THE EFFECT OF GENETIC AND ABIOTIC FACTORS ON THE GEOGRAPHIC VARIATION IN LIFE CYCLE PROCESSES OF *IXODES SCAPULARIS* IN THE EASTERN UNITED STATES

By

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ABSTRACT

THE EFFECT OF GENETIC AND ABIOTIC FACTORS ON THE GEOGRAPHIC VARIATION IN LIFE CYCLE PROCESSES OF *IXODES SCAPULARIS* IN THE EASTERN UNITED STATES

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The blacklegged tick, *Ixodes scapularis*, is the tick vector species responsible for transmitting the Lyme disease pathogen (i.e., *Borrelia burgdorferi*) in the eastern United States (US). Although this vector species is established throughout the southern US, geographical patterns of Lyme disease incidence indicate that cases tend to be localized in the northeastern and north central US. Several hypotheses have been proposed to explain this gradient in incidence, including regional differences in abiotic conditions, host diversity, tick genetics, and tick questing behavior. This dissertation explores how abiotic conditions and tick genetics may contribute to existing and future patterns of Lyme disease incidence, via their effects on *I. scapularis* life cycle processes. Using a four-year common garden experiment, I investigated the existing variation in life cycle processes (i.e., emergence and survival) among four widely-dispersed populations located within northern and southern areas of high and low Lyme disease incidence, respectively (Chapter 1). I then explored the potential mechanistic roles of abiotic and genetic explanatory factors underlying the observed among-population variation in emergence and survival. To address the observed among-site variation in emergence, I evaluated the accuracy of temperature-development models in predicting the emergence of ticks across populations, explored empirical evidence for the effects of genetics and plasticity on emergence, and identified potentially important explanatory factors contributing to the accuracy of temperature-development models in predicting emergence timing (Chapter 2). To address the observed

among-site variation in survival, I explored empirical evidence for differences in survival among local and transplanted ticks, ticks placed at northern and southern sites, and ticks from northern and southern sites of origin; I also identified potentially important explanatory factors contributing to the observed variation in survival (Chapter 3). In Chapter 1, I found that there was significant among-site variation in emergence and survival, and that these life cycles may be extended at northern sites, relative to southern sites. This chapter also provided the first documentation of bimodal emergence for this species. In Chapter 2, I discovered that temperature-development models significantly under-predicted emergence timing across all life stages and populations used in this study, showed evidence of genetics and plasticity affecting emergence among sites, and identified genetics, plasticity, and key abiotic conditions as potentially important explanatory factors influencing the accuracy of these temperature-development models. In Chapter 3, I demonstrated that southern conditions may be less conducive than northern conditions to tick survival, based on observed larval survival patterns; I also showed that ticks of northern origin may be more robust than ticks of southern origin, based on observed nymphal survival patterns. I supported these trends in survival with the identification of the interaction between genetics and plasticity, abiotic conditions, and diapause as important explanatory factors in best-fitting models of *I. scapularis* survival. The findings of this dissertation highlight the potentially important contributions that abiotic and genetic factors have on variation in emergence and survival among *I. scapularis* populations. Understanding how these factors affect the life cycle processes of this may have important implications for predicting disease risk, as populations of this vector species invade new areas and are exposed to new abiotic regimes via climate change.

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KEY TO ABBREVIATIONS

TAME	Tick adverse moisture event
RH	Relative humidity
AICc	Corrected Akaike's information criterion
US	United States
RI	Rhode Island
WI	Wisconsin
TN	Tennessee
FL	Florida

INTRODUCTION

Lyme disease is the most commonly reported vector-borne disease in the United States (US), causing greater than 30,000 confirmed cases annually (Centers for Disease Control and Prevention (CDC 2017). Ixodes scapularis is the primary tick vector species responsible for transmitting the Lyme disease pathogen (i.e., *Borrelia burgdorferi*) in the eastern US. However, although *I. scapularis*, is broadly distributed throughout this region, incidence maps indicate that cases tend to be localized in the northeastern and north central US (Figure 0.1). Several hypotheses may explain this discrepancy, including regional differences in abiotic conditions (Ogden et al. 2004), host diversity (Ostfeld and Keesing 2000), tick genetics (Zee et al. 2015), and tick questing behavior (Arsnoe et al. 2017). This dissertation explores how abiotic conditions and tick genetics may contribute to this pattern, via their effects on *I. scapularis* emergence timing and survival. A greater understanding of these relationships may improve our ability to predict future Lyme disease risk, as abiotic conditions are affected by climate change.

The Disease

In North America, human Lyme disease cases are characterized by three stages; stage is 1 a localized infection that causes an expanding skin lesion in 70-80% of cases, stage 2 is a disseminated infection that is associated with flu-like symptoms such as malaise, fever, headaches, arthralgias, and myalgias in the following days to weeks, and stage 3 is a persistent infection that may cause a variety of chronic symptoms including arthritis and neurological symptoms in the following months to years (Steere et al. 2004). This disease is considered to be an important emerging infection in the US, as it is increasing in incidence over time (CDC 2017) and space (Bacon et al. 2008, Kelly et al. 2014). Part of this emergence is due to widespread forest regrowth efforts and increasing deer populations that began in the 1960's, which have

allowed the vector tick, *I. scapularis*, to expand its range (Steere et al. 2004).

Control measures to reduce human Lyme disease risk are limited; there is currently no human vaccine on the market, the effects of deer culling are mixed, and the application of acaricides is controversial due to their potential effects on humans and other arthropod species (Clark and Hu 2008). Therefore, mitigating human Lyme disease risk requires personal protection methods such as the use of repellents, protective clothing, checking for ticks after time spent in potential tick habitats; it also requires proactive assessments of vector tick activity. The nymphal life stage of *I. scapularis* is of particular epidemiological importance, due to its small size and seasonal activity periods that overlap with human outdoor activity (Fish 1995). To assess human Lyme disease risk, the density of infected nymphs (DIN) serves as a reliable index (Pepin et al. 2012) that can be measured by drag sampling for questing ticks over a standardized field sampling area and testing these ticks for the *B. burgdorferi* pathogen using PCR methods. In areas where nymphal infection prevalence (NIP) estimates are available, or in circumstances where estimating DIN is not cost-effective, the density of nymphs (DON) also provides valuable information about tick-host encounter rates and potential Lyme disease risk. Therefore, the emergence of Lyme disease is a complex phenomenon that has involved pathogen, tick, and host responses to a changing environment. Predicting future changes in Lyme disease risk may prove challenging, as *I. scapularis* is broadly geographically distributed across a wide range of habitats that vary biotically and abiotically.

The Vector

Ixodes scapularis is a medically important vector of the etiological agents of Lyme disease, human anaplasmosis, babesiosis, and Powassan encephalitis. This species belongs to the family of ixodid (i.e., hard-bodied) ticks. This three-host tick is characterized by an egg, larval,

nymphal, and adult life stage, and each questing stage requires a single bloodmeal from a host in order to molt into the subsequent stage or, in the case of adult females, produce an egg clutch (Figure 0.2). *Ixodes scapularis* is a generalist parasite; the immature stages typically feed on a variety of small vertebrate hosts and the adult stage feeds on medium and large-sized mammals, especially White-tailed deer.

Ixodes scapularis emergence timing (i.e., the onset of host-seeking activity) is largely driven by temperature but may also be influenced by temperature-independent factors, including tick genetics and plasticity. Temperature has been shown to be capable of predicting development for *I. scapularis* populations in both northern (Ogden et al. 2004) and southern extremes within this species' range (Mount et al. 1997). However, tick genetics may also play a role in emergence timing. For example, a diapause (i.e., genetically-enabled dormancy) component was included to predict nymphal activity for populations in both Maryland and Ontario, Canada (Ogden et al. 2006). Experimental evidence supports the role of diapause on the delayed emergence timing of related species belonging to the *Ixodes ricinus* species complex (Belozerov et al. 2002, Gray et al. 2016). More broadly, population genetics studies show a pattern in which northern genotypes of *I. scapularis* are invading southwards (Zee et al. 2015). While the ecological traits associated with these lineages have not yet been described, this geographical pattern provides a basis to investigate the potential role of genetic differences on *I. scapularis* emergence. Additionally, phenotypic responses to varying abiotic regimes, such as a shift in emergence timing due to unseasonable temperatures, may play a role in emergence as well. There may also be complex interactions between genetics and plasticity for a given population, where the effect of abiotic conditions varies by the genetic background of the population.

Ixodes scapularis survival is largely driven by temperature and relative humidity but may also be influenced by tick genetics and plasticity. Abiotic conditions may pose several risks to ticks, which are prone to freezing, desiccating, overheating, drowning, and exhausting finite energy reserves while seeking a host (Eisen et al. 2016). *Ixodes scapularis* have a temperature range of approximately -10°C to 30°C (with some variation among life stages and ticks of different engorgement status), below and above which mortality increases due to damage to the integument (Needham and Teele 1991, Burks et al. 1996, Vandyk et al. 1996, Ogden et al. 2004, Eisen et al. 2016). Relative humidity is positively correlated with higher survival (Needham and Teele 1991, Stafford 1994, Vail and Smith 1998, Schulze et al. 2002). Field evidence also indicates that tick adverse moisture events (TAMEs) of >8 hours of exposure to <82% RH are negatively correlated with lower questing nymphal densities later in the season (Rodgers et al. 2007, Berger et al. 2014). However, periods of low relative humidity may be mitigated by the utilization of microhabitats with higher humidity, such as the duff layer (Schulze et al. 2002). With respect to the role of tick genetics, diapause may positively contribute to tick survival, serving as an adaptation that allows ticks to mitigate future predictable (i.e., seasonal) and unpredictable adverse environmental conditions (Belozerov 2009). With respect to among-population genetic patterns, past laboratory experiments found clear differences in the survival of ticks from different geographical regions, but no north-south gradient pattern in survival (Ginsberg et al. 2014). These findings suggest that survival patterns may be site-specific, perhaps due to local adaptation to site-specific environmental factors. Genetics may also influence survival via diapause, which may allow ticks to mitigate future adverse environmental conditions (Belozerov 2009), such as cold winter conditions (Yuval and Spielman 1990) or summer droughts conditions (Padgett and Lane 2001). Or, perhaps via local adaptation, complex

interactions between genetics and plasticity (i.e., non-genetic phenotypic responses to site-specific environmental factors) may contribute to site-specific relationships between survival and surrounding environmental conditions.

The Pathogen

The transmission and maintenance of *B. burgdorferi* depends on a complex cycle between different life stages of the vector tick and its hosts. Host species vary in host competence (i.e., their ability to maintain and transmit the pathogen to ticks). The most widely recognized competent reservoir host species of *B. burgdorferi* is *Peromyscus leucopus* (i.e., the white-footed mouse), which serves as the primary host of immature *I. scapularis* in the north central and northeastern US. In the southeastern US, fossorial lizards of much lower reservoir competence (Swanson and Norris 2007) such as *Plestiodon laticeps* (i.e., the broad-headed skink) and *Sceloporus undulatus* (i.e., the eastern fence lizard) serve as the primary hosts of immature *I. scapularis* (Apperson et al. 1993). *Odocoileus virginianus* (i.e., the white-tailed deer) is an incompetent host (Telford et al. 1988) but serves as an important reproductive host species for adult *I. scapularis*. Therefore, geographic variation in host diversity and species composition could contribute to among-site variation in pathogen transmission and maintenance, where a greater utilization of hosts of lower reservoir competence could prevent pathogen maintenance.

Beyond the role of hosts, B. *burgdorferi* maintenance also depends on the activity patterns of vector ticks. Transovarial transmission of *B. burgdorferi* to the *I. scapularis* larval stage is very rare (Randolph 1999, Gern and Rais 1996); therefore, in order for *B. burgdorferi* to be maintained, the nymphal stage must precede or overlap with the larval stage, whereby nymphs may infect larvae via shared reservoir hosts (Figure 0.3). Infected larvae molt into nymphs and can then infect other uninfected hosts, in what becomes a tag team dynamic between the

immature stages of *I. scapularis* and hosts to perpetuate the enzootic cycle of *B. burgdorferi*. Therefore, the activity periods of the larval and nymphal life stages of *I. scapularis* are critical determinants of Lyme disease risk. The activity periods of these immature stages relative to one another may also contribute to regional differences in strain diversity of *B. burgdorferi*, which vary in clinical presentations and severity (Wormser et al. 1999). Highly synchronous juvenile tick activity periods may promote a greater diversity of long-lived and short-lived *B. burgdorferi* strain types, whereas a longer temporal gap between nymphal and larval activity may confer higher fitness to long-lived, more virulent strain types (Gatewood et al. 2009). Thus, characterizing the life cycle processes of immature *I. scapularis* and identifying the underlying factors governing these periods are essential steps to understanding current and future patterns of Lyme disease risk

In the northeastern US, where the incidence of Lyme disease is the highest, the life cycle of *I. scapularis* is best characterized, based on a combination of laboratory experiments (Yuval and Spielman 1990, Mount et al. 1997, Troughton and Levin 2007) and outdoor enclosure experiments (Daniels et al. 1996, Lindsay et al. 1995, Ogden et al. 2004) that corroborate field questing tick data (Gatewood et al. 2009). In the northeast, the nymphal activity period is from mid-May to early September, peaking in early June; the larval activity period is from early June to early September, peaking in early august; the adult activity period is from mid-September to mid-November, and then resumes from early April to mid-August, peaking in mid-November and mid-April (Yuval and Spielman 1990, Gatewood et al. 2009, Stromdahl et al. 2014). In the north central US, the seasonal activity patterns (i.e., phenologies) of questing life stages (Gatewood et al. 2009, Hamer et al. 2012, Ogden et al. 2018) have provided a basis for making inferences about the *I. scapularis* life cycle. In the north central US, the nymphal activity period

is from mid-May to late September, peaking in early June; the larval activity period is from mid-May to late September, peaking in early July; the adult activity period is from mid-September to mid-November, and then resumes from early April to mid-July, peaking from mid-May to mid-June (Gatewood et al. 2009, Stromdahl et al. 2014). The data on life cycle and phenologies are the sparsest for the southern US, with limited studies on the seasonal activity patterns of immature stages (Ogden et al. 2018), separate studies conducted in different states providing insights on adult phenologies using drag sampling methods (Goddard 1992, Cilek and Olson 2000), and larval and nymphal phenologies (Clark 1998, Kollars et al. 1999) using wildlife capture methods. These phenologies appear to vary among sites as well. For example, in Tennessee (TN), the larval activity period is from early-April to mid-October, peaking in mid-July; the nymphal activity period is from mid-March to mid-June, peaking from early April to early May (Ogden et al. 2018). In contrast, in Florida (FL) the larval activity period is from mid-March to mid-August, peaking from mid-April to late July; the nymphal activity period is from mid-February to mid-September, peaking in mid-June (Ogden et al. 2018). Given the relationship between tick activity patterns and *B. burgdorferi* maintenance, this geographical variation in the activity patterns of *I. scapularis* may have important implications for the existing patterns of disease incidence.

Therefore, the roles of genetic and abiotic factors on the life cycle processes of *I. scapularis*, have important implications for Lyme disease risk, via potential impacts on pathogen transmission and maintenance. A greater understanding of how life cycle processes vary geographically and are shaped by various abiotic and genetic factors is of critical importance to predicting how Lyme disease risk may be affected by climate change. The purpose of this study is to explore how the life cycle processes of this species varies at four sites, widely dispersed

throughout the geographical range of this species (Chapter 1), and to provide insights into the underlying factors contributing to this variation, via their effects on *I. scapularis* emergence (Chapter 2) timing and survival (Chapter 3). We address these topics in greater detail below:

Chapter 1

The objective of this chapter is to explore how critical life cycle processes (i.e., emergence and survival) of this species varies throughout its broad geographical range. We address this topic with the following questions; 1) What is the among-site variation in emergence timing and survival? 2) What is the relationship between temperature and emergence at these sites?

We conducted a microcosm experiment that monitored the emergence and survival of larval, nymphal, and adult life stages of *I. scapularis* at sites in Rhode Island (RI), Wisconsin (WI), Tennessee (TN) and Florida (FL). We also collected temperature data from these sites to investigate the potential relationship between temperature and emergence timing among sites.

Chapter 2

The objective of this chapter is to provide insights into the underlying factors contributing to among-site variation in *I. scapularis* emergence. We address this topic with the following questions; 1) Can existing temperature-development models accurately predict observed emergence patterns of different populations of *I. scapularis*? 2) Is there empirical evidence for an effect of genetics and plasticity on emergence timing? 3) What are the relative roles of genetics and plasticity on the accuracy of temperature-development models in their ability to predict emergence timing?

Using the same microcosm experiment, we monitored the emergence of local and transplanted larval, nymphal, and adult *I. scapularis*. Using temperature data collected from field sites and previously established temperature-development models for this species, we generated

a range of predicted emergence dates for each placement of ticks and compared these estimates with observed emergence dates. We compared subsets of placements that a) shared the same site of origin but were transplanted to different sites or b) were from different sites of origin but were placed at the same placement site, to investigate the potential roles of plasticity and genetics on emergence. Lastly, we identified potentially important factors contributing to the accuracy of these temperature-development models by creating regression models and applying the corrected Akaike's information criterion (AIC) to evaluate these models.

Chapter 3

The objective of this chapter is to provide insights into the underlying factors contributing to among-site variation in *I. scapularis* survival. We address this topic with the following questions; 1) What are the potentially important factors contributing to the survival of different life stages of *I. scapularis* ticks? 2) Do local (i.e., established) ticks have a higher survival than transplanted (e.g. invading) ticks? 3) Do ticks under northern conditions have a higher survival than ticks under southern conditions? 4) Do ticks from northern sites of origin have a higher survival than ticks from southern sites of origin?

Using the same microcosm experiment, we monitored the survival of local and transplanted larval, nymphal, and adult *I. scapularis*. We identified potentially important abiotic and genetic factors contributing to *I. scapularis* survival by creating regression models and applying the corrected Akaike's information criterion (AICc) to evaluate these models. We also compared subsets of placements that a) shared the same site of origin but were transplanted to different sites or b) were from different sites of origin but were placed at the same placement site, to investigate survival patterns among ticks that were local versus transplanted, placed at northern versus southern sites, and from northern versus southern sites of origin.

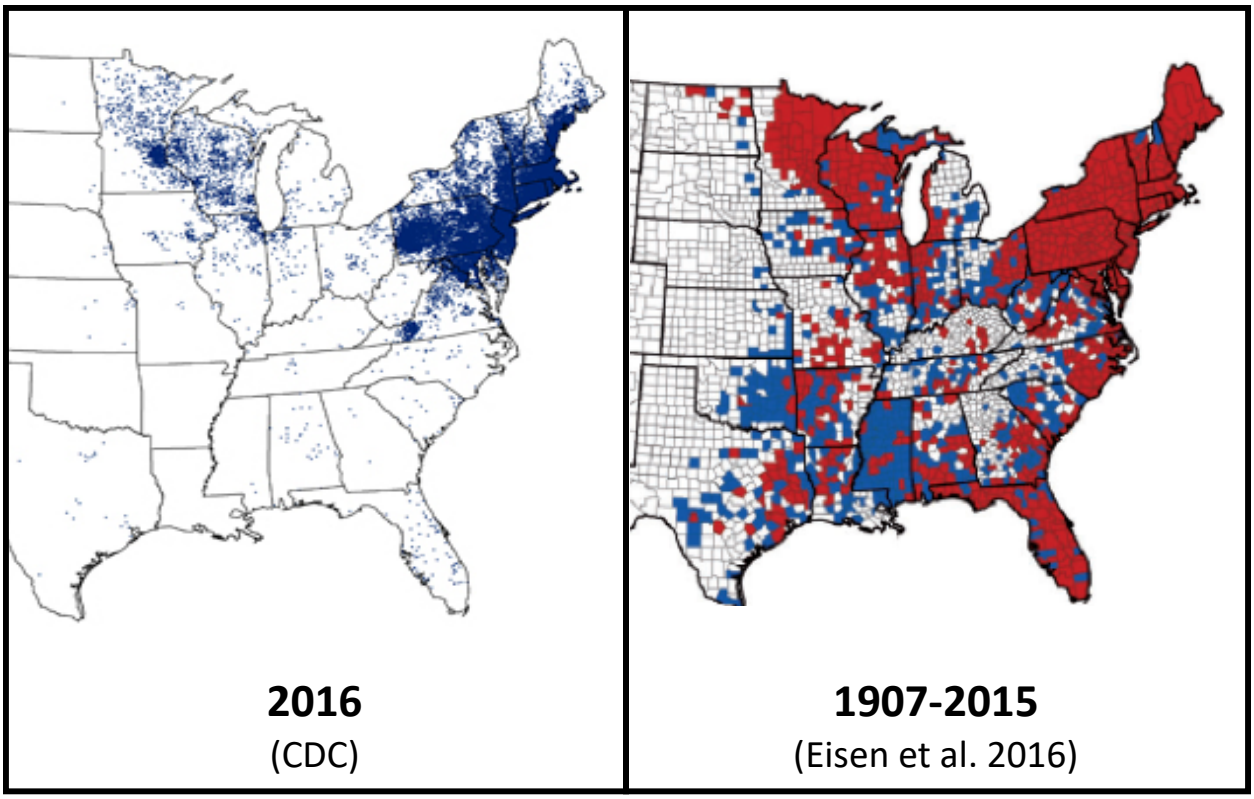

Figure 0.1. Reported cases of Lyme disease in the US in 2016 (left), where each blue dot represents one reported case, and the geographical distribution of the Lyme disease vector *Ixodes scapularis* (right), where red counties show established populations (>6 ticks or 2 life stages reported in a single year) and blue counties show reported populations (<6 ticks or 1 life stage reported in a single year).

