VARIATION IN BLACKLEGGED TICK *IXODES SCAPULARIS* QUESTING BEHAVIOR HAS IMPLICATIONS FOR HUMAN LYME DISEASE RISK IN THE EASTERN UNITED STATES

By

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ABSTRACT

VARIATION IN BLACKLEGGED TICK *IXODES SCAPULARIS* QUESTING BEHAVIOR HAS IMPLICATIONS FOR HUMAN LYME DISEASE RISK IN THE EASTERN UNITED STATES

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The blacklegged tick, *Ixodes scapularis* (Acari: Ixodidae), is the vector of *Borrelia burgdorferi,* the causal agent of Lyme disease (LD) in the eastern United States. Ninety-five per cent of LD cases are reported from the Northeast and upper Midwest, despite the widespread presence of the vector throughout the South. Several hypotheses have been proposed to explain this difference in disease risk. These include geographic variation in large-scale factors such as climatic variables, biodiversity, and tick genetics, which are known and/or believed to influence the abundance of infected ticks in an area. While these factors may explain the ultimate causes of risk variation, they do not reveal the proximate underlying mechanisms maintaining the geographic discrepancy in disease risk. In this dissertation, I propose and demonstrate that a mechanistic factor - the questing behavior of nymphs (the epidemiologically most important life stage of the vector) - is a key factor explaining this gradient in LD risk. I hypothesized that variation in questing behavior is such that nymphs from low LD risk areas (southern regions) rarely host-seek in locations where they come into contact with human hosts, whereas nymphs from high LD risk areas (northern regions) host-seek in locations where human encounters are more likely. I addressed the following specific hypotheses via a 4-year series of field experiments at sites located within the current distribution of *I. scapularis*:

H_1: Nymphal *I. scapularis* host-seeking behavior varies with geographic origin (Chapter 1);

H_2: Behavioral differences exhibited by nymphs from different geographic origins are

maintained when nymphs are translocated to other regions (Chapter 1);

H_3: The broad geographic variation in human LD risk is strongly correlated with geographic variation in nymphal questing behavior (Chapter 2);

H_4: The hybrid offspring of *I. scapularis* from high- and low-risk regions will differ in questing behavior from one or both parental populations (Chapter 3).

In Chapter 1, using outdoor experimental behavior arenas placed at four sites in the eastern US, I demonstrate that nymphal blacklegged ticks derived from a LD-endemic (northern) region were 3.6-11.6 times more likely to be observed questing than were nymphs from non-endemic (southern) regions. Furthermore, the observed behavior trends of northern and southern nymphs were consistent in both years and at all sites where they were tested, implicating a genetic component for questing behavior. In Chapter 2, I confirm the generality of the trends observed in Chapter 1 by using the same experimental approach to observe questing behavior of *I. scapularis* nymphs originating from 15 locations throughout the eastern US. The results of these experiments were consistent with those of Chapter 1; I found that nymphs from LD-endemic regions were much up to 8 times as likely to emerge from underneath the leaf litter versus those from non-endemic regions, regardless of the field site where they were tested. Furthermore, questing behavior was strongly positively related to the LD incidence of the region from which the nymph's mothers were collected which provides further support for the role of questing behavior and human LD risk. In Chapter 3, I explored potential consequences of *I. scapularis* range expansion by mating northern and southern tick and found that hybrid ticks behave more like southern nymphs, but with enhanced survival. Future studies on *I. scapularis* reproductive behavior and biology are necessary to better understanding how local ecology will influence LD risk in areas where northern and southern populations meet.

This dissertation is dedicated to everyone who has loved, supported, believed in and inspired me – thank you.

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KEY TO ABBREVIATIONS

LD – Lyme disease

US – United States

VBD – Vector-borne disease

WNV – West Nile Virus

EID – Emerging Infectious Disease

RH – Relative Humidity

INTRODUCTION

Animal behavior affects pathogen and parasite transmission and disease incidence by directly influencing the contact rates between infected and susceptible individuals (Anderson and May 1981, Keesing et al. 2006). Some behaviors (e.g. group living, sharing food/shelter, promiscuity) increase the probability of disease transmission, while others (e.g. grooming, avoidance, isolation) decrease it (Wasserheit 1994, Wilson-Rich et al. 2009). For vector-borne diseases, which represent a profound public health issue (National Research Council 2008), differences in vector behavior can have large impacts on human disease risk. In the case of West Nile Virus, seasonal changes in mosquito feeding behavior (i.e., host use) are directly linked to increased human West Nile risk in North America (Kilpatrick et al. 2006). Human malaria, the most deadly vector-borne disease, is caused by bites from infected mosquitos of the *Anopheles gambiae sensu lato* complex (World Health Organization 2014) and species within this group exhibit differences in feeding and resting behaviors (Githeko et al. 1994). Control measures can capitalize on these behavioral preferences; for example, in many areas where insecticide treated bed nets have been used, the abundance of the human biting, indoor-resting *A. gambiae* has markedly declined, as have human rates of malaria (Bayoh et al. 2010).

Emerging infectious diseases (EIDs) have increased at alarming rates over the last 70 years and these rates are expected to continue to rise (Jones et al. 2008). These increases pose serious threats to human and animal health, placing more and more organisms at risk. Emerging vector-borne diseases (diseases transmitted by arthropod vectors) constitute almost one-third of the EIDs within the last decade (Jones et al. 2008). Lyme disease (LD) is the most common vector-borne disease in the United States and is considered to be emerging because it is increasing in incidence in space and over time (Bacon et al. 2008, CDC 2014).

In the US, LD is a multisystem disease caused by the bites of *Ixodes scapularis* (*I. pacificus* in the western US) ticks infected with the spirochete *Borrelia burgdorferi* (Barbour and Fish 1993). The maintenance of *B. burgdorferi* in nature involves a complicated cycle among *I. scapularis* ticks and reservoir hosts. Humans are considered to be an incidental host in this cycle and therefore do not directly contribute to the maintenance of *B. burgdorferi* in nature (Piesman and Gern 2004). However, as demonstrated by the increasing trends in human LD cases (CDC 2014), humans can, and do, continue to come into contact with infected *I. scapularis* ticks. Therefore, understanding the ecological factors that contribute to disease transmission is imperative for assessing and predicting tick-borne disease risk. The goal of this dissertation is to examine if a specific mechanism - tick questing behavior (= host-seeking) - is an important factor in determining human LD risk. This question is addressed through a series of field and laboratory studies which closely examine nymphal tick behavior.

The study organism. *Ixodes scapularis* (the 'blacklegged tick' or 'deer tick') is a member of the family Ixodidae - hard-bodied ticks (Sonenshine 1991) and a medically important vector of several pathogens including the agents of Lyme disease and other spirochetoses, human anaplasmosis, erhlichiosis, babesiosis, and Powassan encephalitis. Ticks are obligate parasites, relying on host blood meals to provide necessary energy for physiological processes; this tick is a three-host parasite, feeding on a single, different, vertebrate host during each post-egg life stage (larva, nymph, adult). Adult females are prolific; *I. ricinis* (close relative) lays between 2,000-3,000 eggs per clutch (Randolph 2004). After oviposition, the female dies, leaving the eggs to hatch on their own (Soneneshine 1991). Molting, development, survival, and reproduction are all dependent upon the energy reserves provided from the previous life stages' blood meal (Anderson and Magnarelli 2008).

Tick survival is profoundly regulated by the ability to maintain water balance (Needham and Teel 1991, Sonenshine 1991, Randolph and Storey 1999). The majority of *I. scapularis'* life (up to 98%) occurs off of hosts, during which they are vulnerable to the ambient environment (Anderson and Magnarelli 2008). Ticks have evolved several strategies for conserving their water reserves including, behavioral modifications, utilizing hygroscopic substances to draw water from the air, reabsorption of water from excreta, taking up moisture through the cuticle, regulating gas exchange, and producing guanine for waste (Needham and Teel 1991, Sonenshine 1991).

As obligate parasites, the ability of *I. scapularis* to acquire hosts is crucial for survival. These ticks use an ambush-type (sit and wait) strategy for host-seeking (= questing) and contacting hosts (Anderson and Magnarelli 2008). When a tick comes into direct contact with a host, it grasps their skin/hair with its tarsal claws and climbs on. Carbon dioxide emission, vibration, heat, shadows, and odors all cue *I. scapularis* to host presence (Sonenshine 1991).

The ecology of Lyme disease. There are three necessary elements for the Lyme disease cycle to occur: competent hosts, vectors, and the LD pathogen (Tsao 2009). Without one of these components, the cycle cannot occur. As there is negligible (<1%) transovarial transmission of *B. burgdorferi* (Rollend et al. 2013), it must be maintained by larval and nymphal ticks, of different generational cohorts, that utilize the same hosts (Barbour and Fish 1993). In the northeast, most nymphs host-seek and feed in early summer, infecting the host population with the pathogen. Larval emergence in later summer months allows members of this life stage to become infected by feeding on the previously nymph-infected hosts (Fish 1995). Once infected larvae molt into nymphs, they become capable of infecting naïve hosts in the next summer, thus perpetuating the enzootic cycle of the Lyme disease pathogen. Nymphs are considered to be the most epidemiologically important life stage for human LD transmission because of their small size and spring summer seasonality that coincides with high human outdoor activity (Fish 1995). Hosts differ in the ability to maintain and transmit the Lyme bacterium (LoGiudice et al. 2003), further complicating the understanding of the transmission dynamics of this system. The most recognized and highly competent reservoir for *B. burgdorferi* and primary host for larval and nymphal ticks in northern states is the white-footed mouse, *Peromyscus leucopus* (Levine et al. 1985, LoGiudice et al. 2003). However, in southern regions, juvenile *I. scapularis* more frequently parasitize fossorial lizards (Apperson et al. 1993, Kollars et al. 1999, Durden et al. 2002), which are considered to be much less competent for this pathogen (Levin et al. 1996) . White-tailed deer (*Odocoileus virginianus*) are not competent reservoirs for *B. burdorferi* (Telford et al. 1988) but they are important for perpetuating tick populations, as they are the primary reproductive host for this species.

The problem. Despite widespread presence of *I. scapularis* throughout the eastern US (Dennis et al. 1998, Diuk-Wasser et al. 2006), there is pronounced geographical variation in LD incidence within the region (Figure 1.0). Ninety-five percent of human LD cases are reported from 'Lyme endemic' states in the Northeast and upper Midwest (CDC 2014). Several hypotheses exist to explain this variation. These include large-scale factors such as climatic variables (Brownstein et al. 2005, Ogden et al. 2008, Feria-Arroyo et al. 2014, Ogden et al. 2014), biodiversity (Ostfeld and Keesing 2000a, Schmidt and Ostfeld 2001, Allan et al. 2003, LoGiudice et al. 2003, Keesing et al. 2006, Levi et al. 2012), and tick genetics (Qiu et al. 2002, Humphrey et al. 2010). While these hypotheses describe ultimate causes for variation in LD risk, they do not inform us of the mechanisms driving risk variation. These factors may help us understand the ultimate causes of risk variation, but for planning and executing effective intervention strategies, a mechanistic understanding of factors underlying the Lyme Disease Gradient is key. A mechanistic hypothesis for the Lyme Disease Gradient is that nymphal questing behavior may vary regionally, leading to differences in tick/human contact rates. In this dissertation I investigate this hypothesis as an underlying explanation for the observed variation in LD risk. Specifically, I hypothesized that variation in nymphal questing behavior is such that nymphs from low LD risk areas (southern regions) rarely host-seek in locations where they come into contact with human hosts, whereas nymphs from high LD risk areas (northern regions) host-seek in locations where human encounters are more likely.

Hypotheses tested. I have addressed this hypothesis through a 4-year series of field experiments at sites located within the current distribution of *I. scapularis.* The results of the experiments are presented as the 3 chapters in this dissertation and directly test the following specific hypotheses:

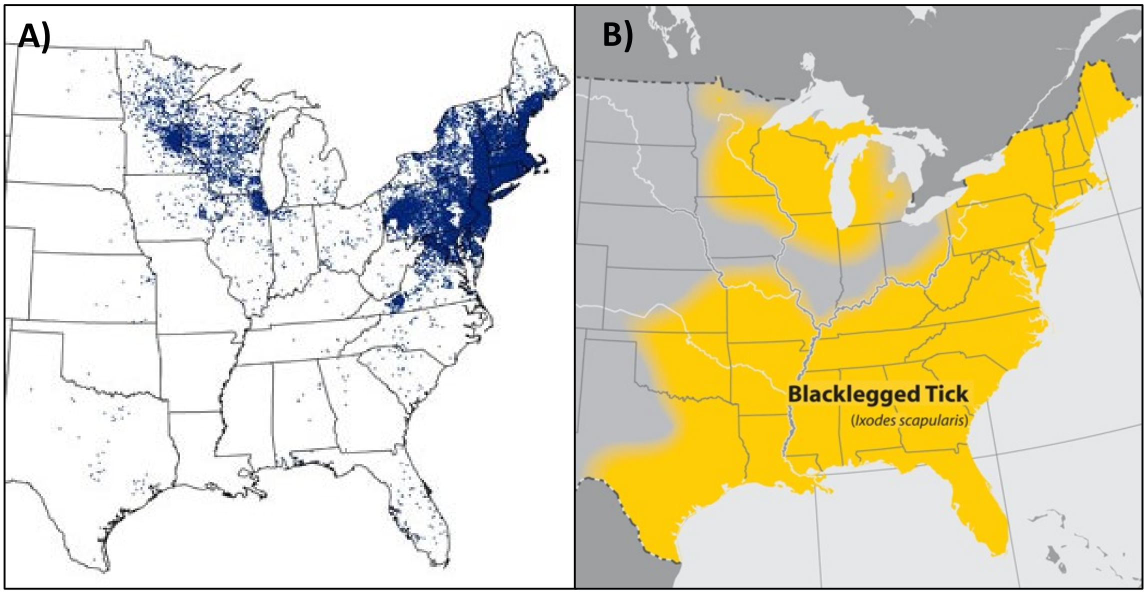
H_1: Nymphal *I. scapularis* host-seeking behavior varies with geographic origin (Chapter 1);

H_2: Behavioral differences exhibited by nymphs from different geographic origins are maintained regardless of environmental influences (Chapter 1);

H_3: The broad geographic variation in human risk of Lyme disease is strongly positively related to geographic variation in nymphal questing behavior (Chapter 2);

H_4: Hybrid offspring of *I. scapularis* from high- and low- risk regions will produce offspring that will differ in questing behavior from one or both parental populations (Chapter 3).

Figure 1.0. Comparison of (A) the distribution of reported human Lyme Disease cases in the United States in 2013 and (B) the geographic distribution of the vector tick *Ixodes scapularis*. Maps from CDC (2014).



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CHAPTER 1

Different populations of blacklegged tick nymphs exhibit differences in questing behavior that have implications for human Lyme disease risk

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Abstract

Animal behavior can have profound effects on pathogen transmission and disease incidence. We studied the questing (= host-seeking) behavior of blacklegged tick (*Ixodes scapularis*) nymphs, which are the primary vectors of Lyme disease in the eastern United States. Lyme disease is common in northern but not in southern regions, and prior ecological studies have found that standard methods used to collect host-seeking nymphs in northern regions are unsuccessful in the South. This led us to hypothesize that there are behavior differences between northern and southern nymphs that alter how readily they are collected, and how likely they are to transmit the etiological agent of Lyme disease to humans. To examine this question, we compared the questing behavior of *I. scapularis* nymphs originating from one northern (Lyme disease endemic) and two southern (non-endemic) US regions at field sites in Wisconsin, Rhode Island, Tennessee, and Florida. Laboratory-raised uninfected nymphs were monitored in circular 0.2 m^2 arenas containing wooden dowels (mimicking stems of understory vegetation) for 10 (2011) and 19 (2012) weeks. The probability of observing nymphs questing on these stems (2011), and on stems, on top of leaf litter, and on arena walls (2012) was much greater for northern than for southern origin ticks in both years and at all field sites (19.5 times greater in 2011; 3.6-11.6 times greater in 2012). Our findings suggest that southern origin *I. scapularis* nymphs rarely emerge from the leaf litter, and consequently are unlikely to contact passing humans. We propose that this difference in questing behavior accounts for observed geographic differences in the efficacy of the standard sampling techniques used to collect questing nymphs.

These findings also support our hypothesis that very low Lyme disease incidence in southern states is, in part, a consequence of the type of host-seeking behavior exhibited by southern populations of the key Lyme disease vector.

Introduction

The blacklegged or deer tick (*Ixodes scapularis*) vectors *Borrelia burgdorferi*, the etiological agent of Lyme disease (LD), which is the most common vector-borne disease in the United States (US) (Bacon et al. 2008). Despite the widespread presence of blacklegged ticks throughout the eastern US (Dennis et al. 1998, Diuk-Wasser et al. 2006), there is pronounced geographical variation in LD incidence in that region. Ninety-five percent of human LD cases in the US are reported from 'Lyme endemic' states in northeast and upper midwest regions (CDC 2014); LD incidence in southeast regions is over an order of magnitude lower (Stromdahl and Hickling 2012). We refer to this latitudinal variation in disease incidence in the eastern US as the "Lyme Disease Gradient".

Ixodes scapularis has three parasitic life stages (larva, nymph, adult), but only the bites of nymphs and adults transmit *B. burgdorferi*. There is neglible transovarial transmission of the pathogen therefore larvae are considered to be born uninfected (Rollend et al. 2013). The nymphal stage is regarded as the most epidemiologically important for LD transmission because of its small size and spring/summer seasonality that coincides with human outdoor activity (Barbour and Fish 1993, Falco et al. 1999). The density of infected nymphs (DIN) is considered a useful predictor of LD risk (Mather et al. 1996, Ostfeld and Keesing 2000b, Schmidt and Ostfeld 2001, Allan et al. 2003, Horobik et al. 2006, Hoen et al. 2009, Diuk-Wasser et al. 2012). Factors that influence DIN (including tick survivorship (Berger et al. 2014), host composition (Swei et al. 2011, Bouchard et al. 2013), and abiotic variables (Berger et al. 2014)) are thought to influence the magnitude of risk (Ostfeld and Keesing 2000a, Allan et al. 2003, Keesing et al. 2006, Ogden and Tsao 2009).

DIN is calculated by multiplying the density of nymphs collected in a given area by the *B. burgdorferi* infection prevalence of those nymphs (Mather et al. 1996, Stafford et al. 1998, Ostfeld and Keesing 2000b, Diuk-Wasser et al. 2012). Ticks in such studies are typically collected by ‘dragging’ or ‘flagging’ a 1 m^2 white cloth through the understory vegetation, as this is considered to be the most reliable and efficient method for sampling nymphal *I. scapularis* populations in the northeastern US (Ginsberg and Ewing 1989, Falco and Fish 1992). These methods intentionally target ticks that are questing (= host-seeking) on or above the leaf litter, as these are the ticks most likely to encounter humans. Flagging and dragging are less likely to collect ticks beneath the surface of the leaf litter; however, since such ticks are unlikely to encounter humans they presumably contribute little to LD risk. Quantification of DIN by flagging/dragging thus provides a useful index of human-nymphal encounter rates. Indeed, DIN is highly correlated with the Lyme Disease Gradient (Diuk-Wasser et al. 2012, Pepin et al. 2012), providing support for its use as an index of human LD risk.

Several hypotheses for the Lyme Disease Gradient have been proposed. These include geographic variation in large-scale predictors such as climatic variables (Brownstein et al. 2005, Ogden et al. 2008, Feria-Arroyo et al. 2014, Ogden et al. 2014), biodiversity (Ostfeld and Keesing 2000a, Schmidt and Ostfeld 2001, Allan et al. 2003, LoGiudice et al. 2003, Keesing et al. 2006), and tick genetics (Qiu et al. 2002, Humphrey et al. 2010), which are known and/or believed to influence the abundance of questing infected nymphs. These predictors help us understand the ultimate causes of risk variation, but for planning and executing effective intervention strategies, a mechanistic understanding of factors underlying the Lyme Disease Gradient is key.

A key mechanistic factor that needs to be considered for the LD system is vector behavior. With vector-borne diseases, it is typically assumed that risk of contact with an infected vector, and therefore risk of disease, is proportional to the abundance of infected vectors (Moore 1993, Mather et al. 1996, Mills et al. 2010). Behavior of both hosts and vectors influences the likelihood of encounter and thus the risk of disease, and these behaviors might differ at different sites. A mechanistic hypothesis is that nymphal questing behavior varies regionally, leading to differences in tick/human contact rates that contribute to the Lyme Disease Gradient.

At least three lines of evidence support this questing behavior hypothesis. First, drag/flag sampling efficacy differs between LD endemic and non-endemic regions. These standard methods readily collect all three tick life stages in northeastern and upper midwestern regions (Falco and Fish 1992, Diuk-Wasser et al. 2006, Hamer et al. 2010), whereas in southeastern regions, they collect very few nymphs even at sites where adult *I. scapularis* are readily flagged or dragged (Rogers 1953, Diuk-Wasser et al. 2006, Goddard and Piesman 2006, Goltz and Goddard 2013). The presence of abundant *I. scapularis* adults at these southeastern sites indicates that nymphs must also be present, and this is confirmed by observations of *I. scapularis* nymphs commonly attached to vertebrate hosts (Rogers 1953, Apperson et al. 1993, Kollars et al. 1999, Durden et al. 2002). Second, small mammals are the primary hosts for juveniles in northern states (Spielman et al. 1985, LoGiudice et al. 2003), whereas lizards fill this role in the South (Apperson et al. 1993, Oliver 1996, Levine et al. 1997, Kollars et al. 1999, Durden et al. 2002); again suggesting differences in questing behavior. Third, nymphal ticks are responsible for the majority of Lyme cases in the northeast and upper Midwest (Falco et al.

1999), but are rarely recorded biting humans in the South (Felz et al. 1996, Oliver 1996, Goddard 2002, Williamson et al. 2010, Stromdahl and Hickling 2012). In combination, these observations motivated us to undertake a series of experiments in which we directly examine the questing behavior of nymphs from LD endemic and non-endemic regions.

Our first experiments aimed to: (i) quantify and compare variation in questing behavior of nymphs from two southern, non-endemic sites with nymphs from one northern, endemic site (2011), and (ii) assess whether observed behavioral differences are maintained when nymphs are translocated between regions (2012), thereby acquiring insight into the relative contributions of proximate environmental conditions and genetics to observed behavioral variation. Here, we tested nymphs derived from mothers collected in Wisconsin (WI), South Carolina (SC), and North Carolina (NC) because these locations represent areas of low, non-endemic (SC, NC) and high, endemic (WI) LD risk in the eastern half of the US: in 2013, Lyme disease incidence was 25.2, 0.7, and 0.4 per 100,000 persons in WI, SC, and NC, respectively (CDC 2014) . Likewise, the sites selected for translocation experiments in 2012 (Wisconsin (WI), Rhode Island (RI), Tennessee (TN) and Florida (FL)) are areas of high and low LD risk (incidence rates per 100,000 persons in 2013 in WI, RI, TN and FL were: 25.2, 42.2, 0.2, and 0.4, respectively (CDC 2014)).

Methods

All mouse and rabbit handling and tick feeding protocols were approved through Michigan State University’s Institutional Animal Care and Use Committee (AUF # 06/09-094-00). Permission for field site use and to conduct the research was obtained from Tall Timbers Research Station and Land Conservancy, the University of Tennessee’s Forest Resources AgResearch and Education Center (FRAEC) and Fort McCoy Military Installation.

Study Sites. In 2011, we measured the climbing behavior of nymphal *I. scapularis* placed at a field site at Fort McCoy Garrison, Wisconsin (latitude 44.01°N), a LD endemic area. In 2012, we expanded our study to include two LD endemic and two non-endemic sites. The 2012 LD endemic sites comprised Fort McCoy plus a site near Kingston, Rhode Island (latitude 41.48°N); the non-endemic sites were near Oak Ridge, Tennessee (latitude 36.01°N) and at Tall Timbers Research Station, Florida (latitude 30.53°N). All sites were located in mixed deciduous forests. The forest at Fort McCoy was dominated by various oaks (*Quercus* spp.), pines (*Pinus* spp.) and red maples (*Acer rubrum*), with a shrub layer of mostly tree saplings. The Rhode Island site was dominated by red maple (*A. rubrum*), white pine (*Pinus strobus*), and white oak (*Q. alba*), with tree saplings in the shrub layer. The Tennessee forest was dominated by upland oaks (*Quercus* spp.), hickory (*Carya* spp.) and yellow poplar (*Liriodendron tulipifera*), with a mixed understory containing various saplings and several invasive understory species. The Florida forest was dominated by oak (*Quercus* spp.), maple (*Acer* spp.), interspersed with shortleaf pine and a shrub layer dominated by tree saplings. Average canopy cover estimates for the sites during the study months ranged from 86%-92%. Meteorological measurements (temperature and relative humidity) were recorded hourly at each site in both years, using paired iButton® data loggers (Hygrochron™, Dallas Semiconductor) placed just below the surface of the leaf litter level (0 cm) and above ground (10 cm).

Rearing Nymphs. The laboratory-reared nymphs used in 2011 originated from 22 (WI = 8, SC = 14) engorged female *I. scapularis* collected in November 2010 from hunter harvested deer at check stations in Monroe County, Wisconsin (latitude 44.01°N) and Aiken County, South Carolina (latitude 33.56° N). Engorged females were allowed to oviposit in individual vials in humidity chambers at 21°C and 98% relative humidity at 16:8 (L:D) hour photoperiod conditions. At 2 to 7 weeks of age, the resulting larvae were fed on female laboratory mice (ICR (CD-1) strain, *Mus musculus*) and allowed to molt into nymphs; at 2 to 4 weeks of age, these nymphs were transferred to the field sites.

