagging and plotted the values against the proportion of females found to be inseminated. We excluded samples from our analyses containing fewer than 3 female ticks and any weekly series of samples when more than one half of the sites yielded no ticks. Tick samples representing all sites that were large enough to analyze were collected between October 16, 1992, and November 6, 1992, as well as November 15–17, 1994.

To derive a predictive equation for tick abundance, nonlinear multiple regression analysis served to correlate collection date with insemination prevalence. Flagging data collected over a period of 7 wk were pooled to derive a mean flag count representing tick abundance that would be less sensitive to weekly variation. Counts from pairs of contiguous sites where ticks were flagged consistently were pooled to form 4 larger sites for analysis. We represented the collection date variable in terms of the number of days elapsed since the onset of adult activity. Although we detected ticks by flagging at a few sites as early as September 25, we assumed that full adult activity began about October 1, when adult ticks could generally be detected. For the variable representing insemination frequency, we regressed arcsine-transformed proportions (Johnson and Kotz 1969) of females inseminated to correct for unequal sample sizes and to normalize the binomially distributed data. Nonlinear and linear multiple regression models were analyzed with DataFit software (Engineered Software 1997). Diverse models were ranked by their residual sum of squares and we chose the simplest model that provided a close description of the data.

To determine how accurately our linear approximation estimates insemination dynamics during the early part of the mating season, we regressed fertility with density on the transformed proportions. Analyses were separated by date of collection to account for variations in weather that might have confounded estimates of density derived from flagging. The null hypothesis that the slope equals zero was tested by means of a *t*-test of the regression coefficient (R^2).

RESULTS

To determine whether insemination frequency increased as the hibernal season progressed, results of all samples taken during 8 months during 1992