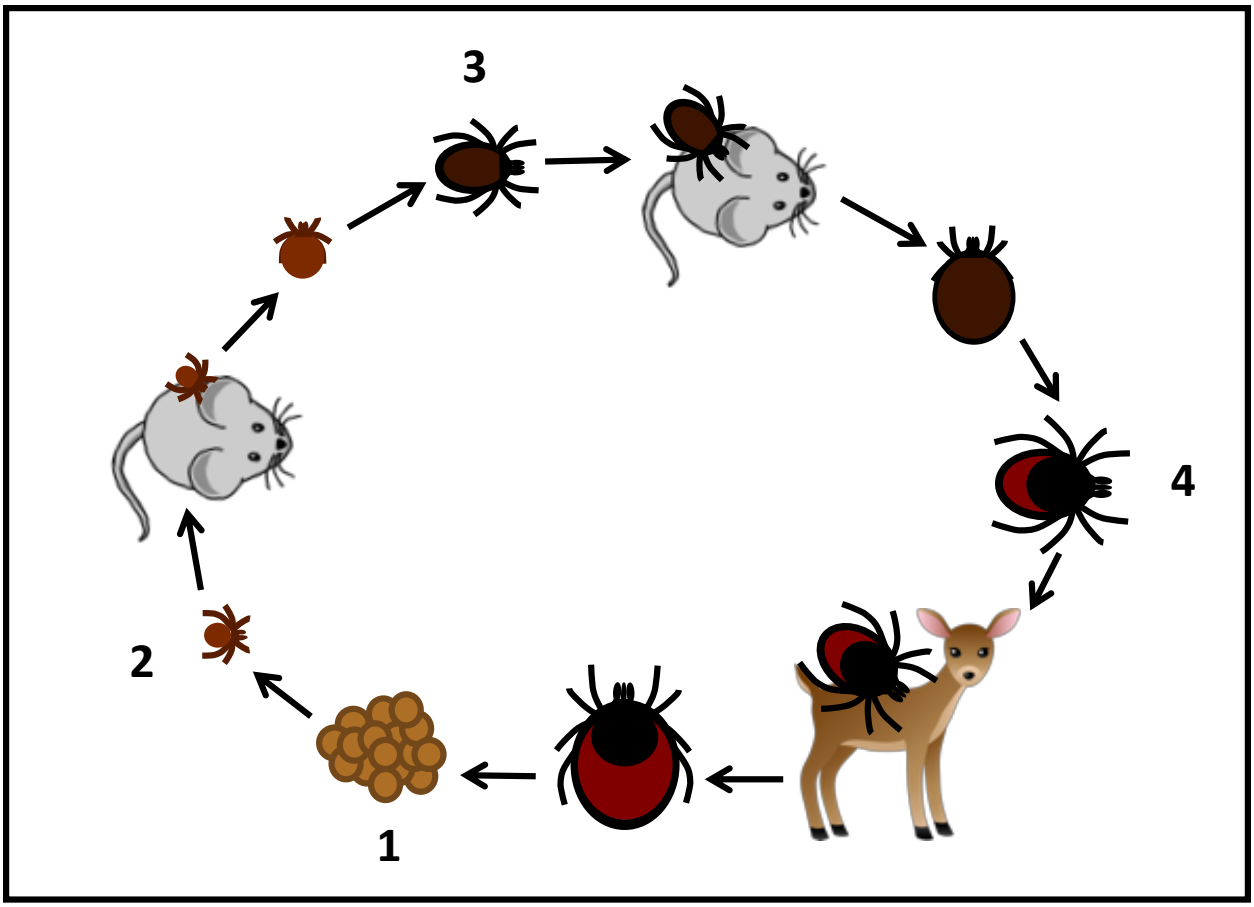

Figure 0.2. The *Ixodes scapularis* life cycle is characterized by four main stages: eggs (1), larvae (2), nymphs (3), and adults (4). Each questing stage requires a single bloodmeal from a host to molt into the next stage or, in the case of adult females, lay an egg clutch.

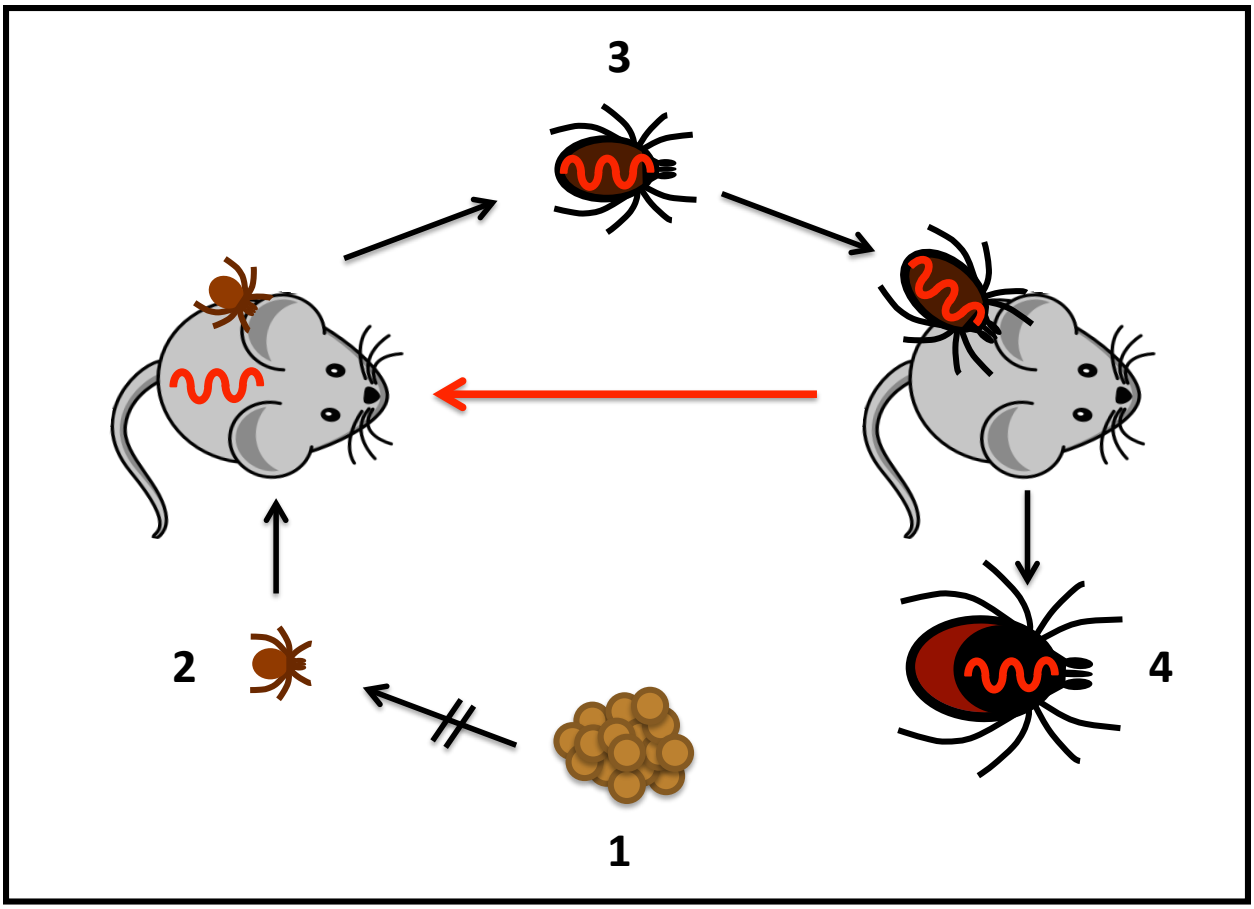

Figure 0.3. Maintenance of the pathogen *Borrelia burgdorferi* via transmission between immature stages of the tick vector *Ixodes scapularis* and reservoir hosts. The *I. scapularis* life cycle is characterized by four main stages: eggs (1), larvae (2), nymphs (3), and adults (4). Lack of transovarial transmission is indicated by the double-hash black lines. The pathogen is indicated by red wavy lines. The red arrow indicates hosts becoming infected and adding to the population of infected hosts that then become available to infect more ticks.

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CHAPTER 1: VARIATION IN ACTIVITY AND SURVIVORSHIP AMONG *IXODES SCAPULARIS* POPULATIONS IN RHODE ISLAND, WISCONSIN, TENNESSEE, AND FLORIDA.

Abstract

An understanding of seasonal activity patterns of *Ixodes scapularis* ticks is fundamental to predicting the dynamics of *I. scapularis* populations and pathogen transmission. However, many questions still exist about the cues and processes driving variation in life-cycle timing among *I. scapularis* populations. We conducted a multi-year microcosm experiment at field sites in Wisconsin, Rhode Island, Tennessee, and Florida, which are located in the four quadrants of this species' range. *Ixodes scapularis* larvae, nymphs, and adults were fed at different times during their active seasons, placed back into the field at their sites of origin, and observed until their activity ceased. Following engorgement, for all life stages there were significant differences among sites in emergence times, with overwintering of ticks at northern sites potentially contributing to longer times to emergence. At our WI site, we observed a bimodal emergence pattern for all life stages, in which a subset of a single placement of engorged ticks emerged before winter and the remainder of the placement emerged the following spring or summer. There was a significant negative relationship between mean placement temperature and time to emergence for all life stages at all sites, suggesting that temperature may play a role in emergence timing. As temperatures rise due to climate change, our ability to predict how *I. scapularis* populations will respond to new abiotic conditions is of critical importance to predicting and mitigating future Lyme disease risk. The results of this study improve our understanding of the existing variation in the life cycle of this species and begin to elucidate the roles of temperature and temperature-independent factors on *I. scapularis* emergence and

survivorship.

Introduction

Several diseases, including Lyme disease, are caused by the bites of infected ticks in the *Ixodes ricinus* complex, including *I. ricinus* (Europe and Asia), *I. persulcatus* (Asia), *I. pacificus* (western North America), and *I. scapularis* (eastern North America). These species are three-host, non-nidicolous ticks, whose populations often comprise multiple overlapping generations, required for the enzootic maintenance of non-transovarially and non-systemically transmitted pathogens (Randolph 1999, Gern and Rais 1996). The seasonal activity patterns (i.e., phenologies) of the immature life stages (Figure 1.1) are important for the enzootic maintenance of the Lyme disease pathogen, *Borrelia burgdorferi* (Mount et al. 1997, Kurtenbach et al. 2006), which requires the nymphs to precede or overlap with the larvae in order to transmit the pathogen from infected nymphs to uninfected larvae via shared reservoir hosts (Figure 1.1). Additionally, differences in the phenological patterns of these immature stages may result in regional differences in strain diversity of *B. burgdorferi* (Gatewood et al. 2009), which vary in clinical presentations and severity (Wormser et al. 1999). For example, a high degree of overlapping activity of immature stages may promote the maintenance of *B. burgdorferi* strains with shorter infectious periods, while more extended periods between nymphal and larval activity periods may select for the persistence of longer-lived strains (Gatewood et al. 2009). Therefore, the seasonal activity patterns of the immature stages, which are governed by each population's life cycle processes (i.e., the emergence timing [Table 1.1] and subsequent activity of all life stages to complete a full life cycle), are critical determinants of enzootic maintenance and Lyme disease risk.

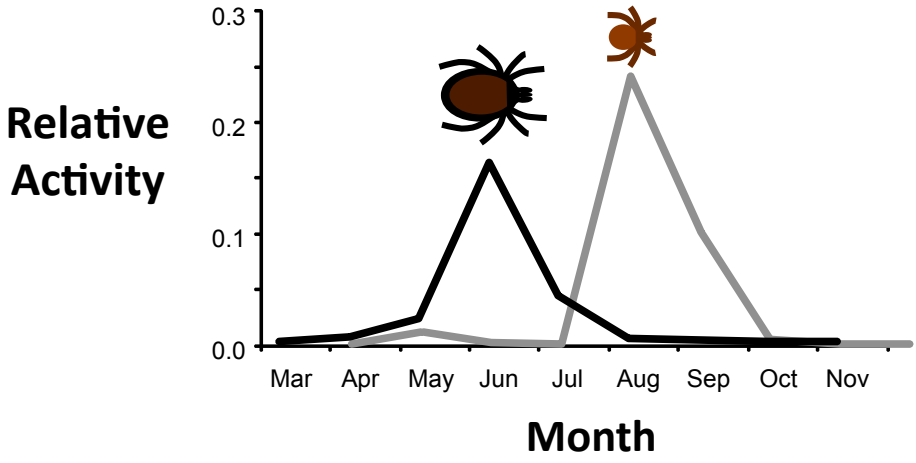

Figure 1.1. An illustration of the phenologies, or relative activities, of questing larval (gray) and nymphal (black) stages of *Ixodes scapularis*. This pattern represents what is typically seen in the Lyme disease-endemic, northeastern United States, where nymphal activity precedes the majority of larval activity. The questing patterns of these immature stages affects pathogen transmission and maintenance, as infected nymphs must feed upon shared hosts prior to when uninfected larval ticks feed, in order for the pathogen-naive larvae to acquire the pathogen.

Although several researchers have studied the life cycle of these ticks (Yuval and Spielman 1990, Mount et al. 1997, Ogden et al. 2004), many questions remain about the geographic variation and causes thereof within this species. The life cycle for northeastern (Supplemental Figure 1.1) *I. scapularis* populations arguably has been studied the most and is characterized by combining phenological data of questing (Table 1.1) and on-host life stages with data from outdoor enclosures monitoring both the onset and duration of development (e.g., Spielman et al. 1985, Yuval and Spielman 1990, Daniels et al. 1996, Lindsay et al. 1995, Ogden et al. 2004). In the central northern US (Supplemental Figure 1.1), phenologies of questing life stages from previous studies have provided a basis for inferring the life cycle processes in the Midwest (Gatewood et al. 2009, Hamer et al. 2012). The data regarding *I. scapularis* life cycle and phenologies are the sparsest for the southern US (Supplemental Figure 1.1), where Lyme disease is rare. Most phenologies from southern states of questing *I. scapularis* ticks focus on adults (Goddard 1992, Cilek and Olson 2000). The limited phenologies for southern larvae and nymphs

that are available have been based on wildlife captures, which require much more effort (Clark 1998, Kollars et al. 1999).

The large variation in life cycle duration and seasonal activity of this species within its range may be due in part to regional differences in climatic conditions (Eisen et al. 2016) and local adaptation affecting how tick populations respond to varying abiotic conditions (Ginsberg et al. 2014, Gray et al. 2016). Temperature and photoperiod affect the development timing of replete stages of *I. ricinus*, and the resulting emergence timing of their subsequent questing stages (Belozerov 2002). Tick survivorship is strongly determined by temperature and relative humidity (Knulle and Rudolph 1982, Stafford 1994, Berger et al. 2014) and, for host-seeking stages, host availability as well (Mount et al. 1997, Ogden et al. 2006). Varying life cycle patterns may impact regional patterns of disease risk (i.e., the probability of encountering an infected questing tick) by affecting vector abundances beyond direct effects on transmission dynamics and survivorship of pathogen-transmitting life stages. For example, and extended life cycle may also reduce reproductive fitness (Ogden et al. 2006, Gray et al. 2016) that, by extension, could contribute to reduced vector abundances (Pepin et al. 2012).

As invading populations of *I. scapularis* expand into non-Lyme endemic regions (e.g., Kelly et al. 2014), and established populations are exposed to the influences of climate change, life cycle processes may vary in ways that can only be predicted by a better understanding of existing relationships between populations and the abiotic conditions to which they are exposed. These life cycle processes, and their resulting effects on phenologies, may have important implications for the enzootic maintenance of the Lyme disease pathogen. Life cycles for *I. scapularis* mainly have been characterized by focusing on activity of the detectable questing stages, with the assumption that when ticks are not detectable by drag cloth sampling or wildlife

capture, it is because they are undergoing development, in developmental or behavioral diapause, or quiescent (Table 1.1). To our knowledge, there have been no experimental studies to characterize the developmental duration and emergence timing of questing stages for populations in either the north central or the southern US. This study provides a unique collection of standardized, longitudinal emergence and contemporaneous abiotic data for populations located in the four main quadrants of this species' range in the US and allows us to explore existing variation in the life cycle of this species.

Methods

Table 1.1. Definitions. The following terms are used to distinguish between commonly terms used to describe tick development and activity (i.e., quiescence, behavioral diapause, and developmental diapause, questing, synchronous emergence, and asynchronous emergence), and terms used that take into consideration the specific design and observations of this study (i.e., active, emergence, bimodal emergence, and unimodal emergence).

Term	Definition
Quiescence	Temporary inactivity or arrested development due to adverse abiotic conditions. Removal of these conditions results in resumed activity or development. Typical abiotic cues: temperature and relative humdiity.
Behavioral Diapause	Delayed *activity* with a genetic basis, usually to triggered by conditions that predict the onset of unfavorable conditions. Typical abiotic cue: photoperiod.
Developmental Diapause	Delayed *development* with a genetic basis, usually to triggered by conditions that predict the onset of unfavorable conditions. Typical abiotic cue: photoperiod.
Active	Observed inside pottle above the leaf litter.
Questing	Actively seeking a host.
Emerging	Observed for the first time as a molted, unfed stage.
Bimodal Emergence	Two distinct pre- and post-winter emergence periods oberved for a single placement of ticks. This type of emergence was only observed at our Wisconsin site.
Unimodal Emergence	One emergence period observed for a single placement of ticks.
Synchronous Emergence	One emergence period observed for two or more placements of ticks.
Asynchronous Emergence	Different emergence periods observed for different placements of ticks.

Summary

The timing of *I. scapularis* development and activity was studied by placing replete ticks in field enclosures (pottles) at various times of the season and observing the timing of emergence and the

duration of survivorship at four sites in the eastern US. Placements (i.e., groups of pottles that represent the same site, life stage, and feeding date) of replete ticks were observed over a period of 2-4 years, depending on the rate at which observed ticks expired (i.e., were no longer observed for at least one year). At least two placements of replete female ticks and three placements of replete larval and nymphal ticks were deployed annually, unless limited by low sample sizes and/or poor survivorship of replete ticks, in order to capture the range of the active season for each life stage at every site. The total number of pottles (i.e., modified specimen containers) per placement of replete ticks ranged from 10-50 for larvae, 3-20 for nymphs, and 10-30 for females. Only pottles that produced molted ticks are included in our analyses (Table 1.2); a small number of pottles that did not produce molted ticks were excluded. For this reason, the number of pottles per placement varied.

Field sites

We observed the emergence timing and activity periods of unfed stages of larval, nymphal, and adult *I. scapularis* at four sites (Supplemental Figure 1.1) in the eastern US – Rhode Island (RI), Wisconsin (WI), Tennessee (TN) and Florida (FL). Field sites were a subset of project sites belonging to a larger study designed to compare the ecology of Lyme disease in different geographic regions of the tick's distribution. All sites supported populations of *I. scapularis*, as evidenced by the ability to collect questing adult blacklegged ticks as well as immature ticks on vertebrate hosts (Tsao unpublished data). Project sites were located in two Lyme endemic regions: Wisconsin (WI) and Rhode Island (RI), and two non-endemic regions: Tennessee (TN) and Florida (FL). Wisconsin is located in northern central US; Rhode Island is in the northeastern US; Tennessee is in the inland southeastern US; Florida is in the southeastern US. Forest at the Rhode Island site (41.5 °N) was dominated by oaks (*Quercus spp*.) and maples

(*Acer spp*.) with an understory containing both shrubby and open spaces. Forest at the Wisconsin site (42.94 °N) was dominated by oaks (*Quercus spp*.) and maples (*Acer spp*.) with a shrubby understory containing mixed saplings and some invasive understory species. Forest at the Tennessee site (36.01°N) 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. Forest at the Florida site near Tall Timbers Research Station, FL (latitude 30.65 °N) was dominated oaks (*Quercus spp*.) and maples (*Acer spp*.) with a dense, shrubby understory containing mixed saplings and invasive understory species.

Microcosm design (Figure 1.2)

Live tick specimens were housed in 60 ml polypropylene specimen containers (i.e., pottles) that were 6.1 cm in diameter x 7.25 cm tall. The lids and bases of these containers were cut using a Dremel 2500, creating a column through which precipitation could pass. An 11 cm x 11 cm piece of synthetic cloth mesh was secured to the base of each pottle using caulk and a cable tie to hold the organdy cloth mesh in place during drying; once dry, the edge of the organdy cloth mesh was further secured by a 5.08 cm wide piece of tape that encircled the bottom circumference of the pottle. A soil core approximately 3.5 cm deep, containing layers of leaf litter, duff and soil, was collected from the surrounding area of each enclosure location and placed into each pottle. Another 11 cm x 11 cm piece of organdy cloth mesh was secured over the top of each container using the screw top lid to hold the organdy cloth mesh in place. An oak dowel that was .25 cm in diameter x 5.08 cm tall was inserted into the center of each soil core to provide ticks with an object to climb and quest from. Pottles were covered with protective plastic crates (44 cm x 36 cm x 27 cm). Pottles belonging to the same placement were housed in the same crate. Crates were arranged haphazardly within the same forest stand at each site.

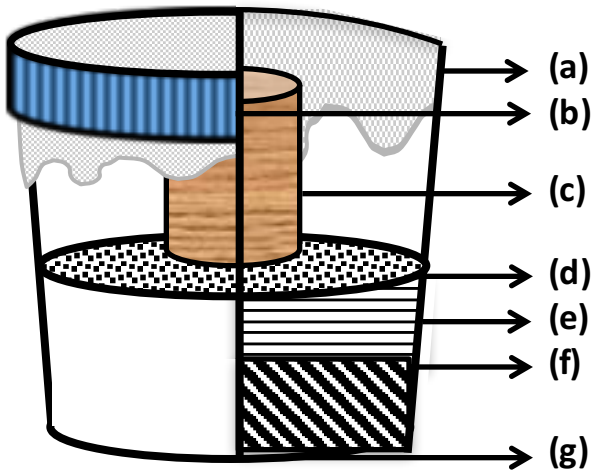

Figure 1.2. A diagram of a pottle, external (left) and internal (right). Components included synthetic cloth mesh (a, g), a pottle lid modified to allow airflow and precipitation to enter each pottle (b), an oak dowel for ticks to ascend (c), and a layer of leaf litter (d) on top of the duff (e) and soil (f).

Figure 1.3. Pottles were haphazardly distributed among crates at each site.

Production of replete ticks to be placed into the field

Live *I. scapularis* adults were collected during peak questing periods at each field site. Within two weeks post-collection, adult ticks were mated and fed on New Zealand White rabbits (Oryctolagus cuniculus) in the lab at Michigan State University following procedures approved through the MSU Institutional Animal Use and Care Committee permit #06/12-103-00. Replete

female *I. scapularis* were returned to each site of origin, placed in modified pottles that exposed them to ambient conditions (Figure 1.3), housed in secure crates in the field, and monitored for oviposition, larval hatch, and larval survivorship. To produce engorged larvae, a subset of pottles representing offspring of different females were subsampled destructively at different time points throughout the larval seasonal activity period. Larvae were fed to repletion on laboratory mice (Mus musculus), divided among pottles, and then returned to the respective field site to be monitored for nymphal molt and nymphal survivorship. Similarly, a subset of pottles with molted, unfed nymphs were then used for producing engorged nymphs (also fed on laboratory mice). Immature stages of ticks were collected from pottles (versus via drag sampling from the field) to standardize the collection method of immature ticks among study sites, where lower densities of questing *I. scapularis* in TN and FL would have not provided enough ticks to conduct this experiment. For each placement (i.e., group of pottles sharing the same site of origin, feeding date, and placement site) of replete larvae or nymphs, pottles were haphazardly selected and destructively sampled until approximately 400 larvae (typically requiring 2-3 pottles) or 120 nymphs (typically requiring 10-15 pottles) were collected, to account for tick mortality during transport and feedings. Laboratory feedings of all life stages at each site were scheduled to coincide with observed questing and on-host phenologies in the field; feeding dates typically ranged from fall through spring for adults, and spring through summer for larvae and nymphs. The number of pottles collected, ticks fed, and laboratory hosts used varied by placement date, depending on the mortality of ticks in pottles throughout the season. Replete ticks were housed at 95% RH at 25° C under a 12:12 light dark cycle and returned to their site of origin as soon as possible, typically within 10 days of detachment. Replete WI adult ticks fed in the late fall and winter were housed in an outdoor chamber in Michigan, where they were

exposed to comparable ambient conditions to those in WI until they could be returned to WI prior to the spring season.