Seven groups of nymphs (3 WI, 4 SC) from the 2011 colonies were used to propagate a second generation of nymphs that were used in the 2012 experiments. Two of these groups were siblings of the nymphs used in the 2011 experiments, while the remaining five groups were from other females collected at the same locations and time as the mothers of the 2011 nymphs (Table 1.1). Nymphs were fed on female laboratory mice and engorged nymphs were housed individually in vials where they were allowed to molt into adults. Adults of a single origin (WI or SC) were then mated together on New Zealand White rabbits (*Oryctolagus cuniculus*) in November 2011. The resulting engorged females were maintained as described above through the ovipositional period. Resulting larvae were reared to nymphs using the 2011 protocol. In November 2011, 5 additional engorged females were collected from hunter killed deer at check stations in Hyde County, North Carolina (latitude 35.50°N) and Aiken County, South Carolina (latitude 33.56°N) to provide additional nymphs for the 2012 experiment.

Table 1.1. Nymph ancestry and experimental design 2011, 2012. Number of clutches (mothers) used to propagate nymphs for behavior experiments in 2011 and 2012. Engorged females were collected from hunter harvested deer in fall of 2010 in Wisconsin (WI_{2010}) and South Carolina (SC_{2010}) and produced nymphs for 2011 experiments. Nymphs for the 2012 experiments were offspring of the nymphal cohort raised from the females collected in 2010. Two of 2012 clutches ($WI_{F2,2010*}$) were directly related to the WI clutches used in 2011 arenas, while the remaining 5 clutches ($WI_{F2,2010}$, $SC_{F2,2010}$) were derived from mothers collected at the same time (but not related to) as those who provided clutches for 2011 arenas. Additional engorged females were collected from deer in North Carolina (NC_{2011}) and South Carolina (SC_{2011}) in fall of 2011 and were used to supplement the 2012 nymph supply. A single arena always contained nymphs from the same geographic origin (WI, SC or NC), however nymphs within an arena could have all been siblings from a single clutch (homogeneous) or a mixture of siblings and non-siblings from multiple clutches (heterogeneous).

Year	**Engorged female origin**	**# clutches propagated**	**Composition of arenas**		**Number of arenas containing nymphs of a given origin (per site)**			
			Homogeneous	**Heterogeneous**	**WI**	**RI**	**TN**	**FL**
2011	WI_{2010}	8	7	1	8	-	-	-
	SC_{2010}	14	4	4	8	-	-	-
			Total number of arenas		**16**			
2012	$WI_{F2,2010*}$	2	7	1	2	2	2	2
	$WI_{F2,2010}$	1	1	11	3	3	3	3
	$SC_{F2,2010}$	4	12	15	8	7	6	6
	SC_{2011}	2	7	0	2	1	2	2
	NC_{2011}	3	12	0	3	3	3	3
			Total number of arenas		**18**	**16**	**16**	**16**

Experimental apparatus and questing observations. Ticks originating from Wisconsin (WI) and South Carolina (SC; 2011 and 2012) and North Carolina (NC; 2012) were placed at field sites in the eastern US (Wisconsin (WI) in 2011; WI, Rhode Island (RI), Tennessee (TN), and Florida (FL) in 2012). The design of the 2011 experiment consisted of 5 blocks, each containing 4 arenas. All naturally-occurring ticks were removed by heat-treating locally-obtained leaf litter before adding it to the arenas. Two arenas were not seeded with nymphs and served as experimental controls for the effectiveness of the arena barrier and leaf litter heat treatment. In 2011, there were 5 individual sightings of a single nymph (which was *not* removed when sighted) in the treated control arenas. No nymphs were recovered from the treated controls during the survival assessments. In 2012, to provide further assurance that all preexisting local ticks were removed from the leaf litter after heat treatment and before the experimental nymphs were released, we conducted microdrags (pressing a 12 cm x 12 cm square of white flannel material against the leaf litter inside of the arenas) and carbon dioxide (CO_2) assays (dry ice baits) in each arena at each site. In 2012, no nymphs were observed in control arenas at any of the four sites, and no nymphs were recovered from these arenas during end-of-study survival assessments.

The arena design was modified from previously published apparatuses used for measuring *Ixodes* spp. questing behavior in natural field conditions (Lane et al. 1995, Dautel et al. 2008). Each arena consisted of a 0.5 m diameter circle of 25 cm high aluminum flashing sunk ~7 cm into the ground. A 6 cm blockade of Tree Tanglefoot Insect Barrier (Contech) was applied to the top inner rim of the arenas to prevent ticks from climbing out (Lane et al. 1995). Inside the arenas we installed 24, 3 mm wide bamboo dowel rods (stems), of three heights, 5 cm, 10 cm, and 20 cm. The stems were spaced in a semi-regular pattern, with the height of stem at each position randomized. These stems served to mimic understory vegetation that ticks can climb in their natural environment. Arenas were grouped in blocks of four and surrounded by a 60 cm wire mesh (2.54 cm) barrier and covered with a wire mesh lid (Figure 1.1). This excluded large and medium-sized terrestrial species and birds from the arenas, while the aluminum flashing walls provided a barrier to deflect smaller terrestrial vertebrates.

Figure 1.1. Experimental arenas for tick questing behavior experiments 2011, 2012. Arenas were grouped in blocks of four and surrounded with wire mesh.

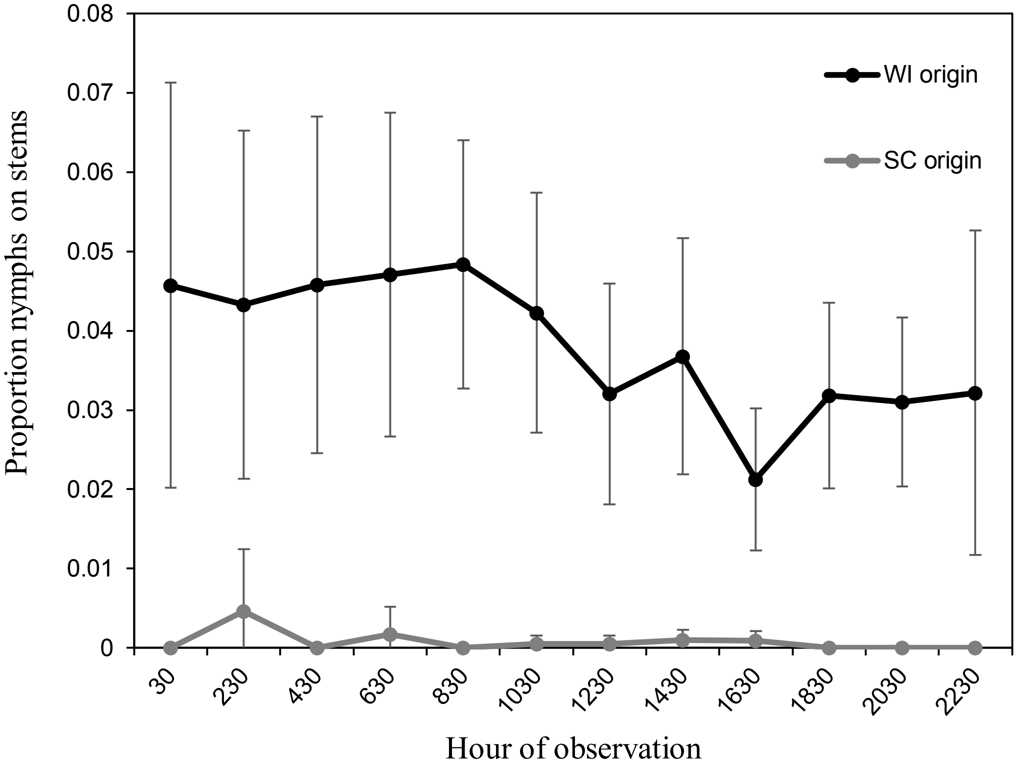
In 2011, 16 arenas in Wisconsin each received 44-60 lab-reared nymphs of a single geographic origin (8 received WI nymphs; 8 received SC nymphs). Due to limited availability of lab-reared nymphs, some arenas contained nymphs from multiple mothers originating from the same geographic origin (Table 1.1). Nymphs were deposited into the arenas on May 23, 2011. Observers, who were blind to the origin of nymphs in the arenas, recorded the number of nymphs visible on the stems (Figure 1.2) during a two minute observation of the arena at bi-hourly intervals during three 24-hour (June 15-16, July 7-8, July 29-30) and one 14-hour (July 5) sampling periods. Control arenas were checked in the same manner during each sampling visit. This sampling design was employed because it was not known whether *I. scapularis* nymphs from different origins might have divergent patterns of diel activity. The questing behavior of the SC versus WI nymphs was compared statistically based on the log-odds of their presence on stems.

Figure 1.2. *Ixodes scapularis* nymph questing on a stem in an experimental arena; approximately 1 cm of a 10 cm dowel is visible. Photo by G. Hickling.

In 2012, the experimental design at each site consisted of four blocks of four arenas, each arena containing 37-62 nymphal ticks from a single geographic origin (5 from WI, 3 from NC, and 8 from SC) and two unseeded additional arenas which served as controls for the leaf litter and arena barriers. The WI site had two additional arenas containing SC derived ticks. Arenas were established using the same protocol as 2011, except that the number of stems used was reduced from 24 to 15 (5 of each of the same heights used in 2011) leaving a larger area of "stem-less" leaf litter adjacent to the arena walls (Figure 1.7; Appendix A). Spacing between stems remained the same in both years. This design was replicated at each of the four field site locations. Nymphs were deposited into the arenas during the 1st week of May, 2012. Nymph questing in arenas was recorded by observers blinded to nymph origin in the morning (approx. 0800 hours) and late afternoon (approx. 1600 hours) at weekly or biweekly intervals from May – September 2012. Additionally, a total of 18 midnight observations were carried out at 3 of the sites (FL, TN, and WI). We chose these times to conduct our observations based the periods of highest-activity of WI and SC nymphs observed in 2011 (Figure 1.3). In 2012, we expanded our definition of nymph questing to include ticks on the leaf litter and arena wall, as well as on stems, after observing nymphs in these locations during the 2011 observations. We hypothesize that all such nymphs emerged from the leaf litter represent a potential risk to human hosts, not just those on stems. As ticks do not jump or fly, they must make direct contact with a host in order to attach and acquire a bloodmeal (Sonenshine 1991). Ticks emerged from the leaf litter can instantly position their forelegs in the air to attach to a passing host. Ticks under the leaf litter (not emerged) would have difficulty making direct contact with hosts walking upon the leaf litter, as the leaf litter would create a barrier between the nymph's forelegs and the host body.

The questing behavior of the nymphs was again assessed by estimating the log-odds of observing emerged nymphs. In 2012, each site had its own set of observers.

Figure 1.3. Bi-hourly questing activity patterns at WI field site in 2011. Proportion of nymphal *I. scapularis* (means ± 95CIs) of northern (WI) and southern (SC) US origin, questing on stems during each observation time, in outdoor arenas in Wisconsin, June-July 2011. For both groups, emergence was highest before 0830 hours and dropped steadily with the exception of a small peak observed in the late afternoon (1230-1630 hours).



Tick Survival. Once behavioral observations were complete (in July 30, 2011 and mid-September 2012, respectively), we assessed the relative survival of the nymphs in each arena by conducting searches of the arena litter. In 2011, we placed air-activated hand warmers (Grabber®, Byron Center, Michigan), wrapped in white flannel into the arenas for 1.5-2 hours. Nymphs attracted to the heat were removed from the flannel and placed in 95% ethanol. A 12 cm x 12 cm square of white flannel material was then pressed against the leaf litter inside of the arenas (= microdrag) and rustling the leaf litter to stir it up and expose subsurface dwelling nymphs. After the initial microdrag, a second round of microdrags was performed, again stirring the leaf litter and with moving the cloth through the leaf litter to contact in the sub-surface layers. Individuals were placed in 95% ethanol to preserve their field collected condition. In 2012, the heat pack method was abandoned because it appeared to preferentially target WI ticks (captured 53.9% of all WI ticks recovered) over SC ticks (captured 6.0% of all SC ticks recovered) and survival was assessed using only the microdragging method.

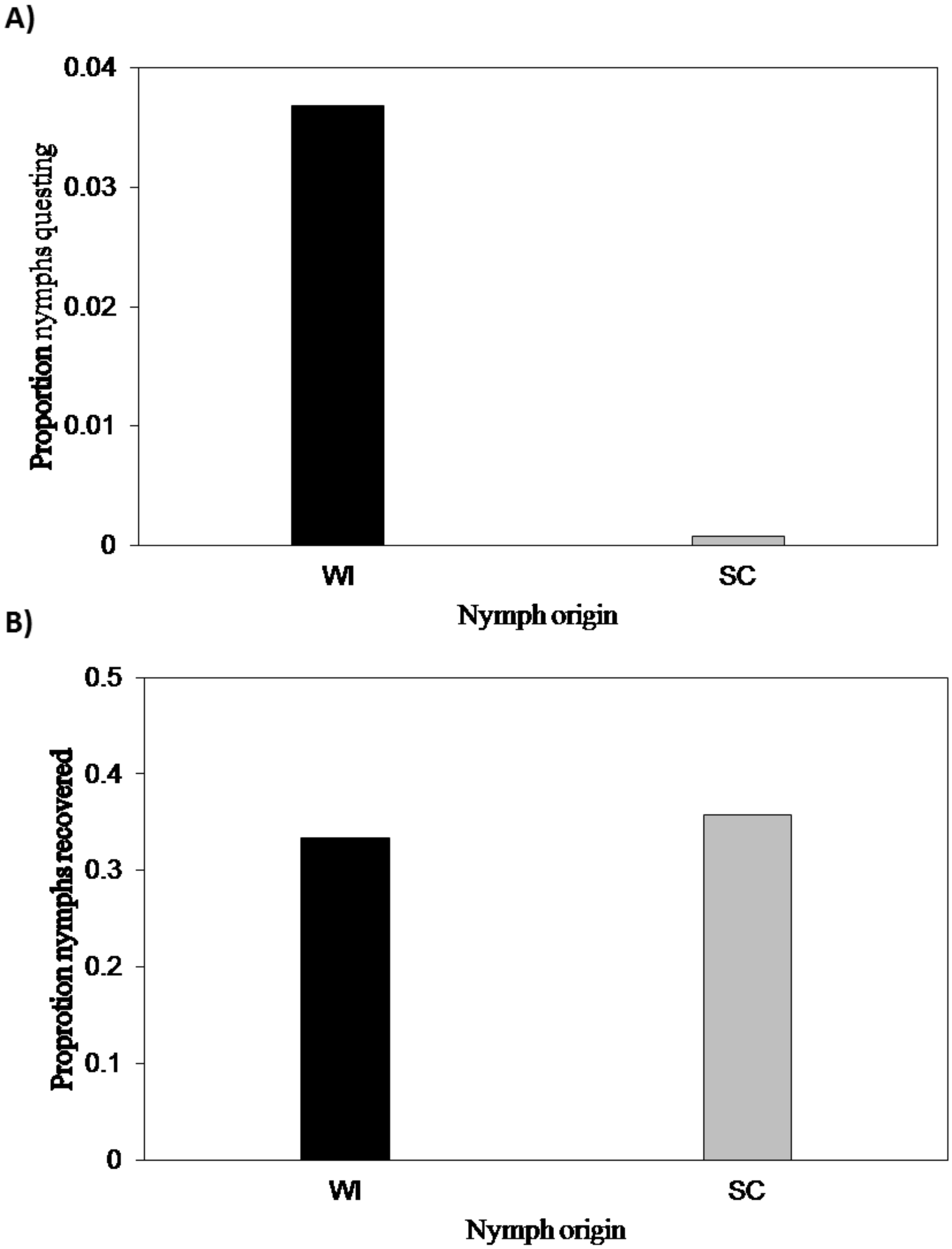
Statistical Analyses. We used a Bayesian approach for predicting the log-odds of nymph questing behavior as a function of nymph geographic origin. No p-values are reported; but rather summaries of the posterior distributions generated from models using the data obtained. These posterior distributions describe the plausibility of possible parameter values generated from the model, given the data we observed (McElreath and Koster 2014). Our goals were to quantify questing behavior and evaluate the strength of evidence for effect of geographic origin on the questing behavior of *I. scapularis* nymphs. We used a multilevel binomial regression model to predict the log-odds of observing questing nymphs in the arenas. Questing behavior was measured by counting the number of nymphs visible on stems (2011), or on stems, leaf litter and arena walls (2012) during a given two-minute observation of an arena. Similarly, survival was measured using a multilevel binomial regression model to predict the log-odds of recovering nymphs from arenas at the end of each study period. Survival was measured by tallying the number of nymphs recovered from arenas at the end of each experiment. We adopted a multilevel modeling approach (see Gelman and Hill (Gelman and Hill 2007) for an overview) for the reasons outlined by McElreath and Koster (McElreath and Koster 2014); briefly, the approach simultaneously addresses our concerns regarding repeated measures and imbalanced sampling. The questing behavior models allowed for nymph questing to vary by individual arena, observation date, state of origin, and (in 2012) site of observation. The survival models allowed for nymph recovery to vary by individual arena, state of origin, and (in 2012) site of observation. Models were fitted using Stan 2.3.0 (Stan Development Team 2014), a Hamiltonian Monte Carlo sampler, to draw samples from the joint posterior density of the parameters. We used weakly informative regularizing priors to analyze the data. The results we present are based on estimates derived from 3,000 samples of each parameter, after 1,000 samples for adaptation. Convergence was assessed by trace plots. To determine if the regression coefficients of the nymph origins were credibly different from one another, we estimated the posterior distribution of the difference between the coefficients. Coefficients were considered to be credibly different if the highest density interval (HDI) of the posterior distribution of their difference did not encompass zero (Kruschke et al. 2012). Model code was generated using a convenience package for Rstan known as map2stan (McElreath 2014). To visualize the results, predicted log-odds and the associated HDIs were back-transformed into probabilities. All statistical analyses were undertaken using R 3.1.0 (http://www.r-project.org).

Results

Questing behaviors differed between ticks from Lyme endemic and non-endemic regions. We used a Bayesian analysis to predict nymph questing behavior by nymph origin while allowing the influence of individual arenas, sites, and weeks to vary. Questing behavior was defined as nymph presence on stems in 2011, or on stems, leaf litter surfaces, and arena walls in 2012. From these field data, statistical models generated predictions of the posterior distribution of questing probabilities for a given nymph origin (i.e., means and 95% HDIs that include the most credible values of the posterior distribution). In addition to the model estimates, we also report summaries of the raw data (proportions of nymphs observed each year).

2011 – Wisconsin nymphs were far more likely than South Carolina nymphs to be observed on stems at a field site in the northern US. These data comprise 752 observations of 16 experimental arenas placed in west central Wisconsin in 2011 over a 10-week time period (May - July). For 521 (69%) observations, no nymphs were observed on stems. In the remaining observations, up to 10 nymphs were observed questing on the stems, and ≤ 8 nymphs were visible for > 95% of non-zero observations. Nymphs tested in the arenas were the F_1 progeny of mothers collected in Wisconsin (WI) or South Carolina (SC). We fit a model to predict questing behavior as a function of tick origin. Arena, hour of observation, and sample session were fit as random effects to account for potential variation among arenas, times of day and seasons. The overall proportion of nymphs observed on stems in arenas in 2011 is displayed in Figure 1.4A; this proportion was much higher for WI nymphs (0.0368) than for SC nymphs (0.0008).

Figure 1.4. Questing behavior data from experimental arenas in Wisconsin (WI) during June-July 2011. (A) Proportion of nymphs from each origin observed questing on stems. A higher proportion of WI nymphs were observed on the stems compared to the South Carolina (SC) nymphs. (B) Proportion of nymphs from each origin recovered when arenas were depopulated on July 30, 2011. Recovery rates were similar for nymphs from both origins.



Model predictions of the probability of observing nymphs on stems as a function of nymph origin are summarized in Table 1.2. On average, WI nymphs were predicted to be 19.5 times as likely as SC nymphs to be observed. There was considerable variation in predicted stem use by nymphs within an origin, as reflected by the broad 95% HDI for each parameter estimate.

Table 1.2. Probability of nymph questing as a function of nymph origin. Posterior distributions for models predicting probability of questing nymphs from each origin (WI, SC, NC) at each field site (WI, RI, TN and FL) in each experimental year (2011, 2012). Posterior distributions are summarized by means and 95% HDIs in parentheses. Effect sizes were calculated as the ratio of the posterior mean questing probability of one origin to another origin. In 2011, nymphs were tested only at one site (WI) and questing behavior was measured as the presence of nymphs on stems. North Carolina (NC) nymphs were not tested in 2011. In 2012, questing behavior was measured as the presence of nymphs on stems, leaf litter, and arena walls. The asterisks and bolded font indicate those comparisons for which a credible difference was evident (Table 1.3).

Year	**Site where tested**	**Posterior probability of questing by origin means (95% HDIs)**			**Effect size (ratio of questing)**		
		WI	**SC**	**NC**	**WI:SC**	**WI:NC**	**SC:NC**
2011	WI	0.034 (0.004, 0.071)	0.002 (8.0e-9, .001)	-	**19.5***	**-**	**-**
2012	FL	0.006 (0.001, 0.011)	0.002 (5e-4, 0.003)	0.001 (7e-5, 2e-3)	**3.6***	**7.0***	2.0
	TN	0.019 (0.005, 0.037)	0.005 (0.001, 0.009)	0.002 (3e-4, 0.004)	**4.0***	**11.6***	3.0
	RI	0.004 (0.001, 0.007)	0.001 (1e-4, 0.002)	4e-4 (4e-5, 1e-3)	**5.0***	**9.9***	2.0
	WI	0.020 (0.005, 0.039)	0.005 (0.002, 0.009)	0.002 (3e-4, 0.004)	**3.9***	**11.3***	2.9

To determine if the regression coefficients of the nymph origins (WI and SC) were credibly different from one another, we estimated the posterior distribution of the difference between the coefficients. Coefficients were considered to be credibly different if the HDI of the posterior distribution of their difference did not encompass zero (Kruschke et al. 2012). This analysis revealed a credible difference in the probability of observing questing WI nymphs versus questing SC nymphs (WI – SC mean = 0.032, 95% HDI = 0.004, 0.076; Table 1.3).

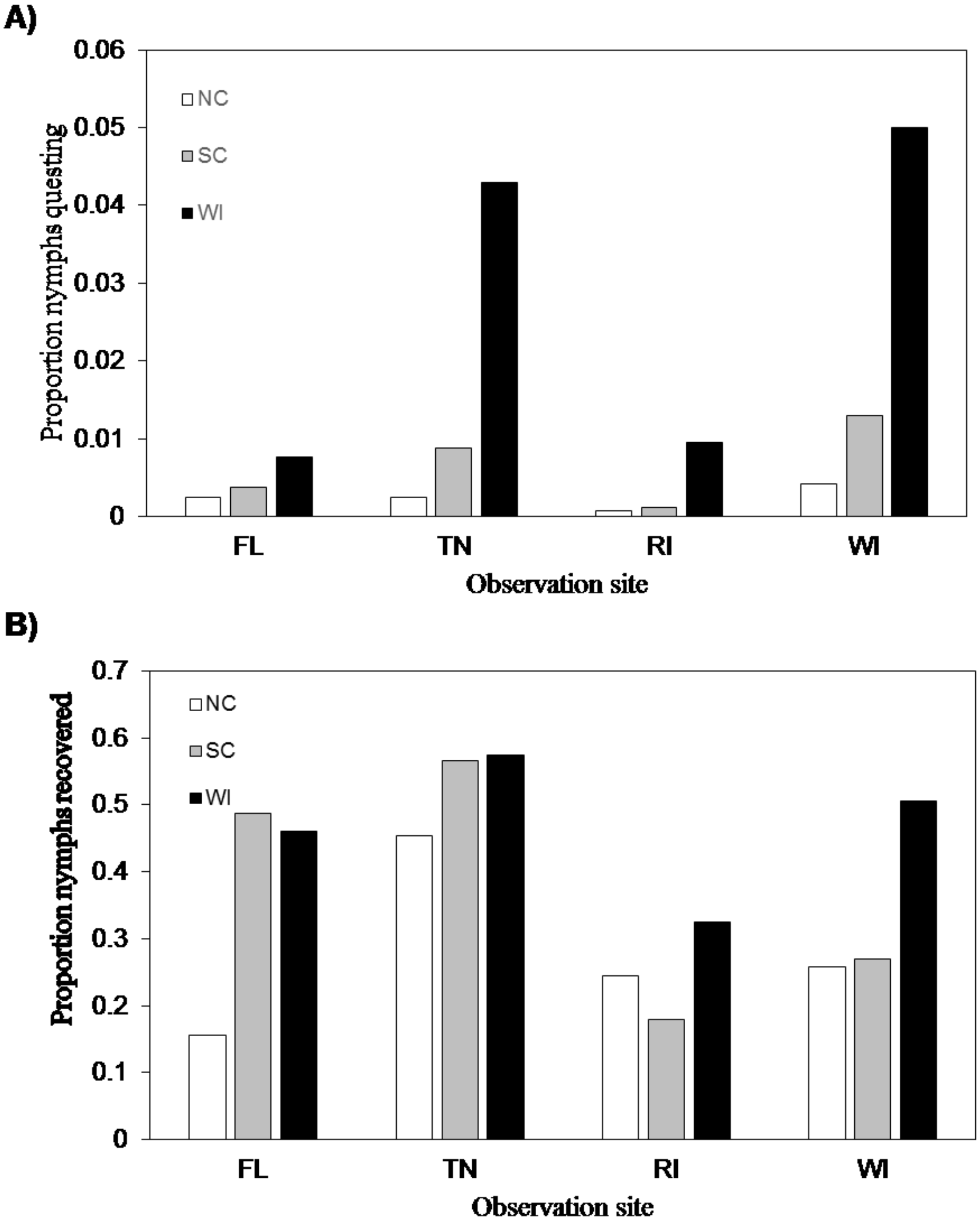
Table 1.3. Determination of nymph questing differences. Posterior mean difference in predicted probability of questing between origins for each site where nymphs were observed. The asterisks and bolded font indicates those comparisons for which a credible difference (HDI does not include zero) has been determined.