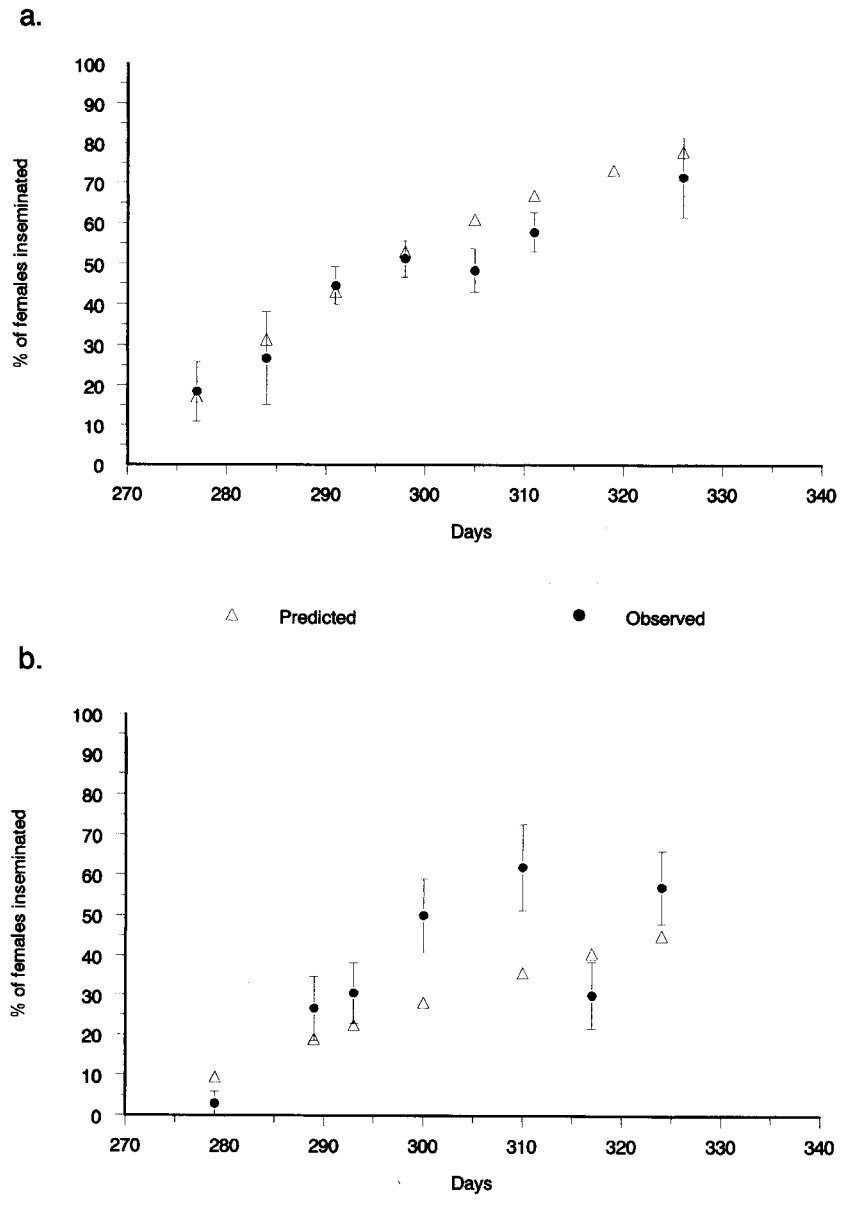

Fig. 2. Predictions of insemination frequency based on estimates of daily mating probability derived from the number of females collected while copulating compared to the observed prevalence of inseminations among female deer ticks flagged during 1992 (a) and 1996 (b). In 1992, 12 of 458 (2.65%) and in 1996, 14 of 736 (1.9%) of females were flagged in copula.

and 1996 were pooled. A steadily increasing trend in insemination prevalence early in the hibernal season was followed by an abrupt decrease in the rate of new inseminations (Fig. 1). Prevalence of preprandial insemination seemed to remain static from December through May. Regression analysis of insemination prevalence in October and November, 1992, revealed a relationship between insemination prevalence and time that approached linearity ($P < 0.001$). Tick density averaged about 4 ticks flagged per minute. We concluded that a derived slope value of 0.0645 describes the weekly increase in the prevalence of insemination.

As a surrogate for the daily probability of insemination, we derived estimates of the daily probability of copulation based on the number of copulating pairs of ticks collected during October and November. In 1992–93, 2.65% of flagged females were copulating (12/458). During 1996–97, 1.9% (14/736) of flagged females were copulating. The scale

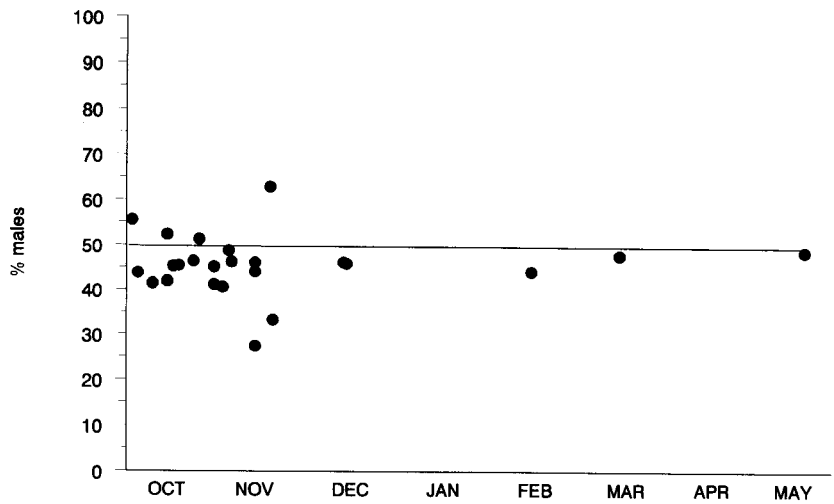

Fig. 3. Percent representation of males in samples collected by flagging in Ipswich, MA. Sex ratios were derived from 24 samples collected from October 1992 to May 1997. Line indicates 1:1 sex ratio.

of these differences reflects the ultimate prevalence of inseminations among females during these years (Fig. 1). The frequency of copulating pairs seems to serve as a crude estimator of insemination dynamics when used in the expression

$$I = (1 - p)^t,$$

where I = the proportion of females inseminated; p = the daily probability of copulation (estimated from the prevalence of copulating pairs); and t = the number of days elapsed since the onset of adult activity.