Placement dates

We fed and placed ticks in the field during ecologically relevant times during their seasonal activity periods. To do so, the relative activity patterns of ticks, based on host burdens and dragging data (Tsao, unpublished) of different life stages were referenced to determine pre-peak, peak, and post-peak placements, such that samples of replete *I. scapularis* were fed and returned to the field during plausible, natural periods of host seeking and feeding for that region.

Data collection and response variables

This study was conducted from May 2012 to September 2015. Annually, at each project site, 2-3 placements of replete ticks for each life stage were placed in the field and monitored. Each pottle received fully replete ticks of one life stage: 20 larvae, 10 nymphs, or 1 adult female. Additional pottles containing only soil cores and no added ticks were observed at each site to confirm that soil cores were not contaminated with ticks already present in the leaf litter.

Visible ticks were noted in pottles the day that they were deployed and then approximately every 2 weeks thereafter. Pottles were checked by searching for ticks on all visible surfaces of the pottle, including the organdy cloth mesh top, the clear sides of the tube, the dowel, and the top of the leaf litter. During spring, summer, and early fall, when ticks emerge and exhibit host-seeking activity at all sites, pottles were monitored every 2-3 weeks. During the late fall and winter, only enclosures in southern sites were monitored every 4 weeks. The number of unfed larval ticks in each pottle was estimated with raw counts if 50 or fewer ticks were observed. Otherwise, a categorical estimate > 50 or > 200 was assigned to that pottle. Pottles were monitored until greater than one year of no activity was observed.

For each pottle, the date in which the subsequent life stage was first observed (e.g., the first date a molted nymph in a particular pottle "emerged" after the engorged larvae were placed into the field) was recorded as the first date of observed emergence. The median date of emergence was estimated from the pool of emergence dates for a given placement, comprised of a single placement and life stage of pottles that were fed at the same time and exposed to those same laboratory and field conditions. Some placements in WI resulted in bimodal emergence (Table 1.1). For placements in WI that exhibited bimodal emergence, we calculated two separate median emergence dates (i.e., pre- and post- winter).

We measured the temperature outside of the crates at each site using HOBO Pro v2 (#U23-001) data loggers programmed to record every 30 min. We also placed two iButton Hygrochron (#DS1923) data loggers programmed to record every 30 min; the first logger was placed inside of a pottle containing no added ticks, and the second logger was placed within the same crate but outside of the pottle. We compared temperature and relative humidity readings among these three data loggers to confirm that within-pottle abiotic conditions were not substantially different from ambient abiotic conditions.

Statistics

Analysis of variance (ANOVA) tests were used to determine whether the observed median emergence timing and periods of detection for each tick life stage varied among our four sites. Tukey's honest significant difference (HSD) post-hoc tests were used to test for differences between groups. Statistics were performed using Program R (R Development Core Team, 2014). The explanatory variable was site, and the outcome variables included the days to emergence and detection periods, post-emergence, of all placements. The days to emergence for each placement were determined from the median emergence date (i.e., the median value of the emergence dates

of all pottles within a placement). The detection period for each placement was determined from the median date of final detection (i.e., the median value of the final detection dates of all pottles within a placement). Given that bimodal emergence was observed at the WI site, within each life stage, ticks that emerged pre-winter were grouped separately from ticks that emerged post-winter.

Linear regression was used to model the time to emergence of larvae, nymphs, and adults using the mean placement temperature as a predictor. Statistics were performed using Program R (R Development Core Team, 2014). The explanatory variable was mean placement temperature, and the outcome variable was the days to emergence. The days to emergence for each placement were determined from the median emergence date (i.e., the median value of the emergence dates of all pottles within a placement). The mean placement temperature was calculated from the mean of HOBO hourly temperatures, from the feeding date until the emergence date of each placement. Given that bimodal emergence was observed at the WI site, within each life stage, ticks that emerged pre-winter were grouped separately from ticks that emerged post-winter.

Results

Among-site variation in emergence timing

Larvae

Larval emergence was earliest in FL, occurring in early and mid-spring (Table 1.2). At the other sites, emergence occurred in late spring (RI, WI, TN), early summer (RI, TN), and late summer (WI). Emergence occurred in as few as 52 days (FL) and as many as 465 days (WI) (Table 1.2). There was significant variation in the time to larval emergence among sites (Figure 1.4a; $F = 29.26$; $df = 4$; $P < 0.001$). A post hoc Tukey test showed significantly ($P < 0.05$) longer times to emergence in WI (post-winter emergence) versus RI, WI (post-winter emergence) versus WI (pre-winter

emergence), WI (post-winter emergence) versus TN, WI (post-winter emergence) versus FL, and WI (pre-winter emergence) versus FL (Figure 1.4a).

Nymphs

Nymphal emergence was earliest in FL (in early spring), and then occurred in mid-spring (WI), late spring (RI), and mid-summer (TN) (Table 1.2). Emergence occurred in as few as 34 days (FL) and as many as 571 days (WI) (Table 1.2). Two instances of bimodal emergence, in which two nymphal emergence periods were observed for one single placement of replete larvae, were only observed in WI, where the emergences spanned 8 months, and occurred nearly 19 months post-placement in the second emergence. There was significant variation in time to nymphal emergence among sites (Figure 1.4b; $F = 30.67$; $df = 4$; $P < 0.001$). A post hoc Tukey test showed significantly ($P < 0.05$) longer times to emergence in RI versus WI (pre-winter emergence), RI versus TN, RI versus FL, WI (post-winter emergence) versus WI (pre-winter emergence), WI (post-winter emergence) versus TN, and WI (post-winter emergence) versus FL (Figure 1.4b).

Adults

Adult emergence was earliest in WI, occurring in early spring, and then occurred in mid-summer (RI), late summer (FL), and early fall (TN) (Table 1.2). Emergence occurred in as few as 54 days (WI) and as many as 342 days (WI) (Table 1.2). Two instances of bimodal emergence, in which two adult emergence periods were observed for one single placement of replete nymphs, were observed in WI. There was significant variation in the time to adult emergence among sites (Figure 1.4c; $F = 147.60$; $df = 4$; $P < 0.001$). A post hoc Tukey test showed significantly ($P < 0.05$) longer times to emergence in WI (post-winter emergence) versus RI, WI (post-winter emergence) versus WI (pre-winter emergence), WI (post-winter emergence) versus TN, WI (post-winter emergence) versus FL, TN versus RI, TN versus WI (pre-winter emergence), FL versus RI, and FL versus WI

(pre-winter emergence) (Figure 1.4c).

Among-site variation in detection period

Larvae (Figure 1.4d)

There was significant variation in the maximum detection period of larvae among sites (df = 4; F = 136.9; P < 0.001; Figure 1.4d). A post hoc Tukey test showed significantly (P < 0.05) longer detection periods of larvae in RI versus WI (pre-winter emergence), RI versus WI (post-winter emergence), RI versus TN, RI versus FL, WI (pre-winter emergence) versus WI (post-winter emergence), TN versus WI (post-winter emergence), and FL versus WI (post-winter emergence) (Figure 1.4).

Nymphs (Figure 1.4e)

There was significant variation in the maximum detection period of nymphs among sites (df = 4; F = 12.12; P < 0.001; Figure 1.4e). A post hoc Tukey test showed significantly (P < 0.05) longer detection periods of nymphs in RI versus TN, RI versus WI (post-winter emergence), FL versus WI (post-winter emergence), and FL versus TN (Figure 1.4).

Adults (Figure 1.4f)

There was significant variation in the maximum detection period of adults among sites (df = 4; F = 18.71; P < 0.001; Figure 1.4e). A post hoc Tukey test showed significantly (P < 0.05) longer detection periods of adults in RI versus WI (pre-winter emergence), RI versus WI (post-winter emergence), RI versus TN, FL versus WI (post-winter emergence), and FL versus TN (Figure 1.4)

Bimodal Emergence (Table 1.2)

Bimodal emergence patterns of replete ticks were observed only at the WI site. For example, the majority of replete females fed in mid-winter (8 of 10 pottles) produced larvae that first emerged in late spring, and the remainder (2 of 10 pottles) produced larvae that first emerged more than a

year later in late spring. For one of the summer placements, nearly half of the pottles of replete larvae (5 of 11 pottles) emerged as nymphs in late summer, and the remainder (6 of 11 pottles) emerged as nymphs the following mid-spring. A portion of the pottles of replete larvae (2 of 8 pottles) that fed in the fall emerged as nymphs in late summer of the following year, and the remainder (6 of 8 pottles) emerged as nymphs the following mid-spring (1.5 years later). Two summer placements of replete nymphs also exhibited bimodal emergence as emerging adults. Adults from the first placement emerged in the late summer (2 of 3 pottles) and the following late spring (1 of 3 pottles). Adults from the second placement (14 days later) emerged in the late summer (2 of 7 pottles) and the following early spring (5 of 7 pottles).

Temperature and Emergence (Figure 1.6)

We observed a significant negative relationship between mean exposure temperatures and the time from feeding to the emergence of larvae [$F(1,116) = 90.78$, $P < 0.001$, $R2 = 0.43$] (Figure 1.5a), nymphs [$F(1,154) = 401.70$, $P < 0.001$, $R2 = 0.72$] (Figure 1.5b), and adults [$F(1,29) = 23.99$, $P < 0.001$, $R2 = 0.43$] (Figure 1.5c).

Table 1.2. Emergence. The median dates for which placements of replete *I. scapularis* were first observed as unfed ticks of the next stage at four sites. Only placements that successfully produced ticks are shown. Placements are arranged by calendar date (not year) of completion (i.e., last day) of feeding, which occurred within two weeks of placement at each site. Each pottle within a placement represents one sample unit (n represents the number of pottles), that contained either one replete female, 20 replete larvae, or 10 replete nymphs. Asterisks (*) represent pottles with the same placement date but bimodal emergence dates.

Site	Transition	n	Feeding			Emergence		SEM
			Month/Day	Year		Median Date	Duration (Days Post Feeding)	
RI	Female -> Larva	4	5/9	2013		7/18/13	70.0	0.00
		4	11/30	2012		5/21/13	172.0	0.00
	Larva -> Nymph	9	5/10	2013		7/31/13	82.0	0.00
		9	5/18	2013		7/31/13	74.0	0.00
		5	7/15	2013		9/11/13	58.0	0.00
		5	8/23	2013		6/9/14	277.0	0.00
		10	9/26	2012		7/17/13	294.0	6.55
	Nymph -> Adult	9	5/18	2013		7/31/13	74.0	0.00
		1	6/7	2013		9/11/13	96.0	-
WI	Female -> Larva	8	2/9	2012	*	5/23/12	104.0	3.09
		2	2/9	2012	*	6/15/13	492.0	-
		9	11/18	2013		8/20/14	275.0	18.66
	Larva -> Nymph	5	7/1	2013	*	9/7/13	68.0	0.00
		6	7/1	2013	*	5/14/14	317.0	9.28
		7	7/25	2013		5/14/14	293.0	0.00
		5	8/23	2013		5/14/14	264.0	0.00
		2	9/26	2012	*	9/7/13	346.0	0.00
		6	9/26	2012	*	5/14/14	595.0	0.00
	Nymph -> Adult	2	7/1	2013	*	9/7/13	68.0	-
		1	7/1	2013	*	6/8/14	342.0	-
		2	7/15	2013	*	9/7/13	54.0	-
		5	7/15	2013	*	4/14/14	273.0	11.21
TN	Female -> Larva	3	1/31	2014		5/19/14	108.0	26.36
		12	2/3	2013		6/21/13	138.0	4.05
		26	2/9	2012		5/24/12	105.0	1.01
		6	12/9	2013		7/10/14	213.0	0.00
	Larva -> Nymph	17	6/23	2014		9/24/14	93.0	5.44
		17	7/1	2013		8/11/13	41.0	23.32
		4	8/23	2013		3/10/14	199.0	0.00
		7	9/15	2014		11/30/14	76.0	0.00
	Nymph -> Adult	5	6/23	2014		10/15/14	114.0	12.24
FL	Female -> Larva	18	2/3	2013		3/21/13	46.0	6.95
		26	3/10	2013		5/6/13	57.0	0.00
	Larva -> Nymph	13	6/7	2014		7/24/14	47.0	0.00
		35	6/22	2013		8/25/13	64.0	0.00
		6	8/25	2013		4/19/14	237.0	0.00
	Nymph -> Adult	1	5/19	2013		8/25/13	98.0	-
		3	6/22	2013		10/20/13	120.0	0.00
		2	7/31	2013		11/8/13	100.0	-

Table 1.3. Final detection. The median dates for which unfed ticks in placements of replete *I. scapularis* were last observed. Only placements that successfully produced ticks are shown. Placements are arranged by calendar date (not year) of completion (i.e., last day) of feeding, which occurred within two weeks of placement at each site. Each pottle within a placement represents one sample unit (n represents the number of pottles), that contained either one replete female, 20 replete larvae, or 10 replete nymphs. Final detection dates for each pottle represent the last date that any unfed ticks were observed for that pottle. Asterisks (*) represent pottles with the same placement date but bimodal emergence dates.

Site	Transition	n	Feeding			Detection			SEM
			Month/Day	Year		Median	Duration (Days Post Feeding	Duration (Days Post Emergence)	
RI	Female -> Larva	4	5/9	2013		7/31/14	448.0	378.0	10.06
		4	11/30	2012		7/31/14	608.0	436.0	0.00
	Larva -> Nymph	9	5/10	2013		7/31/14	447.0	365.0	38.16
		9	5/18	2013		6/25/14	403.0	329.0	0.00
		5	7/15	2013		9/11/13	58.0	42.0	0.00
		5	8/23	2013		7/18/14	329.0	366.0	0.00
		10	9/26	2012		10/21/14	755.0	461.0	0.73
	Nymph -> Adult	9	5/18	2013		6/25/14	403.0	329.0	0.00
		1	6/7	2013		7/31/14	419.0	323.0	-
WI	Female -> Larva	8	2/9	2012	*	10/8/12	242.0	138.0	7.20
		2	2/9	2012	*	6/26/13	503.0	11.0	-
		9	11/18	2013		11/14/14	361.0	86.0	14.48
	Larva -> Nymph	5	7/1	2013	*	9/7/13	68.0	0.0	84.63
		6	7/1	2013	*	7/18/14	382.0	65.0	22.63
		7	7/25	2013		5/14/14	293.0	0.0	0.00
		5	8/23	2013		7/8/14	319.0	55.0	0.00
		2	9/26	2012	*	5/14/14	595.0	249.0	0.00
		6	9/26	2012	*	5/14/14	595.0	0.0	21.92
	Nymph -> Adult	2	7/1	2013	*	2/6/14	220.0	152.0	-
		1	7/1	2013	*	7/8/14	372.0	30.0	-
		2	7/15	2013	*	7/2/14	352.0	298.0	-
		5	7/15	2013	*	6/8/14	328.0	55.0	57.43
TN	Female -> Larva	3	1/31	2014		11/30/14	303.0	195.0	22.33
		12	2/3	2013		8/11/13	189.0	51.0	14.35
		26	2/9	2012		8/12/12	185.0	80.0	5.15
		6	12/9	2013		11/30/14	356.0	143.0	86.29
	Larva -> Nymph	17	6/23	2014		9/24/14	93.0	0.0	7.56
		17	7/1	2013		5/19/14	322.0	281.0	23.83
		4	8/23	2013		5/19/14	269.0	70.0	18.67
		7	9/15	2014		11/30/14	76.0	0.0	0.00
	Nymph -> Adult	5	6/23	2014		11/30/14	160.0	46.0	0.00
FL	Female -> Larva	18	2/3	2013		7/7/13	154.0	108.0	0.00
		26	3/10	2013		7/7/13	119.0	62.0	0.00
	Larva -> Nymph	13	6/7	2014		10/12/14	127.0	80.0	9.94
		35	6/22	2013		6/27/14	370.0	306.0	6.65
		6	8/25	2013		7/12/14	321.0	84.0	29.65
	Nymph -> Adult	1	5/19	2013		6/1/14	378.0	280.0	-
		3	6/22	2013		6/27/14	370.0	250.0	8.67
		2	7/31	2013		5/10/14	283.0	183.0	-

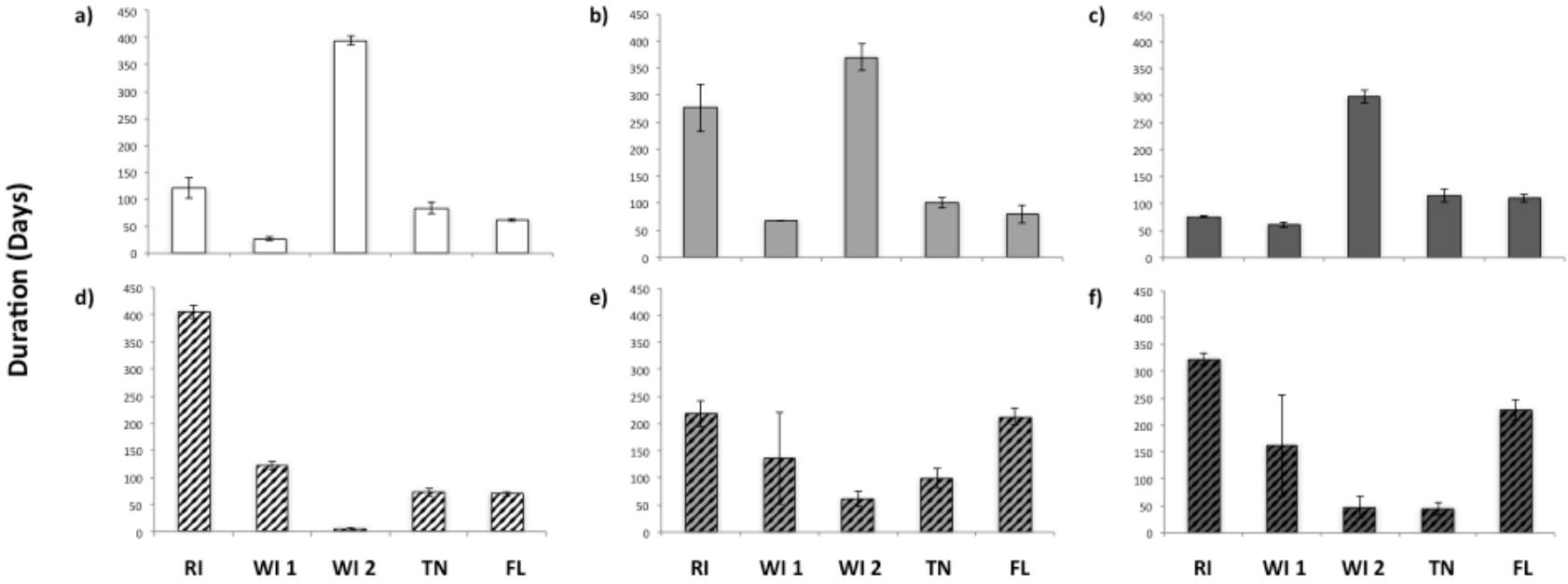

Figure 1.4. Mean days to observed emergence (solid) of larvae from replete females (a), nymphs from replete larvae (b), and adults from replete nymphs (c), and mean detection periods post-emergence (patterned) of unfed larvae (d), nymphs (e), and adults (f) for all placements of *I. scapularis* in Rhode Island (RI), Wisconsin (WI1 and WI2), Tennessee (TN), and Florida (FL). Ticks in WI that emerged pre- (WI1) and post-winter (WI2) were placed into two separate groups. Error bars represent standard error of the mean.

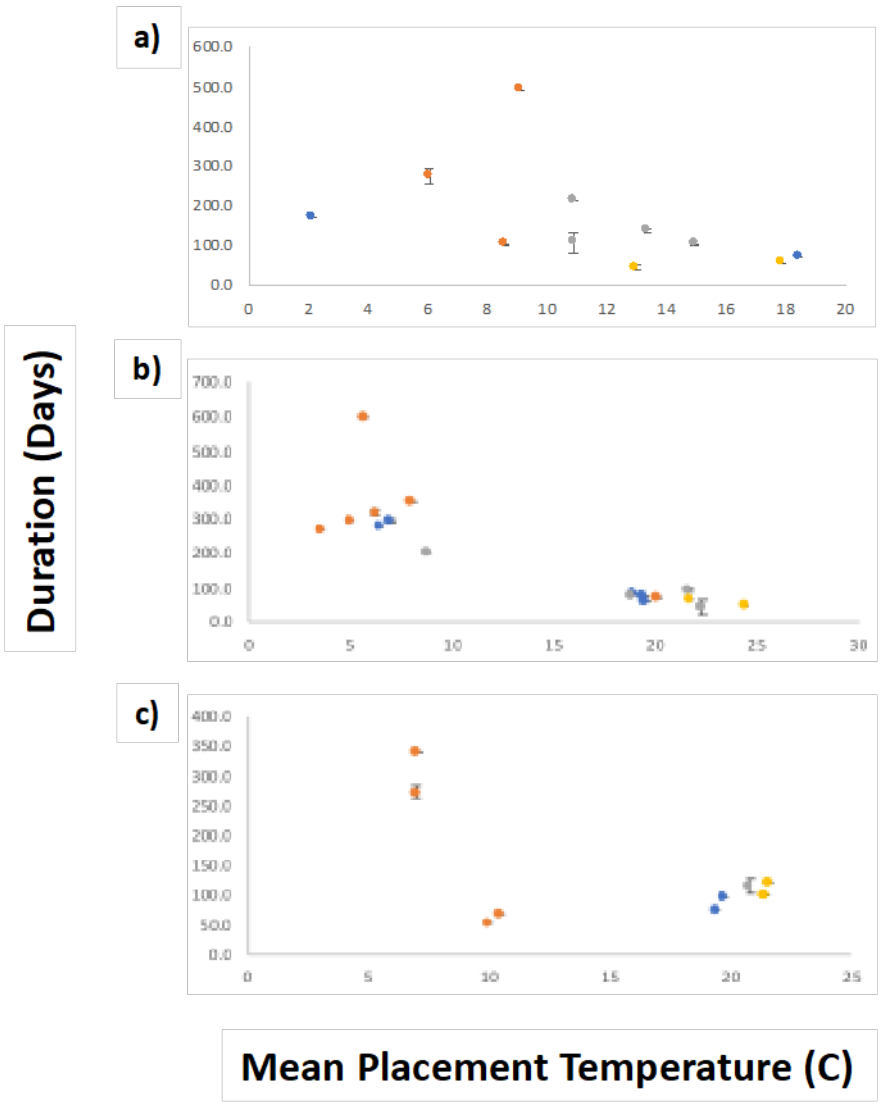

Figure 1.5. Median days to observed emergence of larvae from replete females (a), nymphs from replete larvae (b), and adults from replete nymphs (c), plotted against the mean field temperature for the duration of time that each placement was in the field. Each circle represents a different placement in RI (blue), WI (orange), TN (gray), or FL (yellow). Error bars represent standard error of the mean.