Year	**Site where tested**	**Posterior probability of difference in questing means (95% HDIs)**		
		WI - SC	**WI - NC**	**SC - NC**
2011	WI	**0.032 (0.004,0.076)***	-	-
2012	FL	**0.004 (7e-5, 0.009)***	**0.005 (6e-4, 0.010)***	0.001 (-0.001, 0.003)
	TN	**0.015 (0.002, 0.031)***	**0.018 (0.005, 0.036)***	0.003 (-1e-4, 0.008)
	RI	**0.003 (0.001, 0.006)***	**0.003 (0.001, 0.007)***	4e-4 (-5e-5, 0.001)
	WI	**0.015 (0.002, 0.034)***	**0.018 (0.004, 0.036)***	0.003 (-2e-4, 0.007)

2012 – Wisconsin nymphs were more likely to quest on or above the surface of the leaf litter than North Carolina or South Carolina nymphs at field sites in the northern and southern US. These data comprise 2640 observations of 66 different experimental arenas located at four sites (WI, RI, TN, and FL) in the eastern US in 2012 over a 19-week time period (mid-May - mid-September). For 2059 (78%) observations, no nymphs were observed on stems, leaf litter surfaces, or the arena walls; hereafter, "questing" refers to nymphs observed in any of these locations. In the remaining observations up to 20 nymphs were observed questing, and ≤ 10 nymphs were visible for > 95% of non-zero observations. Nymphs tested in the arenas were the F_2 progeny of mothers collected in WI or SC, or the F_1 progeny of mothers collected in North Carolina (NC) or SC; the two generations were evenly distributed at all sites. We fit a model to predict questing behavior as a function of tick origin. Arena, site, and week were fit as random effects to account for heterogeneity among arenas, locations where nymphs were tested (sites), and time (week in which sample occurred).

The overall proportion of observed questing nymphs at each site (WI, RI, TN and FL) from each origin (WI, SC and NC) in 2012 is displayed in Figure 1.6A. WI nymphs were observed most often at all four sites, with the total proportion questing ranging from 0.008-0.050 among the sites. Nymphs from SC were observed second most often (proportion ranging from 0.001-0.013), and NC nymphs were observed least often (proportion ranging from 0.001-0.004) at all sites.

Figure 1.6. Questing behavior data from experimental arenas at northern and southern field sites; May-September, 2012. (A) Proportion of nymphs at each field site, from each origin, observed questing on stems, leaf litter, and arena walls. Wisconsin (WI) nymphs were observed in higher proportions compared to North Carolina (NC) or South Carolina (SC) nymphs at all four field sites. (B) Proportion of nymphs from each origin recovered at each field site when arenas were depopulated September 13-20, 2012. Recovery rates varied for each nymph origin at the four sites.



Model predictions of the probability of observing nymphs on stems, leaf litter surfaces, or arena walls as a function of nymph origin are summarized in Table 1.2. On average, WI nymphs were 3.6-11.6 times as likely as those from SC or NC to be questing in arenas. As in 2011, considerable within-origin variation was observed, as reflected by the broad 95% HDI estimates.

Comparisons involving WI nymphs (WI - SC; WI - NC) were deemed credibly different at all four sites (Table 1.3). None of the SC - NC comparisons revealed credible differences (i.e. all 95% HDIs overlapped zero), indicating that the questing probabilities of these two origins were not different at any site.

The WI:SC comparison undertaken in WI in 2011 produced an effect size of 19.5 (Table 1.2). When this same comparison was repeated at all four observation sites in 2012, effect sizes were lower than those observed in 2011 (Table 1.2; range 3.6-5.0). This was due, in part, to the 'stricter' definition of questing used in 2011 (counting nymphs on stems) versus 2012 (counting nymphs on stems, leaf litter surfaces, and arena walls). When the 2011 definition was applied to the 2012 data, the effect sizes for the WI:SC comparison rose (range 8.3 - 14.8 for the four observation sites; Table 1.7 & Table 1.8; Appendix B).

Survival rates in 2011 did not differ among nymph origins; 2012 survival varied by origin among sites. *Wisconsin and South Carolina nymphs showed similar survival in arenas at a field site in the northern US in 2011*. The observation of greater questing on stems by WI compared to SC nymphs could have resulted from greater mortality of the southern nymphs in the northern environment. To assess this possibility, we fit a model to predict the probability of recovering nymphs from a given origin at the end of the experiment. Arena was fit as a random effect. The observed recovery of nymphs ranged from 17% - 48% of those initially released into arenas. Furthermore, the overall proportion of live nymphs recovered from arenas at the end of the experiment was similar for nymphs of both origins (WI = 0.333, SC = 0.357; Figure 1.4B). This similarity in recovery rates strongly suggests that the difference in questing behavior of the two groups was not a consequence of differential survival.

If nymphal questing behavior were a function of survival we would anticipate finding a positive relationship between questing behavior and recovery; visual inspection of Figure 1.4A and Figure 1.4B reveals no such pattern. Furthermore, we did not observe a credible difference in recovery between WI and SC nymphs (WI-SC mean = -0.026, 95% HDI = -0.118, 0.080; Table 1.4)**.** Model predictions of the probability of recovering nymphs from arenas as a function of nymph origin are summarized in Table 1.5.

Table 1.4. Determination of nymph recovery differences. Posterior mean difference in predicted probability of recovery between origins for each site where nymphs were observed. The asterisks and bolded font indicates those comparisons for which a credible difference (HDIs do not include zero) has been determined.

Year	**Site where tested**	**Posterior probability of difference in recovery means (95% HDIs)**		
		WI - SC	**WI - NC**	**SC - NC**
2011	WI	-0.026	-	-
		(-0.118, 0.080)		
2012	FL	0.0535	**0.3158**	**0.2623**
		(-0.185, 0.294)	**(0.104, 0.544)***	**(0.038, 0.508)***
	TN	0.058	0.191	0.133
		(-0.198, 0.281)	(-0.137, 0.452)	(-0.160, 0.457)
	RI	0.193	0.172	-0.022
		(-0.009, 0.386)	(-0.078, 0.406)	(-0.279, 0.193)
	WI	**0.195**	0.209	0.014
		(0.001, 0.391)*	(-0.027, 0.433)	(-0.243, 0.214)

Table 1.5. Probability of nymph recovery as a function of nymph origin. Posterior distributions for models predicting probability of recovery of nymphs from each origin (WI, SC, NC) at each field site (WI, RI, TN and FL) in each experimental year (2011, 2012). Posterior distributions are summarized by means and 95% HDIs (in parentheses). Effect sizes are calculated as the ratio of the posterior mean recovery probability of one origin to another origin. In 2011, nymphs were tested in experimental arenas for 69 days; North Carolina (NC) nymphs were not tested that year. In 2012, nymphs were tested for a much longer period (132-137 days). The asterisks and bolded font indicates those comparisons for which a credible difference was evident (Table 1.4).

Year	**Site where tested**	**Posterior probability of recovery by origin means (95% HDIs)**			**Effect size (ratio of recovery)**		
		WI	**SC**	**NC**	**WI:SC**	**WI:NC**	**SC:NC**
2011	WI	0.326	0.357	-	0.9	-	-
		(0.248, 0.396)	(0.283, 0.435)				
2012	FL	0.442	0.392	0.146	1.1	**3.0***	**2.7***
		(0.270, 0.614)	(0.221, 0.574)	(0.014, 0.288)			
	TN	0.553	0.505	0.365	1.1	1.5	1.4
		(0.362, 0.735)	(0.307, 0.688)	(0.152, 0.638)			
	RI	0.385	0.195	0.210	2.0	1.8	0.9
		(0.205, 0.568)	(0.091, 0.315)	(0.065, 0.394)			
	WI	0.455	0.262	0.245	**1.7***	1.9	1.1
		(0.293, 0.631)	(0.146, 0.400)	(0.076, 0.422)			

Wisconsin, South Carolina, and North Carolina nymphs showed varied survival at field sites in the northern and southern US in 2012. Recovery of nymphs from arenas was highly variable in 2012, ranging from 0%-91% of the number initially released. We again fit a model with the outcome variable as number of ticks recovered from an arena, and arena and site as random effects to account for heterogeneity in recovery rates among the arenas and site locations. The overall proportion of nymphs recovered from each origin varied at each site. However, the variation in recovery did not correlate with the questing patterns exhibited by nymphs of each origin at each site (Figure 1.5A and Figure 1.5B). This suggests that differential survival was not responsible for the overall pattern of observed questing differences.

Table 1.5 summarizes the model predictions of the probability of recovering nymphs as a function of nymph origin at each site. WI nymphs were predicted to be 1.1- 2.0 times as likely as SC nymphs, and 1.5 - 3.0 times as likely as NC nymphs, to be recovered at all sites (Table 1.5). Model parameters describing the relationships among recovery and origin were variable within and among sites, indicating that the nymphs in our study experienced a wide range of survival rates. Differences of posterior distribution of WI – SC and WI - NC recovery probabilities were not deemed credibly different at three of four sites. The SC – NC comparison was also not different at three of four sites (Table 1.4). The WI origin nymphs exhibited higher survival probability than SC and NC origin nymphs at the WI site; NC origin ticks did not survive as well as WI or SC nymphs at the FL site.

Taken together, the 2011 and 2012 analyses point to a consistent behavioral pattern whereby nymphs from two southern, non-endemic origins (SC and NC) were far less likely to quest on or above the leaf litter than nymphs from one northern, endemic origin (WI).

Furthermore, our survival analyses suggest these questing differences were not an artifact of differential nymph survival.

Discussion

Southern origin *I. scapularis* nymphs rarely emerge from leaf litter as compared with nymphs from a northern origin, regardless of environment. We report consistent, pronounced differences in the questing behavior of *I. scapularis* nymphs from southern origins (SC, NC) compared with nymphs from a northern origin (WI). The northern-derived nymphs were much more likely (3.6 – 19.5 times) to be observed questing above the leaf litter in experimental arenas during both years and at all sites (northern sites - WI, RI; southern sites - TN, FL) where they were observed. These results support our hypothesis that nymphs from non-endemic, southern regions typically remain in the substrate layers of the forest floor and therefore are unlikely to come into contact with human hosts. Determining the generality of this result will require further study, however, we suggest that this behavior of southern nymphs contributes significantly to the low incidence of human LD cases reported in the southeastern United States. Our results support the hypothesis that the Lyme Disease Gradient in the eastern US is due, in part, to regional differences in vector behavior.

Overall, the northern origin (WI) nymphs were always most likely to be seen questing above the leaf litter (Table 1.2). In 2011 we observed a larger relative difference in questing probability for WI nymphs (19.5 times SC nymphs) than in 2012 (3.6 - 5.0 times SC nymphs). Effect sizes were lower in part because of the more stringent definition of "questing" in 2011. Southern ticks will occasionally venture onto the surface of leaf litter, but they rarely climb above it. Consequently, when "questing" is defined as climbing above the litter (as we did in 2011), the difference in WI vs SC behavior is more pronounced than if questing is defined as "on or above" the litter (as in 2012). However even when corrected for this, effect sizes were still higher in 2011 (Table 1.7 & Table 1.8; Appendix B). That year, observers visited the arenas bi-hourly, up to 13 times during a sampling session, whereas in 2012 arenas were observed at 8-hour intervals, for no more than three times in a sampling session. We speculate that the more frequent observer visits in 2011 may have elevated the questing response of the northern nymphs but not of the southern nymphs, as the former often parasitize small mammals (Spielman et al. 1985, LoGiudice et al. 2003) and other hosts whose cues originate from above the leaf litter, whereas the latter more typically parasitize fossorial lizards (Apperson et al. 1993, Oliver 1996, Levine et al. 1997, Kollars et al. 1999, Durden et al. 2002) whose cues originate from below the leaf litter. Our observation in 2011 that the number of nymphs seen during a sampling session (i.e. 24- or 14-hour period of recurring bi-hourly observations) was typically highest for observations in the latter half of the session supports this hypothesis (Table 1.9; Appendix C). The overall lower rates of questing in 2012 are also consistent with this hypothesis. Natural variability in questing behavior could also have contributed to differences between years.

Differences in questing cannot be explained by differences in survivorship. In 2011, we did not observe a credible difference in probability of recovery (= index of survival) for northern and southern nymphs placed in arenas for approximately 10 weeks (Table 1.5). In 2012, we extended the observation period, from 10 to 19 weeks, in order to explore the possibility that southern ticks might eventually quest more vigorously if given a sufficiently long opportunity to do so or if behavior varies seasonally. In nature, nymphal activity in northern regions commences in mid-spring and tapers off by mid- to late summer (Falco et al. 1999, Kollars et al. 1999, Diuk-Wasser et al. 2006, Gatewood et al. 2009). Although we observed increased activity for all three nymph origins during the second half of the 2012 observation period, the northern origin ticks continued to show greater activity above the leaf litter than southern origin ticks throughout the entire observation period and at all sites (Figure 1.6). Our observations of late-season questing in the arenas, but not in natural conditions (Gatewood et al. 2009), most likely reflect the isolation of ticks in arenas from their hosts and predators. In nature, ticks are finding hosts or being eaten, thus removing them from the system and contributing to the observed declines in questing activity over the season. The longer observation period, however, did accentuate differences in tick recovery rates among groups at each site, but these differences did not correlate with the observed questing patterns at each site. Most striking were patterns observed at the southern sites (TN and FL) where WI and SC nymphs displayed similar recovery rates, but remarkably different questing behaviors (Figure 1.6A and Figure 1.6B). In laboratory trials WI nymphs have higher survival rates than SC nymphs when relative humidity is low (approximately 75%) (Ginsberg et al. 2014). Our field observations confirm this result; WI nymphs survived better than SC nymphs at the northern sites (WI and RI; Figure 1.6B) which had lower ambient relative humidity compared to the southern sites (Table 1.6). Future studies are needed to determine if these findings reflect ecological adaptation by northern origin tick populations.

Figure 1.6. Summary of nymph questing activity and abiotic conditions at each site in 2012. Mean proportion of questing nymphs (by nymph origin-WI, SC, NC) observed in arenas during weekly observations (bar graphs, primary y-axis) at the four field sites in 2012: (A) Florida, (B) Rhode Island, (C) Tennessee and (D) Wisconsin. Mean ambient (10 cm) temperature and relative humidity readings for each observation week are expressed by line graphs with values on the secondary y-axis. The first column (panels A and C) shows data for the southern, non-endemic sites (FL and TN), the second column (panel B and D) shows data for the northern, endemic sites (RI and WI). NOTE: Primary y-axis differs for top and bottom rows.

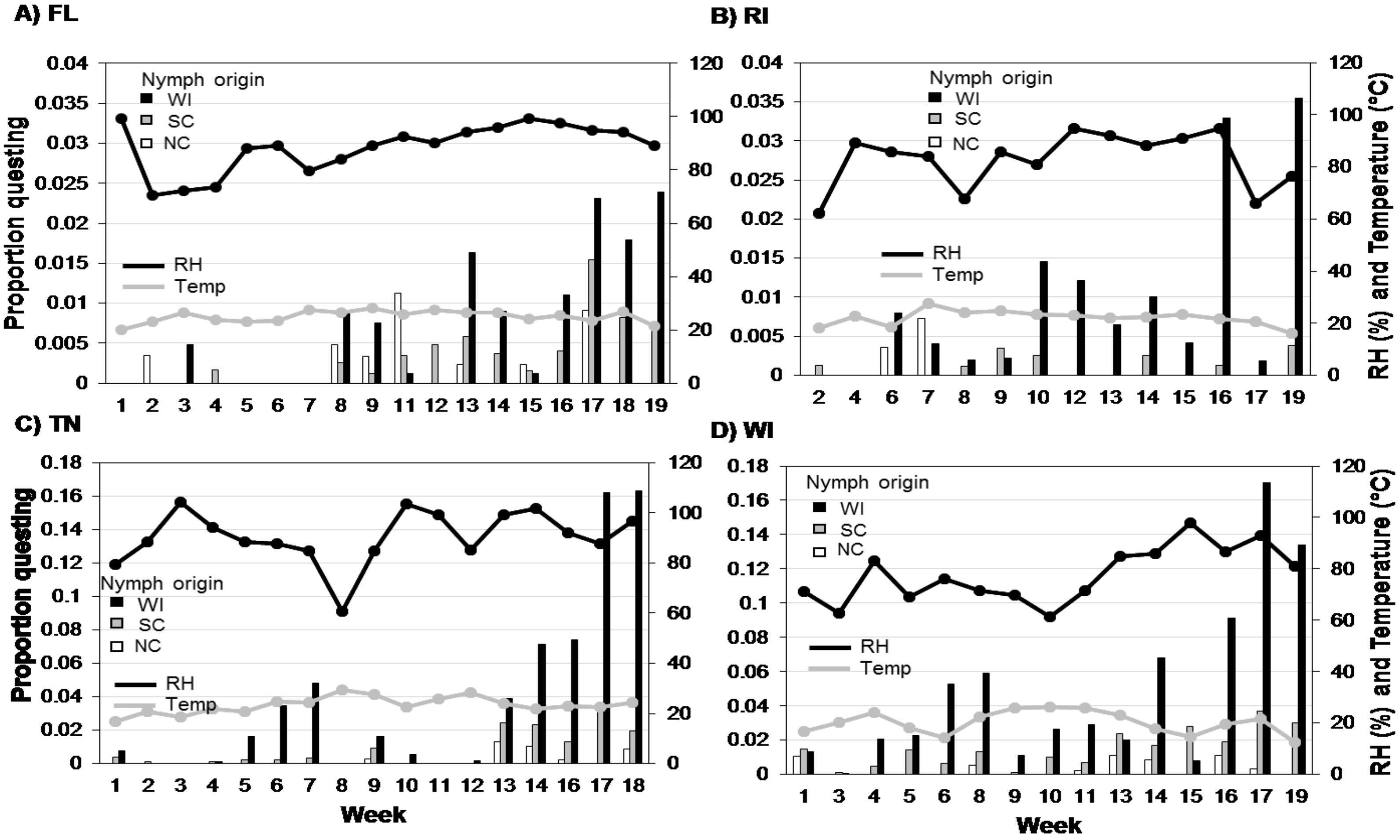


Table 1.6. Environmental conditions at field sites in 2011 and 2012, measured with on-site HOBO dataloggers. Mean temperature and relative humidity (SD in parentheses) at leaf litter (level "0 cm") or above leaf litter (level "10 cm" = ambient) inside arenas at each field site in 2011 and 2012. Fort McCoy, Wisconsin was the only study site in 2011. In 2012, readings were discontinued after the second week of September. Latitude coordinates obtained from nationalatlas.gov.

Field site	**Latitude (°N)**	**Months sampled**	**Temp (°C) 0cm**	**Temp(°C) 10cm**	**Relative humidity (%) 0cm**	**Relative humidity (%) 10cm**
Fort McCoy, WI	44.04	2011: June-July	20.1 (5.1	21.4 (6.7)	93.4 (11.5)	85.1 (19.3)
		2012: May-Sep.	17.9 (4.2)	19.5 (6.9)	101.5 (5.8)	78.8 (19.2)
Kingston, RI	41.48	2012: May-Sep.	18.8 (4.4)	19.5 (5.1)	94.9 (10.8)	90.8 (15.4)
Oak Ridge, TN	36.01	2012: May-Sep.	21.7 (3.9)	22.4 (4.3)	96.6 (7.7)	93.5 (12.6)
Tall Timbers Research Station, FL	30.53	2012: May-Sep.	23.3 (2.5)	24.3 (3.5)	97.0 (9.5)	91.5 (13.7)

Only a small proportion of nymphs are questing at any given time. Another key finding from this study is that on average, the probability of observing nymphs questing at a given time was very low (< 3.5 % in either year; Table 1.2). Questing activity of *I. ricinus* (the LD vector in Europe) nymphs released into field plots was approximately 4-10% (Dautel et al. 2008); drag efficiency of *I. pacificus* (LD vector in the western US) nymphs at a field site in California was determined to be on average 5.4% (Tälleklint-Eisen and Lane 2000); and mark-recapture studies in the northeast found that drag cloths sample approximately 6% of the total *I. scapularis* nymphal population *at a given time* (Daniels et al. 2000). Our results suggest that these are plausible estimates for the proportions of questing nymphs from LD endemic regions, but highly overestimate the proportion of nymphs that will be dragged in non-endemic regions (Table 1.2). An important point is that since >90% of the nymph population will not be dragged at any given time, even small variations in questing behavior between regions with similar population densities will result in large differences in drag sampling success.

Hypotheses for differences in questing behavior of northern and southern *I. scapularis* nymphs. Prior studies have demonstrated there are differences in 'draggability' (likelihood of being collected on drag cloths) of northern and southern nymphs (Rogers 1953, Falco and Fish 1992, Diuk-Wasser et al. 2006, Goddard and Piesman 2006, Hamer et al. 2010, Goltz and Goddard 2013); the current experiment provides a mechanistic explanation for this observation. Equivalent latitudinal trends in draggability, climate, and host use are observed in *I. pacificus* in California, where nymphs are draggable in northern, but rarely in southern California (Lane et al. 2013). As is the case in the eastern US, California's LD incidence is much lower in the south of the state than in the north (Lane et al. 2013). Below we explore non-mutually exclusive hypotheses to explain geographic differences in questing behavior.

Ixodes scapularis nymphs are known to show regional differences in their use of particular classes of vertebrate hosts. Northern nymphs are generally found parasitizing small mammals, particularly mice and chipmunks (Spielman et al. 1985, LoGiudice et al. 2003). Southern nymphs are found mainly on lizards, particularly skinks that utilize habitats below the leaf litter surface (Apperson et al. 1993, Oliver 1996, Levine et al. 1997, Kollars et al. 1999, Durden et al. 2002). While some tick species are known to quest in vertical locations that maximize contact with their primary hosts (Mejlon and Jaenson 1997, Tsunoda and Tatsuzawa 2004) it remains unclear whether *I. scapularis'* associations reflect host preference by nymphs, host-independent questing strategies of nymphs, differences in host ecology (abundance, distribution, behavior), and/or differences in abiotic conditions affecting the interactions between nymphs and hosts.

In general, ticks quest more often and higher when ambient air is less desiccating (i.e., high humidity and/or lower temperature; Vail and Smith 1998, Randolph and Storey 1999, Vail and Smith 2002) presumably due to *I. scapularis'* intolerance to dry conditions (Needham and Teel 1991). Schulze and Jordan (2003) showed that *I. scapularis* nymphs in New Jersey were most readily collected on drag cloths in the early morning hours, when ambient relative humidity (RH) was high and temperatures were low. In 2011, we observed a similar pattern as Schulze and Jordan – nymphs were most active in the morning hours when RH was high and temps low (Figure 1.3). In 2012, nymphs were most active at midnight (Figure 1.8; Appendix D), when RH was higher, but there was little difference in the overall morning (RH similar to midnight) and evening (lower RH than midnight) activity levels. There was a slight trend toward decreased questing activity for all origins when average ambient RH was below 80% (Figure 1.6); there were several weeks, however, when nymph activity remained low despite RH readings well above 80% (Figure 1.6). Questing activity at the sites seemed more influenced by seasonal trends than by day-to-day variation in abiotic conditions - nymphs at all four sites exhibited higher questing activity weeks 10-19 (Figure 1.6) despite fluctuating abiotic conditions. In our study, the ambient conditions at non-Lyme endemic sites (TN and FL) were typically warmer and more humid than the endemic sites (WI and RI; Table 1.6). This suggests that ambient RH is unlikely to be a limiting factor for questing activity by *I. scapularis* ticks at the southern sites. Given that temperature and energy (lipid) consumption in ticks are positively correlated (van Es et al. 1998), the higher temperatures at the southern sites may exert a selection pressure for southern ticks to emerge less from within the leaf litter (where it is generally cooler than above the leaf litter; Table 1.6). Future studies are necessary to delineate the role of temperature on questing behavior of regional tick populations. Ginsberg et al. (2014) studied survival patterns of different genotypes of *I. scapularis* and showed reduced survival of larvae under southern versus northern conditions. There may well be a connection between this finding and ours, since reduced survival under hot conditions in the south may be a selection pressure for reduced questing above the leaf litter. However, we do not know yet whether desiccation stress was the reason for reduced survival of southern nymphs at some of the sites in 2012 – this is being investigated presently.

Environment and genetic differences amongst ticks likely affect tick questing behavior. When populations display phenotypic plasticity (different phenotypes in different environments) for a trait (e.g., questing behavior), common garden experiments - where individuals with different genetic backgrounds are raised and tested together, in the same environment - can be used to determine whether the source of this variation is due to differences in genetics, environment, or an interaction of the two (Conner and Hartl 2004). Genetic influences for a trait are inferred if individuals from different groups (geographic locations, in this case) are reared together in the same environment, yet continue to display phenotypic differences (Conner and Hartl 2004). Our results clearly implicate a genetic component for questing behavior. We observed consistent phenotypic differences at all common garden sites (WI, RI, TN and FL) and in both years (2011 and 2012) of our experiment. In *I. scapularis* populations, mitochondrial lineages representing a widespread "American Clade" and more geographically limited "Southern Clade" have been identified (Qiu et al. 2002), however the behavioral differences in our experiment did not correlate with nymphs' mitochondrial type. Both WI and NC nymphs were American clade, yet WI nymphs quested substantially more often than NC nymphs. As nuclear genetic markers for *I. scapularis* are developed (Van Zee et al. 2013) it may be possible to identify markers correlating with variation in questing behavior. We were unable to directly assess the possibility of a genotype-by-environment interaction, as between site comparisons of questing were confounded by the necessity of employing different observers at the different sites.

Conclusion

Our experiments demonstrate that nymphs originating from southern, non-endemic, low LD risk areas generally stayed below the leaf litter surface while nymphs from a northern, LD endemic, high-risk area were much more likely to quest on or above the leaf litter surface. This result was consistent at all sites and under markedly varying environmental conditions, which suggests that a genetic component is partially responsible for the observed variation in questing behavior.

