
Jenny Dickson
*Georgia Southern University*

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ASSORTATIVE MATING IN *Ixodes scapularis* (Acari: Ixodidae) Ticks? AND *Ixodes scapularis* INFEST MALE DEER MORE OFTEN THAN FEMALE DEER AT SAVANNAH RIVER SITE, (AIKEN, SC)

by

JENNY DICKSON

(Under the Direction of Lorenza Beati)

ABSTRACT

In this study we explored the hypothesis that the mitochondrial genetic diversity observed in *Ixodes scapularis* ticks in the Southeastern United States is maintained through assortative mating. The study of 319 couples of ticks collected *in copula* from deer at the Savannah River Site (Aiken, SC) showed that ticks assigned to mitochondrial (12SrDNA and d-loop genes) phylogenetic monophyletic clades do not chose their mating partner based on genetic similarity or genetic diversity. The genetic composition of couples did not differ significantly from random choice. Our data indicate that if genetic diversity is maintained in this tick population, this cannot be attributed to assortative mating. During this survey of the tick *Ixodes scapularis* collected from deer at the Savannah River Site (Aiken, SC) deer check stations, it became evident that this tick preferentially feeds on male deer. *I. scapularis* prevalence rates on male and female deer were compared and revealed that the sex bias was significant (*p* < 0.0001) and did not depend on deer weight (*r^2* = 0.143). Data also indicated that tick loads on deer were not directly related to tick density in the vegetation (*r^2* = 0.082), which is possibly due to the fact that specific sites where deer are killed during hunts do not always correspond to the normal deer range.

INDEX WORDS: Assortative mating, *Ixodes scapularis*, male bias
ASSORTATIVE MATING IN *IXODES SCAPULARIS* (ACARI: IXODIDAE) TICKS?
AND *IXODES SCAPULARIS* INFEST MALE DEER MORE OFTEN THAN FEMALE
DEER AT SAVANNAH RIVER SITE, (AIKEN, SC)

by

JENNY DICKSON

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Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

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ASSORTATIVE MATING IN *IXODES SCAPULARIS* (ACARI: IXODIDAE) TICKS? AND *IXODES SCAPULARIS* INFEST MALE DEER MORE OFTEN THAN FEMALE DEER AT SAVANNAH RIVER SITE, (AIKEN, SC)

by

JENNY DICKSON

Major Professor: Lorenza Beati
Committee: Lance Durden
Scott Harrison

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DEDICATION

I dedicate this thesis to my parents, Smith and Janet Dickson, who encouraged me to pursue a higher education and pushed me to never give up.
ACKNOWLEDGMENTS

I would like to thank Paul Johns from Savannah River Site for providing us with hunt records and deer information and for facilitating our work at SRS. NSF grant #914390 to L. Beati funded the research, and NSF grant DGE GK12-0841146 (PI: Dr Laura Regassa) provided my stipend. I would also like to thank Dr. Ray Chandler and Dr. Scott Harrison for help with statistical analysis, as well as students who helped with field and laboratory work.
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CHAPTER 1

Introduction:

*Ixodes scapularis*, the blacklegged tick, is an obligate blood-sucking mite found throughout the eastern half of the United States (Brownstein et al., 2003). The tick is economically important as it is the vector of a number pathogenic organisms to humans and animals (Smith et al., 1974; Spielman et al., 1985; Steere et al., 2004; Telford et al., 2011; Krause et al., 2013). *I. scapularis* adults can mate on the vegetation, but they mostly mate while feeding on host (Kiszewski et al. 2001). Population genetic studies of *I. scapularis* based on the analysis of mitochondrial gene sequences all concur in finding a genetically homogeneous population throughout the north-northeast of the tick distribution range (Norris et al., 1996; Qiu et al. 2002). In the Southeast, however, studies have revealed the occurrence of higher levels of genetic diversity. Recent studies showed that *I. scapularis* ticks are subdivided into five major clades: all are found in the Southeast (Southern I, II, and II and American I and II clades), and only one in the Northeast (American I clade) (Chan et al., unpublished data).

The five clades can be found sympatrically in the same area (Norris et al., 1996; Qiu et al., 2002; Chan et al., unpublished data); therefore, the genetic differences cannot be accounted for by geographic separation. Preliminary data on host-race formation, also show that specialization for different host-groups is not at the origin of genetic diversity (Griffin et al., unpublished data), as was demonstrated for other tick species (McCoy et al., 2005; De Meeûs et al., 2010). Among the most frequently evoked reasons for
maintenance of sympatric genetic structure (and also for sympatric speciation) is the occurrence of assortative mating (DeBarre, 2012).