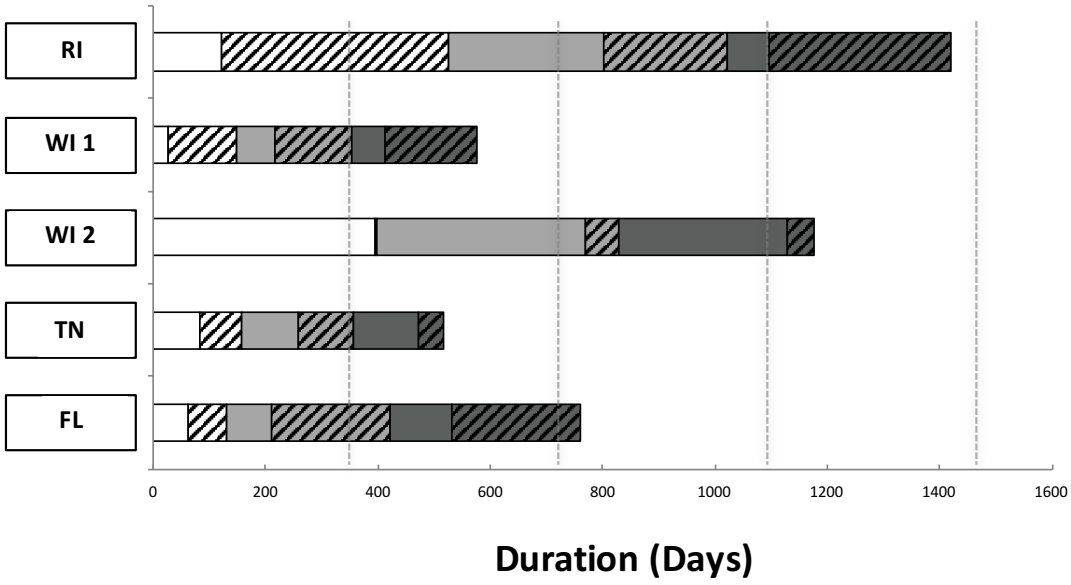

Figure 1.6. Mean days to observed emergence of *I. scapularis* of larvae from replete females (solid white), nymphs from replete larvae (light gray), and adults from replete nymphs (dark gray), and the mean durations of observed activity post-emergence of larvae (patterned white), nymphs (patterned light gray), and adults (patterned dark gray) in Rhode Island (RI), Wisconsin (WI1 and WI2), Tennessee (TN), and Florida (FL) sites. Ticks in WI that emerged pre- (WI1) and post-winter (WI2) were placed into two separate groups. Dashed lines represent yearly increments.

Discussion

The key findings from our work were:

- There is significant variation among sites with respect to both emergence timing and detection period.
- Bimodal emergence (i.e., pre-winter and post-winter emergence of ticks belonging to the same placement) was observed for all life stages in WI but not at other sites.
- There is a significant negative relationship between temperature and days to emergence for all life stages.

In both WI and RI, the life cycle can likely be completed in as little as 2 years but can take 4 or more years. In TN, the life cycle can likely be completed in as little as 1 year but can take 3 or more years. In FL, the life cycle also can likely be completed in as little as 1 year but can take up

to 2 or more years.

Variation among sites

Emergence

Synchronous emergence, in which placements of ticks exposed to varying temperature regimes (being housed in the field for different lengths of time) emerged during the same observation period, may be indicative of temperature-driven quiescence (i.e., an immediate response to adverse environmental conditions), or photoperiod-driven diapause (Table 1.1). We observed synchronous emergence (Table 1.1) at our northern sites for nymphs (RI and WI) and adults (WI). Colder winter temperatures in the north may have resulted in the quiescence of developed ticks, resulting in the synchronous emergence of various placements responding to a short-term abatement of adverse conditions (i.e., warmer ambient temperatures). Or, the observed synchronicity may be due to a behavioral and/or developmental delay in emergence induced by temperature-independent cues such as photoperiod. The role of diapause in the synchronous emergence of ticks has been well documented for various ixodid species, including *I. scapularis* in the northeastern US (Yuval and Spielman 1990), and it has been hypothesized to serve to minimize the exposure of ticks to adverse conditions such as dehydration and freezing (Gray et al. 2016). The absence of pronounced synchronicity at our TN and FL sites may indicate that development in the south is predominantly temperature-driven and lacks a diapause cue or may be a result of the higher temperatures overriding any diapause cue. This diapause cue has been routinely overridden in laboratory populations of *I. scapularis* (Gray et al. 2016). It is also possible that *I. scapularis* populations locally adapted to southern conditions do not undergo diapause, which would unnecessarily extend the life cycle and reduce reproductive fitness (Gray et al. 2016) of southern populations, in the absence of northern adverse winter conditions that

may limit tick survival. Therefore, although the role of temperature-driven quiescence cannot be distinguished from diapause in this study, the observed synchronous emergence at our northern sites suggests that temperature-independent diapause may be occurring at our northern sites. There was also a general trend in which placements of ticks that emerged after the winter season at our northern sites in RI (i.e., emerging larvae and nymphs) and WI (i.e., the second subset of emerging larvae, nymphs, and adults) exhibited longer times to emergence (Figure 1.4). The shorter times to emergence observed for placements of ticks that emerged prior to the winter season in RI (i.e., emerging adults) and WI (i.e., the first subset of emerging larvae, nymphs, and adults) were more similar to emergence times observed at our southern sites with warmer year-round temperatures (Figure 1.4). However, given our inability to determine the precise time of hatching and molting in this study, it is possible that ticks observed to have emerged post-winter developed but were inactive prior to winter, resulting in perceived differences in development timing between pre- and post-winter emergent ticks that were actually attributable to differences in quiescence or behavioral diapause. Whether due to developmental or behavioral factors, this pattern highlights the potential regulatory role of regional temperatures on tick emergence timing, which could contribute to observed regional differences in life cycle lengths. These findings merit a closer investigation of the independent and/or interactive roles of temperature and other abiotic factors on tick emergence timing at these sites.

Survivorship

Our findings reveal the variation in the duration of survivorship (i.e., the duration of time that live ticks were observed post-emergence) that can occur within and among sites for this species (Table 1.3). We observed variation in the duration of survivorship for ticks belonging to different placements for a given site (i.e., within-site differences), ticks from bimodal emergences in WI,

and ticks belonging to different populations (i.e., among-site differences). These comparisons were made based on the assumption that the detectability of ticks within pottles did not vary by site; however, it should be noted that among-site differences in questing tick behavior (e.g. time spent above or below the leaf litter) may have affected the detectability of ticks within pottles and perceived survivorship durations of experimental placements.

Although site was a significant factor regarding post-emergence survivorship, there were no clear trends in comparing the survivorship durations at northern versus southern sites. This finding is supported by past laboratory survivorship experiments, in which differences in the survivorship of larval *I. scapularis* from different geographic regions did not appear to follow a north-south gradient (Ginsberg et al. 2014). Observed differences among sites may be caused in part by the fact that some northern placements diapaused while others did not; the lower metabolic activity maintained by the subsets of ticks undergoing diapause (Belozerov 1982), associated with a protracted life cycle, would presumably reduce energy expenditures and extend a placement's survivorship duration. Alternatively, the abiotic conditions to which different subsets of unfed ticks were exposed may have also played a role in observed differences in survivorship durations. Relative humidity levels, for example, play a major role in the energy consumption of ticks, as energy expenditures involving the uptake of water vapor are very high, relative to other expenditures (Knulle and Rudolph 1982). Such varying relative humidity levels could play a major role in differences observed in the duration of survivorship (Stafford 1994, Berger et al. 2014) for subsets of ticks that emerged at different times. At our WI site, there were no statistically significant differences in the survivorship durations of nymphs and adults that emerged pre- or post-winter. This finding suggests that, perhaps for certain stages, when abiotic conditions are unpredictable and survivorship may not be reliably linked seasonal emergence

timing, greater inter-tick variations in temperature development rates (contributing to a bimodal emergence pattern when interrupted by a cold winter season) may be a developmental strategy that spreads the risk of mortality for a given time of feeding.

Life Cycles

Differences in the duration of survivorship also varied among sites, with the longest periods of detection occurring in RI for all life stages of ticks, followed by WI, FL, and TN (Figure 1.6). It should be noted, however, that due to the bimodal emergence occurring at our WI site, there is more uncertainty regarding the life cycle that has been constructed for this site. One possible explanation for the longer durations of survivorship observed in RI and WI is that these northern sites tend to have protracted life cycles due to the reduced rates of development during their winter seasons (Lindsay et al. 1995, Daniels et al. 1996). Another possibility is that the abiotic conditions associated with these northern sites are more conducive to extended survivorship (Ginsberg et al. 2014). Interestingly, the duration of survivorship appeared to be longer in FL than in TN. This finding implies that the abiotic conditions at our FL site may have been be more conducive to extended survivorship (e.g., higher relative humidity levels and lower temperatures), relative to our TN site. However, temperature and relative humidity data collected from our data loggers showed that FL experienced higher temperatures and lower relative humidity than our TN site (Supplemental Table 1.2), which should have reduced the duration of survivorship (Knulle and Rudolph 1982, MacDonald 2017) of placements in FL. This finding suggests that, beyond the role of abiotic factors, there may be genetic differences among populations contributing to observed differences in life cycle lengths.

The life cycle lengths observed at our RI and WI sites generally corroborate the 2-4 year life cycle previously hypothesized for *I. scapularis* in northeastern populations (Yuval and

Spielman 1990). However, life cycle lengths at our southern sites appear to be shorter, and may perhaps be completed in 1-2 years, depending on the availability of hosts and the host seeking activity of molted ticks throughout the season. This finding suggests that differences in *I. scapularis* life cycle length may follow a north-south gradient, although additional sites would need to be included to account for among-site variation and explore regional patterns.

Bimodal emergence

We observed bimodal emergences for replete larvae, nymphs, and adult females at our WI site, in which a subset of each placement exhibited delayed emergence. Bimodal emergence, in which a subset of ticks delayed emergence relative to other ticks belonging to the same placement and exposed to the same temperature regime, may be attributed to small variations among individual ticks in temperature-development rates (Ogden et al. 2004), or the role of temperature-independent factors on development, such as photoperiod (Gray et al. 2016). A bimodal pattern in WI may be a bet-hedging strategy when current conditions are poor predictors of future conditions, causing some individuals in the population to enter diapause for the winter while others remain active. For example, for ticks that do not diapause, this type of strategy could be selected for when harsh winter conditions might contribute to higher mortality, but milder winter conditions might confer an advantage by allowing these ticks to be active earlier in the spring season.

Temperature and emergence timing

There was a significant negative relationship between mean placement temperature and the median time to emergence. This finding was observed for all emerging life stages and implies a potential regulatory role of temperature (via direct effects on development rate or indirect effects on behavioral diapause or quiescence) on emergence. Although there were no apparent north-

south gradients among placement sites (i.e., where northern or southern sites produced consistently shorter or longer times to emergence), additional placements would be required to further explore these differences.

In the context of other *I. scapularis* populations

Despite high within and among site variation, the seasonal emergence timing in this study corroborates the findings of Ogden et al. (2018), with emergence dates falling within observed activity periods based on drag-sampling and on-host data. However, some notable differences were observed. First, in TN we observed active nymphs in late November, approximately one and two months past the observed and predicted activity period. Second, in RI, which is geographically close to their site, we observed active adults in late July, approximately one month prior to the observed activity period. These differences, however, may be attributable to interannual variation in temperature, since the observations for this study were made one year prior to our experiment.

Beyond these study sites, comparisons in emergence timing may also be made at broader regional scales. At Long Point, Canada, females fed in the fall produced larvae that emerged in mid-to-late summer of the following year (Lindsay et al. 1995), which corresponds with the mid-summer emergence of larvae from fall-fed females that we observed at our nearest site in WI. The emergence times for our RI site generally corroborate previously documented emergence times in the nearby states of Massachusetts (MA) and New York (NY), in which spring fed females produced larvae that emerged in late summer (Yuval and Spielman 1990, Daniels et al, 1996). One difference between these prior studies and our findings, however, is that we also first observed larvae in the spring that were derived from females fed in the winter. Previous findings for northeastern sites suggest that larvae observed in the spring had emerged in the previous

summer/fall, re-emerging in spring after failing to acquire a host (Yuval and Spielman 1990, Daniels et al. 1996). One possible explanation for this difference is that at our RI site, a mild winter in 2012 may have allowed for oviposition and development of larvae to occur much more quickly in a small number of individuals.

The peak activity period for nymphs observed from drag sampling studies for the upper Midwest and the Northeast, occurring from late spring through early summer (Diuk-Wasser 2006, Gatewood 2009) also corroborates the mid-spring and early summer emergences of nymphs that were observed at our WI and RI sites. The similarities in emergence timing between these northeastern sites suggest that, for certain regions with cold winter temperatures or for certain life stages, the emergence timing of ticks may be fairly predictable. Further, the occurrence of high within-site variation in emergence timing may be an adaptive life cycle strategy for certain populations, mitigating the effects of locations where abiotic cues such as photoperiod are not accurate predictors of other factors affecting fitness, such as temperature and relative humidity. In northwestern FL, larval, nymphal, and adult phenologies corroborate the emergence times that we observed at our FL site, with the start in observed questing activity falling within the range of emergence times that we observed for each life stage (Mount et al. 1997, Cilek and Olson 2000). Interestingly, the phenologies of different life stages appeared to be more variable in the south, resulting in some activity periods that corroborated observed emergences at our TN and FL sites, and others that did not. The activity periods described for larvae and nymphs in South Carolina (SC) encompass the emergence times that we observed at our TN and FL sites (Clark 1998). However, in southeastern Missouri (MO), the observed periods of immature activity (Kollars et al. 1999), did not match the emergence times that we observed for our TN site (with no apparent trends in earlier versus later emergence timing across

life stages). Instead immature activity in southeastern MO appeared to be characterized by narrower periods of seasonal activity than that observed at our TN site, although further study would be required to effectively compare the activity observed in the pottles used in this study and questing behavior in the field. The peak questing nymphal densities previously observed at a few southern sites in a large scale effort to detect questing nymphs in the eastern US (Diuk-Wasser et al. 2006) occurred prior to early summer, preceding the array of post-spring emergences of nymphs at our TN and FL sites. These differences may be due to the low sample size of dragged nymphs collected in this prior study, or the fact that these nymphs had developed in the prior year and emerged again in the spring. Previous studies of drag sampled *I. scapularis* in Mississippi (MS) and Louisiana (LA) documented adult activity from fall through spring (Goddard 1992, Mackay and Foil 2005), in contrast to the summer adult emergences observed at our TN and FL sites. Differences between this study and others, in which ticks in project pottles appeared to emerge prior to questing ticks in previous studies, may also be attributable to quiescence or behavioral diapause occurring in pottles, as the activity in pottles does not necessarily imply host-seeking. Pottle design is an unlikely factor in observed differences in emergence timing, as temperatures collected from HOBO (placed outside of pottles) and iButton loggers (placed inside pottles) were not significantly different.

According to Yuval and Spielman (1990) in MA, the majority of northeastern adults emerge in the fall, prior to the onset of winter. Replete nymphs that fed too late in summer failed to molt prior to winter, resulting in poor overwintering survivorship as replete nymphs (Yuval and Spielman 1990). Thus, it is generally believed that, in the Northeast, the majority of adults observed in the spring are the remaining adults from the previous fall that did not successfully locate a host (Daniels et al. 1989). At our RI site, we observed this characteristic fall adult

emergence. We also observed that placements of replete nymphs in the late summer did not survive to the adult stage (i.e., where greater than one year of no activity was observed; see Supplemental Table 1.1). However, further work is needed to corroborate the low overwintering survivorship of engorged nymphs previously observed in the Northeast (Yuval and Spielman 1990). In contrast, the spring adults in WI may be a mixture of adults that first emerged in the fall and overwintered as unfed adults, as well as replete nymphs that had overwintered successfully and molted into adults that emerged the following spring. This spring emergence of overwintering nymphs as unfed adults differs from what was observed for overwintering nymphs in Long Point Ontario, which did not emerge until the fall (Ogden et al. 2004) While the observed fall and spring emergences in WI still represent adults belonging to the same placement, this finding highlights a distinct difference in emergence timing between northeastern, north central, and Canadian populations. Further, differences in the activity periods of adults that emerge in the fall (which are observed through the winter) and those that emerge in spring (which are observed through the summer) could contribute to diverging activity periods of subsequent life stages. At northeastern sites where all adults emerge in fall, one might expect a decline in activity through the spring and summer, either due to adult tick mortality or the successful acquisition of hosts by host-seeking adult ticks. In contrast, at midwestern sites where some adults emerge in the spring, one might expect an extended period of activity through the summer, or even an increase in relative activity in the spring and summer (not necessarily caused by differences in survivorship of adults that emerged in fall versus spring, but due to an influx of newly molted adults throughout the spring). A comparison of adult *I. scapularis* phenologies, based on ticks collected from soldiers in the midwestern states of Minnesota (MN) and WI and the northeastern state of Pennsylvania (PA) documents this pattern, with adult abundances

declining through spring and summer in the northeastern population, peaking in the summer in the midwestern populations (Stromdahl et al. 2014).

Study limitations and future directions

A limitation of this study is that we were unable to determine the precise timing of the end of development or beginning of questing; in an effort to provide ticks with a more natural microhabitat (i.e., soil cores and leaf litter cover), reduce disturbance during sampling (e.g., opening tubes and removing soil cores), and avoid reducing placement sample sizes (i.e., destructive sampling). We also did not distinguish whether observed unfed, active ticks in pottles were actively questing; therefore, it is possible that our emergence dates precede the onset of host-seeking activity for a given placement and that our estimated duration of survivorship extends beyond the period of time that ticks within a placement could successfully acquire and feed from a host. Alternatively, perhaps the active ticks that we observed were host-seeking and are representative of a smaller portion of ticks that quest earlier in the season but are not abundant enough to be detected with conventional levels of effort, via drag sampling and host trapping.

Additional experiments would provide a more detailed understanding of these life cycles, and the relative roles of temperature dependent and independent factors on observed emergence and survivorship. A greater number of placements of replete ticks, encompassing entire seasonal activity periods, would allow one to determine when diapause occurs for each life stage in a season. Transplant experiments exposing ticks from southern and northern populations to different temperature regimes would allow one to further investigate the roles of genetics and temperature on observed emergence timing and survivorship. Additionally, future experiments in which pottles are destructively sampled would allow one to determine when ticks molted, and

thus the role of diapause in the synchronous emergence of different placements of ticks.

Implications for enzootic cycles and Lyme disease risk

The emergence timing and the duration of survivorship of different life stages of *I. scapularis* influence the phenologies of larval and nymphal questing stages, the resulting infection of larvae, and the consequential maintenance of this pathogen in vector populations. Microcosm experiments provide a unique insight to the *I. scapularis* life cycle, as these data enable us to track the origin (i.e., when ticks fed and emerged) of ticks that are observed in the field and observe whether ticks fed at different times synchronize in terms of their emergence timing.

A period of overlapping larval and nymphal activity was observed at all sites, suggesting that current seasonal activity patterns would promote pathogen transmission from nymphs to larvae (as opposed to larvae preceding nymphs, which would reduce transmission). These activity patterns may change in the future, however, as changing abiotic conditions affect both tick development and survivorship. The results of this study indicate that, within a single life stage, ticks fed in different years could become active in the same year and overlap in terms of activity. We observed indications of overlapping placements for nymphs and adults in WI, larvae and nymphs in RI, nymphs in FL, and nymphs in TN. These patterns, in which ticks fed in different years are active during the same time periods, could buffer pathogen maintenance, whereby infected in the previous year maintain and transmit the pathogen via shared hosts to newly-emerged, uninfected larvae in the following year. The Northeast life cycle indicates that the nymphal stage, which has a greater longevity when unfed relative to other life stages, is the only stage in which more than a single age cohort may overlap (Yuval and Spielman 1990). Our findings suggest that the overlapping activity of nymphs from different age cohorts is likely not limited to the Northeast. Further, larvae from different age cohorts may also overlap in activity.

As temperatures rise due to climate change and *I. scapularis* from northern populations expand (Eisen et al. 2016), our ability to predict how *I. scapularis* populations will respond to new abiotic conditions is of critical importance to predicting and mitigating future Lyme disease risk. In southeastern Canada, warming temperatures are predicted to allow for the northward range expansion of *I. scapularis* into warmer territories, while maintaining seasonal activity patterns conducive to pathogen maintenance (Ogden et al. 2006). In the US, however, where *I. scapularis* already is established, how will climate change impact *I. scapularis* populations and enzootic cycles of the Lyme disease pathogen as well as other *I. scapularis*-borne pathogens? As temperature regimes in northern states begin to more closely resemble those in southern states, will Lyme disease incidence decrease in northern states? How will *I. scapularis* from northern regions respond to warmer temperatures as they invade southern regions? This investigation of *I. scapularis* life cycles demonstrates that there exists high variation within and among populations with respect to emergence timing and durations of survivorship, which may make the effects of increasing temperatures difficult to predict. Further, there appears to be a genetic component affecting *I. scapularis* development, suggesting the potential role of temperature-independent factors as well. Studies investigating the relative roles of temperature and temperature-independent factors on *I. scapularis* survivorship and development are required to further address the potential role of climate change on Lyme disease risk.

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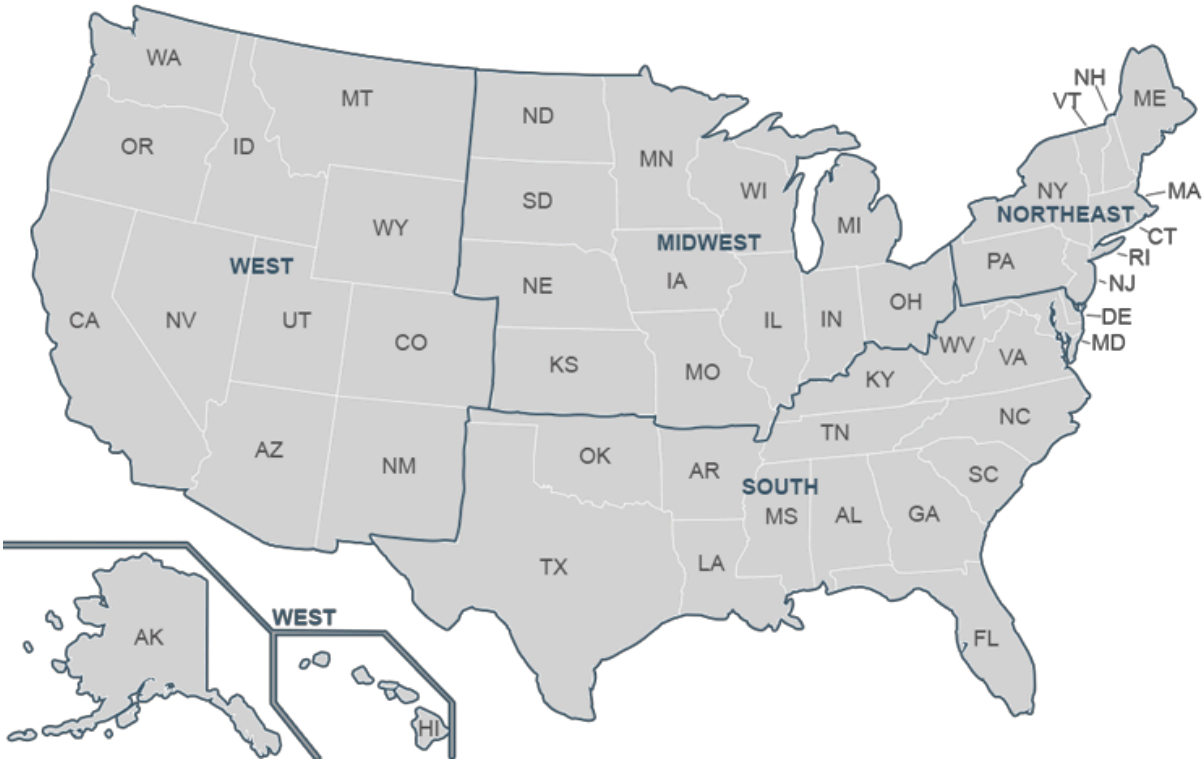
Muller, and Eric Rulison, Seungeun Han, Marc Wiseman, Samantha Zohr, and Marisa Albert for assisting with the collection of field data and tick infestations. This work was supported by National Science Foundation EID award EF-0914476, National Science Foundation Graduate Research Fellowship Program, and the Hal and Jean Glassen Memorial Foundation.