Climatic variables (Brownstein et al. 2005, Ogden et al. 2008, Feria-Arroyo et al. 2014, Ogden et al. 2014), biodiversity (Ostfeld and Keesing 2000a, Schmidt and Ostfeld 2001, Allan et al. 2003, LoGiudice et al. 2003), and tick genetics (Qiu et al. 2002, Humphrey et al. 2010) have all been proposed as explanations for the Lyme Disease Gradient in the eastern US. Such hypotheses focus on the ultimate causes of the observed geographic variation. Here we describe questing behavior of ticks from selected northern and southern locations and demonstrate that a mechanism – differences in questing behavior – is likely contributing to the observed geographic variation in human LD risk. The following chapter will address the generality of these behavior trends for nymphs collected from geographically scattered northern and southern sites which will help to explain the variation in human LD risk throughout *I. scapularis'* distribution; this is critical information to inform the public and health workers. Future studies are needed to unravel the ultimate causes for such differences in behavior as well as how plastic and adaptable questing behavior may be. This will be especially important to help to predict disease risk in areas where blacklegged tick populations are becoming established. Specifically, as northern populations of blacklegged ticks expand southward (Cortinas and Kitron 2006, Kelly et al. 2014, Wang et al. 2014), will the risk of Lyme disease also expand? Or, will factors currently suppressing 'risky' nymphal questing behavior prevail?

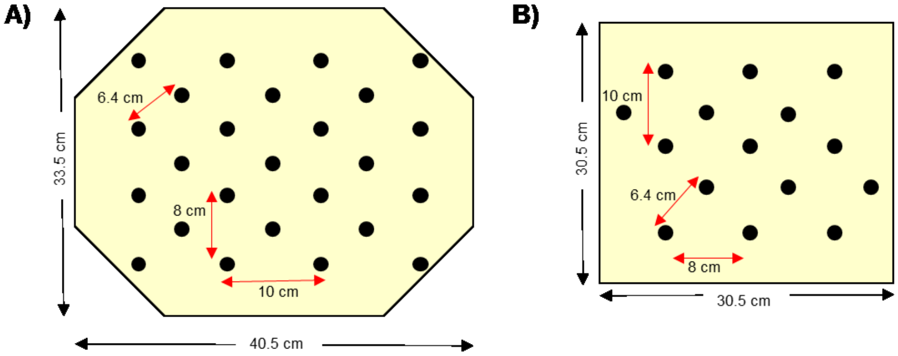
More broadly, this study highlights the importance of vector behavior in disease ecology. Here we provide an example of how a shift in one aspect of vector behavior can translate into profound differences in transmission of disease to humans. The impacts of vector behavior on disease transmission to humans are noted in other vector-borne disease systems including malaria (Day 2005, Mahande et al. 2007), West Nile virus encephalitis (Kilpatrick et al. 2006), and Chagas disease (Canals et al. 1999, Martínez-Ibarra et al. 2001). Continued research on the role of vector behavior and disease transmission is a critical foundation upon which to build improved approaches for mitigating disease risk. In the case of LD, *I. scapularis* ranges continue to expand (Cortinas and Kitron 2006, Diuk-Wasser et al. 2006, Ogden et al. 2008, Hamer et al.

2010, Wang et al. 2014), and so a better understanding of regional nymph behavior will be critical for forecasting change in human risk.

APPENDICES

Appendix A. Experimental apparatus

Figure 1.7. Stem arrangement patterns in experimental questing arenas. (A) 2011 stem arrangement pattern. There were 24 stems of three heights in each arena: 8 small (~ 2.5 cm), 8 medium (~ 10.0 cm) and 8 tall (~20.0 cm). The stems were spaced in a regular pattern, with the height of stem at each position randomized. (B) 2012 stem arrangement pattern. There were 15 stems of three heights: 5 small (~ 2.5 cm), 5 medium (~ 10.0 cm) and 5 tall (~20.0 cm) spaced in a regular pattern with the height of stem at each position randomized.



Appendix B. Statistical tables

Table 1.7. Probability of nymph questing on stems as a function of nymph origin. Posterior distributions for models predicting probability of questing nymphs from each origin (WI, SC, NC) at each field site (WI, RI, TN and FL) in each experimental year (2011, 2012). Posterior distributions are summarized by means and 95% HDIs in parentheses. Effect sizes were calculated as the ratio of the posterior mean questing probability of one origin to another origin. Questing behavior was measured as the presence of nymphs on stems. In 2011, nymphs were tested only at one site (WI) and North Carolina (NC) nymphs were not tested in 2011. The asterisks and bolded font indicate those comparisons for which a credible difference was evident (see Table 1.8. Appendix B).

Year	**Site where tested**	**Posterior probability of questing by origin means (95% HDIs)**			**Effect size (ratio of questing)**		
		WI	**SC**	**NC**	**WI:SC**	**WI:NC**	**SC:NC**
2011	WI	0.034 (0.004, 0.071)	0.002 (8.0e-9, .001)	-	**19.5***	**-**	**-**
2012	FL	7.7e-4 (3.3e-5, 2.2e-3)	5.2e-5 (2.6e-7, 1.5e-4)	2.8e-6 (3.9e-23, 1.1e-5)	**14.8***	**278.0***	18.8
	TN	0.01 (1.4e-e, 2.4e-2)	9.0e-4 (1.1e-4, 2.2e-3)	1.3e-5 (1.7e-21, 5.9e-5)	**11.2***	**791.8***	**70.1***
	RI	4.1e-3 (5.1e-4, 1.1e-2)	3.8e-4 (3.6e-5, 9.1e-4)	5.7e-5 (4.8e-12,2.3e-4)	**10.8***	**71.6***	6.7
	WI	6.1e-3 (6.9e-4, 1.5e-2)	7.4e-4 (7.5e-5, 1.7e-3)	1.0e-5 (5.0e-30, 4.7e-5)	8.3	**606.6***	**73.4***

Table 1.8. Determination of nymph questing on stems differences. Posterior mean difference in predicted probability of recovery between origins for each site where nymphs were observed. The asterisks and bolded font indicates those comparisons for which a credible difference (HDIs do not include zero) has been determined.

Year	**Site where tested**	**Posterior probability of difference in questing means (95% HDIs)**		
		WI - SC	**WI - NC**	**SC - NC**
2011	WI	**0.032 (0.004,0.076)***	-	-
2012	FL	**7.2e-4 (2.9e-5, 2.1e-3)***	**7.6e-4 (3.3e-5, 2.2e-3)***	4.9e-5 (-3.2e-6, 1.6e-4)
	TN	**9.2e-3 (5.5e-4,2.3e-2)***	**1.0e-2 (1.4e-3,2.4e-2)***	**8.9e-4 (6.9e-5, 2.1e-3)***
	RI	**0.004 (1.8e-4, 1.0e-2)***	**0.004 (4.1e-4, 1.1e-2)***	3.2e-4 (--1.1e-4, 9.8e-4)
	WI	5.4e-3 (-1.8e-5, 1.4e-2)	**6.1e-3 (6.9e-4, 1.5e-2)***	**7.3e-4 (7.3e-5, 1.7e-3)***

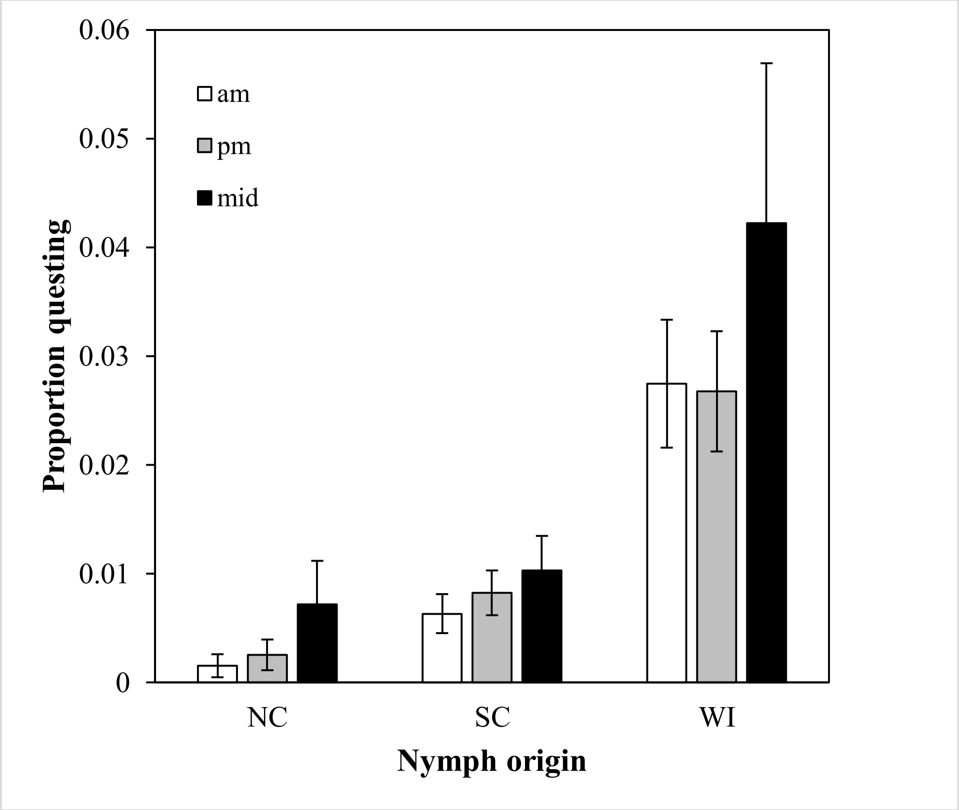
Appendix C. Summary of 2011 raw questing data

Table 1.9. Number of nymphs observed during individual sampling session in 2011. Observations listed 1-13, this indicates that observation 1 was the first performed in the sample and 13 was the last. Sampling events were initiated at different times of day. The total number of nymphs observed on stems was typically higher in the latter half of the observations during each the sampling session.

Observation no.	**Sample no. (date)**				**Total seen per observation (samples 1,3,4)**
	1 (6/15-6/16)	**2 (7/5)**	**3 (7/7-7/8)**	**4 (7/29-7/30)**	
1	0	12	26	44	**70**
2	4	13	20	30	**54**
3	0	12	19	28	**47**
4	4	12	16	26	**46**
5	1	15	16	17	**34**
6	1	12	22	18	**41**
7	7	14	26	22	**55**
8	6	18	25	30	**61**
9	8	-	33	37	**78**
10	10	-	32	28	**70**
11	11	-	20	28	**59**
12	7	-	10	33	**50**
13	7	-	4	11	**22**
Total seen per sample	**66**	**108**	**269**	**352**	

Appendix D. Questing activity by time 2012

Figure 1.8. Questing activity patterns (all sites) by observation time, 2012. Proportion of nymphal *I. scapularis* (means ± 95 CIs) of northern (WI) and southern (NC, SC) origin, observed questing at all four sites during each observation hour (am = ~0800 hours, pm = ~1600 hours, mid= ~0000 hours), in outdoor arenas at 4 sites (WI, RI, TN, FL) in 2012. Questing was highest during the midnight observations; am and pm observations yielded similar numbers of ticks for all 3 origins.



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CHAPTER 2

Geographical differences in nymphal *Ixodes scapularis* questing behavior are strongly associated with regional variation in Lyme disease risk

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Abstract

The majority of human Lyme disease cases in the eastern United States are caused by the nymphal stage of the blacklegged tick, *Ixodes scapularis*. We observed the questing behavior of nymphs raised from female ticks collected at locations previously classified as 'high', 'transitional', or 'low' for human Lyme disease risk (N = 4, 3 and 8 locations respectively). Nymphs were observed from May to October 2013 in replicated field arenas in Michigan and Tennessee. At both field sites, the log-odds of questing on or above the surface of the leaf litter was much higher for nymphs from the high and transitional risk regions vs. those from low risk regions ($z = -5.32$, $P<0.0001$). High risk nymphs were 8 times as likely to emerge from underneath the leaf litter vs. nymphs from low risk regions, regardless of whether they were in a northern (Michigan) or southern (Tennessee) climate. Furthermore, there was a strong positive relationship between the predicted probability of observing questing nymphs (questing index) from a given geographic locale (county) and the Lyme disease incidence of that locale ($R^2 = 0.79$, $P < 0.0001$); nymphs with low questing indexes were derived from mothers collected in counties with low Lyme disease incidence and nymphs with high questing indexes were derived from mothers collected in areas with high Lyme disease incidence. These results support and expand our previous findings that profound regional differences in tick questing behavior contribute to geographic variation in human Lyme disease risk in the eastern United States. The propensity of southern nymphs to spend most of their time under the leaf litter is a sufficient, although not necessarily exclusive, explanation for the low incidence of locally acquired Lyme

disease in the southern United States. Future research should investigate the selective pressures underlying this difference in behavior. Public health workers in southern states need to be aware that recent reports of southwards expansion of northern populations of *I. scapularis* may herald an increase in risk of locally acquired Lyme disease.

Introduction

The factors contributing to the risk of acquiring an infectious disease are complex and can increase when vectors are involved. For a vector-borne disease (VBD), pathogen transmission occurs when infected vectors contact susceptible hosts (Anderson and May 1981, Keesing et al. 2006) and contact rates depend on the behaviors of vectors and hosts (Keesing et al. 2006). Even small changes in vector feeding behavior can result in extreme consequences. Kilpatrick et al. (2006) demonstrated that human West Nile Virus (WNV) epidemics in the US were directly associated with shifts in the feeding behavior of *Culex spp.* mosquitos (vector for WNV) from birds to humans.

The blacklegged tick, *Ixodes scapularis* is the vector for Lyme disease (LD) in the eastern United States (Bacon et al. 2008). This tick exhibits variation in behavior across its geographic range. Nymphal *I. scapularis* from northern and southern regions differ in their host utilization (Spielman et al. 1985, Apperson et al. 1993, Kollars et al. 1999, Durden et al. 2002, LoGiudice et al. 2003), rates of human biting (Felz et al. 1996, Oliver 1996, Stromdahl and Hickling 2012), and ability to be collected using standard sampling methods (Falco and Fish 1992, Diuk-Wasser et al. 2006, Goddard and Piesman 2006). These observations are particularly relevant for understanding LD transmission dynamics because the nymphal stage is responsible for the majority of human LD cases in the US (Barbour and Fish 1993, Falco et al. 1999).

There is disproportionate occurrence of human LD cases in the eastern US (Figure 2.1B, in Methods). More than 95% of cases are concentrated in the Northeast and upper Midwest, whereas very few are reported from the Southeast (CDC 2014a). This geographic variation in disease incidence (hereafter referred to as the "Lyme Disease Gradient") remains a controversial subject among researchers, doctors, and patients with symptoms indicative of a tick-borne illness (Stromdahl and Hickling 2012, Orent 2013). A better understanding of the causes and consequences of the Lyme Disease Gradient is needed for clear insight regarding tick-borne disease risk and diagnosis, especially in areas where disease is considered nearly absent.

LD risk measures and risk maps are useful tools for guiding public health resources. The standard method for measuring LD risk is the dragging or flagging method, which is considered a direct measure of human contact with host-seeking (= questing) ticks (Ginsberg and Ewing 1989, Mather et al. 1996). Human LD risk is specifically linked to the presence of infected nymphs detected using this sampling method (Mather et al. 1996, Diuk-Wasser et al. 2006, Diuk-Wasser et al. 2012, Pepin et al. 2012). Though risk and human incidence are highly correlated, estimating risk directly from case data has limitations (Bacon et al. 2008, Diuk-Wasser et al. 2012) and therefore is a less informative measure than quantifying the presence of infected nymphs through drag sampling. Indeed, the most recent LD risk map (Diuk-Wasser et al. 2012) was developed using an unprecedented standardized dragging effort to collect infected nymphs throughout *I. scapularis*' range. Human LD risk was modeled using nymph collection data and environmental co-variates for contiguous 8 km x 8 km plots throughout the eastern US. To our knowledge this is the most comprehensive and objective spatial assessment of Lyme risk to date, and therefore we use the risk designations delineated in this map to guide our current research.

Previously, we demonstrated that there are reliable differences in the questing behavior of nymphs from different LD risk areas (Arsnoe et al. 2015). *I. scapularis* nymphs originating from a region of high LD risk (Monroe County, Wisconsin), were *much* more likely to be observed questing (defined as being emerged from the leaf litter) versus nymphs from lower risk regions (Hyde County, North Carolina and Aiken County, South Carolina). We postulate that this difference in questing behavior alters the contact rates between humans and ticks in these regions and drives the observed variation in LD incidence observed in the eastern US. This hypothesis provides a direct explanation for the Lyme Disease Gradient, and in particular, why LD risk is much lower in the South.

Here we build on our earlier studies, which assessed the questing behavior of *I. scapularis* nymphs in field arenas experiencing natural abiotic conditions. These studies were limited by the small number of geographic origins from which nymphs were collected; thus our results may have been restricted to site-related differences rather than indicative of more general north – south questing patterns. Here we greatly extend the number of populations tested (nymphs from 15 states) to assess if the behavior differences that were previously observed are widespread throughout *I. scapularis'* range and consistent with the predictions of the LD risk map. Furthermore, we consider the utility of using tick questing behavior as a measure of human LD risk by assessing the relationship between regional nymph behavior and regional human Lyme disease incidence. To assess consistency in questing behavior, we conducted replicate studies at two sites that differed in latitude and abiotic conditions.

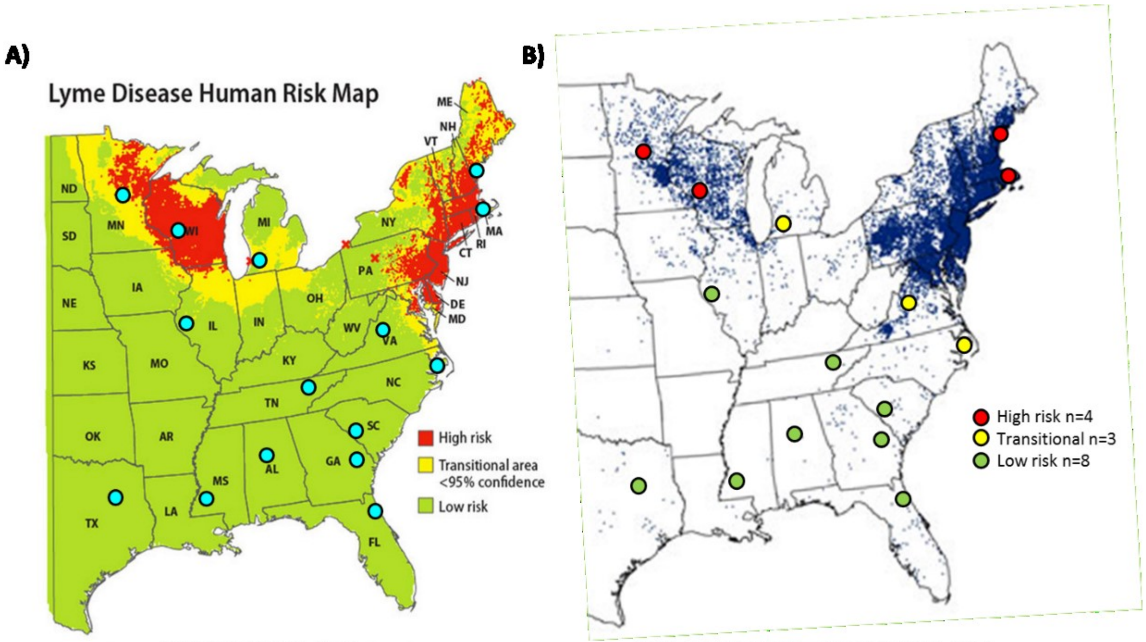
Methods

Study Sites. In 2013, we measured the questing behavior of nymphal *I. scapularis* placed at deciduous forest field sites in Barry County (42.49 latitude), Michigan (MI) and

Anderson County (36.01 latitude), Tennessee (TN). We selected these sites to represent northern (MI) and southern (TN) environmental conditions in which nymphs were tested. The TN field site was utilized in 2012 for similar studies and thus previously described (Arsnoe et al. 2015). Briefly, this site was dominated by upland oaks (*Quercus* spp.), hickory (*Carya* spp.) and poplar (*Liriodendron tulipifera*). The MI site was dominated by oaks (*Quercus* spp.), maples (*Acer* spp.), and beech (*Fagus* spp.). Environmental conditions (temperature and relative humidity) were recorded hourly at each site using iButton® data loggers (Hygrochron™, Dallas Semiconductor) placed at the surface of the leaf litter level (0.0 cm) and 10.0 cm above the leaf litter level. One “pair” (0 cm and 10 cm) of loggers was placed inside a randomly selected arena, another pair was placed outside of the selected arena. Conditions were recorded at hourly intervals throughout the study. Latitude coordinates for the sites were obtained from: http://earthexplorer.usgs.gov/ ™.

Nymphs for Experiments. The laboratory-reared nymphs used in the experiment originated from engorged female *I. scapularis* collected October-December 2012 from deer harvested by hunters at 15 locations (states) across *I. scapularis*’ range in the eastern US (Figure 2.1A). Collection locations were classified as high risk (n=4), transitional (n=3), or low risk (n=8) based on the LD risk map produced by Diuk-Wasser et al. (2012). This map classifies Lyme disease risk based on predicted density of questing infected nymphs. Regions predicted to harbor ≥ 0.3 infected nymphs per 1,000m^2 are classified as high risk, those ≤ 0.3 low risk, and those where 95% confidence spans these values are considered transitional. We reclassified one of the locations (Nelson County, Virginia) from low risk to transitional because of the dramatic increase in the abundance of infected nymphs collected from this location in recent years (Brinkerhoff et al. 2014).

Figure 2.1. Lyme disease risk map, tick collection locations, and human Lyme disease incidence. (A) Collection sites of engorged females used to produce nymphs for experiments in 2013 overlaid on Lyme disease risk map (Diuk-Wasser et al. 2012) and (B) Lyme disease case map for 2012 (CDC 2014a). In comparing the two maps, it is evident that human risk corresponds to areas of high disease incidence. Engorged female *I. scapularis* were collected from hunter harvested deer in locations (states) of high (n=4), transitional (n=3), and low (n=8) risk status for human Lyme disease. We reclassified one of the locations (Nelson County, VA) from low risk to transitional because of the dramatic increase in the abundance of infected nymphs collected from this location in recent years (Brinkerhoff et al. 2014). The clutch of a single female from each state was reared in the lab and resulting nymphs were placed in outdoor arenas in Michigan and Tennessee.



Diuk-Wasser et al. 2012

Reported cases of Lyme disease 2012 - CDC

The engorged females were brought into the lab and allowed to oviposit in individual vials in humidity chambers at 21°C, 98% relative humidity, and 16:8 light:dark photoperiod. At 1 - 9 weeks of age, the resulting larvae were fed on laboratory mice (*Mus musculus*; strain ICR CD-1) and allowed to molt into nymphs. At 3 – 6 weeks of age, these nymphs were transferred to the field sites and released into experimental arenas. Species identification was confirmed using taxonomic keys (Durden and Keirans 1996) prior to release into field arenas. The collection of *I. scapularis* females and subsequent feeding of larval ticks on *M. musculus* was done under protocol 06-12-103-00 approved by the Institutional Animal Care and Use Committee of Michigan State University and United States Department of Agriculture Veterinary Permit for Importation of Controlled Materials and Organisms and Vectors – Permit No. 121368.

Questing Observations. The experimental design consisted of four blocks of four arenas (n=16) in MI, and eight blocks of four arenas (n=32) in TN, from which all naturally-occurring ticks had been removed by heat-treating locally-obtained leaf litter before leaves were added to the arenas. Arena construction and litter treatment were as described in Chapter 1 (Arsnoe et al. 2015). Briefly, the arenas consisted of a 0.5 m circular aluminum flashing boundary containing leaf litter and 15 bamboo skewers (= stems) of three heights emerging from the leaf litter. Arenas were surrounded by an outer barrier to exclude wildlife.

In May 2013, 17-59 nymphal ticks derived from a single female (total of 15 females from 15 origins (states)) were released into arenas in MI and TN. Eight origins were tested in both TN and MI (listed in order of decreasing latitude): Wisconsin (WI), Massachusetts (MA), Michigan (MI), Virginia (VA), North Carolina (NC), Tennessee (TN), South Carolina (SC), and Florida (FL). The remaining 7 origins were tested only in TN due to limited availability of nymphs:

Minnesota (MN), Maine (ME), Alabama (AL), Georgia (GA), Mississippi (MS), and Texas (TX). Origin locations, LD risk classification, and LD incidence of counties of tick origin are summarized in Table 2.1.

Table 2.1. Lyme disease incidence and risk status for the 15 nymph origins (location of engorged female collection). Estimation of county incidence described in Methods. LD risk status based on Diuk-Wasser et al. (2012) classifications, with VA reclassified from low to transitional as described in Methods.

State of Origin	State abbreviation	County of origin	Latitude (degrees)	LD risk status	County incidence per 100,000
Florida	FL	St. John's	30.02	low	2.7
Texas	TX	Freestone	31.71	low	< 0.001
Mississippi	MS	Jefferson	31.74	low	< 0.001
Georgia	GA	Bulloch	32.42	low	1.4
Alabama	AL	Bibb	33.04	low	< 0.001
South Carolina	SC	Aiken	33.25	low	< 0.001
Tennessee	TN	Anderson	35.93	low	<0.001
Illinois	IL	Adams	39.97	low	4.5
North Carolina	NC	Hyde	35.50	transitional	<0.001
Virginia	VA	Nelson	37.79	transitional	98.6
Michigan	MI	Cass	41.92	transitional	5.9
Massachusetts	MA	Plymouth	41.98	high	304.9
Maine	ME	York	43.38	high	434.8
Wisconsin	WI	Monroe	44.01	high	581.4
Minnesota	MN	Morrison	46.09	high	218.2

Nymphs were released into the arenas on May 8 (MI) and May 10 (TN). Tick questing behavior was recorded by observers (blinded to tick origin) in the morning (approx. 0800 hours) and late afternoon (approx. 1600 hours) at weekly or biweekly intervals from May – October 2013. Questing behavior was quantified as the number of nymphs present on stems, leaf litter surfaces, and arena walls during a 2 minute observation period (Arsnoe et al. 2015).