Assortative mating occurs when organisms with similar phenotypes or genotypes mate more often than would be expected under a model of random mating (Burley, 1983). For instance, similar size (Brown, 1993; McKaye, 1986; Pack et al; 2012), age range (Farrell et al. 2011), or vocalization (Moravec et al., 2006) can determine mate choice in a number of organisms. Assortative mating can have a profound impact on populations’ genetics and demographics (Crespi 1989; Farrell et al. 2011; Pack et al. 2012).

A study conducted in Europe on Ixodes ricinus, a close relative of I. scapularis, found tick populations lacking heterozygosity (De Meeûs et al., 2002). Heterozygous deficits can sometimes be explained by the occurrence of assortative mating in genetically similar individuals. If assortative mating takes place, it can create inbreeding in subpopulations (Wahlund effect). Kempf et al. (2009) detected a signal for assortative mating in the tick I. ricinus, a tick with life cycle, host association, and questing behavior similar to that of I. scapularis. In their study, assortative mating was found only in small foci within the distribution range of I. ricinus, but this focality could not be explained.

In this study, we test the hypothesis that ticks belonging to the distinct mitochondrial clades mate assortatively in the southeastern United States, in order to establish if this could explain the important genetic diversity observed in this area.
Methods:

Tick sample:

The Savannah River Site (SRS) Environmental Monitoring Section holds lottery deer hunts each October-December and we were allowed to collect ticks in 2010 and 2011 from some of the killed deer. Each year, hunts took place in separate portions of the SRS compound. We had access to a total of 12 hunting events and a total of 118 deer (86 males and 32 females).

Tick couples were removed from freshly killed deer by using forceps to grasp the tick as closely to the deer skin as possible and placing them into vials of 90% ethanol.

In order to compare the genetic composition of the ticks collected from deer to the genetic composition of ticks found on the vegetation, adult *I. scapularis* were collected at each hunt site by dragging for one hour over an area of approximately 8100 m$^2$. All ticks collected, both from deer and from vegetation, were identified according to taxonomic keys as *I. scapularis* (Keirans and Litwak, 1989). Tick sex and date of collection were recorded for each specimen.

DNA extraction:

A Qiagen Tissue Kit (Valencia, CA) with a 2-day DNA extraction protocol modified by Beati and Keirans (2001) were used to extract DNA. On day one, ticks were placed in separate vials and vacuum dried for 10 minutes. A total of 180 µl of ATL buffer were added to each vial. Each tick was cut with a scalpel blade in such a way, that at least 2/3 of the tick body was intact. Proteinase K (Roche Applied Science, Indianapolis, IN) (40 µl of a 14.3mg/ml solution) was added to each vial, which was rapidly vortexed. The
vials were incubated overnight at 55°-56°. On day two, 220 µl of AL buffer were added to each vial and vortexed. The vials were heated at 72°C for 10 minutes and supplemented with 250 µl of molecular grade ethanol. The cuticle of each tick was preserved in 70% ethanol as a morphological voucher specimen, while the lysis solution was transferred to a Qiagen column and centrifuged at 12,000 rpm for 1 min. Washing steps were performed according to the manufacturer’s instructions. DNA was eluted in 100 µl of hot (72°-C) molecular grade H2O and stored at 4°C until used.