APPENDICES

APPENDIX A: Supplemental Material



Supplemental Figure 1.1. Map of states belonging to regional designations in the United States. In this study, WI is in the Midwest, RI is in the Northeast, and TN and FL are in the South.

Supplemental Table 1.1. The proportion of placements of replete *I. scapularis* that successfully produced molted ticks. Each pottle within a placement represents one sample unit, and contained either 1 replete female, 20 replete larvae, or 10 replete nymphs.

	Transition	Feeding Date		n (Total)	n (Detected)	Proportion Detected
		Month	Year			
RI	Female -> Larva	5/9	2013	13	4	0.31
		5/18	2013	10	0	0.00
		11/10	2013	15	0	0.00
		11/30	2012	11	4	0.36
	Larva -> Nymph	5/10	2013	10	9	0.90
		5/18	2013	9	9	1.00
		7/15	2013	5	5	1.00
		8/23	2013	5	5	1.00
		9/26	2012	10	10	1.00
	Nymph -> Adult	5/18	2013	9	9	1.00
		6/7	2013	8	1	0.13
		6/23	2014	3	0	0.00
		7/25	2013	9	0	0.00
		8/20	2013	1	0	0.00
WI	Female -> Larva	5/2	2012	15	10	0.67
		11/18	2013	25	9	0.36
	Larva -> Nymph	7/1	2013	11	11	1.00
		7/25	2013	8	7	0.88
		8/23	2013	5	5	1.00
		9/26	2012	10	8	0.80
	Nymph -> Adult	7/1	2013	9	3	0.33
		7/15	2013	8	7	0.88
		8/20	2013	1	0	0.00
TN	Female -> Larva	1/31	2014	4	3	0.75
		2/3	2013	15	12	0.80
		5/2	2012	27	26	0.96
		8/20	2013	4	0	0.00
		12/9	2013	21	6	0.29
	Larva -> Nymph	6/23	2014	17	17	1.00
		7/1	2013	27	17	0.63
		7/30	2013	5	0	0.00
		8/23	2013	4	4	1.00
		9/15	2014	27	7	0.26
	Nymph -> Adult	6/23	2014	5	5	1.00
FL	Female -> Larva	2/3	2013	20	18	0.90
		3/10	2013	30	26	0.87
	Larva -> Nymph	6/7	2014	19	13	0.68
		6/22	2013	35	35	1.00
		8/25	2013	10	6	0.60
	Nymph -> Adult	5/19	2013	2	1	0.50
		6/1	2013	3	0	0.00
		6/22	2013	3	3	1.00
		7/31	2013	3	2	0.67

Supplemental Table 1.2. Mean monthly temperatures (C) and relative humidity in Florida and Tennessee. Readings were collected from Pro v2 (#U23-001) data loggers programmed to record every 30 min. Loggers were placed inside crates, at leaf litter level next to pottles.

Temperature and Relative Humidity in TN and FL				
Month	Mean Temperature (C)		Mean %RH	
	TN	FL	TN	FL
5/2013	17.47	20.87	91.63	62.93
6/2013	21.55	20.97	97.75	54.95
7/2013	22.19	21.23	98.61	55.08
8/2013	22.27	23.00	98.75	57.96
9/2013	19.78	20.93	98.23	67.99
10/2013	14.72	20.65	95.39	59.36
11/2013	6.69	19.87	90.01	51.44
12/2013	4.56	15.31	97.33	46.26
1/2014	-1.20	16.63	88.71	40.66
2/2014	4.28	-2.20	85.22	48.55
3/2014	8.55	2.34	78.96	50.69
4/2014	15.15	19.14	78.67	95.52
5/2014	17.81	21.04	92.98	94.17
6/2014	21.35	24.16	98.38	97.00
7/2014	21.29	24.43	96.64	96.09
8/2014	21.80	25.17	99.01	96.33
9/2014	20.11	23.35	98.42	98.14
10/2014	14.07	18.20	96.93	94.58
Mean	15.14	18.62	93.42	70.43

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CHAPTER 2: TEMPERATURE UNDER-PREDICTS *IXODES SCAPULARIS* DEVELOPMENTAL TIMING IN THE EASTERN UNITED STATES - AN INVESTIGATION OF GENETICS AND PLASTICITY ON OBSERVED EMERGENCE PATTERNS.

Introduction

The geographic distribution of reported Lyme disease cases indicates that incidence is highest in the north central and northeastern United States (US), although populations of vector *Ixodes scapularis* are established throughout the southeastern US as well (Bishopp and Trembley 1945, Tugwell and Lancaster 1962, Barnard 1981, Koch 1982, Oliver 1993, Clark 1998). There are several non-mutually exclusive ecological hypotheses that may contribute to this discrepancy, including regional differences in host composition and densities, tick behavior, tick genetics, and climate. Given the wide range of regional abiotic and biotic differences, this study focuses specifically on the roles that temperature and genetics (i.e., via local adaption) play on the seasonal activity patterns of immature stages (Gern and Rais 1996, Diuk-Wasser et al. 2006, Gatewood et al. 2009, Randolph et al. 1999), which can have important implications for the transmission efficiency and maintenance of non-transovarially transmitted pathogens such as *Borrelia burgdorferi*, the causative agent of Lyme disease.

The effect of temperature on development rates and emergence timing (i.e., the seasonal timing that molted, flat ticks of a given life stage are first observed) is believed to be one of the main drivers of *I. scapularis* seasonal activity patterns, because, as with other poikilotherms (Damos and Savopoulou-Soultani 2012), temperature affects development rates and plays a critical role in the emergence timing (i.e., the onset of host-seeking activity) of different stages within a population (Ogden et al. 2004). Temperature-development relationships have been shown to be

capable of predicting development for *I. scapularis* populations in both northern (Ogden et al. 2004) and southern extremes within this species' range (Mount et al. 1997). However, temperature-based models alone do not accurately predict the emergence patterns of all stages at different times of the year, suggesting that, for certain populations or at certain times of the year, other factors may play a greater role in emergence patterns. For example, in order to accurately predict nymphal activity for populations in both Maryland and Ontario, Canada, temporal constraints to account for diapause (i.e., a genetically programmed developmental or behavioral delay in emergence that relies on a temperature-independent photoperiod cue; Eisen et al. [2016]) had to be included (Ogden et al. 2006). In addition, to diapause, relative humidity can also affect the activity periods of *I. scapularis* (Berger et al. 2014, Burtis et al. 2016). These results suggest that while a large component of observed emergence may be accounted for using only temperature, other temperature-independent factors may play roles in these activity patterns as well. Further, when this model was used to simulate the seasonality *I. scapularis* populations located in different regions of the eastern U.S., the inclusion of temperature-independent diapause (i.e., photoperiod) improved the fit of the model at certain sites (Ogden et al. 2018). There is also well-established experimental evidence for the role of photoperiod on the development timing and emergence of related species belonging to the *Ixodes ricinus* species complex (Belozerov and Naumov 2002, Gray et al. 2016).

Geographical differences in *I. scapularis* emergence patterns may be caused by genetic differences among locally adapted populations as well as plasticity (i.e., non-genetic phenotypic responses to site-specific environmental factors). Populations may exhibit different activity patterns due to genetic differences in temperature-dependent (i.e., population-specific temperature-development rates [Ogden et al. 2018]) and temperature-independent responses (i.e.,

diapause [Gray et al. 2016]). Alternatively, phenotypic differences among populations may have no genetic basis and may be due to responses to varying abiotic regimes, where each regime should elicit a different but predictable response. For example, during the summer of 1976, unseasonably warm summer temperatures in Wicklow, Ireland were postulated to have shifted the nymphal activity of its *I. ricinus* population from a bimodal pattern (with nymphs documented in the spring/summer and fall) to one in which >90% of nymphs were observed in the spring/summer (Gray et al. 2009). There may also be complex interactions between genetics and plasticity for a given population, where the effect of abiotic conditions varies by the genetic background of the population. Therefore, the emergence patterns of immature *I. scapularis* stages may be caused by genetic differences among populations as well as plastic responses of this species when exposed to different abiotic regimes.

To investigate the factors contributing to the variation in emergence timing among populations, we used a common garden experimental design to compare ticks from four different sites that were raised under the same conditions and transplanted to different sites. This experimental setup allowed for us to evaluate for the effects of temperature versus temperature-independent factors, as well as genetics versus plasticity, on the observed emergence timing of different *I. scapularis* populations. We address the following questions:

1) Can existing temperature-development models (Ogden et al. 2004) accurately predict observed emergence patterns of different populations of *I. scapularis* found throughout the broad geographic distribution of this species? Approach: In field microcosms at four widely-dispersed tick populations, we evaluated whether emergence dates based on temperature-development models were significantly different from observed emergence dates.

2a) Is there empirical evidence for an effect of genetics on emergence timing? Approach: We compared the emergence timing of placements from different sites of origin that were placed at the same placement site on the same date.

2b) Is there empirical evidence for an effect of phenotypic plasticity on emergence timing? Approach: We compared the emergence timing of placements from the same site of origin that were placed at different placement sites on the same date.

3) What are the roles of genetics and on the accuracy of temperature-development models in their ability to predict emergence timing? Approach: We investigated the effect of site of origin and placement site on the differences between the observed and predicted emergence timing (i.e., generated using temperature-development models) of each stage.

This study provides a unique collection of standardized, longitudinal emergence and contemporaneous abiotic data for populations located in northern, southern, coastal, and inland regions within this species' range in the eastern United States. These data provide an opportunity to evaluate the capacity of temperature to predict the emergence of populations that are widely geographically dispersed and exposed to a wide range of abiotic conditions. We also simultaneously collected longitudinal emergence data for transplanted specimens, to determine the roles of genetics and plasticity on emergence for this species.

Methods

Field Sites

We observed the emergence timing of larval, nymphal, and adult *I. scapularis* stages at four forested sites in the eastern US. Field sites were a subset of project sites belonging to a larger study

designed to compare the ecology of Lyme disease in different geographic regions. All sites supported populations of *I. scapularis*, as evidenced by the ability to collect questing adult blacklegged ticks as well as juvenile ticks on vertebrate hosts (Ogden et al. 2018). Project sites were located in two Lyme endemic regions: Wisconsin (WI) and Rhode Island (RI), and two non-endemic regions: Tennessee (TN) and Florida (FL). Wisconsin is located in northern central US, Rhode Island is in the northeastern US, Tennessee is in the inland southeastern US, and Florida is in the southeastern US (Supplemental Figure 2.1). Forest at the Rhode Island site (41.5 °N) was dominated by oaks (*Quercus spp*.) and maples (*Acer spp*.) with an understory containing both shrubby and open spaces. Forest at the Wisconsin sites near Barneveld, WI (42.94 °N) and Fort McCoy, WI (44.04 °N) were dominated by oaks (*Quercus spp*.) and maples (*Acer spp*.) with a shrubby understory containing mixed saplings and some invasive understory species. Forest at the Tennessee site (latitude 36.01°N), near Oak Ridge, Tennessee, 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. Forest at the Florida site near Tall Timbers Research Station, FL (latitude 30.65 °N) was dominated oaks (*Quercus spp*.) and maples (*Acer spp*.) with a dense, shrubby understory containing mixed saplings and invasive understory species.

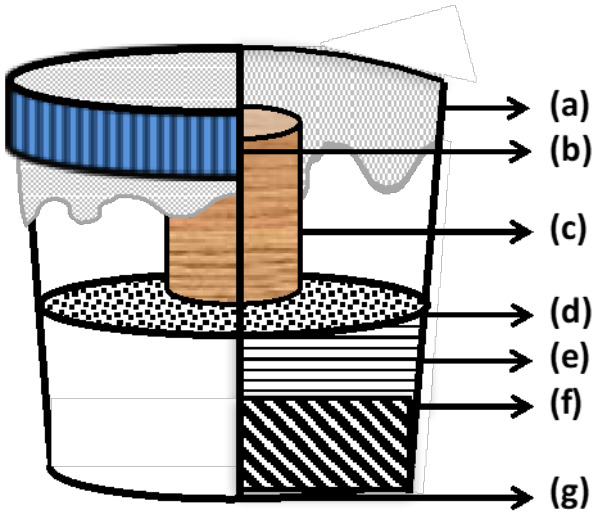

Figure 2.1. External (left) and internal (right) views of a plastic pottle. Components included synthetic cloth mesh (a), a modified pottle lid (b) and base (g) allowing for air flow and precipitation to enter each pottle, an oak dowel for ticks to ascend (c), leaf litter (d), duff (e), and soil (f).

Microcosm design

Live tick specimens were housed in 60 ml polypropylene specimen containers (i.e., pottles) that were 6.1 cm in diameter x 7.25 cm tall. The lids and bases of these containers were cut to create a column through which precipitation could pass. An 11 cm x 11 cm piece of synthetic cloth mesh was secured to the base of each pottle using caulk and a cable tie to hold the organdy cloth mesh in place during drying; once dry, the edge of the organdy cloth mesh was further secured by a 5.08 cm wide piece of tape that encircled the bottom circumference of the pottle. A soil core approximately 3.5 cm deep, containing layers of leaf litter, duff and soil, was collected from the surrounding area of each enclosure location and placed into each pottle. Another 11 cm x 11 cm piece of organdy cloth mesh was secured over the top of each container using the screw top lid to hold the organdy cloth mesh in place. An oak dowel that was .25 cm in diameter x 5.08 cm tall was inserted into the center of each soil core to provide ticks with an object to climb and quest from. Pottles were covered with protective plastic crates (44 cm x 36 cm x 27 cm). Pottles

belonging to the same placement were housed in the same crate. Crates were arranged haphazardly within the same forest stand at each site.

Producing replete ticks to be placed into the field

Live *I. scapularis* adults were collected during peak questing periods at each field site. Within two weeks post-collection, adult ticks were mated and fed on New Zealand White rabbits (Oryctolagus cuniculus) using procedures approved through Michigan State University's Institutional Animal Use and Care Committee permit #06/12-103-00. Replete female *I. scapularis* were returned to sites, placed in modified pottles that exposed them to ambient conditions (Figure 2.1), housed in secure crates in the field, and monitored for oviposition, larval hatch, and larval survivorship. A subset of pottles were subsampled destructively at different time points throughout the seasonal emergence period and used to produce engorged larvae. Larvae were fed on mice (Mus musculus), divided among pottles, and then returned to the respective field site to be monitored for nymphal molt and nymphal survivorship. Similarly, a subset of pottles with molted, flat nymphs were then used for producing engorged nymphs (also fed on lab mice). Replete ticks were housed at 95% RH at 25 C under a 12:12 light dark cycle and returned to their site of origin as soon as possible, typically within 10 days of detachment. At our WI site, replete ticks fed in the fall and winter were housed in an outdoor chamber where they were exposed to comparable ambient Michigan conditions until they could be returned to their site of origin prior to the spring season. Subsets of placements were transplanted to different sites, except in cases where low sample sizes in which all ticks were returned to their sites of origin (Table 2.2).

Placement dates

We fed and placed ticks in the field during ecologically relevant times in their seasonal emergence period. To do so, the relative activity patterns based on host burdens and dragging data

(unpublished) of different stages were referenced to create pre-peak, peak, and post-peak placements, such that samples of replete *I. scapularis* were fed and returned to the field during plausible, natural periods of host seeking and feeding for that region.

Data collection and response variables

This study was conducted from May 2012 – September 2015. Annually, at each project site, 2-3 cohorts of replete ticks for each life stage were placed in the field and monitored until no activity was observed for at least one year. Each pottle received fully replete ticks of one life stage: 20 larvae, 10 nymphs, or 1 adult female. Additional pottles containing only soil cores and no added ticks were observed at each site to confirm that soil cores were not contaminated with ticks already present in the leaf litter.

Visible ticks were noted in pottles the day that they were deployed and then approximately every 2 weeks thereafter. Pottles were checked by searching for ticks on all visible surfaces of the pottle, including the organdy cloth mesh top, the clear sides of the tube, the dowel, and the top of the leaf litter. During spring, summer, and early fall, when ticks emerge and exhibit host-seeking activity at all sites, pottles were monitored every 2-3 weeks. During the late fall and winter, only enclosures in southern sites were monitored every 4 weeks. The number of flat larval ticks in each pottle was estimated with raw counts if 50 or fewer ticks were observed. Otherwise, a categorical estimate > 50 or > 200 was assigned to that pottle. Pottles were monitored until greater than one year of no activity was observed, after which all ticks in the pottle were recorded as expired.

For each pottle, the date in which the subsequent stage was first observed (e.g., the first date a molted nymph in a particular pottle "emerged" after the engorged larvae were placed into the field) was recorded as the first date of observed emergence. The average date of emergence was determined for each placement of ticks, where a 'placement' comprises a single cohort and life

stage, fed at the same time, and exposed to that same laboratory and field conditions. Some placements in WI resulted in "bimodal emergence," (i.e., a subset of pottles produced molted, flat ticks before winter and the remainder of the pottles produced their first molted, flat ticks the following spring or summer). For placements that exhibited bimodal emergence, we calculated two separate average emergence dates (i.e., pre- and post- winter) because calculating the average emergence date across all pottles would have produced the misleading result that molted juvenile ticks emerged in the middle of winter.

We measured the temperature and RH at leaf litter level inside one crate at each site using HOBO Pro v2 (#U23-001) data loggers (i.e., HOBO loggers) programmed to record the temperature every 30 min. To determine whether the pottles had a significant effect on abiotic conditions, we also placed one iButton Hygrochron (#DS1923) data logger (i.e., iButton logger) inside one haphazardly placed pottle containing no added ticks, and one iButton logger inside one haphazardly selected crate external to the pottles; iButton loggers were programmed to record every 30 min over a period of several months. We compared readings among these three data loggers and confirmed that within-pottle abiotic conditions were not substantially different from within-crate abiotic conditions. Readings from HOBO loggers were converted to daily means. In the event of a logger malfunction due to freezing or inundation, a linear relationship (Berger et al. 2014) was established between the logger and a backup logger installed at each site, using hourly data points from 5 days before and 5 days after the gap in logger data (using a total of 240 data points). When both loggers at a site malfunctioned, we used local airport weather station data.

Generating Predicted Emergences

Laboratory-generated temperature-development rates and associated error terms for each life stage have been estimated for a population of *I. scapularis* from Ontario, Canada (Ogden et al. 2004),

and were used to generate a predicted emergence date for each experimental placement of ticks. Mean daily temperatures were generated from HOBO logger temperature data (collected at 30-minute intervals, beginning with the feeding date of each placement), and used as input for the temperature-development models.

Statistics

Evaluating the accuracy of temperature-development models. To investigate general patterns in the accuracy of predicted emergence dates using temperature-development models, we compared the observed and predicted emergence timing of each stage. A range of predicted emergence dates was calculated for each placement, using previously-estimated standard error values and temperature-development relationships (Ogden et al. 2004) for larvae (days to oviposition = 1,300 x Temp-1.427 with SEs 1.26 for intercept and 0.083 for coefficient, and days to eclosion = 34,234 x Temp-2.271 with SEs 1.30 for intercept and 0.090 for coefficient), nymphs (days to eclosion = 101,181 x Temp-2.547 with SEs 1.38 for intercept and 0.104 for coefficient), and adults days to eclosion = 1,596 x Temp-1.208 with SEs 12.44 for intercept and 0.080 for coefficient). If the observed emergence date fell within the predicted range, the observation was assigned a value of zero (i.e., zero days difference between predicted and observed days to emergence). If the observed emergence date fell outside the predicted range, the difference (in days) between the observed and predicted emergence date was calculated, using the day within the predicted range of emergences that was nearest in value to the observed emergence date; positive values indicated that the temperature-development model under-predicted time to emergence, whereas negative values indicated that the temperature-development model over-predicted time to emergence. In order to account for potential hardening of each life stage (in which ticks may have developed but delayed activity), a separate set of analyses were run, with 21 days added to the end of each predicted

range. If the observed emergence date fell outside this modified predicted range, the difference (in days) between the observed and predicted emergence date was calculated, using the day within the predicted range of emergences that was nearest in value to the observed emergence date. The residuals of these values appeared to be normally-distributed, and thus did not require transformations or alternative analyses. All placements belonging to the same life stage were grouped together, including placements from different sites of origin and placement sites (Table 2.1). For each group, a one-sample two-tailed T-test was used to determine whether the mean absolute differences between predicted and observed days to emergence were significantly different from the value of zero.

Effects of genetics and plasticity on observed emergence. To evaluate the potential effects of genetics (i.e., site of origin) and plasticity (i.e., site of placement) on emergence timing, we compared the emergence timing of transplanted placements fed at the same time of year (to account for temporally-varying abiotic factors, such as photoperiod). Emergence periods were log-transformed to normalize the data and allow comparisons of the emergence periods of ticks by site of origin and placement site. Placements that shared the same feeding date were grouped together, such that each group either 1) shared the same site of origin but were placed at different sites or 2) were placed at the same site but came from different sites of origin (Table 2.2). For each group, an analysis of variance (ANOVA) test was used to determine, for that specific feeding period, whether the observed median emergence periods varied by site of origin or placement site; the explanatory variable was either placement site or site of origin, and the outcome variable was the log-transformed observed emergence period. Post-hoc comparisons were made using Tukey honest significant difference (HSD) tests to evaluate differences between groups. Statistics were performed using Program R (R Development Core Team, 2014). The above analyses were

conducted separately for larval and nymphal stages.

Effects of genetics and plasticity on the accuracy of temperature-development model-based predictions. To evaluate for the potential effect of genetics (i.e., site of origin), plasticity (i.e., site of placement) on the accuracy of temperature-development models, we determined the effect of each factor on the differences between the observed and predicted emergence timing of each stage. All pottles that produced successfully molted ticks were included in this analysis. Differences between observed and predicted emergence periods were calculated using the same methods described above. We applied a multiple regression model to estimate the potential effects of genetics (i.e., site of origin) and plasticity (i.e., site of placement) on differences between predicted and observed emergence among sites. For active larvae and nymphs, explanatory variables were site, origin, a site-by-origin interaction, and feeding season (i.e., the season in which ticks were fed a bloodmeal, which was included as a factor to account for temperature-independent abiotic conditions that vary by feeding date). For active adults, the site-by-origin interaction term was excluded due to limited sample sizes. The outcome variable for all models was the difference between predicted and observed days to emergence. The most parsimonious model representing the emergence timing of each stage was selected using the corrected Akaike's information criterion (AICc). Statistics were performed using Program R (R Development Core Team, 2014).