Tick Survival. In October of 2013 we assessed the relative survival of the nymphs by conducting intensive searches of the arena litter. We adopted the method used in Chapter 1 (Arsnoe et al. 2015), performing three microdrag searches. Nymphs were recovered at the MI and TN sites on October 16 and October 23, 2013, respectively.

Lyme disease incidence. Human Lyme disease incidence for each county from which nymphs were derived was calculated (Equation 1) from a national surveillance dataset of confirmed cases by county (CDC 2014b) in 5-year intervals, for the years 1992-2011. Annual reported incidence rates fluctuate (CDC 2014b), so we used the most recent 5-year average as our measure of each county's typical incidence. Likewise, county population estimates were calculated as the average of the yearly population estimates from 2007-2011 as obtained from US census data (U. S. Census Bureau 2014a and U.S. Census Bureau 2014b).

$$\text{LD incidence per 100,000} = \frac{\text{confirmed LD cases in county 2007-2011} * 10^5}{\text{mean county population estimate 2007-2011}} \qquad \textbf{(Equation 1)}$$

Statistical Analysis. We applied a Generalized Linear Mixed Model (GLMM) using the function *glmer* in the R package *lme4* (Bates et al. 2014) to analyze the log-odds of observing nymphs questing as a function of the Lyme risk status of the geographic location from which they were derived (LD risk). Questing behavior was measured as the number of nymphs visible during a given 2-minute observation of an arena. Lyme risk status was a fixed factor while arena, site, state, and week were modeled as random factors to account for heterogeneity among arenas, location of studies (sites), location of female collection (state), and over time (week). Fit statistics were calculated using the function *r.squaredGLMM* in the R package *MuMIn* (Barton 2014). This function calculates a conditional and marginal coefficient of determination (R^2) for GLMMs based on the method established by Nakagawa and Schielzeth (2013). Marginal R^2 ($R^2_{(m)}$) quantifies the explanatory power for fixed effects while the conditional R^2 ($R^2_{(c)}$) quantifies the explanatory power of the fixed and random effects of the model. The difference between $R^2_{(c)}$ and $R^2_{(m)}$ describes the explanatory power of the random effects in the model. To assess the change in variance components when the predictor was added, we calculated the proportion change in variance (PCV) between the null (intercept) model and full model fitted for the dataset. PCV is defined as 1 minus the ratio of the value of a variance component in the full model to the value of that variance component in the null model (Nakagawa and Schielzeth 2013). Positive PCV values indicate that the addition of a predictor(s) has reduced variance at a specific level, while negative values indicate an increase in variance.

We used a GLMM to analyze the log-odds of recovery of nymphs (survival) from arenas as a function of Lyme risk status (fixed factor). Survival was measured by tallying the number of nymphs recovered from arenas at the end of each study period. Arena, site, and nymph origin were modeled as random factors for the reasons described above; fit statistics were calculated for this model as described above. To visualize the results, predicted log-odds and their associated standard errors were back-transformed to probabilities.

To assess if regional questing behavior may be a useful measure for predicting disease risk, we used linear regression to examine the relationship between probability of questing (questing index) and our estimates of county Lyme disease incidence from each location where mothers were collected. To evaluate the influence of genetics on questing behavior, we used a Pearson's product moment correlation to compare the questing behavior (mean proportion questing over entire observation period) of siblings (n=8 sibling groups) at each site. Relationship of abiotic conditions with questing behavior was assessed by including each abiotic variable (temperature or relative humidity at 0 cm or 10 cm) in the questing behavior model and examining the fit statistics. Abiotic variables were centered prior to statistical analysis to facilitate interpretation and model fitting (McElreath 2014). All statistical analyses were performed using R 3.1.0 (http://www.r-project.org).

Results

Environmental conditions at field sites. Temperature and humidity readings for the field sites are shown in Table 2.2. The average ambient (10 cm) conditions at the TN site were warmer and more humid, than the MI site. Average leaf litter (0 cm) temperatures were warmer in TN, but average leaf litter humidity was slightly lower in TN than in MI.

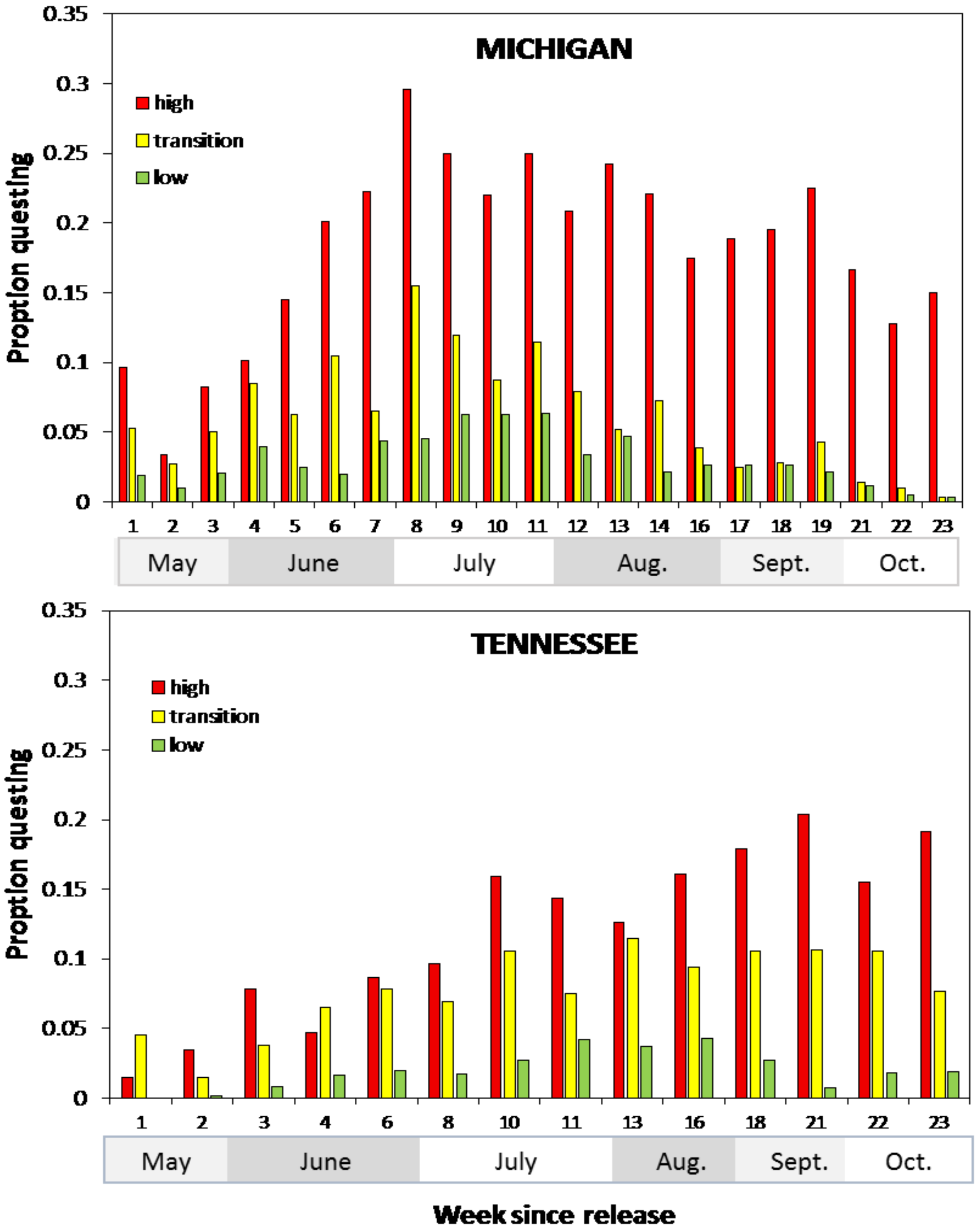
Table 2.2. Environmental conditions at field sites in 2013, measured using on-site HOBO dataloggers. Temperature and relative humidity (means ± SD) at leaf litter level ("0 cm") or above leaf litter level ("10 cm") at each field site in 2013. Readings are the average of the 2 loggers placed in each position at each site. Readings were collected at hourly intervals from May 10, 2013 through October 16, 2013.

Field site	**Latitude (°N)**	**Months sampled**	**Temp (°C) 0cm**	**Temp (°C) 10cm**	**Relative humidity (%) 0cm**	**Relative humidity (%) 10cm**
Barry County, MI	42.49	May-Oct.	17.5 (3.53)	18.4 (5.23)	99.5 (8.87)	86.4 (15.4)
Anderson County, TN	36.01	May-Oct.	20.1 (3.29)	20.3 (3.76)	98.9 (6.93)	96.1 (9.49)

Questing behavior of high risk, transitional, and low risk nymphs. These data comprise 1330 observations of 46 experimental arenas located at two sites in the eastern US over a 23 week time period (mid- May through mid-October, 2013). For 520 (39%) of the observations, no nymphs were observed questing in arenas. In the remaining observations 1 to 26 nymphs were observed questing, with >95% of non-zero observations being ≤ 16 nymphs.

High risk nymphs were observed questing more often than low risk nymphs at both sites and during every observation week (Figure 2.2). Nymphs from transitional zones also quested more than low risk nymphs at both sites and over all weeks (Figure 2.2). A genetic influence on behavior was detected as the behavior of siblings was highly correlated between the two sites ($r = 0.93$, $P < 0.001$). Questing behavior of nymphs at each site throughout the observation period is summarized in Figure 2.2.

Figure 2.2. Summary of nymph questing behavior at each site in 2013. Mean proportion of questing nymphs from high risk (red bars), transitional (yellow bars), and low risk (green bars) regions observed in arenas during each observation week at field sites in Michigan (top) and Tennessee (bottom). Nymphs were released at the Michigan site on May 8, 2013, and at the Tennessee site on May 10, 2013.



There was a significant effect of LD risk status on questing behavior (Figure 2.3). High risk and transitional nymphs (mean probability questing = 11.7%, 5.0%, respectively) were observed questing more often than low risk nymphs (mean probability questing = 1.4%; Figure 2.3; $z = -5.3$, $P<0.0001$). High risk nymphs were 8 times as likely to emerge from underneath the leaf litter vs. low risk nymphs. This model accounted for a third of the observed variation in questing behavior ($R^2_{(c)} = 0.34$) with LD risk status, explaining 18% of the variance ($R^2_{(m)}=0.18$; Table 2.3). Addition of the LD risk predictor reduced the variance at all levels in the model (indicated by positive PCV values for all random effects; Table 2.3).

Figure 2.3. Probability of observing nymphs from high, transitional, and low risk regions questing in arenas at sites in Michigan and Tennessee. Mean probability of questing (±SE; number of arenas tested for each risk category in parenthesis) for nymphs from each Lyme disease risk status group. Nymphs were derived from 15 different locations, classified as high, transitional, and low risk for human Lyme disease. Questing probability was estimated by observing questing behaviors of nymphs in secure outdoor arenas at field sites stationed in Michigan and Tennessee from early May through mid-October in 2013. Bar colors correspond to those of the LD risk map in Figure 2.1. Similar letters above bars indicate coefficients not different among groups.

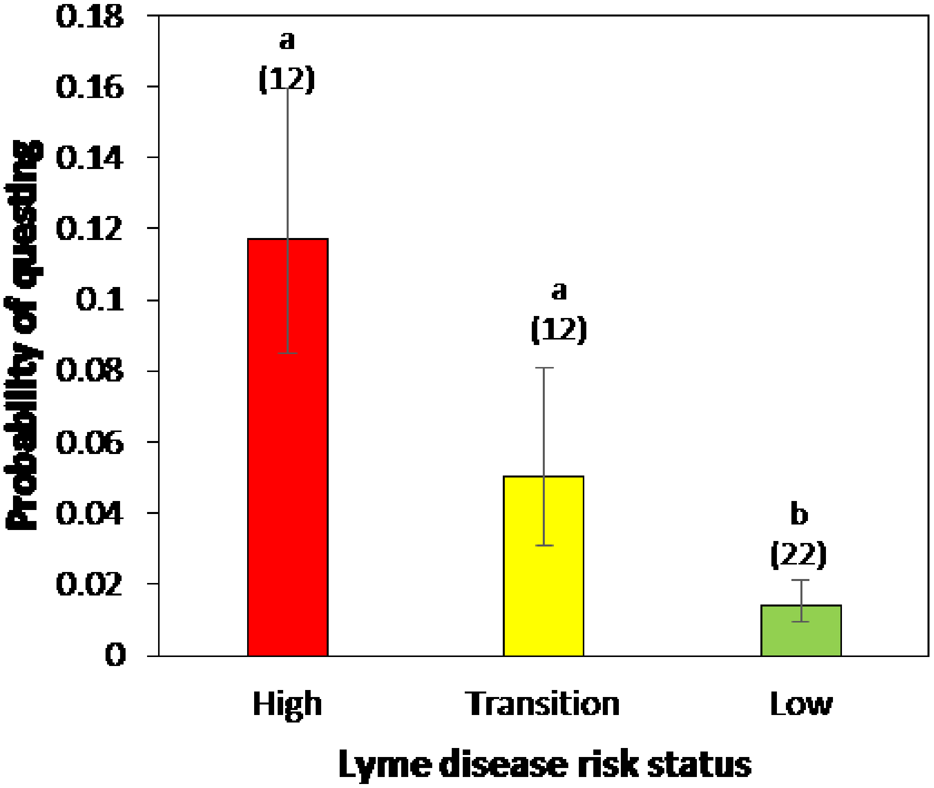


Table 2.3. Model summaries for effects of: Lyme disease risk status (LD risk-high, transition, low), ambient relative humidity (RH10), recovery (% recover), and interactions on questing behavior of nymphs originating from mothers collected at 15 locations across the eastern US. Intercept and coefficients are listed for modeling the effect of Lyme disease risk status on log-odds of observing questing of nymphs in arenas; $N_{[site]} = 2$, $N_{[week]} = 21$, $N_{[arena]} = 46$, $N_{[state]} = 15$. Asterisk indicates significance at P<0.0001. CI, confidence interval; VC, variance component; PCV, proportion change in variance, AIC, Akaike Information Criterion; BIC Bayesian Information Criterion. AIC and BIC values estimated using Maximum Likelihood.

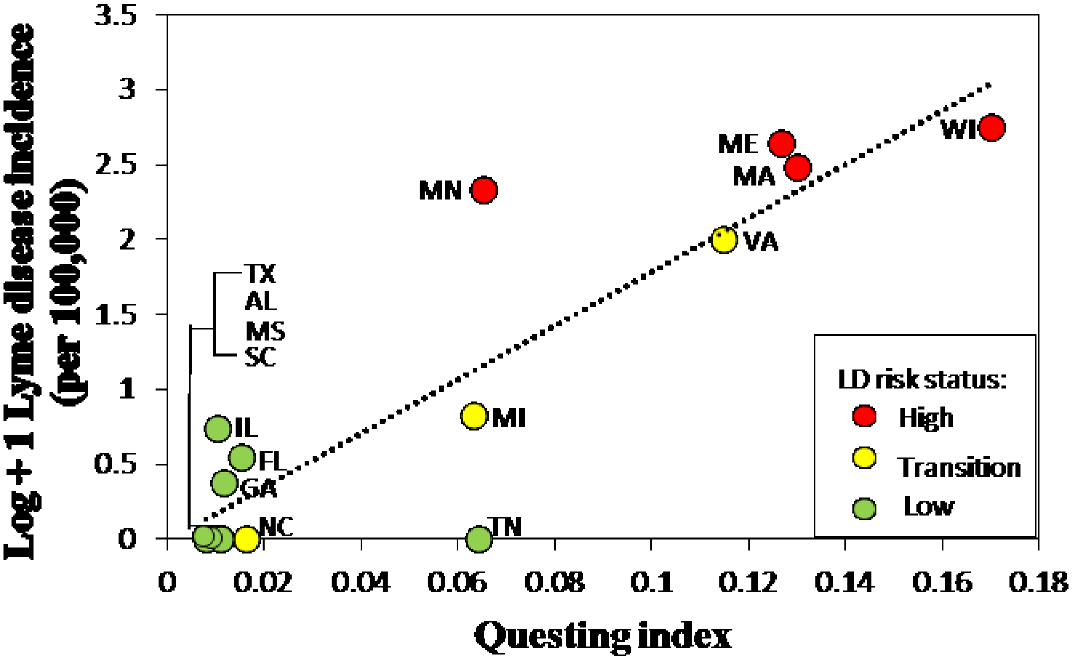
	Questing Models	
	Null Model	**LD risk**
Fixed effects	[95%CI]	[95%CI]
Intercept[1]	-3.41 [-4.06,-2.76]	-1.94 [-2.44,-1.43]
LD risk (trans)	-	-1.00 [-1.66, -0.34]
LD risk (low)	-	-2.22 [-2.81,-1.64]*
Random effects	**VC**	**VC**
Site	0.010	0.003
Week	0.220	0.219
Arena	0.207	0.205
State	1.299	0.377
PCV[Site]	-	0.670
PCV[Week]	-	0.008
PCV[Arena]	-	0.009
PCV[State]		0.709
$R^2_{(m)}$	-	17.6%
$R^2_{(c)}$	-	33.8%
AIC	5715	5702
BIC	5741	5739

[1] The intercept represents “high” Lyme risk status. 95% CI was estimated by assuming an infinite degree of freedom (t = 1.96).

Relationship between probability of questing and human Lyme disease incidence.

We observed a strong positive relationship between the predicted probability of observing questing nymphs (questing index) from a given geographic locale (county) and the Lyme disease incidence of that locale ($R^2 = 0.79$, $P<0.0001$); nymphs with low questing indexes were derived from mothers collected in counties with low Lyme disease incidence and nymphs with high questing indexes were derived from mothers collected in areas with high Lyme disease incidence (Figure 2.4).

Figure 2.4. Nymphal questing behavior is strongly positively related with Lyme disease incidence across the eastern US. Relationship between county-level Lyme disease incidence from 2007 - 2011 (log + 1 transformed) and 'questing index' (calculated as mean questing probability for nymphs from each origin) of nymphs derived from 15 geographic origins (indicated by state abbreviations from where nymph mothers were collected) across the eastern US. The color of each point corresponds to the human Lyme risk status of each origin (designations described in Methods). Dotted line shows line of best-fit ($y = 17.949x - 0.0076$, $R^2=0.79$).



Abiotic conditions, nymph recovery rates and questing behavior. There was a significant effect of ambient relative humidity (humidity reading at 10cm above ground) on overall questing behavior (Table 2.4). Questing appeared to decrease slightly when relative humidity was very high (above average). This effect reached significance for the transitional nymphs (significant interaction of LD risk (transition) * rh; $z = -2.7$, $P = 0.007$). Only one abiotic variable (ambient RH) was selected for the model because all abiotic variables measured were highly collinear. Ambient RH was selected because it had the highest explanatory power ($R^2_{(m)} = 0.003$) of the four abiotic variables measured (Table 2.6; Appendix A).

Survival (proportion of nymphs recovered in arenas) was not a significant predictor of questing behavior ($z = 0.76$, $P = 0.53$; Table 2.4).

Table 2.4. Model summaries for effects of: Lyme disease risk status (LD risk), ambient relative humidity (RH10, centered at the mean), recovery (% recovered), or interaction on questing behavior of nymphs originating from mothers collected from 15 locations across the eastern US. Intercept and coefficients listed for modeling effect of Lyme disease risk status on log-odds of observing questing nymphs in arenas (main research question); $N_{[site]} = 2$, $N_{[week]} = 21$, $N_{[arena]} = 46$, $N_{[state]} = 15$. Asterisks indicate significant difference from intercept at $P \leq 0.05$. CI, confidence interval; VC, variance component; PCV, proportion change in variance, AIC, Akaike Information Criterion; BIC Bayesian Information Criterion. AIC and BIC values estimated using Maximum Likelihood.

	Questing Models				
	Null Model	**RH10 x LD risk**	**RH10**	**Recovery x LD risk**	**Recovery**
Fixed effects	[95%CI]	[95%CI]	[95%CI]	[95%CI]	[95%CI]
Intercept[1]	-3.41 [-4.06,-2.76]	-2.05	-3.42 [-4.12,-2.73]	-2.20 [-3.10,-1.30]	-3.59 [-4.21,-2.96]
LD risk (low)	-	-2.22 [-2.81,-1.30]*	-	-2.08 [-3.09,-1.01]*	-
LD risk (trans)	-	-0.9 [-1.92, 0.12]	-	-0.84 [-2.03, 0.35]*	-
RH10 <u>or</u> Recovery	-	-0.005[-0.007,-0.002]*	-0.007[-0.009,-0.005]*	0.76 [-1.58, 3.09]	1.71 [-0.21, 3.63]
LD risk (low) inxn	-	-0.003 [-0.009, 0.002]	-	-0.13 [-5.88, 5.62]	-
LD risk (trans) inxn	-	-0.006 [-1.01,-0.002]*	-	0.52 [-3.79, 4.83]	-
Random effects	**VC**	**VC**	**VC**	**VC**	**VC**
Site	0.010	0.027	0.035	0.000	0.000
Week	0.220	0.252	0.253	0.219	0.220
Arena	0.207	0.213	0.207	0.197	0.205
State	1.299	0.390	1.319	0.383	1.089
PCV[Site]	-	-1.868	-2.681	1.00	1.000
PCV[Week]	-	-0.142	-0.149	0.01	0.001
PCV[Arena]	-	-0.030	-0.001	0.05	0.011
PCV[State]		0.699	-0.016	0.71	0.161
R^2 (m)	-	17.9%	0.3%	17.5%	0.8%
R^2 (c)	-	35.2%	35.8%	33.6%	32.1%
AIC	5715	5654	5670	5707	5714
BIC	5741	5706	5702	5759	5745

[1] In modes with LD Risk as interaction term, the intercept represents “high” Lyme risk status. 95% CI was estimated by assuming an infinite degree of freedom (t = 1.96).

Survival of nymphs from arenas from high, transitional, and low Lyme disease risk areas. Our recovery data originate from 46 experimental arenas located at two sites (MI, n=16; TN, n=30). A total of 2,291 nymphs were released in 2013 (MI, n=802; TN, n=1489) and a total of 11.8% (MI, n=68; TN n=203) of these were recovered approximately 5 months later (161 days in MI; 166 days in TN). There was a significant effect of LD risk status on the log-odds of recovering nymphs in arenas (Figure 2.5). Nymphs from high risk (mean probability = 19.8%) regions were recovered more often than nymphs from transitional (mean probability = 5.7%; z = -2.9, P = 0.004) and low (mean probability = 4.1%; z = -4.2, P<0.0001) risk regions. The model accounted for 29% of the observed variation in nymph recovery ($R^2_{(c)} = 0.29$) with the predictor 'LD risk status', explaining 12% of the variance ($R^2_{(m)}=0.12$; Table 2.5). Addition of the risk predictor reduced variance (positive PCV) for the site and origin levels, but increased variance in the arena level (negative PCV value; Table 2.5).

Figure 2.5. Probability of recovering nymphs from high, transitional, and low risk regions from arenas at sites in Michigan and Tennessee. Mean probability of recovery (±SE) for nymphs from each Lyme disease risk status group. Nymphs were derived from 15 different locations, classified as high, transitional, and low risk for human Lyme disease. Recovery probability is estimated as the number of nymphs recovered from arenas at the end of the experiment. Nymphs were released in early May 2013 (MI – May 8; TN – May 10) and were recovered in mid-October 2013 (MI – October 16, 161 days; TN – October 23, 166 days). Bar colors correspond to those of the LD risk map in Figure 2.1. Number of arenas tested for each risk category are in parentheses. Similar letters above bars indicate coefficients not different among groups.

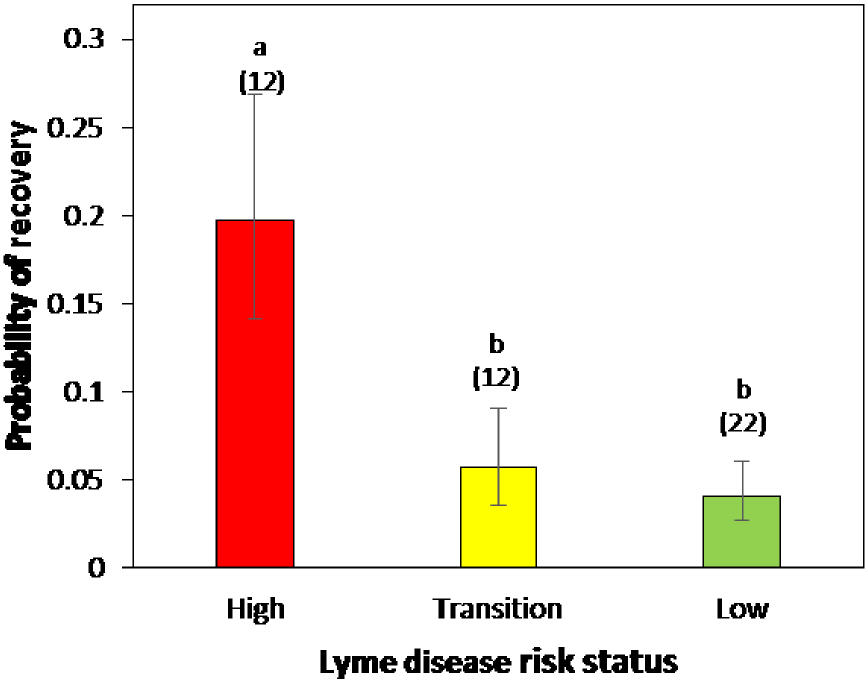


Table 2.5. Model summary of nymph recovery. Modeling effect of Lyme disease risk status on log-odds of recovery of nymphs from arenas vs. null (intercept) model; $N_{[site]} = 2$, $N_{[arena]} = 46$, $N_{[state]} = 15$. Asterisks indicate significance at $P \leq 0.01$. CI, confidence interval; VC, variance component; PCV, proportion change in variance, AIC, Akaike Information Criterion; BIC Bayesian Information Criterion. AIC and BIC values estimated using Maximum Likelihood.