**Polymerase chain reaction (PCR), and sequencing:**
All PCR were performed with 5-Prime PCR kits (Fisher Scientific, Pittsburgh, PA) in a Brinkman Eppendorf Master Cycler (Brinkmann Instruments, Westbury, NY). All PCR solutions contained 10.3 µl of H2O, 2.5 µl of 10x Taq buffer (with 15mM Mg2+), 5 µl of 5x Taq Master Enhancer, 1.5 µl of 25mM Mg(OAc)2, 0.2 µl of Taq polymerase (5U/µl), 1.25 µl of a 10pmol/µl solution of each primer, 0.5 µl of a deoxinucleoside triphosphate mixture (10mM of each), and 2.5 µl of tick DNA sample. A 350 bp fragment of the mitochondrial 12SrRNA of the ticks was amplified by using primers T1B and T2A (Beati and Keirans, 2001; Beati et al, 2012). For 12SrDNA amplification, an initial step of denaturation at 94°C for 5 minutes, was followed by 5 cycles of denaturation at 94°C for 20 sec, annealing at 65°C -2.5°C/cycle for 20 sec, and elongation at 72°C -0.8°C/cycle for 30 sec, and 35 cycles of denaturation at 94°C for 20 sec, annealing at 49°C for 45 sec, and elongation at 68°C for 30 sec. The program was completed by a 5-minute elongation at 68°C. Only DNA samples positive in the 12SrRNA PCR, were used to amplify the mitochondrial control region (D-loop) of the same tick, by using primers DL3-IX and
DL4-IX (Beati et al., 2012). The amplification conditions were as follows: 94°C for 5 minutes, followed by 30 cycles each consisting of 1 min denaturation at 94°C, 1 min annealing at 60°C, 1 min of extension at 72°C and a final 5 min extension at 72°C.

The ticks showing positive results for 12SrRNA and D-loop were sent to the High Throughput Genomics Unit (Washington University, Seattle) for purification and sequencing. The two strands of each amplicon were and assembled by using Sequencher 4.10.1 (Ann Arbor, MI). Sequences representing the known I. scapularis clades were used for comparison (GenBank accession numbers not yet available). The reference sequences and our sequences were aligned by using MacClade 4.08 (Corvallis, OR). Fixed characters defining the 5 clades had previously been identified (Chan et al, unpublished data). These were used to assign ticks to either one of the 5 clades. In addition, we analyzed phylogenetically a concatenated dataset of DL and 12SrDNA gene fragments of the ticks collected from deer, in order to verify that the clade assignment was done correctly. Briefly, the DL and the 12SrDNA alignments were concatenated in MacClade. Identical sequences were identified in PAUP 4.0b10 (Swofford, 2000) and only one representative for each haplotype was kept in the data matrix. PAUP was used to generate a neighbor joining (NJ) tree, which was used as starting tree for a maximum likelihood search after a substitution model was estimated, also in PAUP. Homologous sequences of Ixodes affinis were used as outgroup for the analysis.

Statistical analyses. A Wilcoxon (ranked sums) test was used to compare the genetic composition of ticks found in the vegetation to the genetic composition of ticks collected from deer. Number of female vs. male ticks in the 4 clades found questing on the vegetation were compared by $\chi^2$. Contingency tables were generated in Jmp v. 10,
(SAS, Cary, NC) and used to verify by the Pearson test if clade composition in questing ticks varied significantly between sites and to test if clade composition varied significantly by hunting event. A contingency table listing the number of each possible clade combination (25 possibilities) and the Pearson test were used to verify if any of the combinations was significantly more frequent than others. In order to verify whether assortative mating would become more evident after grouping southern ticks in a single cluster and American ticks in a second cluster, a contingency table with the number of couples matching each of the 4 possible combinations was similarly analyzed.
Results:

Tick sample:

A total of 160 (71 males and 89 females) ticks were collected from vegetation in the sites where deer were killed in 2010 (63 ticks; 29 males and 34 females) and 2011 (97 ticks; 42 males and 55 females). Deer collections, from 81 deer, yielded a total of 638 ticks (319 couples), 199 couples in 2010 and 120 couples in 2011 (Table 1).

Tick genotyping: After eliminating the ticks for which only one of the two genes could be amplified, our data matrix included 619 concatenated sequences. Identical sequences were eliminated resulting in a data matrix containing 230 total sequences. The $-\ln L$ score of the NJ tree was of 6687.55 whereas the score for the final ML tree was of 6618.77. The tree confirmed that I. scapularis is subdivided in 5 distinct mitochondrial clades (shown with different colors in Fig. 1) and that the clade assignment, based on fixed characters of both genes was consistent and correct. Only three lineages did not fit in any of the clades but those corresponded to incomplete sequences.