Although this model provided accurate predictions of insemination prevalence early in the 1992 mating season (Fig. 2), insemination prevalence late in the 1992 season was overpredicted, and was underpredicted throughout the 1996–97 season.

The relative abundance of male and female ticks throughout the hibernal season was then compared. Males comprised 46% of the samples, taken in the course of 24 separate collections spanning 1992–97. No trend in sex ratio over time was detected that would suggest that one or the other sex might quest more successfully or live longer (Fig. 3). Sex ratio seems to remain stable throughout the period of adult activity.

Table 1. Linear regressions of mean tick abundance as determined by flagging against the percent of females inseminated across collection sites. Analyses were separated by date to control for variations in weather between collection dates. Unequal sample sizes were corrected for by means of arcsine transformations of percentages (Johnson and Kotz 1969). The P values are derived from a t-test of the regression coefficient (r).

Collection date (1992)	R^2	P
Oct. 16	0.9434	0.0287
Oct. 23	0.9889	0.0056
Oct. 30	0.8449	0.0808
Nov. 6	0.9508	0.0249

To determine whether insemination frequency correlates with tick density, we compared endospermatophore prevalence between each of our collection sites. Linear regression analysis indicated that endospermatophore prevalence increased in conjunction with increasing tick abundance across sites on 3 of the 4 collection dates ($P < 0.05$) when ticks were most active and weather conditions between collections were least variable (Table 1). Insemination prevalence in sites where ticks were more abundant generally exceeded that in sites where density was lower. The prevalence of insemination among female deer ticks depends on local tick density and the relationship is linear, at least for the 1st part of the mating season.

Multiple regressions describing the relationship between tick density and mating frequency over time were compared. Insemination frequency was expressed as the arcsine-transformed proportion (Johnson and Kotz 1969) of inseminated females collected at each site on a given date. Candidate equations were ranked in order of closeness of fit as indicated by their residual sum of squares. The most accurate solutions required as many as 7 parameters, but did not differ from much simpler solutions in their predictive value. The following equation provided a simple nonlinear relationship that also proved to predict relatively accurately (Fig. 4):

$$y = 45.68 - 2498.13/x_1 + 30049.35/x_1 + 136.78/x_2$$

$$(R^2 = 0.928)$$

where y = the mean number of ticks flagged per minute; x_1 = the arcsine-transformed percent fe-