	Survival Models	
	Null Model	**LD risk model**
Fixed effects	b [95%CI]	b [95%CI]
Intercept[1]	-2.64 [-3.43,-1.86]	-1.39 [-2.18,-0.61]
LD risk (trans)	-	-1.41 [-2.36,-0.45]*
LD risk (low)	-	-1.76 [-2.59,-0.94]*
Random effects	**VC**	**VC**
Site	0.134	0.105
Arena	0.517	0.531
State	0.921	0.178
PCV[Site]	-	0.217
PCV[Arena]	-	-0.028
PCV[State]	-	0.806
$R^2_{(m)}$	-	11.8%
$R^2_{(c)}$	-	29.3%
AIC	257	249
BIC	264	260

[1] In LD risk model, the intercept represents “high” Lyme risk status. 95% CI was estimated by assuming an infinite degree of freedom (t = 1.96).

Discussion

These results provide direct evidence of widespread differences in *I. scapularis* questing behavior: nymphs derived from high LD risk regions quested up to 8 times more often in outdoor field arenas than nymphs from low risk regions. Questing behavior trends were consistent among LD risk groups at each field site and over all weeks that they were observed. High risk nymphs quested more than low risk nymphs during every week at both sites. Moreover, the probability of nymphal questing was highly positively correlated with the LD incidence of the county from which the nymphs originated, providing further strong support for a link between questing behavior and LD risk. Taken together, these results strengthen the hypothesis that very low LD incidence in the southeastern US is, in part, a consequence of altered host-seeking behavior of the key LD vector.

The nymphs tested in this study were derived from female *I. scapularis* collected from 15 states (designated as high risk, transitional, or low risk for LD) across the eastern US. This extensive collection allowed us to achieve replication across *I. scapularis*' geographical distribution and within each risk status level thus providing strong evidence for the generalizability of our results on a large scale.

The risk map used to classify nymph origins is based on questing *I. scapularis* nymphs actively surveyed in the eastern US from 2002-2006 (Diuk-Wasser et al. 2012). Consequently, some regions of the map have changed status as a result of recent invasion of *I. scapularis* into previously uncolonized areas (Brinkerhoff et al. 2014, Wang et al. 2014). Assessing tick-borne disease risk is a dynamic process because tick populations continue to expand in space and time. Indeed, two of the three transitional groups we tested (MI and VA) represent ticks at the leading edge of actively invading fronts of northern populations (Hamer et al. 2010, Brinkerhoff et al.

2014, Kelly et al. 2014) and would be expected to have questing behaviors similar to the high risk group. The third transitional group, NC, is likely an extension of the southern population (Qiu et al. 2002). *Ixodes scapularis* have been collected from hunter harvested deer in NC as early as the 1970s (Apperson et al. 1990), decades before the northeastern populations began to expand from their foci (Barbour and Fish 1993). Questing behavior of these individual groups conform to what we would expect for ticks closely related to high risk and low risk regions (Figure 2.4).

In 2011 (Chapter 1), 2012 (Chapter 1), and 2013, we demonstrated reliable differences in regional nymphal questing behavior. For all years we were able to test nymphs derived from the same locations, but different families, in WI and SC. Consistent differences were observed for all three years between these nymph groups (this chapter and Arsnoe et al. 2015). WI nymphs always quested more often than SC nymphs -in every year and at every site – further confirming that true differences exist between these populations.

These results implicate a genetic basis for questing behavior because the behavior differences were maintained regardless of variable environmental conditions. Our prior studies involved nymphs of multiple lineages from a single location and these studies indicated that questing behavior is variable within these populations (Arsnoe et al. 2015). In our current study, nymphs from each origin (state) placed at the two sites were siblings (derived from a single female collected from that state). This design allowed us to evaluate evidence for a genetic contribution to questing behavior by controlling for questing variation among lineages from the same location. We observed consistent trends for the 8 sibling groups that were placed in both sites; questing behavior of sibling groups was highly correlated between the two sites (r = .93). Additionally, similar questing patterns were observed between replicate sibling arenas within each site (Figure 2.6; Appendix B), providing further support of a genetic basis for questing behavior in *I. scapularis* nymphs.

I. scapularis exhibits genetic structuring in 16s mitochondrial genes, which reflects a phlyogeographic history whereby northern tick populations were established by migrants from the southeast following the recession of the Pleistocene glaciers (Norris et al. 1996, Qiu et al. 2002, Humphrey et al. 2010). This genetic structuring has resulted in the identification of two mitochondrial lineages, the "American Clade," which is distributed in both the northern and southern US and the less genetically diverse "Southern Clade," which is found only in the Southeast (Qiu et al. 2002). It has been suggested that genetic differences among these lineages could account for observed phenotypic differences (e.g. questing behavior) among *I. scapularis* populations (Van Zee et al. 2013, Kelly et al. 2014). If mitochondrial clade lineages correspond to behavior differences, we would expect American clade ticks from southern regions to exhibit increased questing behavior versus those from the Southern clade. We did not find this to be the case: in 2012, American clade nymphs from North Carolina quested less than Southern clade nymphs from South Carolina (Arsnoe et al. 2015). It would appear, at least in our studies, that the mitochondrial genetic structure is not useful for describing behavioral differences among *I. scapularis* populations. Similar deficiency of mitochondrial genetic differences have been reported for *I. scapularis* from Virginia across an east-west gradient where LD risk is higher inland versus the coast (Brinkerhoff et al. 2014, Kelly et al. 2014). Molecular analysis using more diverse nuclear DNA markers (e.g., SNPs - single nucleotide polymorphisms, microsatellites) may reveal insight for identification of genetic basis for behavior (Van Zee et al. 2013).

Questing activity in arenas at the Michigan site peaked in early and mid-summer (Figure 2.2). This is consistent with the typical nymphal activity levels recorded in the Northeast and upper Midwest (Fish 1995, Gatewood et al. 2009). After peaking in the first week of July, the high risk group remained fairly active through the remainder of the study. Declines in activity were observed for the transitional and low risk groups, which may reflect differences in survival past late summer of these two groups (see below). *I. scapularis'* lifecycle is largely temperature dependent (Ogden et al. 2004) and it has been hypothesized that seasonal questing activity of nymphs differs among northern and southern nymphal populations. However, the few southern field studies describing southern nymphal phenologies indicate that activity of southern nymphs is similar to northern nymphs (Kollars et al. 1999, Goltz and Goddard 2013; G. Hickling, unpublished data), perhaps slightly extended in both directions (earlier and later). In Tennessee, questing in arenas peaked in mid- and late-summer and remained fairly stable through the end of the study (Figure 2.2). Similar patterns were observed in Canada, where captive *I. scapularis* nymphs in outdoor mesocosms remained active from April to November (Lindsay et al. 1995).

Nymphal survival did not explain a significant proportion of the variance in questing behavior (P = 0.53; Table 2.5) providing further support for the role of questing behavior as a determinant of LD risk. However, the log-odds of recovering nymphs from high risk areas was significantly higher than recovering transitional (P = 0.01) or low risk (P = 0.0001) nymphs (Figure 2.5). In laboratory trials WI nymphs have higher survival rates than SC nymphs when relative humidity is low (approximately 75%; Ginsberg et al. 2014), whether northern nymphs exhibit better survival than southern nymphs is currently under investigation.

Nymphal density is a key component of human risk – the more nymphs there are, the more likely one is to come into contact with them (Mather et al. 1996). It is estimated that *I.*

scapularis densities in the Southeast may be 1/10 of those reported in the Northeast (Goddard and Goddard 2008, Stromdahl and Hickling 2012). We detected differences in questing behavior which, when combined with the observed differences in population density, should intensify human LD risk in the North and abate risk in the South. The mechanism would work by influencing tick human contact rates, if there are high densities of above leaf litter surface questing ticks in North, and low densities of below surface dwellers in the South. The difference in risk is may be even more extreme if infection prevalence of *B. burgdorferi* found in questing *I. scapularis* varies drastically among regions, which is thought to be the case.

Knowledge of the relationship between nymphal questing behavior and LD risk is fundamental for assessing the current threat in areas where *I. scapularis* already is established and for predicting how risk will change in the future. Populations of *I. scapularis* have been detected in new areas since the LD risk map was generated (Brinkerhoff et al. 2014, Wang et al. 2014). It is estimated that *I. scapularis* ranges will continue to expand (Cortinas and Kitron 2006, Diuk-Wasser et al. 2006, Ogden et al. 2008, Hamer et al. 2010, Kelly et al. 2014, Wang et al. 2014). Northern populations are currently moving westward and southward into uncolonized areas (Brinkerhoff et al. 2014, Kelly et al. 2014, Wang et al. 2014). When northern and southern populations meet, what will be the outcome? Will these populations remain reproductively isolated or will they produce hybrids? What sort of questing behaviors will the hybrids display? Northern and southern populations can interbreed and produce viable offspring in the lab (Oliver et al. 1993), but their behavioral phenotypes remain unknown. Future studies are crucial to determine the consequences of northern population movement into southern regions. Anticipating questing behavior changes as nymphs move into new environments will be important for accurate assessment of local tick-borne disease risk.

APPENDICES

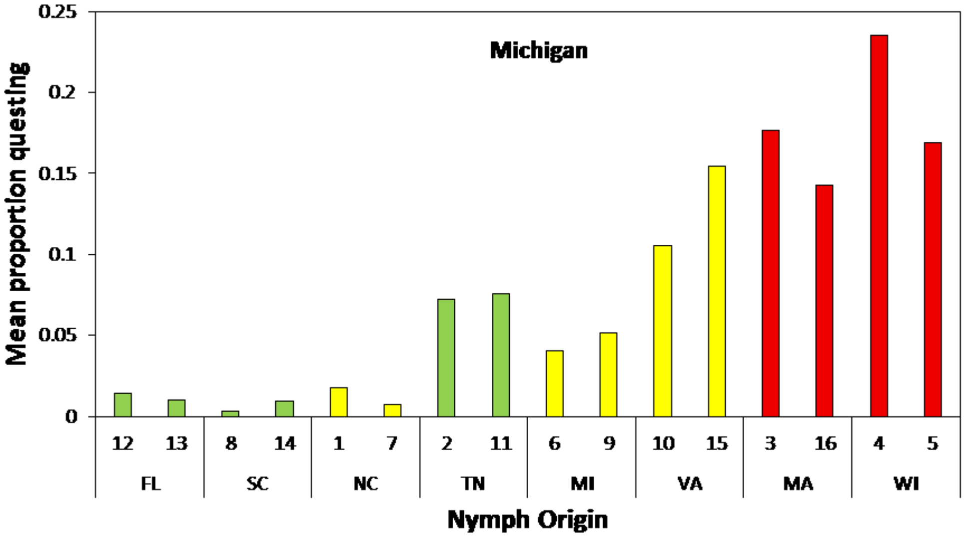
Appendix A. Model summaries for 2013 experiments

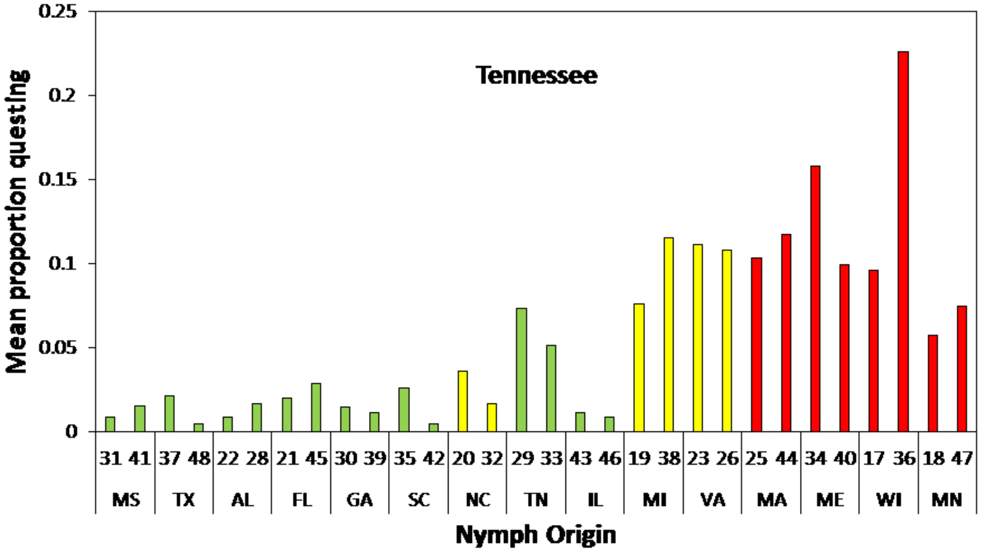
Table 2.6. All questing models constructed with random effects on arena, site, week, nn.origin (state). All survival models constructed with random effects on arena, site, nn.origin (state).

Questing Models (log-odds of observing ticks in the area - success/failures)		**Model comparison**-fit stats *glmer* output									
model	**fixed effects**	**AIC**	**Δ AIC**	**BIC**	**logLik**	**deviance**	**sig.effect of:**	**marginal R^2 (fixed)**	**conditional R^2 (random&fixed)**	**R2c-R2m (random)**	**comments**
m.null	-	5715	0	5741	-2853	5705	-	0.000	0.345	0.345	NULL MODEL
m.full (LD risk)	LD.risk	5702	13	5739	-2844	5688	LD.risk	0.176	0.338	0.162	low sig different from high and trans
m.full.rh10	rh10.c*LD risk	5654	61	5706	-2817	5634	LD risk, rh10.c, inxn	0.179	0.352	0.174	coef for rh10 is -0.004; neg relation to log odds of emergence of high
m.rh10	rh10.c	5670	45	5702	-2829	5658	rh10.c	0.003	0.358	0.354	rh10 coef = -0.007085
m.rh0	rh0.c	5710	5	5741	-2849	5698	rh0.c	0.001	0.346	0.345	rh0 coef = -0.008402
m.temp10	temp10.c	5710	5	5741	-2849	5698	temp10.c	0.001	0.344	0.343	temp slope = 0.009513
m.temp0	temp0.c	5716	-1	5747	-2852	5704	-	0.000	0.348	0.348	
m.full.recover	prop.recovered*LD risk	5707	8	5759	-2844	5687	LD.risklow	0.175	0.336	0.161	NO significant inxn of recovery and risk, sig effect of risk
m.recover	prop.recovered	5714	1	5745	-2851	5702.1	-	0.008	0.321	0.313	
Survival models (log-odds of recovering ticks from arenas – success/failures)											
m.surv.null	-	257.1	0	264.4	-124.5	249.1	-	0.000	0.323	0.000	
m.surv.full	LD.risk	249	8	260.3	-118.7	237.3	LD.risk only	0.118	0.293	0.175	high sig different from low and trans

Appendix B. Summary of questing data

Figure 2.6. Comparison of sibling arenas at each site. Mean proportion of questing nymphs observed in arenas at MI (top) and TN (bottom) sites in 2013. Nymphs from each origin (state abbreviations) were placed in replicate at each site (MI and TN). Numbers indicate anonymous arena IDs for sibling arenas during the study. Red bars represent nymphs classified as "high risk", yellow bars "transitional", and green bars "low risk".





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CHAPTER 3

Hybridization of *Ixodes scapularis* behavioral phenotypes: observations of offspring behavior and survival

Abstract

Populations of *Ixodes scapularis*, the blacklegged tick, are expanding in geographic range. Consequently, these distributional shifts are changing the dynamics of human tick-borne disease risk at local scales. We have previously demonstrated that the questing behavior of *I. scapularis* nymphs varies among populations derived from different geographic origins. Nymphs from northern regions display elevated questing behavior compared to nymphs from southern regions, and these behavioral phenotypes are positively related to local Lyme disease risk. Here we present data from matings among four geographic populations representing the two distinct questing phenotypes: high questing (n=2) and low questing (n=2). Crosses were successful for all hybrid pairs, but not for inbred crosses from southern regions. Offspring questing behavior and survival rates were observed and compared among crosses. In general, when both parents were high questing, offspring also exhibited high questing phenotypes; comparatively, when both parents were low questing, offspring were low questing. Hybrid offspring with parents of different phenotypes (high parent x low parent = "mixed phenotype") displayed questing behavior more similar to hybrids with both low questing parents than to hybrids with both high questing parents. Survival of mixed phenotype hybrids was higher than all other hybrid groups, indicating a possible heterozygote advantage for these offspring. These results emphasize the need for ongoing research of ecological interactions among disease vectors in order to predict how human risk will change as vector populations expand.

Introduction

Vector presence directly determines whether or not a vector-borne disease cycle can be maintained within a region (Gage et al. 2008). Thus, changes in the geographic range of vector populations can put new regions of humans at risk for vector-borne diseases (Mills et al. 2010). Given the mounting concern regarding climate change and the potential effects on human health (Patz et al. 2005), investigations of the consequences of shifting vector distributions are paramount. Pleas for research in vector ecology and biology have been issued (by Gage et al. 2008 and Mills et al. 2010), which further highlights the importance of acquiring this basic knowledge in order to understand the ecology of vector-borne diseases (Brownstein et al. 2005, Gage et al. 2008, Mills et al. 2010, Léger et al. 2013, Estrada-Peña et al. 2014).

The blacklegged tick, *Ixodes scapularis*, is the vector of the etiological agent of Lyme disease (*Borrelia burgdorferi*) in the eastern United States (US). Human Lyme disease (LD) cases have increased significantly in the eastern US since standardized surveillance began in 1991 (Bacon et al. 2008). This increase in LD cases has been associated with the demographic and spatial expansion of *I. scapularis* from population foci in the Northeast and upper Midwest (Barbour and Fish 1993, Steere et al. 2004, Brinkerhoff et al. 2014). Studies citing recent invasions of *I. scapularis* populations (Hamer et al. 2010, Brinkerhoff et al. 2014, Wang et al. 2014) support the predictions of habitat suitability models which forecast northern movement of *I. scapularis* through Canada (Ogden et al. 2008) and southern movement through the mid-central US (Brownstein et al. 2005) eventually connecting northern and southern tick populations.

The current distribution of *I. scapularis* in the US is thought to reflect historical shifts in this species' range due to glaciation events followed by subsequent establishment by founder populations at foci in the Northeast and Midwest (Qiu et al. 2002, Humphrey et al. 2010, Van Zee et al. 2013). Southern populations are considered to be ancestral to those in the North and this is supported by the relatively high genetic diversity of southern versus northern populations consistently detected through both mitochondrial and nuclear genetic markers (Qiu et al. 2002, Humphrey et al. 2010, Van Zee et al. 2013). Despite their apparent genetic diversity, ticks from northern and southern regions are considered to be a single species which have been shown to readily mate and produce viable offspring in the lab (Oliver et al. 1993).

Our previous investigations of nymphal *I. scapularis* questing behavior substantiate claims for the existence of 'northern' and 'southern' behavioral phenotypes associated with differential levels of human LD risk (Oliver 1996, Goddard and Piesman 2006, Stromdahl and Hickling 2012). We found geographic variation in questing behavior such that nymphs from northern regions quest on or above the leaf litter surface much more often than nymphs from southern regions, which tend to stay hunkered down in the leaf litter, limiting their contact with human hosts (Arsnoe et al. 2015 and Chapter 2). These common garden studies strongly suggest a genetic component for questing behavior because relative questing activity between groups remained similar at various testing locations (environments) and among sibling groups tested at different locations.

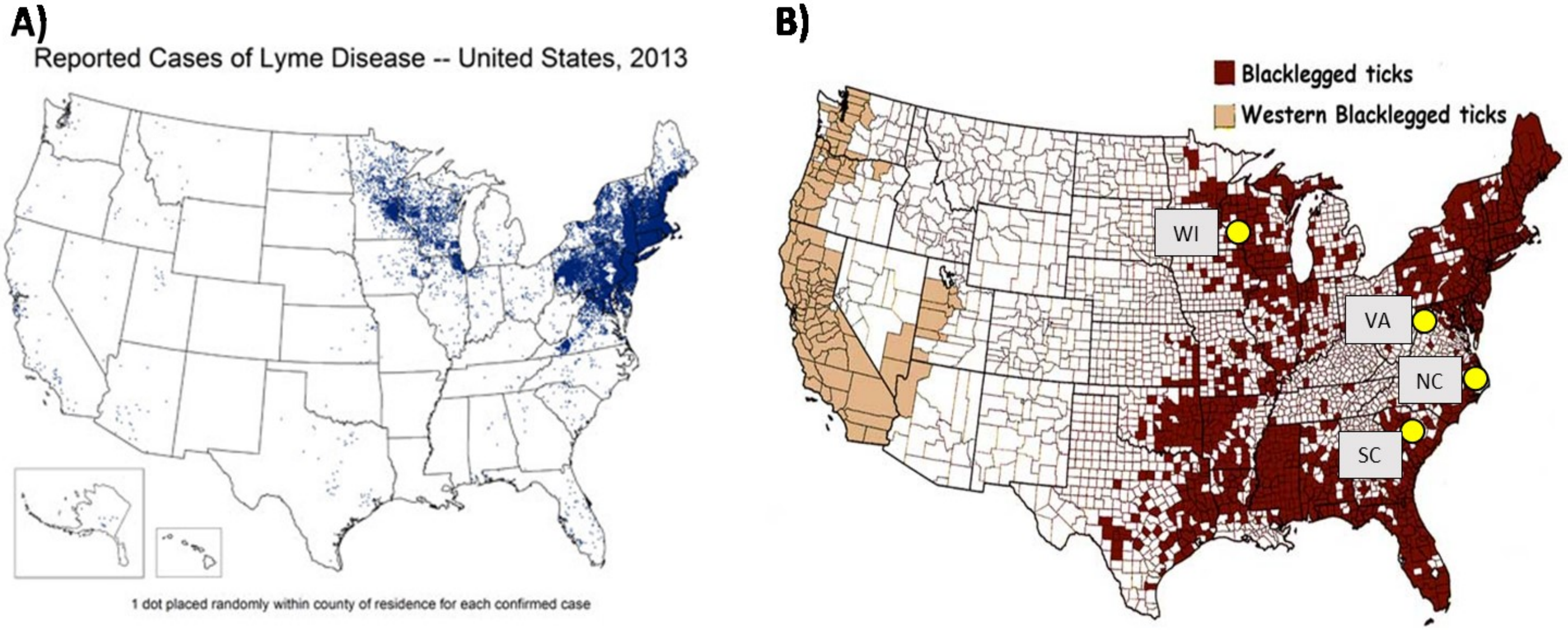
These findings raise important questions about the nature of inheritance of these behavioral traits and the behavior of hybrid individuals. *Ixodes scapularis* ranges are expected to continue to expand (Brownstein et al. 2005, Ogden et al. 2014) resulting in a situation where northern and southern populations will eventually meet, and possibly, interbreed. Information about the reproductive traits, offspring behavior, and survival of crosses between northern and southern populations is crucial for assessing if and how LD risk will change in hybrid zones in the future. Furthermore, by studying the behavior of hybrid offspring, insight regarding the mode of inheritance of behavioral traits can be assessed (Messina and Slade 1997, Summers et al. 2004). We present results from preliminary experiments in which we cross populations of *I. scapularis* with distinct questing behavior phenotypes to better understand 1) reproductive development between hybrid crosses, 2) questing behavior of hybrid offspring, and 3) survival of hybrid offspring. Individuals originating from four geographic origins (Wisconsin = WI, Virginia = VA, North Carolina = NC, South Carolina = SC) were selected for a mating (source populations). We selected these populations because: 1) they represent the behavioral phenotype extremes of within the region (very high or low questing behavior); 2) we had previously obtained behavioral and survival data on relatives of these groups with which we can make comparisons; and 3) because at least two of these groups represent populations that are likely to be sympatric in the near future and thus the characteristics of the behavior and survival of resulting offspring are of immediate interest to local public health agencies for public health preparedness. Using our previously tested field methodology, we assessed the questing behavior and survival of these hybrids and provide insight into how risk may change when tick populations meet.

Methods

Selecting and rearing ticks for crossing experiments. For our previous work on *I. scapularis* nymphal behavior, in Fall 2012 we obtained engorged female *I. scapularis* from hunter harvested deer at 15 locations across the eastern US. From May – September of 2013, the progeny from these females (nymphs) were tested in experimental outdoor arenas where behavioral and survival data were recorded. Given the results from those experiments (Chapter 2), we selected siblings of four groups to be used in the current crossing study (see Figure 3.9,

Appendix A). By using nymphs from families of known behavioral phenotypes to produce offspring for the current study, we are able to make comparisons between traits of relatives which provides insight into the inheritance patterns of these traits (Messina and Slade 1997). We selected four origins (= 4 families of ticks, each derived from a single female) for crossing: Wisconsin (WI), Virginia (VA), North Carolina (NC) and South Carolina (SC). The four origins represent groups which 1) displayed very high or very low questing behavior (the trait of interest) in our 2013 studies and 2) originated from regions of high or low LD incidence (Figure 3.1). In addition, two of these groups (VA and NC) appear likely to meet in the future. The VA population is rapidly expanding and exhibiting a southwest expansion trend (Brinkerhoff et al. 2014, Kelly et al. 2014) towards the NC population. If the current expansion trends continue, VA ticks could be expected to reach NC within the next decade. Expansion of *I. scapularis* populations can occur rapidly, invasion of *I.scapularis* populations were documented traversing a 200 mile distance along the west coast of Michigan within a four year period (Hamer et al. 2010).