Results

Evaluating the accuracy of temperature-development models (Table 2.1).

Temperature-development models accurately predicted the emergence (i.e., the observed emergence date fell within the range of predicted emergence dates) of 93/327 (28.4%), 46/265 (17.4%), and 3/45 (6.7%) pottles of larval, nymphal, and adult stages, respectively. There was a general pattern in which predicted days to emergence were significantly shorter than the

observed days to emergence of larval (M = 36.17 days, df = 326; t = 7.97; P < 0.001), nymphal (M = 69.46 days, df = 264; t = 11.41; P < 0.001), and adult stages (M = 92.20 days, df = 44; t = 5.99; P < 0.01). When predicted ranges were modified to account for potential hardening (i.e., adding 21 days to the end of the predicted window), temperature-development models accurately predicted the emergence (i.e., the observed emergence date fell within the range of predicted emergence dates) of 110/327 (33.6%), 57/265 (21.5%), and 8/45 (17.8%) pottles of larval, nymphal, and adult stages, respectively. There was a general pattern in which predicted days to emergence were significantly shorter than the observed days to emergence of larval (M = 16.79 days, df = 326; t = 5.35; P < 0.001), nymphal (M = 37.48 days, df = 264; t = 7.93; P < 0.001), and adult stages (M = 42.56 days, df = 44; t = 3.42; P < 0.01). The results of both sets of analyses indicate that the temperature-development relationships developed for an Ontario population of *I. scapularis* under laboratory conditions (Ogden et al. 2004) tended to under-predict emergence timing.

Effects of genetics and plasticity on observed emergence (Table 2.2).

Only pottles that produced successfully molted ticks were included in this analysis; the placements of ticks that did not survive to molt were excluded, which prevented us from comparing every combination of sites. There were fewer instances where we observed a significant effect of origin (i.e., a genetic effect) of larvae (1/8 comparisons significant) and nymphs (1/10 comparisons significant) simultaneously placed at the same site, relative to instances where placement site had a significant effect (i.e., indicating an effect of plasticity) on the emergence periods of larvae (5/6 comparisons significant) and nymphs (6/7 comparisons significant) belonging to the same origin (Table 2.2). There was no clear north-south gradient regarding the effect of placement site on observed emergence.

Larvae

Site of origin had a significant effect ($P < 0.001$) on the mean larval emergence periods of ticks placed at the same site: RI > FL (placed in WI, fed on 6/15/13). In this example, the mean number of days before larvae were first observed in pottles was significantly greater for pottles of RI origin than FL origin; these pottles were compared because they belonged to the same placement, sharing the same placement site (WI) and date (6/15/13). The following comparisons indicate that placement site had a significant effect ($P < 0.05$) on the mean larval emergence periods of ticks belonging to the same origin: TN > WI (RI origin fed on 5/2/12), TN > FL > RI (RI origin fed on 11/30/12), RI > TN (WI origin fed on 5/2/12), TN > FL (WI origin fed on 11/30/12), and RI > TN > WI (FL origin fed on 5/2/12).

Nymphs

Site of origin had a significant effect ($P < 0.01$) on the mean nymphal emergence periods of ticks placed at the same site: WI > FL = TN (placed in WI, fed on 7/25/13). The following comparisons indicate that placement site had a significant effect ($P < 0.01$) on the mean nymphal emergence periods of ticks belonging to the same origin: RI > FL (RI origin fed on 9/25/12), FL > WI > TN (WI origin fed from 8/23/13-8/25/13), RI > TN (TN origin fed on 7/1/13), FL > WI (TN origin fed on 7/25/13), RI > WI = TN (FL origin fed on 7/1/13), and RI > WI (FL origin fed from 7/25/13-7/29/13).

<u>Effects of genetics and plasticity on the accuracy of temperature-development model-based predictions.</u>

Using multiple regression models, we identified potentially important factors affecting the accuracy of temperature-development models (Table 2.3). For larval and nymphal stages the most parsimonious model was the full model that included all variables (i.e., placement site, site

of origin, the interaction of placement site of site of origin, and feeding season), and for the adult stage the most parsimonious model included only placement site and feeding season (Table 2.4). These findings suggest that both genetics (i.e., site of origin) and plasticity (i.e., placement site, abiotic conditions, and feeding season) play an important role in the observed emergence timing of *I. scapularis* populations.

Table 2.1. Emergence timing (i.e., the median dates for which placements of replete *I. scapularis* were first observed as unfed ticks of the next stage) at four sites. Only placements in which ticks successfully molted are shown. Placements are arranged by calendar date (not year) of completion (i.e., last day) of feeding. Each pottle within a placement represents one sample unit (n represents the number of pottles), that contained either one replete female, 20 replete larvae, or 10 replete nymphs. Asterisks (*) represent pottles with the same placement date but bimodal emergence dates. Predicted emergence dates for each placement were calculated using contemporaneous field temperature data as input; the lowest and highest SE values of the relationships between temperature and development observed in the laboratory (Ogden 2004) were used to generate a range of predicted dates.

Site	Transition	Origin	n	Feeding		Emergence				
				Month/Day	Year	Range of Predicted Dates			Observed (Median)	SEM
RI	Female -> Larva	WI	13	5/2	2012	6/30/12	-	7/23/12	3/27/13	10.29
		FL	7	5/2	2012	6/30/12	-	7/23/12	3/27/13	7.86
		RI	4	5/9	2013	7/4/13	-	7/22/13	7/18/13	0.00
		RI	4	11/30	2012	6/9/13	-	7/2/13	5/21/13	0.00
		WI	2	11/30	2012	6/9/13	-	7/2/13	7/31/13	-
		TN	1	11/30	2012	6/9/13	-	7/2/13	7/31/13	-
	Larva -> Nymph	RI	9	5/10	2013	6/12/13	-	6/30/13	7/31/13	0.00
		RI	9	5/18	2013	7/3/13	-	7/24/13	7/31/13	0.00
		WI	4	6/7	2013	7/11/13	-	8/7/13	9/11/13	0.00
		TN	1	6/7	2013	7/11/13	-	8/7/13	9/11/13	0.00
		FL	5	6/7	2013	7/11/13	-	8/7/13	9/11/13	0.00
		TN	5	7/1	2013	7/25/13	-	8/31/13	6/9/14	7.80
		FL	5	7/1	2013	7/25/13	-	8/31/13	6/9/14	0.00
		RI	5	7/15	2013	8/17/13	-	10/2/13	9/11/13	0.00
		FL	3	7/29	2013	9/8/13	-	4/22/14	7/31/14	0.00
		RI	5	8/23	2013	11/17/13	-	6/10/14	6/9/14	0.00
		RI	10	9/25	2012	5/27/13	-	6/29/13	7/17/13	6.55
	Nymph -> Adult	FL	3	3/20	2013	5/30/13	-	6/20/13	7/31/13	0.00
		RI	9	5/18	2013	6/27/13	-	7/15/13	7/31/13	0.00
		RI	1	6/7	2013	7/10/13	-	7/29/13	9/11/13	-
		FL	5	7/1	2013	7/29/13	-	8/22/13	6/9/14	0.00

Table 2.1 (cont'd)

		RI	11	5/2	2012		7/14/12	-	7/1/13	8/6/12	1.68
		WI	8	2/9	2012	*	5/20/12	-	6/20/12	5/23/12	3.09
		WI	2	2/9	2012	*	5/20/12	-	6/20/12	6/15/13	-
		FL	8	5/2	2012		7/14/12	-	7/1/13	8/9/12	2.91
	Female -> Larva	RI	10	5/20	2013		7/9/13	-	8/7/13	8/10/13	0.00
		RI	4	6/15	2013		7/22/13	-	8/27/13	9/7/13	0.00
		FL	22	6/15	2013		7/22/13	-	8/27/13	8/10/13	0.00
		WI	9	11/18	2013		6/17/14	-	7/13/14	8/20/14	18.66
		RI	3	12/11	2013		6/17/14	-	7/13/14	8/20/14	0.00
		WI	5	7/1	2013	*	8/2/13	-	9/1/13	9/7/13	0.00
		WI	6	7/1	2013	*	8/2/13	-	9/1/13	5/14/14	9.28
		FL	4	7/1	2013		8/2/13	-	9/1/13	8/10/13	0.00
WI		WI	7	7/25	2013		8/30/13	-	10/12/13	5/14/14	0.00
		TN	5	7/25	2013		8/30/13	-	10/12/13	9/7/13	49.80
	Larva -> Nymph	FL	4	7/25	2013		8/30/13	-	10/12/13	9/7/13	7.00
		WI	5	8/23	2013		9/29/13	-	5/28/14	5/14/14	0.00
		FL	2	9/5	2013		5/9/14	-	6/17/14	5/14/14	-
		RI	5	9/5	2013		5/9/14	-	6/17/14	5/14/14	19.93
		WI	2	9/26	2012	*	6/15/13	-	7/13/13	9/7/13	0.00
		WI	6	9/26	2012	*	6/15/13	-	7/13/13	5/14/14	0.00
		WI	2	7/1	2013	*	8/2/13	-	8/25/13	9/7/13	-
		WI	1	7/1	2013	*	8/2/13	-	8/25/13	6/8/14	-
	Nymph -> Adult	WI	2	7/15	2013	*	8/19/13	-	9/7/13	9/7/13	-
		WI	5	7/15	2013	*	8/19/13	-	9/7/13	4/14/14	11.21
		FL	1	7/27	2014		9/26/14	-	5/2/15	12/20/14	-

Table 2.1 (cont'd)

Site	Transition	Origin	n	Feeding		Emergence				
				Month/Day	Year	Range of Predicted Dates			Observed (Median)	SEM
TN	Female -> Larva	TN	3	1/31	2014	7/29/13	-	5/25/14	5/19/14	26.36
		TN	12	2/3	2013	6/12/13	-	6/15/13	6/21/13	4.05
		FL	29	3/27	2013	6/18/13	-	6/25/13	6/21/13	3.33
		RI	10	5/2	2012	6/14/12	-	7/3/12	9/10/12	0.00
		WI	11	5/2	2012	6/14/12	-	7/3/12	9/9/12	4.87
		TN	26	2/9	2012	4/27/12	-	5/27/12	5/24/12	1.01
		FL	7	5/2	2012	6/14/12	-	7/3/12	9/10/12	0.00
		RI	9	6/21	2013	8/5/13	-	8/19/13	7/22/13	0.00
		RI	11	11/30	2012	6/2/13	-	6/4/13	6/21/13	0.00
		WI	13	11/30	2012	6/2/13	-	6/4/13	6/21/13	3.77
		TN	6	12/9	2013	5/22/14	-	6/16/14	7/10/14	0.00
	Larva -> Nymph	TN	17	6/23	2014	7/22/14	-	8/18/14	9/24/14	5.44
		RI	2	7/1	2013	7/27/13	-	8/21/13	10/25/13	-
		TN	16	7/1	2013	7/27/13	-	8/21/13	8/11/13	23.32
		FL	5	7/1	2013	7/27/13	-	8/21/13	8/11/13	0.00
		WI	3	8/6	2013	9/1/13	-	10/4/13	3/10/14	0.00
		WI	5	8/23	2013	9/21/13	-	3/1/14	4/10/14	12.92
		TN	4	8/23	2013	9/21/13	-	3/1/14	3/10/14	0.00
		TN	7	9/15	2014	5/6/14	-	3/24/15	11/30/14	0.00
		RI	5	10/25	2013	5/14/14	-	6/14/14	4/10/14	12.92
		FL	5	10/25	2013	5/14/14	-	6/14/14	4/10/14	33.40
	Nymph -> Adult	TN	5	6/23	2014	7/23/14	-	8/12/14	10/15/14	12.24

Table 2.1 (cont'd)

FL	Female -> Larva	FL	18	2/3	2013	4/18/13	-	5/12/13	3/21/13	6.95
		FL	26	3/10	2013	5/16/13	-	5/26/13	5/6/13	0.00
		RI	4	11/30	2012	3/10/13	-	4/16/13	6/4/13	3.47
		WI	11	11/30	2012	3/10/13	-	4/16/13	5/26/13	0.00
		TN	8	11/30	2012	3/10/13	-	4/16/13	5/6/13	8.86
		RI	9	12/11	2013	4/25/14	-	5/22/14	7/27/14	15.84
		WI	9	12/11	2013	4/25/14	-	5/22/14	6/27/14	7.72
	Larva -> Nymph	FL	13	6/7	2014	6/28/14	-	7/17/14	7/24/14	0.00
		RI	6	6/21	2014	7/11/14	-	7/31/14	10/12/14	0.00
		WI	1	6/21	2014	7/11/14	-	7/31/14	8/31/14	-
		FL	35	6/22	2013	7/22/13	-	8/18/13	8/25/13	0.00
		TN	8	7/25	2013	8/21/13	-	9/12/13	3/9/14	9.83
		RI	1	7/25	2013	8/21/13	-	9/12/13	6/27/14	-
		RI	5	8/25	2013	9/21/13	-	10/18/13	4/19/14	37.35
		WI	4	8/25	2013	9/21/13	-	10/18/13	6/1/14	6.50
		FL	6	8/25	2013	9/21/13	-	10/18/13	4/19/14	0.00
		RI	19	9/25	2012	11/1/12	-	1/16/13	6/10/13	6.66
	Nymph -> Adult	FL	1	5/19	2013	6/19/13	-	7/9/13	8/25/13	-
		FL	3	6/22	2013	7/23/13	-	8/12/13	10/20/13	0.00
		RI	1	7/27	2014	8/21/14	-	9/7/14	10/12/14	-
		WI	4	7/27	2014	8/21/14	-	9/7/14	10/21/14	0.00
		FL	2	7/31	2013	8/28/13	-	9/18/13	11/8/13	-

Table 2.2. Placements with a shared feeding date and significantly different emergence periods (i.e., the median dates for which placements of replete *I. scapularis* were first observed as unfed ticks of the next stage). Placements from different sites of origin represent comparisons where there was a significant genetic effect on emergence timing. Placements at different placement sites represent comparisons where there was a significant effect of plasticity on emergence timing. Only significant comparisons ($P < 0.05$) are shown. Each pottle within a placement represents one sample unit (n represents the number of pottles), that contained either one replete female, 20 replete larvae, or 10 replete nymphs. Asterisks (*) represent placements fed <5 days apart.

Instar	Origin	Placement Site	Feeding Date		N	Mean Days to Emergence	SE
Larva	RI	WI	6/15/13		4	84	0
	FL	WI	6/15/13		22	56	0
	RI	WI	5/2/12		11	96	1.68
	RI	TN	5/2/12		10	131	0
	RI	RI	11/30/12		4	172	0
	RI	TN	11/30/12		11	203	0
	RI	FL	11/30/12		4	186	3.47
	WI	RI	5/2/12		13	329	10.29
	WI	TN	5/2/12		11	130	4.87
	WI	TN	11/30/12		13	203	3.77
	WI	FL	11/30/12		11	177	0
	FL	RI	5/2/12		7	329	7.86
	FL	WI	5/2/12		8	99	2.91
	FL	TN	5/2/12		7	131	0
Nymph	WI	WI	7/25/13		7	293	0
	TN	WI	7/25/13		5	44	0
	FL	WI	7/25/13		4	44	7
	RI	RI	9/25/12		10	295	6.55
	RI	FL	9/25/12		19	258	6.66
	WI	WI	8/23/13		5	264	0
	WI	TN	8/23/13	*	5	230	12.92
	WI	FL	8/25/13		4	280	6.5
	TN	RI	7/1/13		5	343	7.8
	TN	TN	7/1/13		16	41	23.32
	TN	WI	7/25/13		5	44	0
	TN	FL	7/25/13		8	227	9.83
	FL	RI	7/1/13		5	343	0
	FL	WI	7/1/13		4	40	0
	FL	TN	7/1/13		5	41	0
	FL	RI	7/29/13	*	3	367	0
	FL	WI	7/25/13		4	44	7

Table 2.3. Regression models of the accuracy of temperature-development models in predicting larval, nymphal, and adult *I. scapularis* emergence. AICc is the corrected Akaike information criterion, ΔAICc is the difference in AICc from the best model, and w_i is the Akaike weight for each model (Burnham and Anderson 2002). Results for the model with the lowest AICc are shown in boldface.

Transition	Model	AICc	△AICc	w_i
Female -> Larva	**Site + origin + site*origin + feed season**	**2446.5**	**0.0**	**1.00**
	Site + origin + site*origin	2649.4	202.9	<0.01
	Site + origin + feed season	2662.4	215.9	<0.01
Larva -> Nymph	**Site + origin + site*origin + feed season**	**2231.8**	**0.0**	**1.00**
	Site + origin + site*origin	2244.1	12.3	<0.01
	Site + origin + feed season	2328.4	96.6	<0.01
Nymph -> Adult	Site + origin+ feed season	392.7	0.9	0.02
	Site + origin	408.6	16.8	0.44
	Site + feed season	**391.8**	**0.0**	0.00
	Origin + feed season	412.4	20.6	0.54

Table 2.4. Parameter estimates and SEs for the best-fitting regression models, with the lowest AICc values, of the accuracy of temperature-development models in predicting larval, nymphal, and adult *I. scapularis* emergence.

Transition	Parameter		Parameter Estimate	SE
Female -> Larva	Placement site			
	RI		239.87	23.32
	WI		-14.219	18.961
	TN		7.903	18.713
	Origin			
	RI		61.992	18.366
	WI		36.984	17.088
	TN		29.059	20.45
	Feed Season			
	Spring		11.669	9.775
	Summer		-265.062	16.992
	Winter		-11.543	13.024
	Placement site x origin interaction			
	RI	RI	-320.514	28.494
	RI	WI	-50.085	25.421
	RI	TN	-220.247	49.363
	WI	RI	-54.794	21.321
	WI	WI	53.257	27.527
	TN	RI	36.851	22.145
	TN	WI	-5.927	20.902
	TN	TN	-23.286	31.91
Larva -> Nymph	Placement site			
	RI		143.578	20.456
	WI		-22.499	23.977
	TN		-37.987	23.706
	Origin			
	RI		141.437	17.361
	WI		181.728	30.675
	TN		154.098	25.18
	Feed Season			
	Spring		-50.443	15.869
	Summer		-1.421	14.701
	Winter		181.728	30.675
	Placement site x origin interaction			
	RI	RI	-263.165	26.509
	RI	WI	-261.561	49.161
	RI	TN	-62.449	40.721
	WI	RI	-122.215	40.32
	WI	WI	-16.001	39.601
	TN	RI	-163.748	38.802
	TN	WI	-83.017	44.131
	TN	TN	-107.001	34.508
Nymph -> Adult	Placement site			
	RI		181.899	35.737
	WI		64.603	31.616
	TN		-6.234	39.917
	Feed Season			
	Summer		214.369	34.701

Discussion

This study uses a common garden experiment to provide novel insights in the role of genetics and plasticity on *I. scapularis* emergence at sites representative of the wide geographical range of this species. The findings of this study allowed us to explore whether the application of existing temperature-development relationships (Ogden et al. 2004) could accurately predict the emergence timing of different stages of widely-dispersed populations of *I. scapularis*, and investigate the potential roles of site-specific and genetic factors on observed emergence when controlling for abiotic conditions, while considering the seasonal timing of feeding (and associated temporally-varying temperatures and temperature-independent factors).

Evaluating the accuracy of temperature-development models.

Generally, temperature-development models under-predicted the emergence timing of all stages across all sites of origin, even when accounting for potential delays in observation due to hardening. This finding suggests that temperature-development rates, which in this case were generated under laboratory conditions for *I. scapularis* originating from Ontario, Canada, cannot necessarily be applied to other populations within this species' range without modification. These findings are generally consistent with the findings of Ogden et al. 2018, which used the same sites as this study to explore the fit of temperature-development models with various modifications (e.g. different diapause scenarios, temperature-development rates, and questing timing), and fitted these models using dragged and on-host ticks. Their study found that the inclusion of a diapause component to the temperature-development model (resulting in longer predicted times to emergence) generally improved the model fit at most sites, with the exception of TN larvae and FL nymphs, for which a temperature-development model without a diapause component appeared to best fit the observed larval and nymphal activity. This particular finding

for TN larvae and FL nymphs does not match the findings of this study, which indicate that temperature-development models under-predicted larval and nymphal emergence in TN and FL, respectively. A possible explanation for this discrepancy is that, given that best fit models in Ogden et al. 2018 are based on on-host phenologies, unfed TN larvae and FL nymphs surviving from the previous year could have become active earlier in the season, emerging in place of the diapausing larvae and nymphs.

A possible cause for the observed discrepancies between predicted and observed emergence is that the *I. scapularis* populations used in this study have site-specific temperature-development rates that are different from the Ontario population due to, for example, adaptation of populations to local abiotic regimes. If so, one might expect that, relative to the Ontario population which is located at a higher latitude in the lower temperature threshold of this species' geographical range (Leighton et al. 2012, Ogden et al. 2006), the temperature-development rates of locally-adapted populations at lower latitudes may diverge from those of the Ontario population, as average annual temperatures increase. However, we did not observe a north-south gradient regarding how well the Ontario temperature-development relationship predicted observed emergence among our sites, suggesting that site-specific temperature development rates may not follow a north-south gradient, requiring the consideration of factors such as elevation, aspect, coastal and inland effects, or simply additional project sites in order to identify broad regional patterns in temperature-development rates. Another explanation for the observed discrepancies between predicted and observed emergence is that the temperature-development rate is the same across populations, but that temperature-independent diapause is causing delays in emergence. The role of diapause for *I. scapularis* and other *Ixodes* species has been well documented (Gray et al. 2016, Ogden et al. 2004, Ogden et al. 2006) and is thus a

likely factor driving temperature-independent delays in emergence. Diapause allows populations to mitigate exposure to adverse cold winter temperatures (Gray et al. 2016) and, potentially, hot summer temperatures, and is a plausible mechanism differentially affecting the emergence timing of populations throughout the latitudinal range of this species.

Effects of genetics and plasticity on observed emergence.

The comparisons of placements sharing the same feeding date, which allow us to directly compare emergence periods while controlling for temporally-varying temperatures and temperature-independent factors, indicate that both genetics and plasticity appear to play a role in observed emergence. The number of comparisons showing statistically significant effects of placement site for emerging larvae and nymphs (5/6 and 6/7 comparisons, respectively) were higher than the number of instances where origin had a significant effect (1/8 and 1/10 comparisons, respectively); thus, there is stronger evidence for consistent effects of exposure environment than effects of genetic background on the observed emergence patterns of immature stages (Table 2.2). The conditions under which genetic differences may affect emergence may be site-specific, depend on the interaction of multiple factors, or require a greater number of comparisons in order to elucidate broad scale patterns; factors such as seasonality of feeding date, placement site, and the combination of sites being compared did not appear to affect whether site of origin had a significant effect on the emergence periods of larvae and nymphs. Therefore, when controlling for feeding date, although there are instances where genetics and plasticity significantly affect emergence timing, these effects do not appear to follow a north-south gradient, suggesting that data at a finer scale (e.g. at a site or population level) are required in order to accurately predict emergence.