Of the 160 ticks collected on the vegetation, 56 (35.00%) were assigned to clade Am1, 20 (12.50%) to clade Am2, 23 (14.38%) to clade South1, 61 (38.13%) to clade South2, and 0 (0.00%) to clade South3 (Table 1). Of the 638 ticks collected from deer, 212 (33.23%) belonged to clade Am1, 42 (6.58%) to Am2, 88 (13.79%) to South1, 286 (44.83%) to South2, and 10 (1.57%) to South3. For both the questing and the feeding ticks, South2 and Am1 ticks were predominant, followed by South1 and Am2. South3 ticks were not collected questing, and only a few of them (10 ticks) were collected from deer. The percentage of the I. scapularis clades found on vegetation versus those found in deer hunts were comparable (Fig. 2).
**Statistical tests:**

A $\chi^2$ test showed that the numbers of male and female ticks in clades Am1, Am2, South1, and South2 were not significantly different ($p = 0.136$). The Wilcoxon test showed that the genetic composition of ticks found on the vegetation did not differ significantly from the genetic composition of the tick sample collected from deer ($p = 0.406$). The Pearson test showed no significant difference in the diversity of clades found at each sweep site ($p = 0.170$), and no significant difference in the diversity of clades found on deer at each hunt site ($p = 0.441$).

In order to determine if ticks were mating assortatively in the 2010/2011 hunt seasons, the observed number of couples from each possible clade combination (of a total of 25 possible pairs) were totaled (Fig. 3) and listed in a contingency table. The Pearson test showed that no combination was observed significantly more often than expected ($p = 0.706$), indicating that neither intra- nor inter-clade assortative mating were occurring.

Another contingency table was created, after the American I and II ticks were clustered in a single group, as were the Southern I, II, and III (four possible clade pairings) (Fig. 4) to see if *I. scapularis* ticks were mating assortatively based on looser genetic similarities. The Pearson test showed that there was no significant difference in the ways ticks paired ($p = 0.120$).
Discussion:

Mitochondrial gene sequences have been used in the past, not only to investigate the evolutionary history (Black and Piesman, 1994; Beati & Keirans, 2001), but also to draw conclusions on the taxonomic status of ticks (Norris et al, 1996; Norris et al, 1997). In particular, Norris et al. (1996) concluded that *I. scapularis* was a single species based on 12S and 16SrDNA sequence analysis. Although there is now a large consensus in considering *I. scapularis* to be a single taxon, the fact that the genetic diversity of the tick is much higher in the southeastern portion of its distribution still needs to be fully explained. In particular, the occurrence of all known haplotypes of *I. scapularis* in sympatry in many areas of the Southeast (Beati, unpublished data) indicates that present geographical isolation is not maintaining genetic diversity in the tick. Biogeographical hypotheses ascribing this genetic to ancient biogeographical events (glaciation in particular) (Qiu et al., 2002), which could have caused the tick to evolve for a long time in geographical refugia, are well founded. Nevertheless, genetic diversity could also be sustained by assortative mating.

In this study we analyzed the genetic composition of mating couples of ticks collected on deer at SRS in order to investigate whether or not the observed genetic diversity could be ascribed to assortative mating. We also analyzed the genetic composition of ticks collected questing on the vegetation at approximately the same sites where deer were killed, in order to compare the genetic composition of the local population of ticks with that found feeding on deer. Ticks were assigned to clearly identified mitochondrial clades based on results obtained by phylogenetic analysis of large samples of *I. scapularis* from its whole distribution range.
Our data indicate that the overall mitochondrial genetic structure in the local population of tick is fairly stable with two clades (Am1 and South2) being consistently predominant, followed by clades Am2 and South1 which are found in moderate numbers, while ticks belonging to clade South3 are only collected sporadically and, in our case, only from deer. The genetic composition of the populations of ticks collected from the vegetation at the different sites was not significantly different, nor was the composition of ticks collected from deer during the different hunts. However, tick densities at site J, for instance, was consistently lower than at other sites.

Our data indicate that there is no evidence for assortative mating, whether with genetically similar or genetically different ticks in our tick couples. Mating with genetically similar was not more frequent than mating with genetically distinct ticks. Therefore, the choice of mates in *I. scapularis* does not appear to be genetically determined.