Figure 3.1. Lyme disease incidence and *Ixodes scapularis* distribution in the US. (A) Reported cases of Lyme disease in the United States, CDC 2013 and (B) reported distribution of *I. scapularis* ticks from surveys conducted in the 1990s (Dennis et al. 1998). Reports of established *I. scapularis* populations in Virginia and Ohio have been published within the last year (Brinkerhoff et al. 2014, Wang et al. 2014). Yellow dots represent origin locations of adults used for crosses in these experiments: Wisconsin (WI), Virginia (VA), North Carolina (NC), and South Carolina (SC).



The selected groups of nymphs were fed on female white lab mice (ICR(CD-1) strain, *Mus musculus*). To ensure we would be working with virgin females, all engorged nymphs were maintained in individual vials to prevent preprandial (= off –host) matings post-moult. Adults were grouped into 16 crosses and mated on New Zealand White rabbits (*Oryctolagus cuniculus*) in December 2013. Four crosses were fed on a rabbit at a time, and were isolated from contact with each other by containment in mesh bags that were attached to the rabbit's skin. Engorged females which dropped from the rabbit were collected, placed in individual vials, and weighed within one day of repletion. Reproductive and development data for each female was checked and recorded at least two times per week throughout the oviposition and larval hatch period.

Hybrid crosses. Adults from the four origins were grouped into four reciprocal crosses, and four inbred crosses for a total of 16 individual crosses (see Table 3.1, column 1 in Results section). Because we were unsure if females crossed with males from a different geographic origin would produce eggs, and subsequent larvae, inbred crosses were performed as controls for the potential effects of laboratory rearing on development (unnatural hosts, constant environmental conditions). For reciprocal crosses, each parent originated from a different origin (source population), in the inbred crosses, both parents are from the same family. For two of the inbred crosses, SC x SC and NC x NC, females failed to attach or if attached, failed to engorge. This was unexpected as detrimental effects of inbreeding are not expected to occur for 3-4 generations (Troughton and Levin 2007). Crosses were further grouped into categories either by the questing behavior phenotypes (High = H, Low = L) of their parents.

Nymphs for behavior and survival studies. A single clutch from each successful cross (n = 14) was selected (based on estimating if there were enough larvae hatched to end up with # of nymphs needed to fill two behavior arenas; n ~ 100) and larvae from these clutches were fed on white lab mice as described above. Engorged larvae were collected and maintained in individual vials in humidity chambers at 21° C and ~ 98% relative humidity at 16:8 (Light:Day) hour photoperiod conditions. At 1 to 7 weeks of age, the resulting larvae of the 14 crosses chosen for field studies were fed on lab mice and allowed to molt into nymphs; at 2.5 – 3.5 weeks of age, these nymphs were transferred to the field site in Tennessee (late-May 2014). All ticks were reared and housed under identical environmental conditions throughout the entire time in the lab. All mouse and rabbit handling and tick feeding were done under protocol 06-12-103-00 approved by the Institutional Animal Care and Use Committee of Michigan State University and United States Department of Agriculture Veterinary Permit for Importation of Controlled Materials and Organisms and Vectors – Permit No. 121368.

Study site. Questing behavior of nymphal *I. scapularis* were observed at a deciduous forest field site in Anderson County (36.01 latitude), Tennessee (TN). The TN field site was utilized in 2012 and 2013 for similar studies and is thus previously described (Arsnoe et al. 2015). Briefly, this site was dominated by upland oaks (*Quercus* spp.), hickory (*Carya* spp.) and poplar (*Liriodendron tulipifera*). Environmental conditions (temperature and relative humidity) were recorded hourly at each site using iButton® data loggers (Hygrochron™, Dallas Semiconductor) placed at the surface of the leaf litter level (0.0 cm) and 10.0 cm above the leaf litter level. One "pair" (0 cm and 10 cm) of loggers was placed inside a randomly selected arena; another pair was placed outside of the selected arena. Conditions were recorded at hourly intervals throughout the study. Latitude coordinates obtained from http://earthexplorer.usgs.gov/™.

Questing behavior observations. The experimental design consisted 28 arenas grouped in blocks of four from which all naturally-occurring ticks had been removed by heat-treating locally-obtained leaf litter before leaves were added to the arenas. Arena construction and litter treatment were as described in Arsnoe et al. (2015). Briefly, the arenas consisted of a 0.5 m circular aluminum flashing boundary containing leaf litter and 15 bamboo skewers (= artificial stems) of three heights (5 cm, 10 cm, and 20 cm) emerging from the leaf litter. Dowels were scored at 2.5 cm increments in order to provide a point of reference for measuring questing heights. Arenas were surrounded by an outer barrier to exclude wildlife.

On May 31 2014, 49-57 nymphal ticks derived from an individual clutch representing thirteen individual crosses were released into arenas in TN. Each cross was replicated (n=2 arenas), except for a fourteenth cross (NC x SC) in which only 19 nymphs were available from lab rearing. Upon release, it was noted that the NC x SC cross did not move around much compared to all other nymphs in arenas. Nymphs were never observed questing in this arena during the entire study and no nymphs were recovered from this arena at the end of the study. Therefore, the NC x SC cross was excluded from the analysis because observations of behavior would be confounded by rapid mortality. Crosses are described in Table 3.1 in Results section.

Nymph questing behavior was recorded throughout the study period as previously described (Arsnoe et al. 2015 and Chapter 2) by a single observer, who was blinded to the identity of the crosses in arenas. Observations occurred in the morning (approx. 0800 hours) and late afternoon (approx. 1600 hours) at weekly intervals from June 1 – August 11, 2014. Questing behavior was quantified as the number of nymphs present on stems, leaf litter surfaces, and arena walls during a two minute observation period (Arsnoe et al. 2015 and Chapter 2). The questing heights of all individual nymphs in the arenas was estimated by the observer by using the markings on the dowels as points of reference. An average questing height was calculated for all questing (i.e. visible) nymphs in a single arena during each observation.

Survival analysis. At the completion of observations, we assessed the relative survival of the nymphs by conducting intensive searches of the arena litter. We adopted the method used in Arsnoe et al. 2015 by performing seven microdrag searches over 26 days in 2013 and 6 microdrag searches over 19 days in 2014. Nymph recovery by microdragging began on October 23, 2013 for the parental generation and on August 11, 2014 for the cross generation.

Statistical Analysis. Linear fixed effects models were used to analyze the relationships between parent origin or parent questing phenotype on engorgement success, oviposition timing, and larval hatching for successful crosses. Pairwise comparisons (Tukey-Kramer) were made using *lsmeans* in the R package version 2.12 (Lenth 2014). We applied Generalized Linear Mixed Models (GLMMs) using the function *glmer* in the R package *lme4* (Bates et al. 2014) to analyze the log-odds of observing questing nymphs as a function of 1) parental origin, 2) cross by parent questing phenotype, and 3) cross by parent origin (3 different models). Questing behavior was measured as the number of nymphs visible during a given two minute observation of an arena. Arena, week, and clutch ID were modeled as random factors to account for heterogeneity among arenas, clutches of individual females, and over time (week). GLMMs were also used to analyze the log-odds of recovery of nymphs (survival) from arenas as a function of 1) parental origin, 2) cross by parent questing phenotype, and 3) cross by parent origin (each modeled as a single, fixed factor). Survival was measured by tallying the number of nymphs recovered from arenas at the end of each study period. Arena and clutch ID (for 'cross' survival only) were modeled as random factors for the reasons described above. We used linear mixed effects models in the R package *nlme* (Pinheiro et al. 2014) to examine questing height as a function of the same 3 predictors (fixed effects, modeled separately) used for the questing behavior and survival analysis. Random effects were modeled for arena, week, and clutch ID.

Visual inspection of residual plots did not reveal violations of homoscedacity or normality. P – values were obtained by likelihood ratio tests of the full model which included the predictor of interest against the model without the predictor of interest. To visualize the results, predicted log-odds and their associated standard errors were back-transformed into probabilities. All statistical analyses were performed using R 3.1.0 (http://www.r-project.org).

Results

Hybrid crossing experiments. The reproductive and development data for female *I. scapularis* (n = 119) representing 16 crosses is summarized in Table 3.1. Engorgement success was 36.1% with 43 females achieving full repletion and dropping from rabbit hosts 6-14 days (mean = 10.1, sd = 1.73) after attachment. An additional live female (NC x SC cross), was removed from the rabbit 14 days following attachment after taking a small bloodmeal (slightly inflated idiosoma) but showing no further signs of engorgement (enlarged body size) for over a week. Females from the L-pure crosses were unsuccessful in attaching to the rabbits with the exception of a single SC x SC female, which attached and expired without engorging.

Table 3.1. Reproductive and development data for crosses of *I. scapularis* adults originating from four geographic origins (WI-Wisconsin, VA-Virginia, NC-North Carolina, SC-South Carolina). Crosses were grouped by 1) questing phenotypes of the parents: L pure – inbred cross, both parents low questing; L x L – hybrid cross, both parents low questing; H x L – hybrid cross, 1 low, 1 high questing parent; H x H – hybrid cross, both parents high questing; H pure – inbred cross, both parents high questing, and by 2) parent origin (WI, VA, NC, SC). Engorgement success calculated as # females placed on rabbits for feeding/# females successfully replete. Fecundity calculated at # females that oviposited/# females that successfully engorged. Days to oviposition calculated as # of days from drop off from rabbit to first eggs seen in female housing tube. Days to larvae emergence calculated as the number of days from the first day of oviposition to the first larva observed emerged from the clutch. All females which were fecund, were also fertile – only fecund (n=40) are included in engorgement weight, ovipoistion time, and larval emergence time analyses.

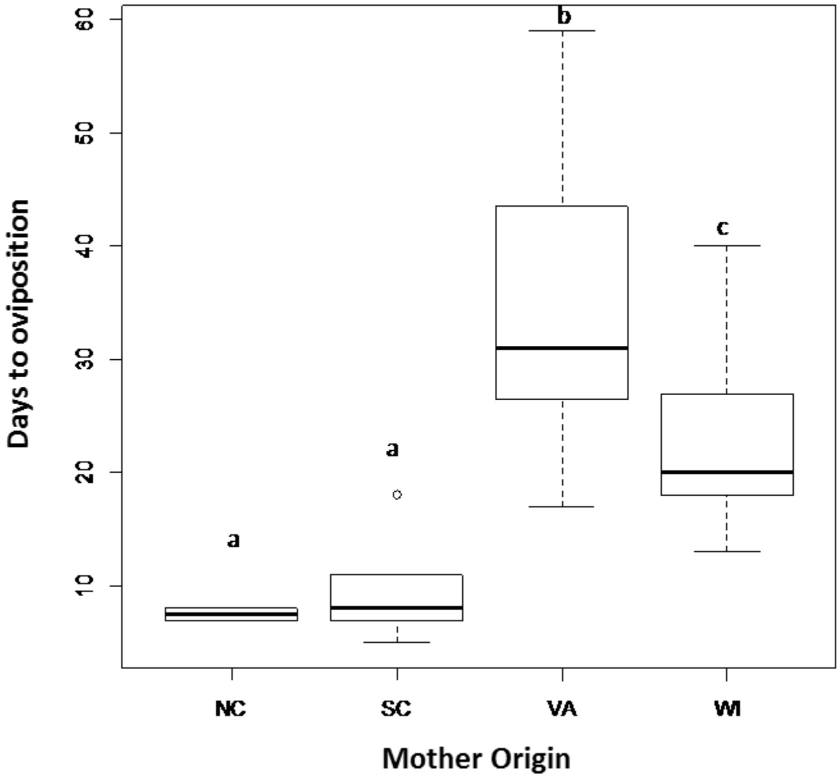
Unique cross (♀ x ♂)	Cross by parent origin	Parent questing phenotype	# ♀♀ on rabbits	# replete	Engorge success	Engorge wt. (g) mean(sd)	% fecund	Days to oviposit mean(sd)	Days to larvae emerge means(sd)
NC x NC	NC x NC	L pure	8	0	0	-	0	-	-
SC x SC	SC x SC	L pure	6	0	0	-	0	-	-
SC x NC	SC x NC	L x L	6	3	0.5	0.10 (0.067)	1	12 (5.1)	48 (0.7)
NC x SC		L x L	8	1*	0.13	< 0.01 (-)	1	18 (-)	44 (-)
VA x WI	VA x WI	H x H	6	2	0.33	0.15 (0.010)	1	36 (26.9)	47 (4.9)
WI x VA		H x H	7	7	1.0	0.24 (0.071)	0.86	17 (2.3)	45 (3.1)
NC x VA	NC x VA	H x L	8	1	0.13	0.08 (-)	1	7 (-)	49 (-)
VA x NC		H x L	9	4	0.44	0.12 (0.050)	0.75	39 (7.9)	53 (8.1)
NC x WI	NC x WI	H x L	8	5	0.63	0.14 (0.053)	1	8 (0.5)	46 (3.0)
WI x NC		H x L	8	2	0.25	0.13 (0.002)	1	30 (0.7)	52 (6.4)
SC x VA	SC x VA	H x L	6	3	0.5	0.23 (0.061)	1	8 (1.0)	46 (2.1)
VA x SC		H x L	9	3	0.33	0.17 (0.054)	1	38 (18.1)	50 (6.2)
SC x WI	SC x WI	H x L	7	4	0.57	0.22 (0.024)	1	8 (2.5)	48 (3.1)
WI x SC		H x L	8	1	0.13	0.08 (-)	1	20 (-)	50 (-)
VA x VA	VA x VA	H pure	8	3	0.38	0.14 (0.079)	1	30 (6.8)	51 (4.0)
WI x WI	WI x WI	H pure	7	5	0.71	0.18 (0.072)	0.8	28 (8.4)	51 (3.8)

* pulled from rabbit on D14 after attachment; not included in analysis, neither were L pure crosses since they didn't successfully attach or feed

Engorgement weights of successful females ranged from 0.8 – 2.4 g (mean = 0.17, sd = 0.065), the NC x SC female removed from the rabbit was <0.01g. A total of 40 females and their respective clutches are included in the following analysis of the cross reproductive data; three females were excluded because no eggs were laid; additionally the NC x SC female was excluded due to premature detachment. Weights were not significantly different among the four questing phenotypes ($F_{3,36} = 2.23$, $p = 0.10$; Table 3.1) or when grouped by mother or father origin ($F_{3,36} = 1.93$, $p = 0.14$, father $F_{3,36} = 2.39$, $p = 0.08$). Oviposition success of crosses ranged from 75% - 100%. All crosses that produced eggs also had high (100%) hatch success.

The number of days from female detachment from rabbits to the first day of oviposition varied widely among the crosses (range 5 – 59 days, mean = 20.55, sd = 13.88). A significant effect of mother origin was observed ($F_{3,36} = 23.39$, $p = 1.4$ e-8; Figure 3.2); northern mothers (VA and WI) exhibited longer oviposition times (mean 29.1 days, sd = 9.56) versus southern mothers (SC and NC; mean = 8.3 days, sd = 1.13). No effect of father origin on oviposition times ($F_{3,36} = 2.64$, $p = 0.07$) or of father questing phenotypes ($F_{3,36} = 1.43$, $p = 0.25$) were observed.

Figure 3.2. Ovipostion time (in days) of clutches by mother's geographic origin. Shared letters indicate populations that did not differ in mean number of days from repletion to oviposition (Tukey p > 0.05). Number of clutches for each origin were N= 6, 10, 11, 13 for NC, SC, VA, and WI respectively.



In contrast to oviposition timing, larval hatch times were similar among clutches regardless of parent origin or questing phenotype (range: 41-60 days; mean = 48.1, sd = 4.41). No significant effects were observed for either parent origin, or questing phenotype of cross ($F_{3,36} = 2.36$, $p = 0.09$; $F_{3,36} = 1.46$, $p = 0.24$; $F_{3,36} = 1.76$, $p = 0.17$ for phenotype, mother, father, respectively).

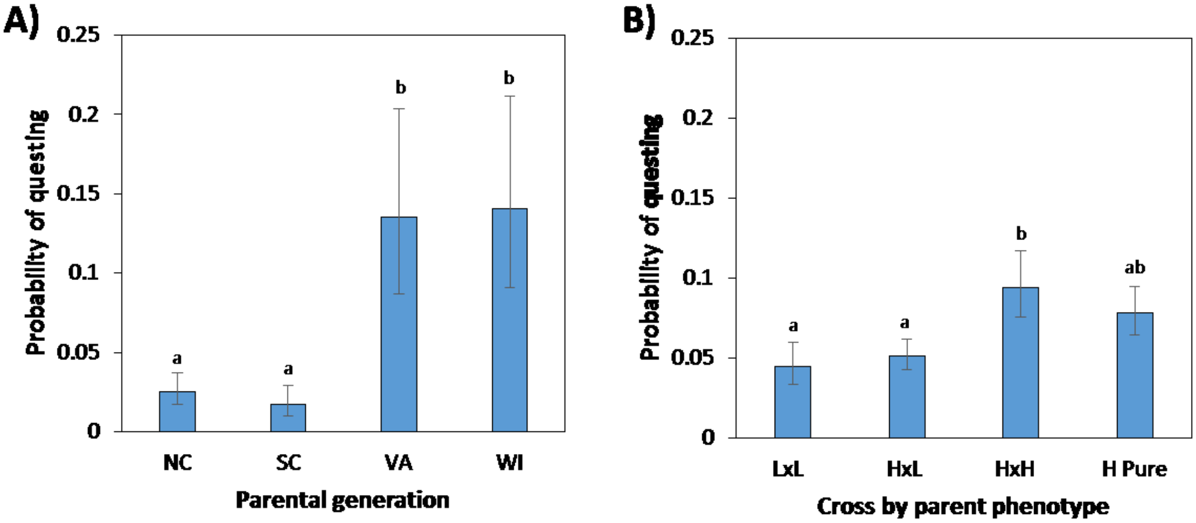
Environmental conditions for each year. Temperature and humidity readings at the field site for each year of behavior testing are shown in Table 3.2. The ambient (10 cm) conditions in 2014, the year crosses were tested, tended to be less humid (drier) and slightly warmer than in 2013 when parental crosses were tested.

Table 3.2. Comparison of environmental conditions at Tennessee field site in 2013 (when parental generation experiments occurred) and 2014 (when F_1 generation experiments occurred). Temperature and relative humidity (means ± SD) at leaf litter level ("0 cm") or above leaf litter level ("10 cm") for each year of observations are shown. Readings are the average of the 2 loggers placed in each position (0cm and 10 cm). Readings were collected at hourly intervals.

Year	**Dates sampled**	**Temp (°C) 0cm**	**Temp (°C) 10cm**	**Relative humidity (%) 0cm**	**Relative humidity (%) 10cm**
2013 (Parental Generation)	6/1 – 8/29	21.4 (2.26)	21.6 (2.69)	99.9 (4.41)	97.6 (6.45)
2014 (F1 Generation)	6/1 – 8/11	21.9 (2.65)	21.9 (3.50)	101.3 (5.31)	91.0 (14.26)

Questing behavior analysis. *Parental generation in 2013.* These data represent the nymphal questing behavior of siblings of the parental ticks used for the crosses (uncles and aunts of the F_1 generation) and are hereafter referred to as the "parental generation". These data comprise 112 observations of 8 experimental arenas located at a field site in eastern Tennessee from June 1 – August 29, 2013 (approximately 12 weeks). Questing behavior by the nymphal stage of the parental generation was consistent with our previous findings in there was a significant effect of nymph origin on questing ($X^2(3) = 13.0$, $p = 0.0046$). The odds ratio of northern nymphs (= VA and WI, "H" phenotypes) questing to southern nymphs (=SC and NC, "L" questing phenotypes) questing was ~6:1. Northern origins (log-odds: WI mean (se) = -1.8 (0.49); VA = -1.9 (0.49)) quested more often than the southern origins (log-odds: SC = -4.1(0.53); NC = -3.6 (0.38)), but not different from one another (Figure 3.3A).

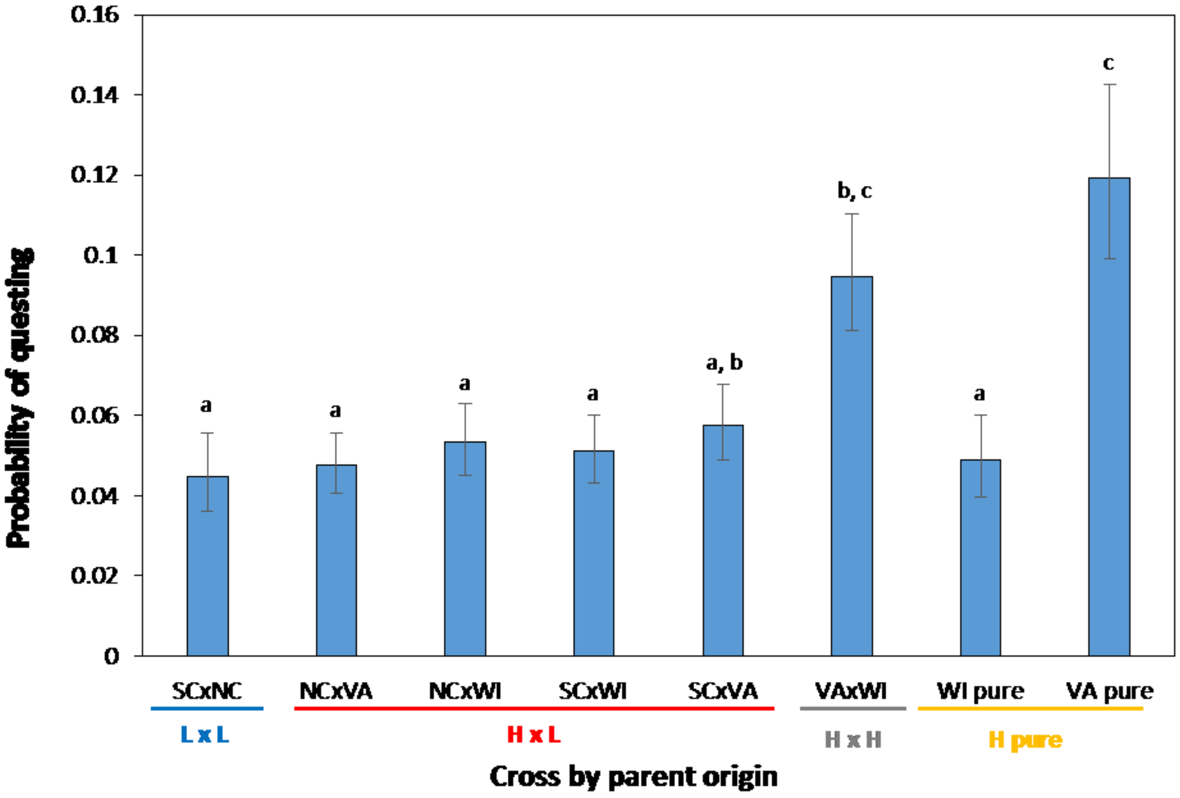
Figure 3.3. Probability (±SE) of observing questing nymphs in arenas in Tennessee: (A) parental generations from 4 geographic origins, observed June 1 – August 29, 2013. Each origin was represented by 2 arenas and (B) F_1 crosses by parent phenotype, observed June 7-August 11, 2014. Each cross represented by 2, 18, 4, 4 areas for LxL, HxL, HxH, and H pure respectively. Shared letters above bars indicate that populations did not differ in log-odds of questing (Tukey p > 0.05).



F_1 generation – Hybrids and inbreds. These data represent the nymphal questing behavior of the offspring (F_1) of the brothers and sisters from the "parental generation". These data comprise 448 observations of 28 experimental arenas from June 7 – August 11, 2014 (approx. 9 weeks) located at the same field site in eastern Tennessee that was used for questing behavior experiments in 2013. Questing behavior among nymphs from the F_1 generation produced by crossing adults from four geographic origins designated as having high or low questing phenotypes is shown in Figure 3.3B. Questing phenotypes of parents had a significant effect on the questing behavior of offspring ($X^2(3) = 10.4$, $p = 0.015$). The odds ratio of HxH questing to HxL and LxL questing was ~ 2:1. The odds of H pure questing was similar to HxH questing, and approx. 1.5 greater than the odds of questing by LxL and HxL (Figure 3.3B). Model estimates of the log-odds of questing for each group were: LxL mean(se) = -3.1(0.31), HxL = -2.9(0.19), HxH = -2.3(0.24) and H pure = -2.5(0.21).

A closer examination of among cross differences was conducted by using "cross" (representing the two parental origins involved in the cross) as a predictor. A significant difference of cross by parent origin was found ($X^2(7) = 22.4$, $p = 0.002$). Crosses of WI x VA and VA x VA quested more often than all other crosses (Figure 3.4).

Figure 3.4. Probability (±SE) of observing questing nymphs in arenas in Tennessee by F_1 crosses by parent origin, observed June 7-August 11, 2014. Each cross represented by 2, 6, 4, 4, 4, 4, 2, 2 areas left to right, respectively. Questing phenotype for each cross is denoted by colored bars and corresponding colored text under cross labels. Shared letters above bars indicate that populations did not differ in log-odds of questing (Tukey $p > 0.05$).



Questing height observations. We calculated the average questing heights of nymphs for each cohort because we were interested in learning more about the questing heights of the nymphs that do emerge from the leaf litter (quest) as this will influence the types of hosts in which they come into contact with. These data represent the means of questing heights of *emerged* nymphs in arenas: for the parental generation n=80 observations, for the F_1 generation, n = 386 observations. In general, questing heights followed the same trends as the questing probabilities for the parental (L.ratio = 13.1, p = 0.004) and F_1 generations (L.ratio = 9.02, p = 0.029). Low questers had lower questing heights; high questers had higher questing heights (Figure 3.5A and Figure 3.5B). Hybrids of high and low questers (H x L) had slightly higher heights, but more similar to L x L group than to the H x H or H pure groups. Questing heights (means(sd))were: 1.24(0.705), 1.21(0.593), 2.67(1.128), and 2.93(1.43) for SC, NC, VA, and WI of the parental generation, and 0.77(0.715), 1.02(1.124), 1.64(1.032), and 1.96(1.833) for LxL, H x L, H x H, and H pure groups, respectively. Variation among crosses by parent origin for questing height was observed (Figure 3.6).