Effects of genetics and plasticity on the accuracy of temperature-development model-based predictions.

The best fitting models indicate that the site of origin, placement site, and feeding season play a potentially important role in the emergence timing of *I. scapularis*. These findings indicate that for all life stages, plastic responses to different environments may influence the accuracy of temperature-development models, and that genetic differences among populations may affect the ability of these models to predict larval and nymphal emergence as well. Further, the interaction between site and origin on larval and nymphal emergence indicates that the emergence patterns based on how *I. scapularis* respond to external abiotic conditions may be complex, where larvae and nymphs of different genetic backgrounds respond to environmental cues in ways that may be difficult to predict. The role of feeding season also suggests that, beyond site-specific factors, temporally varying temperature and temperature-independent factors within a site may play an important role in *I. scapularis* emergence timing. These results highlight the degree to which site-specific genetic, abiotic, and ecological data are necessary to accurately predict the emergence patterns of a specific population.

In summary, 1) in certain cases, temperature-development relationships accurately predicted observed emergence patterns of different stages within each population. However, for the majority of cases for all stages, temperature-based predictions tended to under-predict emergence by approximately one to three months, depending on the stage, and thus likely require the inclusion of both site-specific temperature-development rates as well as temperature-independent diapause in order to accurately predict emergence at other sites. 2) We found evidence that populations from different sites of origin respond differently to abiotic cues (i.e., a genetic effect) and that for the same population, exposure to different abiotic regimes affects emergence timing as well (i.e., an effect of plasticity). The specific conditions under which

temperature and temperature-independent factors, genetics, and plasticity regulate *I. scapularis* emergence play a direct role in observed emergence patterns and thus have important implications for pathogen transmission efficiency and maintenance.

Implications.

In conclusion, it is unlikely that temperature-development relationships for a single *I. scapularis* population can be used to accurately describe widely-dispersed populations without modifications to account for site-specific responses to temperature as well as temperature-independent factors. The results of this study support a previous finding that diapause appears to play an idiosyncratic role in the activity patterns of different *I. scapularis* populations throughout the Eastern U.S. (Ogden et al. 2018). Future studies using standardized laboratory experiments, such as those conducted for Ontario ticks (Ogden et al. 2004), would allow us to estimate temperature-development relationships among different populations, determine how these relationships vary among populations, and investigate potential spatial patterns across a latitudinal gradient. Also, additional life cycle field experiments that include multiple feeding dates within a season for all life stages (such as those conducted by Gray 1982, Estrada-Peña 2004, and Ogden et al. 2004) are needed to elucidate the role of temperature-independent diapause on the observed emergence of each stage and understand how these effects may vary temporally within a season for a given population. These experiments would provide an improved understanding of the potential roles that temperature and temperature-independent factors play in the seasonal activity patterns of different *I. scapularis* populations and lend insight to the type and scale of data needed to accurately predict the emergence patterns of a given population of *I. scapularis*. As climate change alters the existing abiotic regimes for established populations, and invading populations are exposed to new abiotic regimes, an

improved understanding of the underlying factors driving observed emergence, and whether the impacts of these factors vary among populations, will improve our ability to determine how generalizable existing models are in their ability to accurately predict the dynamics of different populations for this species.

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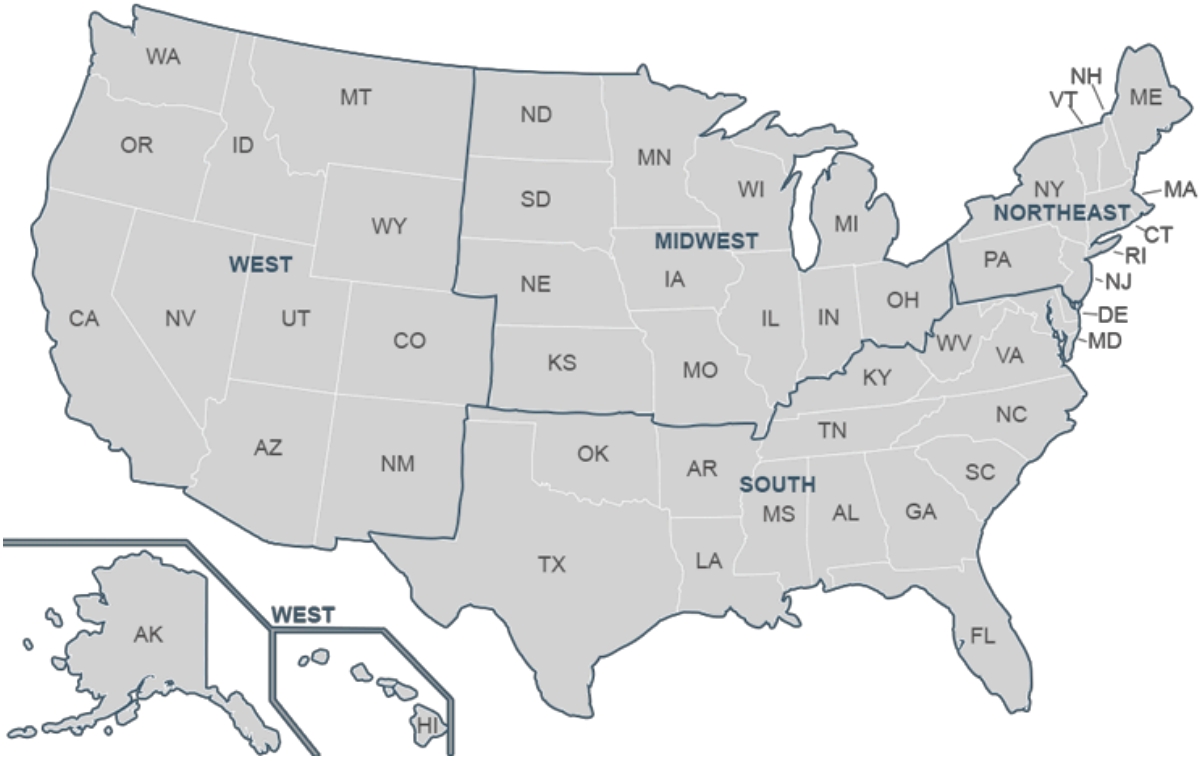
APPENDICES

APPENDIX B: Supplemental Material



Supplemental Figure 2.1. Map of states belonging to regional designations in the United States. In this study, WI is in the Midwest, RI is in the Northeast, and TN and FL are in the South.

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CHAPTER 3: AN INVESTIGATION OF ABIOTIC FACTORS, GENETICS, LIFE-CYCLE PLASTICITY, AND DIAPAUSE ON THE SURVIVAL PATTERNS OF IMMATURE *IXODES SCAPULARIS* IN THE EASTERN UNITED STATES.

Abstract

Although *Ixodes scapularis*, the primary North American tick vector of the Lyme disease pathogen, is widely established throughout the eastern United States (US), Lyme disease incidence is localized in the north central and northeastern US. These differences may be due in part to variation in survival among tick populations, influenced by key underlying biotic and abiotic factors. To address this problem, we conducted a field experiment monitoring the maximum and post-peak days of survival of immature *I. scapularis* collected from and redistributed among four sites (Rhode Island, Wisconsin, Tennessee, and Florida) that are located throughout this species' range in the eastern US. We compared the survival patterns of local and transplanted ticks, ticks that were placed at northern and southern sites, and ticks of northern and southern origin. We then investigated the potentially important explanatory genetic and abiotic factors contributing to observed tick survival patterns. Nymphal survival was higher for transplanted nymphs than for local nymphs; larval survival was higher at northern placement sites than at southern placement sites; and nymphal survival was higher for nymphs from northern sites of origin than nymphs from southern sites of origin. Temperature, relative humidity, an interaction between site of origin and placement site, and diapause were selected as important factors in larval and nymphal survival, suggesting that site-specific abiotic factors and geographic genetic differences among tick populations may be contributing to the survival patterns observed in this study. The results of this study indicate that southern conditions may be generally less conducive to tick survival relative to northern conditions and that the survival of

northern genotypes may be higher relative to southern genotypes, although further studies with greater samples sizes would be necessary in order to fully explore these preliminary patterns. These findings and similar future studies have important implications for understanding the relatively lower Lyme disease incidence in the southern regions of this species' range and predicting how Lyme disease risk may change as populations of *I. scapularis* are exposed to new abiotic regimes via invasion and climate change.

Introduction

The geographic distribution of reported Lyme disease cases indicates that incidence is highest in the upper midwestern and northeastern United States (US) although the vector tick, *Ixodes scapularis*, is found throughout the south central and southeastern regions (Eisen et al. 2016). There are several non-mutually exclusive hypotheses that may contribute to lower incidence rates in the southern regions (defined as south of 37° N latitude) relative to northern regions (defined as north of 37° N latitude). First, greater host diversity in the south may result in the dilution of competent reservoir hosts (Ostfeld and Keesing 2000). Second, there is a greater abundance and utilization of incompetent lizard hosts in the south (Apperson et al. 1993). Third, genetic differences between ticks of northern and southern origin may lead to differences in host-seeking behavior, resulting in higher encounter rates between humans and northern nymphs and humans (Arsnoe et al. 2015, Arsnoe et al. 2019). Fourth, regional differences in abiotic conditions may reduce pathogen transmission via their effects on tick phenologies (Ogden et al. 2004) that may contribute to lower tick densities (Tugwell and Lancaster 1962, Barnard 1981, Koch 1982, Oliver et al. 1993, Clark et al. 1998, Stromdahl et al. 2014). These differences in density may be due, in part, to less hospitable abiotic conditions in the south compared to those in the north and contribute to lower survival of ticks in southern populations (Ginsberg et al. 2014).

Abiotic factors affecting *I. scapularis* survival

Abiotic factors, particularly temperature and relative humidity (Vail and Smith 1998, Ogden et al. 2004), play a critical role in the survival of *I. scapularis*, which are prone to freezing, desiccating, overheating, drowning, and exhausting finite energy reserves while seeking a host (Eisen et al. 2016). There exists a temperature range above and below which temperature affects survival, and within which relative humidity affects survival (Eisen et al. 2016).

Vandyk et al. (1996) found that the lower temperature threshold of immature *I. scapularis*, as defined by increased mortality after 8 hours of exposure, is approximately -10° C, with some variation based on life stage, engorgement status, and the duration of exposure. Temperatures below this threshold can contribute to higher mortality rates (Eisen et al. 2016) by causing cold damage to the tick (Burks et al. 1996, Vandyk et al. 1996). Ogden et al. (2004) found that the upper temperature threshold of immature *I. scapularis*, as defined by longer pre-molt periods and induced pathological effects, is approximately 30° C. Temperatures above this threshold can contribute to higher mortality rates (Ogden et al. 2004) by damaging the integument (i.e., cuticle), thereby increasing rates of desiccation (Needham and Teele 1991, Eisen et al. 2016). Relatedly, higher temperatures may also require ticks to modify host-seeking behaviors and spend a greater amount of time rehydrating in the duff layer to mitigate the risk of desiccation (Vail and Smith 1998), resulting in a higher likelihood that finite energy reserves will be expended prior to finding a host (Eisen et al. 2016). Therefore, *I. scapularis* populations that are more frequently exposed to higher temperatures may experience higher mortality rates either directly or indirectly through not obtaining a bloodmeal.

Between the *I. scapularis* upper and lower temperature thresholds, relative humidity is positively correlated with higher survival (Needham and Teele 1991, Stafford 1994, Vail and

Smith 1998, Schulze et al. 2002). *Ixodes ricinus* (a species related to *I. scapularis* that is distributed throughout Europe) requires a relative humidity of at least 80%, below which prolonged exposure results in mortality due to desiccation (Gray et al. 2009). However, the threshold relative humidity for survival of *I. scapularis* may be higher than 80%, as experimental evidence indicates a pronounced difference in the survival between *I. scapularis* unfed larvae held at 100% and 93% (where 50% mortality occurred after 67.1 versus 26.6 days, respectively) and unfed nymphs held at 93% and 85% (where 50% mortality occurred after 168.5 versus 118.8 days, respectively) relative humidity (Stafford 1994, Rogers et al. 2007). Field evidence also indicates that tick adverse moisture events (TAMEs) of >8 hours of exposure to <82% RH are negatively correlated with densities of questing *I. scapularis* nymphs later in the season (Berger et al. 2014). In nature, the risk of desiccation during periods of low relative humidity may be mitigated by the utilization of microhabitats with higher humidity, such as the duff layer (Schulze et al. 2002). Some evidence suggests that the refuge provided by these microhabitats may contribute to a non-random distribution of host-seeking ticks within a site, where tick densities are positively correlated with shrubs, leaf litter, and duff (Schulze et al. 2002). In summary, the relative humidity and available microhabitats of different sites play a critical role in the mortality rates of *I. scapularis* populations, as individual ticks expend energy to use these habitats in search of hosts while conserving finite energy reserves and mitigating the risk of fatal desiccation.

Genetic factors affecting *I. scapularis* survival

Population genetics studies show a pattern in which *I. scapularis* genotypes in northern regions differ from those southern regions; further, these genotypes are expanding southward (Zee et al. 2015). While the biological traits associated with these genotypes have not yet been described, this geographical pattern provides a basis for the consideration of regional genetic differences in the

investigation of *I. scapularis* survival patterns. Previous laboratory experiments that exposed immature *I. scapularis* from northern and southern origins to northern and southern light:dark and temperature conditions found that, while there were clear differences in survival of ticks from different populations, there was no north-south gradient pattern in survival (Ginsberg et al. 2014). These findings suggest that survival patterns may be site-specific, perhaps due to local adaptation to site-specific environmental factors or genetic drift, and merit further validation with empirical field data. A key way in which genetics may contribute to tick survival is via diapause (i.e., genetically-enabled dormancy), which is an adaptation that allows ticks to mitigate future adverse environmental conditions (Belozerov 2009). Developmental and behavioral diapause cause adaptive arrests in development and activity, respectively, that potentially stall progression at multiple points throughout the life cycle (e.g., egg-laying, hatching, molting, hardening, and questing), and thus plays a critical role in regulating the activity periods of each life stage (Belozerov 2009). One commonly accepted role of diapause in the *I. scapularis* life cycle is the arrested development of overwintering ticks from northern sites (Yuval and Spielman 1990) to mitigate harsh winter conditions. However, the role of diapause at southern sites is less clear. Based on the best-fitting models for field-collected tick data, Ogden et al. (2018) hypothesized that despite much warmer winter temperatures relative to northern sites diapause may reduce the winter activity of immature *I. scapularis* at southern sites. Therefore, while regional genetic differences exist for this species, the ecological consequences of these differences may be complex and idiosyncratic.

In this study, we compare the survival of immature *I. scapularis* collected from and redistributed among four sites (Rhode Island, Wisconsin, Tennessee, and Florida) that are located throughout this species' range in the eastern US. We conducted a field experiment to investigate

the relative roles of genetics, plasticity, diapause, and abiotic factors on survival. Based on the evidence documenting the invasion of *I. scapularis* from established populations into new areas (Kelly et al. 2014) we also investigate the following questions. (1) Do local (i.e., established) ticks have a higher survival than transplanted (e.g. invading) ticks? Ticks that are locally adapted may survive longer than transplanted ticks under local conditions. (2) Do ticks under northern conditions have a higher survival than ticks under southern conditions? Temperatures at northern sites are cooler than in the south during the spring and summer questing season, reducing metabolic activity and the consumption of energy reserves and thereby allowing ticks placed at northern sites to live longer than ticks placed at southern sites. (3) Do ticks from northern sites of origin have a higher survival than ticks from southern sites of origin? Ticks of northern origin may be more robust and live longer than ticks of southern origin and only found in the South.

Methods

Field Sites

We observed the changes in abundance of larval, nymphal, and adult *I. scapularis* instars from emergence to death at four sites in the eastern US. All sites supported populations of *I. scapularis*, as evidenced by the ability to collect questing adult blacklegged ticks and immature ticks on vertebrate hosts (Ogden et al. 2018). Project sites were located in two Lyme endemic regions: Wisconsin (WI) and Rhode Island (RI), and two non-endemic regions: Tennessee (TN) and Florida (FL). Wisconsin is located in northern central US; Rhode Island is in the northeastern US; Tennessee is in the inland southeastern US; Florida is in the southeastern US. Forest at the Rhode Island site (41.5 °N) was dominated by oaks (*Quercus spp.*) and maples (*Acer spp.*) with an understory containing both shrubby and open spaces. Forest at the Wisconsin site (42.94 °N) was dominated by oaks (*Quercus spp*.) and maples (*Acer spp*.) with a shrubby

understory containing mixed saplings and some invasive understory species. Forest at the Tennessee site (36.01°N) 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. Forest at the Florida site at Tall Timbers Research Station, FL (latitude 30.65 °N) was dominated oaks (*Quercus spp*.) and maples (*Acer spp*.) with a dense, shrubby understory containing mixed saplings and invasive understory species.

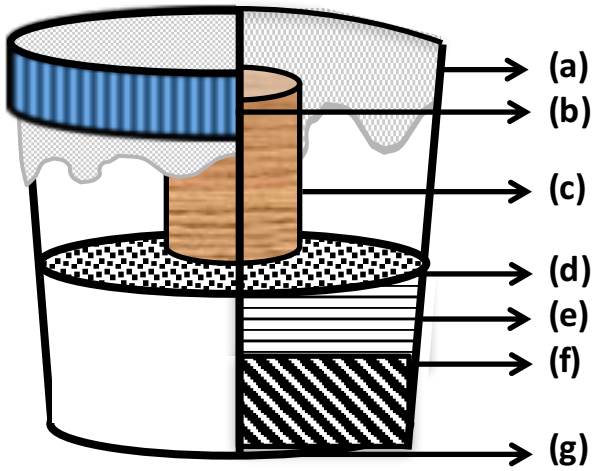

Figure 3.1. A diagram of a pottle, external (left) and internal (right) that contained ticks that were monitored. Components included synthetic cloth mesh (a, h), a modified pottle lid allowing for air flow and precipitation to enter each pottle (b), an oak dowel for ticks to ascend (c), leaf litter (d), duff (e), soil (f), and tape (g).

Microcosm design (Figure 3.1)

Live tick specimens were housed in 60 ml polypropylene specimen containers (i.e., pottles) that were 6.1 cm in diameter x 7.25 cm tall. The lids and bases of these containers were cut to create a column through which precipitation could pass. An 11 cm x 11 cm piece of synthetic cloth mesh was secured to the base of each pottle using caulk and a cable tie to hold the organdy cloth mesh in place during drying; once dry, the edge of the organdy cloth mesh was further secured

by a 5.08 cm wide piece of tape that encircled the bottom circumference of the pottle. A soil core approximately 3.5 cm deep, containing layers of leaf litter, duff and soil, was collected from the surrounding area of each enclosure location and placed into each pottle. Another 11 cm x 11 cm piece of organdy cloth mesh was secured over the top of each container using the screw top lid to hold the organdy cloth mesh in place. An oak dowel that was .25 cm in diameter x 5.08 cm tall was inserted into the center of each soil core to provide ticks with an object to climb and quest from. Pottles were covered with protective plastic crates (44 cm x 36 cm x 27 cm). Crates were arranged haphazardly within the same forest stand at each site.

Producing replete ticks to be placed into the field

Live *I. scapularis* adults were collected during peak questing periods at each field site. Within two weeks post-collection, adult ticks were mated and fed on laboratory New Zealand white rabbits (Oryctolagus cuniculus) using procedures approved through Michigan State University's Institutional Animal Use and Care Committee permit #06/12-103-00. Replete female *I. scapularis* were returned to sites, placed in modified pottles that exposed them to ambient conditions (Figure 3.1), housed in secure crates in the field, and monitored for oviposition, larval hatch, and changes in larval survivorship. A subset of pottles were subsampled destructively at different time points throughout the seasonal emergence period and used to produce engorged larvae. Larvae were fed on laboratory mice (Mus musculus), divided among pottles, and then returned to the respective field site (i.e., site of origin) to be monitored for nymphal molt and changes in nymphal survivorship. Similarly, a subset of pottles with molted, flat nymphs were then used for producing engorged nymphs (also fed on laboratory mice). Replete ticks were housed at 95% RH at 25 C under a 12:12 light dark cycle and returned to their site of origin as soon as possible, typically within 10 days of detachment. At our WI site, replete ticks fed in the

fall and winter were housed in an outdoor chamber where they were exposed to comparable ambient Michigan conditions until they could be returned to their site of origin prior to the spring season. Subsets of replete females and larvae were transplanted to other sites. Replete nymphs were not transplanted to other sites due to low numbers produced.

Placement dates

We fed and placed ticks in the field during ecologically relevant times in their seasonal emergence period, based on drag sampling and tick burden data at these field sites (Ogden et al. 2018). The relative activity patterns of different instars were referenced (Ogden et al. 2018) to create pre-peak, peak, and post-peak placements, such that samples of replete *I. scapularis* were fed and returned to the field during plausible, natural periods of host seeking and feeding for that region.

Data collection and response variables

This study was conducted from May 2012 – September 2014. Annually, at each project site, 2-3 cohorts of replete ticks for each life stage were placed in the field and monitored. Each pottle received fully replete ticks of one life stage: 20 larvae or 1 adult female. Additional pottles containing only soil cores and no added ticks were observed at each site to confirm that soil cores were not contaminated with ticks already present in the leaf litter.

Visible replete ticks were noted in pottles the day that they were deployed and then approximately every 2 weeks thereafter. Pottles were checked by searching for ticks on all visible surfaces of the pottle, including the organdy cloth mesh top, the clear sides of the tube, the dowel, and the top of the leaf litter. During spring, summer, and early fall, when ticks emerge and exhibit host-seeking activity at all sites, pottles were monitored every 2-3 weeks. During the late fall and winter, only enclosures in southern sites were monitored every 4 weeks.

The number of flat larval ticks in each pottle was estimated with raw counts if 50 or fewer ticks were observed. Otherwise, a categorical estimate > 50 or 50> x > 200 was assigned to that pottle. Pottles were monitored until greater than one year of no activity was observed, after which all ticks in the pottle were recorded as expired.

For each pottle, the date in which the subsequent life stage was first observed (e.g., the date a molted nymph was first observed in a given pottle of engorged larvae) was recorded as the date of observed emergence. The date on which the maximum observed abundance was observed for each pottle was determined to be the date of peak abundance for that pottle. In cases when the same maximum abundance estimate was observed for multiple dates (e.g. abundance plateaued), the first date of maximum abundance was counted as the date of peak abundance. In cases where declining abundance estimates increased in a later observation, the higher abundance estimate was assigned to the lower previous observations, creating a steady decline in abundance post-peak, based on the assumption that not all live ticks were observed in some post-peak observations.

We measured the temperature outside of the crates at each site using HOBO Pro v2 (#U23-001) data loggers programmed to record every 30 min. We also placed two iButton Hygrochron (#DS1923) data loggers programmed to record every 30 min; the first logger was placed inside of a pottle containing no added ticks, and the second logger was placed within the same crate but outside of the pottle. We compared temperature and relative humidity readings among these three data loggers to confirm that within-pottle abiotic conditions were not substantially different from ambient abiotic conditions. Readings from HOBO loggers were converted to daily means. In the event of a logger malfunction due to freezing or inundation, a linear relationship (Berger et al. 2014) was established between the logger and a backup logger

installed at each site, using hourly data points from 5 days before and 5 days after the gap in logger data (using a total of 240 data points). When both loggers at a site malfunctioned, we used weather station data from the nearest local airport.