Nevertheless, we are aware that by using mitochondrial genes for genetic differentiation, we are dealing with markers that are maternally transmitted and may not represent the whole evolutionary history and genetic structure of a diploid species. Mitochondrial genes are inherited maternally in a clonal fashion and are not subjected to recombination. They are excellent markers for the evolutionary study of phylogeographical or demographical events, but are not ideally suited for the study of taxonomic issues. Our samples should, therefore, also be studied with biparentally inherited makers such as nuclear genes and microsatellites, in order to further investigate the genetic structure of populations of *I. scapularis*. 
We know that multiple paternity has been observed in other tick species (Cutullé et al., 2010; Ruiz-López et al., 2012) and in particular in *Ixodes* species (McCoy and Tirard, 2002; Hasle et al., 2008). If that is the case also for *I. scapularis*, the males we collected *in copula* from the deer may not be the only males which have mated with the corresponding female. If multiple mating can occur, one can imagine different possible outcomes: (a) only one male spermatophore succeeds in fertilizing eggs and the offspring has a single paternal genetic origin (sperm competition); (b) sperm from multiple males succeed in fertilizing eggs and the offspring is of mixed paternal origin. In the latter case, assortative selection can occur after mating if some eggs are more viable than others or if the offspring of one male has better fitness and survives through subsequent generations, while the offspring of a less suitable male may hatch from the eggs, but not survive.

The possible occurrence of genetically mixed offspring from single females should also be investigated by using microsatellite markers. In addition, cross-mating experiments should be carried in order to compare the long term success of intra- versus inter-clade cross-breeding.

The differences between clades and groups within clades possibly play a role in the ticks’ ability to carry certain pathogens (Lin et al 2005). A prominent difference found between the *I. scapularis* in the Southeast and those in the Northeast is their capacity of carrying *B. burgdorferi*, as the northeastern region of the U.S. is responsible for over 80% of cases of Lyme disease in the United States (Qiu et al 2002). Therefore, the study of the genetic composition of tick populations and of their mating patterns can have an impact on our understanding of pathogen transmission mechanisms.
CHAPTER 2

Introduction:

*Ixodes scapularis*, commonly known as the blacklegged tick, is an economically important ectoparasite and vector of a number of pathogens (Smith et al., 1974; Spielman et al., 1985; Steere et al., 2004; Telford et al., 2011; Krause et al., 2013), including the causative agent of Lyme disease in the United States (Piesman and Gern, 2004; Steere et al., 2004). The tick has a geographic range that extends throughout most of the eastern half of the United States (Keirans et al., 1996; Dennis et al., 1998; Diuk-Wasser et al., 2006).

In its two-year life cycle, *I. scapularis* goes from egg to larval, nymphal, and finally adult stages, with a single bloodmeal preceding each molting event. The life cycle can be shorter in the southeastern portion of the tick distribution, where it can be completed within a single year. Hosts in the Southeastern U.S. include mice, lizards, and birds for immatures (Bishopp and Trembley 1945; Cliford et al. 1961) and deer, cows, bobcats, hogs, and humans for adults (Anderson, 1989; Barbour and Fish 1993; Keirans, 1996). The availability of potential vertebrate hosts can have a profound impact on *I. scapularis* population densities (Oorebeek and Kleindorfer, 2008). One of the most prominent hosts for *I. scapularis* in the southeast is *Odocoileus virginianus*, the white-tailed deer (Kellogg et al., 1971; Durden et al., 1991; Barbour and Fish, 1993), and the densities of tick populations are known to be closely related to the densities of populations of *O. virginianus* (Wilson et al. 1985, 1990). It has been shown that the removal of deer or acaricide treatment of deer can cause significant decrease in *I.*
*scapularis* population densities in nature (Wilson et al., 1988; Daniels et al. 2009; Stafford, 1993).

While conducting a study on the population genetics of *I. scapularis* at the Savannah River Site (SRS) in Aiken (South Carolina), we collected and counted ticks on deer shot during the 2010/2011 deer hunting seasons. The hunts are organized on a yearly basis to control deer populations at SRS. In this study we report findings demonstrating that ticks in this area and in the October-December period infest preferentially male, and much less frequently female deer. Prevalence rates on deer were also compared to tick densities at the site where deer were killed.
Methods:

Tick sample:

The Savannah River Site Environmental Monitoring Section holds yearly lottery deer hunts during the October-December period. During the 2010-2011 hunting seasons we were allowed to collect ticks from some harvested deer at the SRS check stations. Each hunt took place in a separate portion of SRS. Records of sex, weight, killing site, and date were recorded by SRS personnel for each deer.

Ticks were collected during a total of 5 hunts in 2010 and 7 hunts in 2011. Although our objective was to sample as many male as female deer, it became rapidly apparent that this would be impossible because of the marked preference for buck vs. doe trophies among hunters. Ticks were removed from freshly killed deer by closely inspecting the entire deer body and using forceps to grasp the tick as closely to the deer skin as possible and placing them into 90% ethanol. Date of collection, tick numbers and sex were recorded.