Figure 3.5. Mean questing heights (±95%CI) for nymphs observed in arenas in Tennessee in: (A) parental generations from four geographic origins, observed June 1 – August 29, 2013. Each origin was represented by two arenas and (B) F_1 crosses, observed June 7-August 11, 2014. Each cross represented by 2, 18, 4, 4 areas for LxL, HxL, HxH, and H pure respectively. Shared letters above bars indicate that population mean questing heights (Tukey $p > 0.05$).

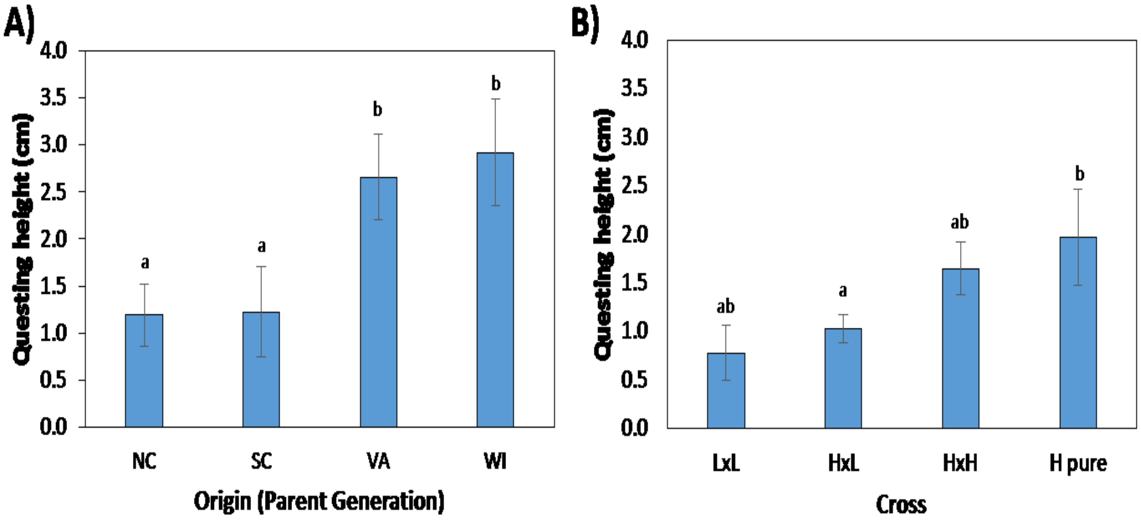
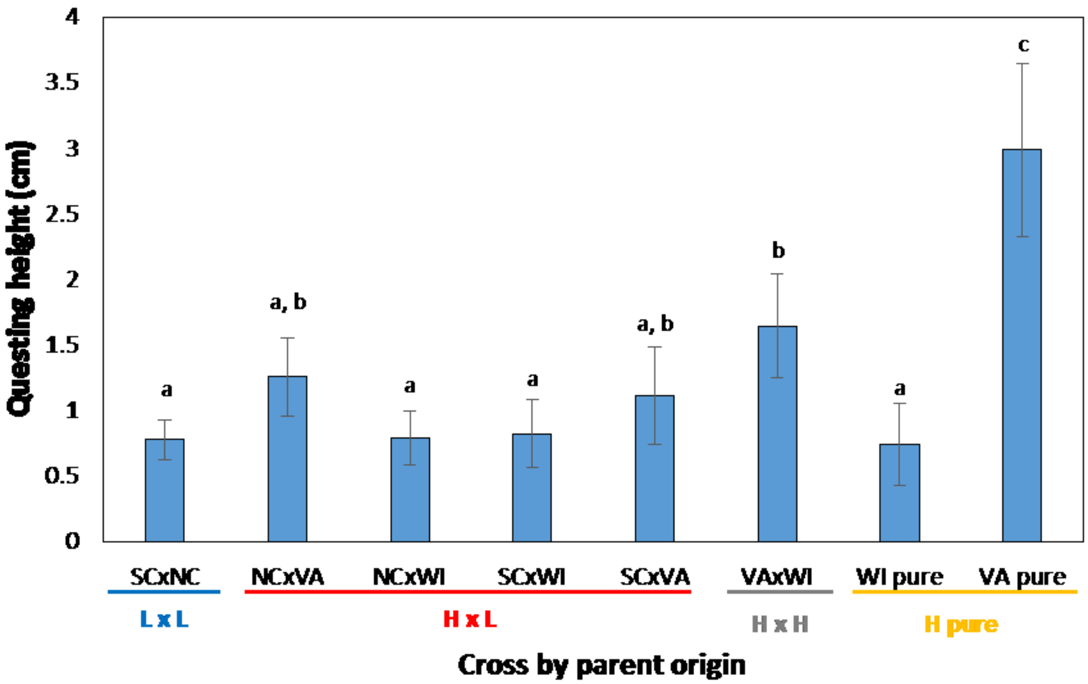


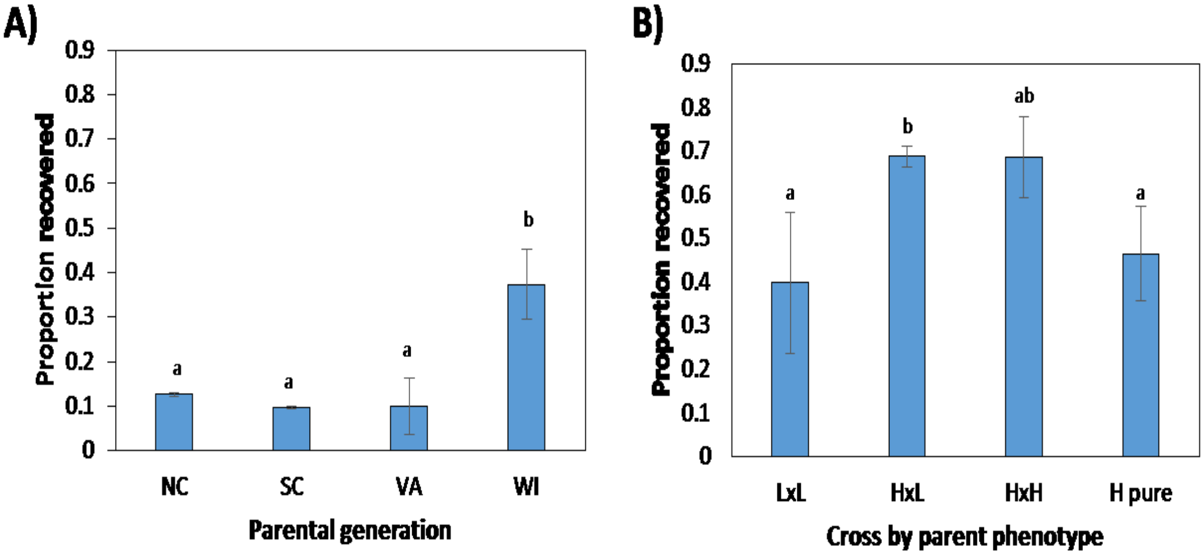
Figure 3.6. Mean questing heights (±95%CI) for F_1 crosses by parent origin. Each cross represented by 2, 18, 4, 4 areas for LxL, HxL, HxH, and H pure respectively. Each cross represented by 2, 6, 4, 4, 4, 4, 2, 2 arenas left to right, respectively. Questing phenotype for each cross is denoted by colored bars and corresponding colored text under cross labels. Shared letters above bars indicate that population mean questing heights did not differ (Tukey $p > 0.05$).



Survival of parent and hybrid generations. *Parental generation.* These survival data originate from 8 experimental arenas observed in Tennessee in 2013. A total of 413 nymphs from the four origins were released on May 10, 2013 and a total of 71 (17.2%) of these were recovered approximately 5 months later (166 days). There was a significant effect of parent geographic origin on survival ($X^2(3) = 12.32$, $p = 0.006$). The odds of WI nymph recovery was ~ 4 times greater than the odds of VA, NC, or SC nymphs recovery (Figure 3.7A).

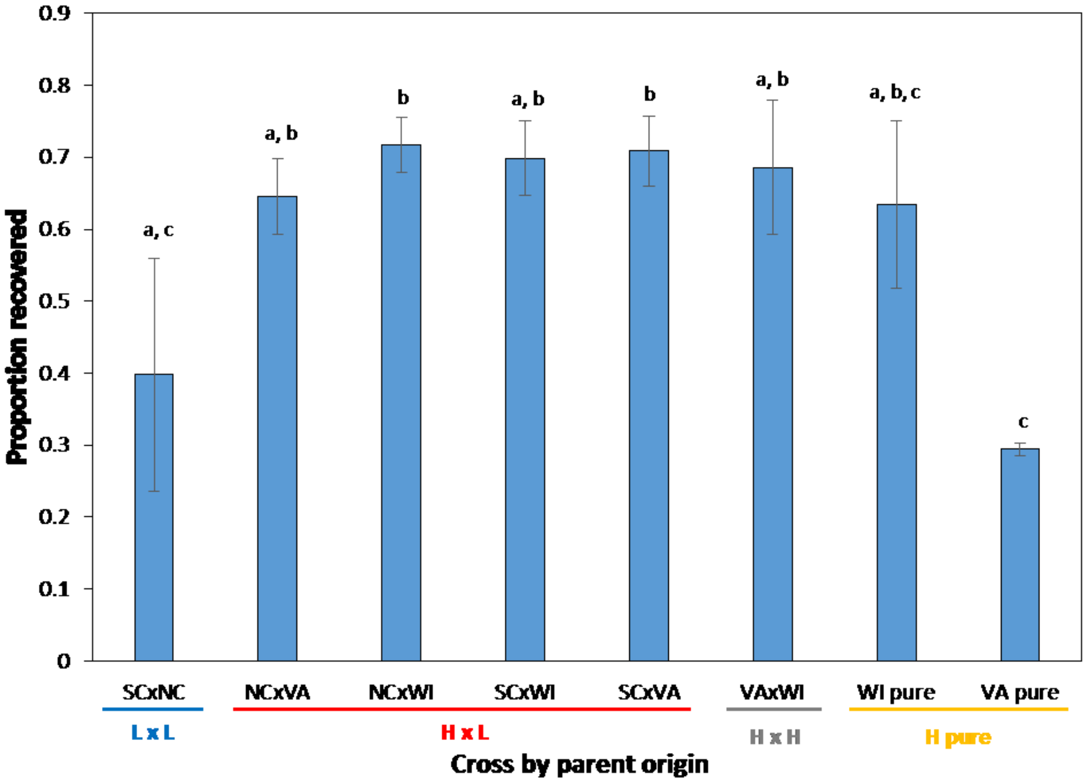
F1 generation. These survival data originate from 28 experimental arenas observed in Tennessee in 2014. A total of 1532 nymphs representing 13 individual crosses were released on May 31, 2014 and a total of 971 (63.4%) of these were recovered approximately 2.5 months later (73 days). There was a significant effect of cross by parent phenotype on survival ($X^2(3) = 9.83$, $p = 0.02$). The odds of recovery for hybrid crosses with one high questing parent (H x L) versus crosses with both low questing (L x L) or inbred (H pure) parents were ~ 3:1 (Figure 3.7B).

Figure 3.7. Mean proportion (±SE) nymphs recovered from arenas in Tennessee (A) parental generations from four geographic origins, released on 5/10/13 and recovered on 10/23/13, 166 days later. Each origin was represented by two arenas and (B) F_1 crosses, released on 5/31/14 and recovered 8/13/14, 73 days after release. Each cross represented by 2, 18, 4, 4 areas for LxL, HxL, HxH, and H pure respectively. Shared letters above bars indicate that populations did not differ in log-odds of recovery (Tukey $p > 0.05$).



Closer examination of among cross differences revealed further information regarding effect of parent origin on recovery. A significant difference for cross by parent origin was found ($X^2(7) = 17.3$, $p = 0.016$). Crosses of SC x NC and VA pure were recovered at lower proportions than the other crosses (Figure 3.8).

Figure 3.8. Mean proportion (±SE) nymphs recovered from arenas in Tennessee for F_1 crosses by parent origin, released on 5/31/14 and recovered 8/13/14, 73 days after release. Each cross represented by 2, 6, 4, 4, 4, 4, 2, 2 areas left to right, respectively. Questing phenotype for each cross is denoted by colored bars and corresponding colored text under cross labels. Shared letters above bars indicate that populations did not differ in log-odds of recovery (Tukey p > 0.05).



Discussion

Our laboratory and common garden experiments provide evidence for genetic differences in reproductive behavior among mothers from different geographic origins and evidence for differences in the questing behavior and survival of hybrid crosses resulting from mating these mothers with fathers of different geographic origins. The behavior of nymphs from crosses where both parents had the same behavioral phenotype, was similar to that of their parents with the exception of the WI pure cross, which exhibited low questing behavior when compared with the WI parental generation. Nymphs from crosses with parents of different behavioral phenotypes (H x L), exhibited low questing behavior similar to L x L crosses, and different from H x H and VA pure crosses. Mean survival was high for H x L, H x H, and WI pure crosses, but reduced for L x L and VA pure crosses.

The observed low questing behavior of H x L crosses could indicate that the propensity for low questing is a dominant trait within the population, since all H x L crosses quested lower relative to H x H and VA pure crosses. Another explanation is that questing behavior is phenotypically plastic, meaning that different questing phenotypes are expressed in different environments (Via and Lande 1985). Tick activity has been shown to be directly affected by temperature and relative humidity in the lab (Randolph and Storey 1999, Vail and Smith 2002), and the field (Eisen et al. 2002, Schulze and Jordan 2003) indicating that questing behavior is a plastic trait (because ticks do not exhibit constant activity levels in all conditions). It is possible that behavioral plasticity varies between northern and southern populations, with northern populations exhibiting higher levels of plasticity. The results from our previous studies indicate that northern ticks exhibit greater plasticity for questing behavior relative to southern ticks because they utilize leaf litter surfaces, twigs and within leaf litter areas, whereas southern ticks tend to remain within in the leaf litter (Arsnoe et al. 2015 and Chapter 2). Plasticity in questing behavior would likely to provide the northern nymphs with more opportunities to contact a wider range of hosts in their environment, yet mediate the threat of changing environmental conditions (i.e. highly dessicating air). In the South, plasticity of questing behavior may not be as useful if there is an abundance of hosts on which to feed present within the leaf litter environment. This may be the case at least in some areas of the south, where fossorial lizards outnumber rodents 2:1 (Apperson et al. 1993).

The observation that H x H crosses quested high (and were exposed to more desiccating conditions - Table 3.2) and survived well, but the L x L cross exhibited low questing and survived poorly, could imply that northern nymphs may be better physiologically equipped to handle prevailing environmental conditions. Laboratory studies of larval *I. scapularis* survival indicated that northern genotypes may be more robust to environmental conditions than southern genotypes (Ginsberg et al. 2014), with northern genotypes surviving better in all environmental conditions tested in the lab. Future studies which examine physiological differences (e.g. size, fat resources, cuticle composition) among nymphs of northern and southern populations would provide useful information regarding the effects of biological differences on questing behavior and survival.

The survival data suggest that H x L hybrids exhibit higher fitness than L x L or H pure individuals. When the fitness of heterozygotes is greater than that of homozygotes, this is known as heterozygote advantage (or overdominance) (Conner and Hartl 2004). Tick populations are likely to experience heterozygote deficit due to genetic drift that occurs within isolated populations (Kempf et al. 2010, Chan 2012); therefore the meeting and merging of northern and southern populations (with high and low questing behavior, respectively) could increase overall fitness in the new population by increasing genetic variation. Overall fitness increases in a vector population would have drastic consequences for human vector-borne disease risk if vectors become more prolific. However, it is possible that assortative mating or other mechanisms that promote reproductive isolation between northern and southern populations may persist and prevent the mixing of genotypes. This phenomenon has been observed in other *Ixodes* species (McCoy et al. 2005, Kempf et al. 2011) and is thought to occur as a result of host association (i.e. "host races"). Northern *I. scapularis* nymphs primarily parasitize small mammals (Spielman et al. 1985, LoGiudice et al. 2003) while those in the south are associated with fossorial lizards (Apperson et al. 1993, Kollars et al. 1999, Durden et al. 2002); therefore, it is possible these populations may segregate instead of mating with each other.

We observed that the geographic origin of the mother affects oviposition timing. Northern mothers took longer to oviposit than southern mothers. The longer ovipostion time for ticks from northern regions is consistent with our previous experience for engorged females collected from hunter harvested deer from various regions in the US (IMA-personal observation). Oviposition timing appears to be genetically controlled, as differences were evident among the females reared in a controlled laboratory environment. Other studies have indicated that oviposition timing is directly influenced by temperature (Ogden et al. 2004). Our observations suggest that environment and genes likely interact to influence these rates. If southern moms (who oviposit relatively quickly) mate with northern dads, northern genes could move rapidly through the population compared to southern genes.

The major limitations of our study is that we were unable to assess the reproductive viability of the offspring from our crosses. However, previous work with crosses of colony reared *I. scapularis* from Massachusetts and Georgia indicates that northern and southern crosses will breed and produce viable offspring (Oliver et al. 1993). Investigations are currently underway to determine the viability of the hybrids used in these experiments. Unlike arthropods with relatively short generation times (e.g. mosquitos, fruit flies) *Ixodes* ticks have an extended lifecycle that may last many years in a natural environment (Lindsay et al. 1995). In the lab, rearing ticks through an entire generation takes 204-219 days (Troughton and Levin 2007) and therefore has obvious implications for the feasibility of conducting multigenerational studies on these organisms. The duration of multiple lifecycles can easily span longer than the time needed to complete a typical graduate degree.

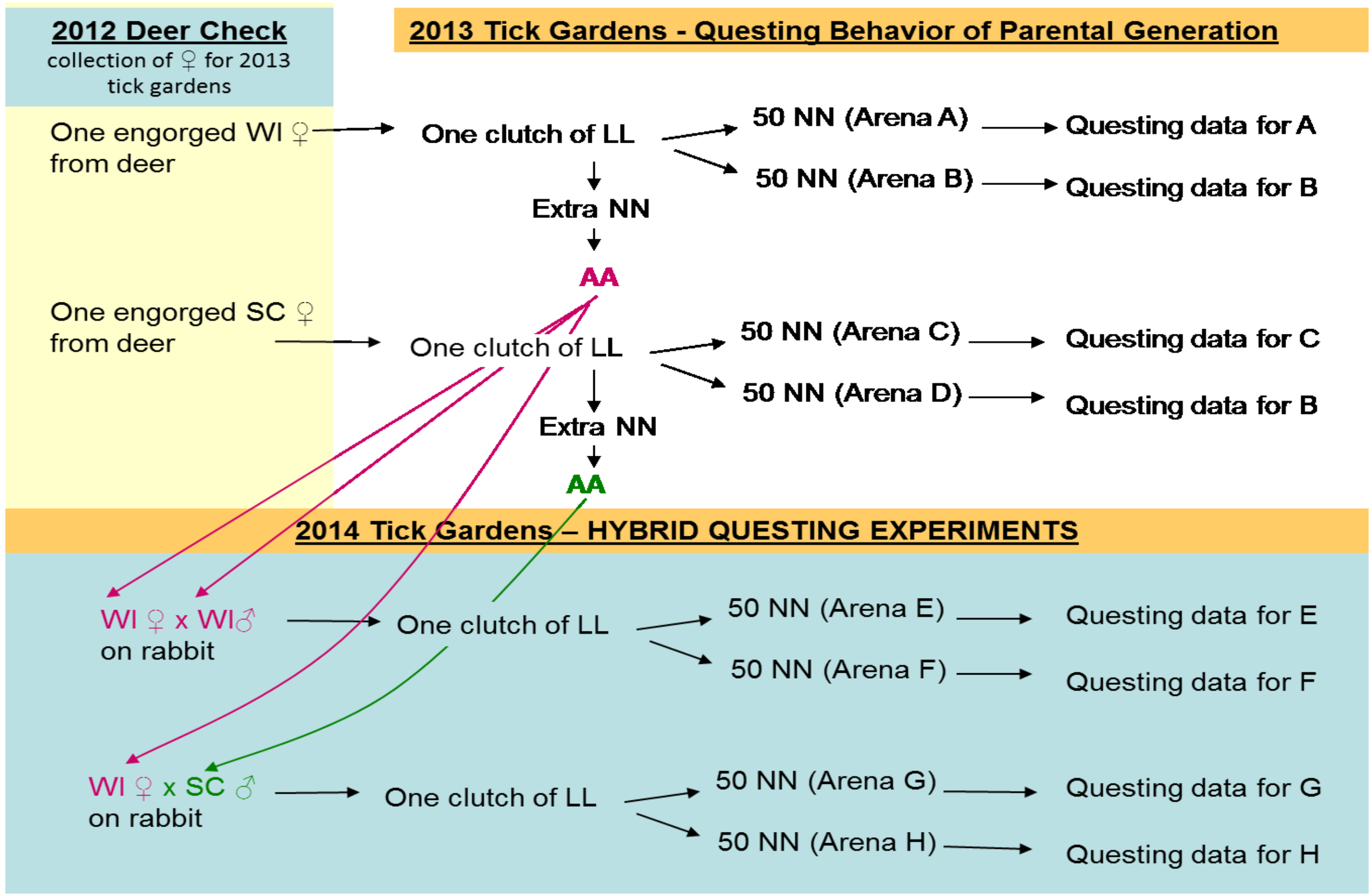
The generalizability of these results are limited due to the low number of crosses generated and tested from each location. Each origin by parent cross is represented by a single mother and father. Given that the WI and VA pure groups performed quite differently in questing behavior and survival, it appears that the effects of parental geographic origin are likely to vary within and among northern and southern regions.

The effects of merging northern and southern tick populations remains largely unknown. Ecological interactions such as competition and predation could have unforeseen effects on northern populations moving southward (or vice versa). The results of this pilot study provide a basic foundation from which future, more elaborate, population genetic studies may be conducted. These studies will be essential for understanding the outcomes of *I. scapularis* population shifts on human risk for vector-borne disease. It is possible that if behavioral plasticity for high and low questing comes to dominate the genetic landscape in areas where these populations will meet, LD risk could increase if some individuals begin to use surface areas to alleviate competition for fossorial vertebrates. Field and laboratory studies are needed to understand more about the interactions among those populations which are likely to soon meet.

APPENDIX

Experimental design, nymph relationships

Figure 3.9. Schematic showing relationship of parental generation (source populations) to hybrid populations used for the current experiments (2014 Tick Gardens). Parental populations were derived from engorged females collected from hunter harvested deer in 2012. Questing behavior data was obtained from these populations during experiments performed in 2013 (Chapter 2; 2013 Tick Gardens). Siblings of NN tested in 2013 ('Extra NN'-nymphs from the same clutch but not placed in 2013 tick gardens) were reared to adults, these were the adults mated for the 2014 Tick Gardens. LL = larvae, NN = nymphs, and AA = adults.



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CONCLUSIONS

Lyme disease continues to be a looming public health threat. In 2013, the Centers for Disease Control and Prevention released a statement acknowledging that the true number of Lyme cases in the US may be 10 times that officially reported each year (CDC 2013). The CDC contends that these cases are within current Lyme risk regions and do not represent undiagnosed disease in the southeastern US (CDC 2013). This continues to fuel contentious debates regarding the status of human Lyme disease in southern states. In 2013, Discover Magazine published a 59-page investigative narrative discussing the on-going controversy over tick-borne illness in southern regions (Orent 2013).

The conclusions from this dissertation inform this debate: I found that southern *I. scapularis* nymphs were not likely to quest above the leaf litter, and thus, it would be expected that nymphal *I. scapularis* rarely come into contact with human hosts in the southeastern US. This is borne out by the very low proportion of human *I. scapularis* bites that are from nymphs in the South, where as the majority of *I. scapularis* bites in the North are from nymphs (Stromdahl and Hickling 2012).

Summary of findings. In Chapter 1, using field studies, I demonstrated that nymphal questing behavior varies for *I. scapularis* nymphs derived from a northern and two southern regions and that the behavior differences were influenced by a genetic component. Although this pattern was suspected, direct observations of these behavioral differences had not been made prior to this study. In Chapter 1, I concluded that southern *I. scapularis* nymphs spend most of their time within the leaf litter where they are unlikely to contact human hosts.

In Chapter 2, I examined the scope of these behavioral differences by testing nymphs from a number of locations across *I. scapularis*' geographic range. The result of this experiment revealed that these questing differences are a geographically widespread phenomenon and are strongly related to the human Lyme risk in a region. These findings provide compelling evidence that differences in nymph questing behavior contribute to the variation in observed human Lyme disease risk throughout the eastern US (Diuk-Wasser et al. 2012).

In Chapter 3, I probed the genetic aspects of questing behavior by mating ticks from various geographic regions and subsequently monitoring the progeny to understand the inheritance of behavior. This was an important direction to take with the research because *I. scapularis* populations are expanding and it is anticipated that northern and southern populations will meet. Using laboratory observations, I determined that mothers from northern and southern regions differ in their ovipositional timing, and this timing is not affected if a northern female is mated with a southern male (and vice versa). In subsequent field studies, I also found that crosses between northern and southern populations (with different questing behavior) result in progeny that survive well in the field and behave more like southern parents (low questing) rather than northern parents, or intermediate. Understanding how hybrids will behave is critical for predicting how human LD risk will change in hybrid zones.

This dissertation project is part of a large-scale effort funded through the National Science Foundation Emerging Infectious Disease Award EF-0914476 which seeks to understand the ecological drivers of Lyme disease in the eastern US. My dissertation research complements the efforts of this project by providing information about a mechanism directly related to human LD risk. This work also contributes to the field of vector ecology by demonstrating the importance of vector behavior as a driver of disease transmission to humans.

Much work remains for disease ecologists and so it is an exciting time to be a part of this field. As vector populations redistribute with climate changes (Mills et al. 2010), there will be inevitable impacts on the transmission of vector-borne diseases. Ecological studies will help us anticipate these threats by providing information that is useful for predicting, preventing, and mitigating future risks.

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