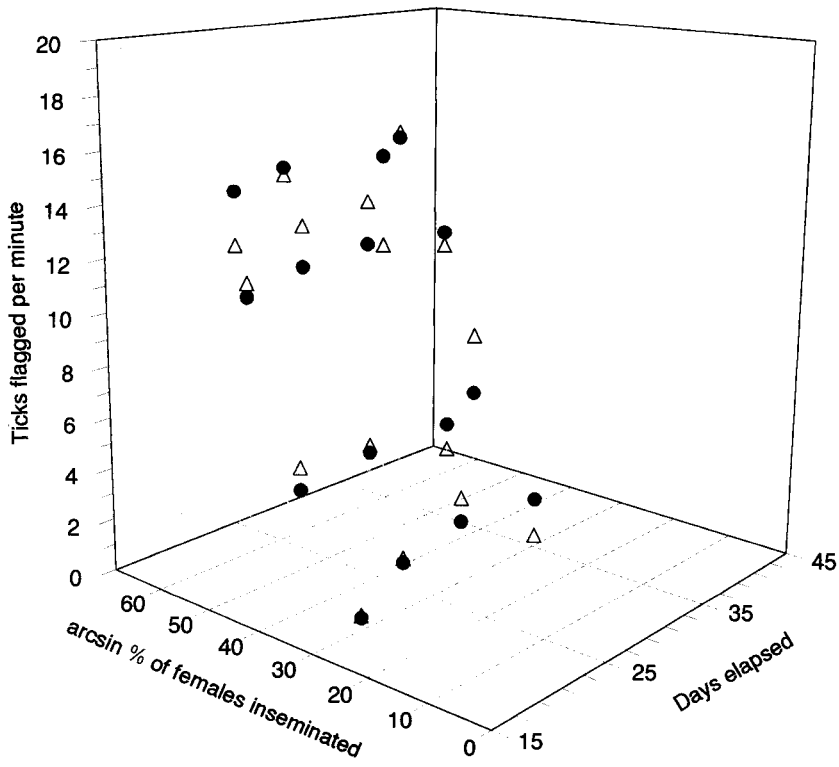

Fig. 4. Comparison of observed deer tick flag counts in Ipswich, MA, with predictions by an estimator derived from nonlinear multiple regression of arcsine percent of females inseminated and days elapsed since onset of questing ($y = 45.68 - 2{,}498.13/x_1 + 30{,}049.35/x_1 + 136.78/x_2$ [F = 4.645; df = 3,12; $P < 0.05$]). Closed circles represent observed numbers of deer ticks flagged per minute. Open triangles represent model predictions. Percentages were arcsine transformed to correct for uneven sample sizes (Johnson and Kotz 1969).

males inseminated (Johnson and Kotz 1969); and x_2 = the number of days elapsed since the onset of adult questing activity.

Variation in weekly prevalence of insemination tends to be less than that that occurs in flag counts between collection dates, illustrating the sensitivity of flagging results to environmental conditions. Thus, relatively simple nonlinear regression models based on insemination prevalence can be used to predict the relative abundance of deer ticks in a given area.

DISCUSSION

Estimates of flagging-derived tick density frequently are confounded by environmental conditions that affect the ticks as well as the efficiency of the flagging operation itself. Wind velocity, ambient temperature, snow cover, and moisture conditions all affect the ability of flags to harvest deer ticks from vegetation. Flagging-based estimates of the densities of deer ticks are particularly constrained by this limitation because these ticks are exceptionally sensitive to dehydration and their ability to imbibe atmospheric water is temperature dependent. Estimates based on the density of adult ticks feeding on their definitive hosts provide a somewhat more robust index of adult abundance because these ticks feed for about a week. Each deer would harbor a week-long cumulative sample of questing females, and an even wider accumulation of males. However, several consecutive days of unfavorable weather would negate this buffering effect. Because deer generally become available for such a monitoring operation solely during a relatively brief hunting season, little latitude is available to sample during some standardized weather conditions. Indirect measures of tick density that are less sensitive to short-term weather variations would facilitate efforts to determine tick abundance and to anticipate the impact of ticks on human health.

The frequency of preprandial copulation may provide a relatively weather-independent index of the density of deer ticks because endospermatophores accumulate in females over the entire hibernal season. If mate-finding probability is proportional to the number of male as well as female ticks patrolling a particular space, the frequency of intersexual encounters would correlate linearly with tick density, as suggested in the case of *I. ricinus* (Gray 1987). Insemination prevalence would ac-

cumulate, as virgin ticks progressively became inseminated. Although the observed frequency of copulation reflects tick density, the utility of this measure for estimating tick density is limited by its immediate sensitivity to weather conditions.

The nature of the vegetation covering a site inevitably affects conventional estimates of tick density. Where the vegetational substrate is exceptionally branched, chance encounters of females with males would occur less frequently than in less complex sites. Indeed, plant community structure strongly influences the burdens of immature ticks feeding on white-footed mice (Adler et al. 1992). The Ipswich study site was mainly comprised of upland hardwood forest but deer ticks can be found in diverse habitats. Models using insemination prevalence as an estimator of abundance should be calibrated to reflect unusual floristic conditions.

Because the probability of insemination increases with the density of available mates, and because sperm and spermatophores remain evident in females for at least a year, insemination frequency provides a stable, cumulative record of insemination events. The strong, positive correlation between density and fertility make it possible to estimate local tick abundance simply from the number of days elapsed from the onset of adult activity and the prevalence of insemination among females. Late October to early November may provide the optimum time to apply such a survey technique in the northeastern United States. Insemination prevalence provides a robust, weather-independent indicator of tick abundance.

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