In order to compare the density of tick populations in the vegetation with infestation prevalence on deer we went to each hunt site and collected ticks by dragging for one hour over the vegetation with a 1m\(^2\) flannel drag cloth over an area of approx. 8100 m\(^2\). Ticks were immediately placed in vials containing 90% ethanol. All ticks collected, both from deer and from vegetation, were identified as *I. scapularis* male or female (Keirans and Litwak, 1989).

Statistical methods:

A \(\chi^2\) approximation was used to test whether there was a significant difference in the number of ticks by hunt-year. A contingency analysis of variance was conducted to
determine if there was a significant difference between the number of ticks found on female versus male deer (significance was evaluated with both the Pearson and a likelihood test). In addition, in order to establish whether weight rather than sex was responsible for the observed differences in tick loads, the relationship between the number of ticks found on deer and the deer weight was evaluated through regression analysis. The relationship between tick prevalence on deer and tick density on the vegetation was also investigated by linear regression.
Results:

Tick sampling:

Collection data are listed in Table 2. A total of 118 deer, 86 males (72.9%) and 32 females (27.1%), were inspected for the presence (prevalence) of *I. scapularis*. We collected a total of 1001 ticks of which 54.8% were females (n = 549), and 45.2% were males (n = 452). Of the 86 male deer, 81 (94.2%) carried ticks (showed prevalence), whereas only 17 of the 32 does (53.1%) showed prevalence for ticks. Of the 1001 ticks, 964 (96.3%) were collected from male and 37 (3.7%) from female deer. The overall infestation intensities in males was of 11.9 ticks/deer carrying ticks (from 0 to 41 ticks/deer), and in females of 2.2 ticks/deer carrying ticks (0 to 8 ticks/deer). Overall tick infestation intensities (deer not carrying ticks included) were of 13.0 ticks/buck, and 2.5 ticks/does in 2010, 10.1 ticks/buck and 0.9/does in 2011, and 11.2 ticks/buck and 1.2 ticks/does for both years (Fig. 5). A total of 232 ticks were collected from vegetation at the approximate sites were deer were killed in 2010 (63 ticks; 34 females and 29 males) and 2011 (169 ticks; 75 males and 94 females). The tick sex ratio in all collections was fairly constant and approached 1:1. Tick densities in the areas where the deer were killed are listed in Table 2. They varied from 0.12 to 8.27 ticks/1000m² (average: 2.39ticks/1000m²) depending on the collection site, with site D yielding in both years more ticks than any other site.

Statistical analysis

The $\chi^2$ approximation indicated that the difference in the number of ticks collected in 2010 and 2011 was not statistically significant (p-value = 0.44). The contingency analysis of variance revealed a significant difference between the number of ticks found
on female versus male deer (p-value < 0.0001 with both the Pearson and likelihood tests).

A regression analysis showed that only ~14% of the number of ticks found on deer was directly related to deer weight \( (r^2 = 0.143) \), thus indicating that the higher tick load found on male deer was not simply due to larger size and weight (Fig. 6). Linear regression also showed that only 2.4% \( (r^2 = 0.024) \) of the number of ticks on deer was related to density of ticks on the vegetation.
Discussion:

Our findings indicate that at SRS the tick infestation intensity on male deer in the October-December period is consistently more important than on female deer. Our data also indicate that tick infestation intensities are not simply related to deer size (weight), as larger males do not necessarily carry more ticks than smaller ones. In general, prevalence rates in our male deer sample (94.2%) is similar (74-100%) (French et al., 1992) or slightly higher (71.3%) (Main et al, 1981) than that observed in some northern areas. The overall infestation prevalence in female deer was of 53.1%, which is consistent with data (45%) recorded in Connecticut by Main et al (1981), but lower than rates (20-83.3%) recorded by French et al. (1992) in Wisconsin.

Several hypotheses can be formulated in order to explain the differences in tick prevalence rates. First, male biases in parasitism of mammals has sometimes been shown to be related to the immune depression induced by male hormones (Schalk & Forbes, 1997). Tick attachment certainly benefits from decreased host immune responses, as tick are also known to provoke immune depression through inoculation of a number of salivary immuno-modulatory molecules in order to facilitate their own feeding process (Wang and Nuttall, 1994; Gang et al., 2012). Such a mechanism would, however, not explain why this male bias is mostly known in the southeastern states (Kollars et al., 1997) and not in the Northeast.

Ticks, in particular female ticks, are known to show arrestant response secretions produced by different groups of scent glands on the deer legs. Nevertheless, it has been shown that male or female deer are equally effective at eliciting this kairomonal response in ticks (Carroll et al. 1995; Carroll, 1998). A later study, showed that urine from
dominant reproductive bucks and does in oestrus elicited a similar response in ticks (Carroll 2000). Does, at SRS, are in oestrus in November (D’Angelo et al. 2004), a period which corresponds to our sampling season. As we did not record the reproductive status of the killed does, it is impossible for us to establish *a posteriori* if female deer in their reproductive phase were more likely to carry ticks.

Another possible reason for finding more ticks on males versus female deer could be the gender-related differences in dispersal ranges. Male deer roam further and more widely than female deer (Ishmael 1984; French et. al. 1992) particularly during the mating season. When tick densities in the environment are moderate, it has been noted that more ticks are found on male deer and that this can be ascribed to their superior mobility (French et. al, 1992). Average adult tick density at SRS can certainly be considered to be low (2.39 ticks/1000m²) when compared to densities in most northeastern states were it can reach an average of 330 adult ticks/1000m² (Daniels et al., 2000). As our collection periods matched the reproductive season in deer, increased mobility in male deer and low tick density may account for sex biased parasitism.

The typical habitat of both *I. scapularis* and *O. virginianus* consists of wooded areas, both hardwood and pine, and areas densely covered with low-lying shrubs (Harlow 1984; Daniels et al., 2009), making *O. virginianus* a readily available host in many tick habitats. *I. scapularis*, however, is known to prefer deciduous forests and to be less abundant in pine-dominated forests (McShea 2012). At SRS, the growth of shrub in pine forests is controlled through prescribed periodic burns. There is some evidence for variation in habitat selection in white-tailed deer being related to sexual segregation. In Minnesota, female deer appear to seek more open habitats during the winter months and
more protected habitats during the summer months (DePerno et al., 2003). If this were the case at SRS, a pine-dominated forest would be perceived as an open habitat when compared to a undergrowth-rich deciduous forest. It would, therefore, be interesting to investigate whether does at SRS prefer to congregate in pine forests during the Fall-Winter month at SRS.

The lack of relationship between tick infestation prevalence and tick density in the vegetation is not surprising, as deer are often dislodged from their original home range during hunts even if for brief periods (Downing & McGinnes, 1976; D’Angelo et al. 2003).

In conclusion, *I. scapularis* appears to infest male deer more readily than female deer and this might be due a combination of factors: low tick densities, decrease in immune response in male deer during the mating season, wider roaming from the home range in males, or different preference of habitat in female deer (pine vs. deciduous forests). However, it must be noted, that we collected ticks from deer during a short period which corresponds only to the first part of the activity season of adult *I. scapularis* ticks and we can, therefore, not be sure that our data will apply to the whole seasonal activity peak of the tick which lasts usually until March.


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<th>SOUTH 3</th>
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Table 1. Clade diversity collection data from hunts and vegetation
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<th>Ticks on males</th>
<th>Infestation (ticks/deer)</th>
<th>Prevalence Range min</th>
<th>Prevalence Range max</th>
<th>Female Deer</th>
<th>Ticks on Females</th>
<th>Infestation Prevalence (ticks/deer)</th>
<th>Prevalence Range min</th>
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<td>29.00</td>
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Table 2. Tick collection data from SRS 2010/2011
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<th>Ticks total</th>
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<th>Prevalence Range max</th>
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<th>Drag ticks Female</th>
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Table 2 (continued). Tick collection data from SRS 2010/2011
Fig. 1

South I

South II

South III

Am I

Am II

0.005 substitutions/site
Figure 2. Percentages of clades making up *Ixodes scapularis* populations in vegetation compared to percentages of clades making up the deer hunt populations.
Figure 3. The 319 tick couples found on deer, classified by clade. (The top represents the male and the bottom represents the female in each couple).
**Figure 4.** Couples grouped by American and Southern clade classifications. (The top represents the male and the bottom represents the female in each couple).
Figure 5. Average number of ticks per male vs. female deer
Figure 6. Regression analysis of number of ticks found on deer by deer weight.