Geographical patterns of survival

We investigated three questions about survival patterns of immature ticks. 1) Do local ticks had a higher survival than transplanted ticks? 2) Do ticks under northern conditions have a higher survival than ticks under southern conditions? 3) Do ticks from northern sites of origin had a higher survival than ticks from southern sites of origin? To address these questions, we created separate cumulative mortality distributions for placements that were fed and placed at field sites at the same time of year (to account for temporally-varying abiotic factors, such as photoperiod). For each cumulative mortality distribution, post-peak abundance estimates over time were added from all pottles belonging to the same placement. Maximum differences in cumulative mortality between placements were then grouped into relevant pairwise comparisons to investigate broad patterns in survival.

Factors affecting tick survival

We fit regression models to evaluate which potential predictive variables related to abiotic factors, diapause, and genetics best explained observed survival of ticks from emergence to death (i.e., from the first to the final date that active ticks were observed), and from peak activity to death (i.e., from the date of peak abundance to the final date that active ticks were observed). The residuals of these values appeared to be approximately normally-distributed, and thus did not require transformations or alternative tests. Emergence to death and peak activity to death were modeled separately, the former to account for periods of pre-peak activity in the maximum measure of survival, and the latter to investigate the post-peak declines in abundance (of which

negative changes in abundance were assumed to be largely attributable to tick mortality, without the pre-peak, positive changes in abundance caused by emerging ticks).

Maximum survival

The outcome variable was the maximum number of days that ticks were observed in each pottle, from emergence to death. Explanatory variables were the number of days from the date of feeding to the date that emergence was first observed, the number of TAMEs (i.e., the number of tick adverse moisture events of >8 hours exposure to <82% RH) from the first to final date that active ticks were observed, mean temperature of the season in which emergence occurred, mean temperature of the season in which death occurred, the interaction between site of origin and placement site, and diapause as a binary term.

Post-peak survival

The outcome variable was the number of days that active ticks were observed in each pottle, from peak abundance to death. Explanatory variables were the number of days from the date of feeding to the date that emergence was first observed, the number of TAMEs (i.e., the number of tick adverse moisture events of >8 hours exposure to <82% RH) from the first to final date that active ticks were observed, mean temperature of the season in which emergence occurred, mean temperature of the season in which death occurred, the interaction between site of origin and placement site, and diapause as a binary term.

For maximum and post-peak survival of active larvae and nymphs, the occurrence of diapause was determined based on whether the observed emergence date for a given pottle fell within an interval of predicted emergence dates, estimated using previously established temperature-development relationships (Ogden et al. 2004) and field temperature data. The soil cores in pottles prevented the observation of hardening ticks, so a 21-day period was added to the

upper bound of each interval to account for ticks that may have developed but were not yet active.

For both maximum and post-peak survival models, there were seven potential explanatory models; possible models included six models with five explanatory factors and one model with all factors (Table 3.1). Each pottle represents one sample unit. The most parsimonious models were selected using Akaike's information criterion corrected for sample size (AICc; Burnham and Anderson 2002), which measures how well a model fits the data relative to the number of parameters included in that model (Table 3.1). Statistics were performed using Program R (R Development Core Team 2014).

Results

Geographical patterns of tick survival

Local versus transplanted larvae and nymphs (Figure 3.2).

The numbers of cases where local larvae had higher (6/11 cases) and lower (5/11 cases) survival than transplanted larvae were similar. In the majority of cases, transplanted nymphs (5/6 cases) had higher survival than local nymphs (1/6 cases).

Northern versus southern placement sites (Figure 3.3).

In all cases (7/7 cases), larvae placed at northern sites had higher survival than larvae placed at southern sites. However, the numbers of cases where nymphs placed at northern sites had higher (2/4) and lower (2/4 cases) survival than nymphs placed at southern sites were similar.

Northern versus southern sites of origin (Figure 3.4).

The numbers of cases where larvae from northern sites of origin had higher (5/8 cases) and lower (3/8 cases) survival than larvae from southern sites of origin larvae were similar. In the majority of cases, nymphs from northern sites of origin had higher (5/6 cases) survival than nymphs from

southern sites of origin (1/6 cases).

Factors affecting tick survival

Maximum survival. According to the most parsimonious models (Table 3.1) TAMEs (i.e., the number of tick adverse moisture events of >8 hours exposure to <82% RH from the first to final date that active ticks were observed), mean temperatures of the season in which emergence occurred, mean temperatures of the season in which death occurred, and diapause were important explanatory factors of larval maximum survival (Table 3.2). Days between placement and emergence, TAMEs, mean temperatures of the season in which death occurred, and diapause were important explanatory factors of nymphal maximum survival (Table 3.2). The interaction between placement site and site of origin was also included in the best-fitting models of the maximum survival of both life stages.

Post-peak survival

According to the most parsimonious models (Table 3.1), days between placement and peak abundance, TAMEs, mean temperatures of the season on which emergence occurred, mean temperatures of the season in which death occurred, and diapause were important explanatory factors of larval post-peak survival (Table 3.2). Days between placement and peak abundance, TAMEs, mean temperatures of the season in which death occurred, and diapause were important explanatory factors of nymphal post-peak survival (Table 3.2). The interaction between placement site and site of origin was also included in the best-fitting models of the post-peak survival of both life stages.

Several explanatory factors were shared among the best-fitting models for active larval and nymphal maximum and post-peak survival (Table 3.2), with some notable differences. First, the duration of time from placement to emergence and peak abundance appeared to affect the

maximum survival of nymphs as well as the post-peak survival of larvae and nymphs, respectively, but not the maximum survival of larvae. Second, the mean temperatures of the season in which emergence occurred affected the maximum and post-peak survival of larvae, but not nymphs.

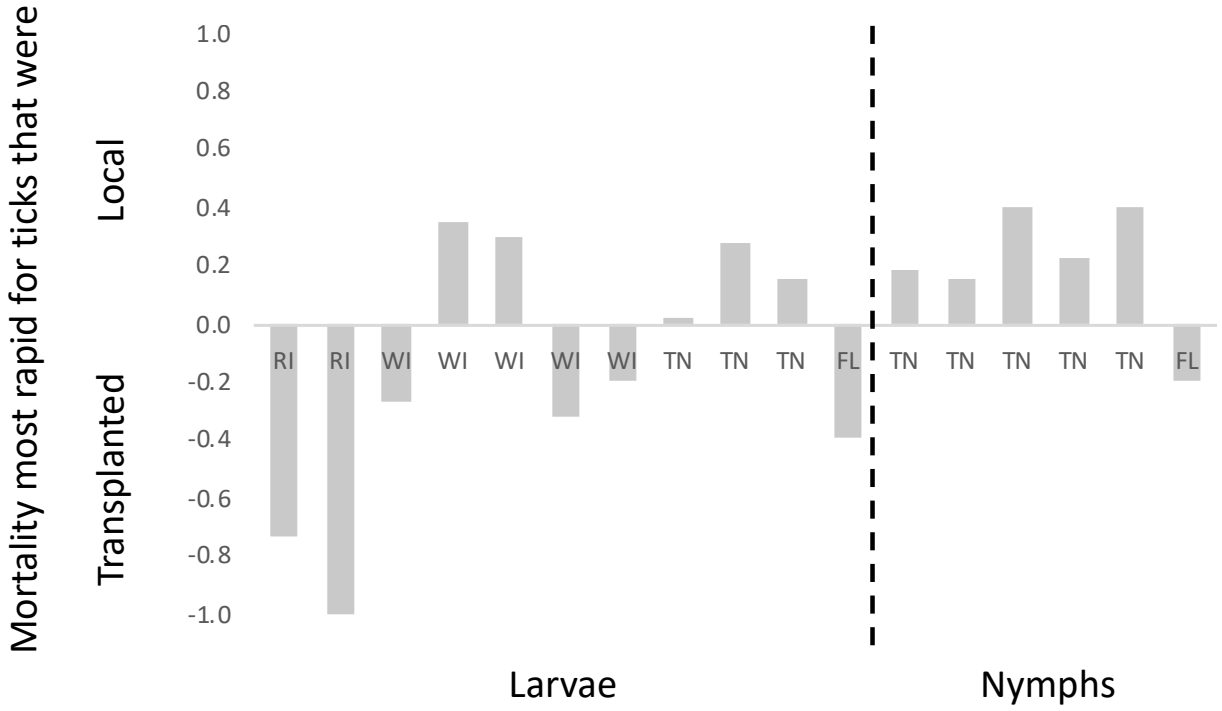

Figure 3.2. The maximum differences in cumulative mortality did not show a pattern for *I. scapularis* larvae but indicated that cumulative mortality may be lower for transplanted versus local active nymphs. Negative values of maximum differences in cumulative mortality between two placements (one local placement and one transplanted placement) indicate that maximum cumulative mortality was higher for transplanted versus local ticks. Site abbreviations for Rhode Island (RI), Wisconsin (WI), Tennessee (TN), and Florida (FL) indicate the placement site of the ticks in each comparison.

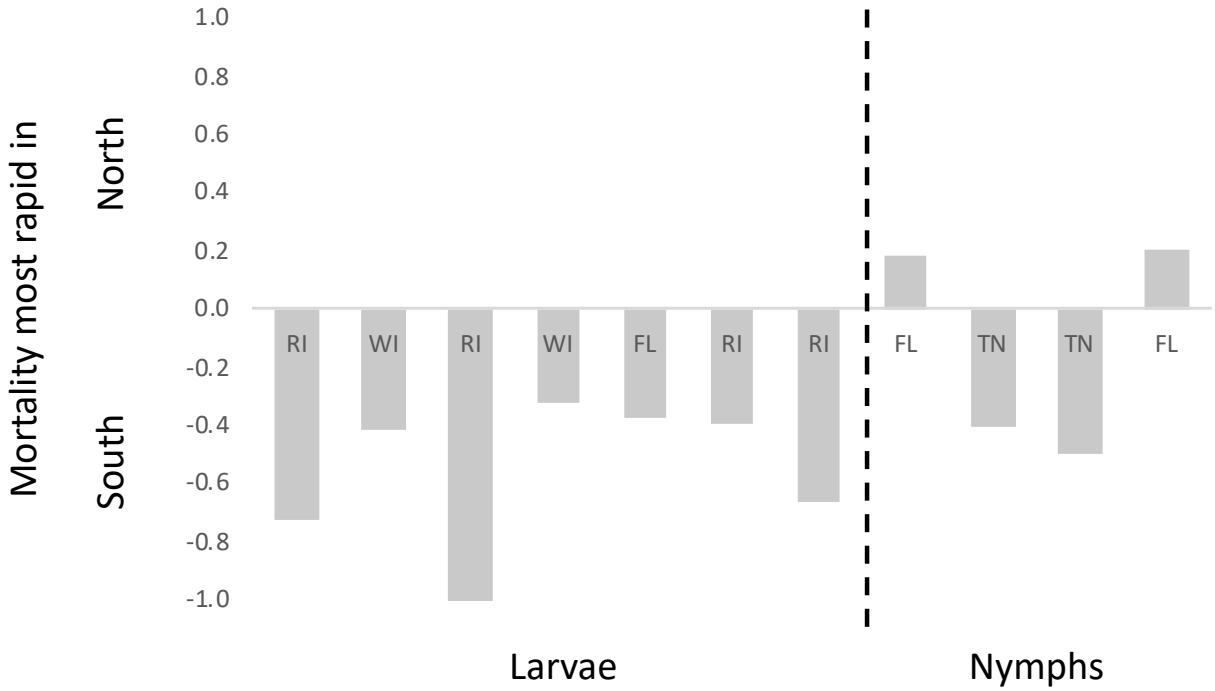

Figure 3.3. The maximum differences in cumulative mortality indicated that cumulative mortality may be lower for active *I. scapularis* larvae placed at northern versus southern sites but did not show a pattern for nymphs. Negative values of maximum differences in cumulative mortality between two placements (one northern and one southern placement site) indicate that maximum cumulative mortality was higher at southern sites versus northern sites. Site abbreviations for Rhode Island (RI), Wisconsin (WI), Tennessee (TN), and Florida (FL) indicate the site of origin of the ticks in each comparison.

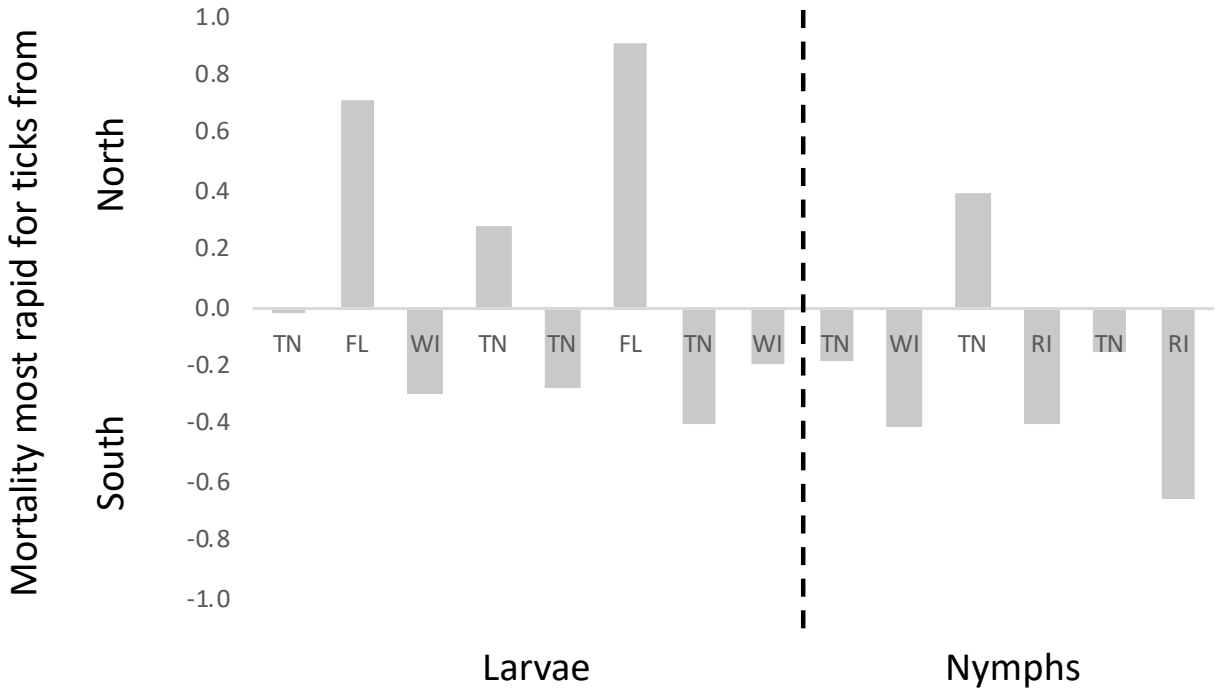

Figure 3.4. The maximum differences in the cumulative mortality of *I. scapularis* from northern versus southern sites of origin did not show a pattern for active larvae or nymphs. Negative values of maximum differences in cumulative mortality between two placements (taken from one northern and one southern site of origin) indicate that maximum cumulative mortality did not consistently differ based on site of origin. Site abbreviations for Rhode Island (RI), Wisconsin (WI), Tennessee (TN), and Florida (FL) indicate the placement site of the ticks in each comparison.

Table 3.1. Evidentiary support for models of the maximum and post-peak survival of active larval, nymphal *I. scapularis*. AICc is the corrected Akaike information criterion, ΔAICc is the difference in AICc from the best model, and w_i is the Akaike weight for each model (Burnham and Anderson 2002). Results for the best-fitting model with the lowest AICc are shown in boldface. Days feed to emerge, the number of days between feeding and when active molted ticks were first observed; Days feed to peak, the number of days between feeding and peak activity of active molted ticks; TAME's, the number of events of >8 hours at <82% RH; T_{avg} Season of emergence, the mean temperature of the season in which ticks emerged; T_{avg} Season of death, the mean temperature of the season in which ticks were last observed; Placement site x origin interaction, the interaction between placement site and site of origin; Diapause, a binary term, determined by whether observed emergence fell within a range of expected dates using temperature-development models (with 21 days added to predicted ranges to account for larval or nymphal hardening).

Transition	Outcome Variable	Model	AICc	ΔAICc	w_i
Female -> Larva	Maximum Survival	Days feed to emerge + TAMEs + Tavg Season of emergence + Tavg Season of death + Placement site*origin + Diapause	2004.8	1.5	0.31
		TAMEs + Tavg Season of emergence + Tavg Season of death + Placement site*origin + Diapause	**2003.3**	**0.0**	**0.66**
		Days feed to emerge + Tavg Season of emergence + Tavg Season of death + Placement site*origin + Diapause	2098.7	95.4	0.00
		Days feed to emerge + TAMEs + Tavg Season of death + Placement site*origin + Diapause	2009.4	6.1	0.03
		Days feed to emerge + TAMEs + Tavg Season of emergence + Placement site*origin + Diapause	2024.7	21.4	0.00
		Days feed to emerge + TAMEs + Tavg Season of emergence + Tavg Season of death + Diapause	2132.5	129.2	0.00
		Days feed to emerge + TAMEs + Tavg Season of emergence + Tavg Season of death + Placement site*origin	2028.6	25.3	0.00
Larva -> Nymph	Post-peak Survival	**Days feed to peak + TAMEs + Tavg Season of emergence + Tavg Season of death + Placement site*origin + Diapause**	**1349.8**	**0.0**	**1.00**
		TAMEs + Tavg Season of emergence + Tavg Season of death + Placement site*origin + Diapause	1548.6	198.8	0.00
		Days feed to peak + Tavg Season of emergence + Tavg Season of death + Placement site*origin + Diapause	1494.7	144.9	0.00
		Days feed to peak + TAMEs + Tavg Season of death + Placement site*origin + Diapause	1377.9	28.1	0.00
		Days feed to peak + TAMEs + Tavg Season of emergence + Placement site*origin + Diapause	1361.0	11.2	0.00
		Days feed to peak + TAMEs + Tavg Season of emergence + Tavg Season of death + Diapause	1382.4	32.6	0.00
		Days feed to peak + TAMEs + Tavg Season of emergence + Tavg Season of death + Placement site*origin	1449.3	99.5	0.00
Female -> Larva	Maximum Survival	Days feed to emerge + TAMEs + Tavg Season of emergence + Tavg Season of death + Placement site*origin + Diapause	653.6	1.9	0.28
		TAMEs + Tavg Season of emergence + Tavg Season of death + Placement site*origin + Diapause	660.9	9.2	0.01
		Days feed to emerge + Tavg Season of emergence + Tavg Season of death + Placement site*origin + Diapause	754.8	103.1	0.00
		Days feed to emerge + TAMEs + Tavg Season of death + Placement site*origin + Diapause	**651.7**	**0.0**	**0.71**
		Days feed to emerge + TAMEs + Tavg Season of emergence + Placement site*origin + Diapause	664.1	12.4	0.00
		Days feed to emerge + TAMEs + Tavg Season of emergence + Tavg Season of death + Diapause	736.0	84.3	0.00
		Days feed to emerge + TAMEs + Tavg Season of emergence + Tavg Season of death + Placement site*origin	664.2	12.5	0.00
Larva -> Nymph	Post-peak Survival	Days feed to peak + TAMEs + Tavg Season of emergence + Tavg Season of death + Placement site*origin + Diapause	615.1	1.7	0.20
		TAMEs + Tavg Season of emergence + Tavg Season of death + Placement site*origin + Diapause	649.7	36.3	0.00
		Days feed to peak + Tavg Season of emergence + Tavg Season of death + Placement site*origin + Diapause	782.6	169.2	0.00
		Days feed to peak + TAMEs + Tavg Season of death + Placement site*origin + Diapause	**613.4**	**0.0**	**0.48**
		Days feed to peak + TAMEs + Tavg Season of emergence + Placement site*origin + Diapause	614.2	0.8	0.32
		Days feed to peak + TAMEs + Tavg Season of emergence + Tavg Season of death + Diapause	716.6	103.2	0.00
		Days feed to peak + TAMEs + Tavg Season of emergence + Tavg Season of death + Placement site*origin	626.7	13.3	0.00

Table 3.2. Parameter estimates and standard errors (SE) for the models with the lowest AICc of the maximum and post-peak survival of active larval, nymphal *I. scapularis*. Days feed to emerge, the number of days between feeding and when active molted ticks were first observed; Days feed to peak, the number of days between feeding and peak activity of active molted ticks; TAME's, the number of events of >8 hours at <82% RH; Tavg Season of emergence, the mean temperature of the season in which ticks emerged; Tavg Season of death, the mean temperature of the season in which ticks were last observed; Placement site x origin interaction, the interaction between placement site and site of origin; Diapause, a binary term, determined by whether observed emergence fell within a range of expected dates using temperature-development models (with 21 days added to predicted ranges to account for larval or nymphal hardening).

Transition	Outcome Variable	Parameter		Parameter Estimate	SE
Female -> Larva	Maximum Survivorship (days from emergence to death)	TAMEs		0.91	0.08
		T_{avg} Season of emergence		5.84	1.95
		T_{avg} Season of death		-11.15	2.16
		Placement site x origin interaction			
		TN	RI	299.06	37.83
		TN	WI	344.42	38.82
		TN	FL	258.55	36.16
		FL	RI	284.24	48.90
		FL	WI	269.66	45.95
		FL	FL	160.77	41.71
		Diapause		-73.75	13.27
	Post-peak Survivorship (days from peak abundance to death)	Days feed to peak		0.61	0.03
		TAMEs		0.71	0.05
		T_{avg} Season of emergence		5.19	0.94
		T_{avg} Season of death		-2.20	0.62
		Placement site x origin interaction			
		TN	RI	59.68	11.70
		TN	FL	47.05	10.81
		FL	RI	54.63	16.48
		FL	WI	57.97	14.46
		FL	FL	43.58	14.14
		Diapause		-75.57	6.78
Larva -> Nymph	Maximum Survivorship (days from emergence to death)	Days feed to emerge		0.18	0.06
		TAMEs		2.05	0.16
		T_{avg} Season of death		-3.34	0.98
		Placement site x origin interaction			
		RI	RI	-159.10	40.73
		RI	TN	-152.85	41.99
		RI	FL	-151.26	38.52
		TN	TN	-77.60	25.10
		TN	FL	91.24	25.27
		Diapause		52.37	15.43
	Post-peak Survivorship (days from peak abundance to death)	Days feed to peak		0.24	0.04
		TAMEs		1.74	0.08
		T_{avg} Season of death		-1.27	0.82
		Placement site x origin interaction			
		RI	RI	-175.66	25.58
		RI	TN	-62.99	22.33
		RI	FL	-68.33	18.22
		TN	TN	-75.16	18.07
		FL	TN	-77.98	25.50
		Diapause		40.50	11.35

Discussion

This study used a common garden experiment to explore broad geographical patterns in *I.*

scapularis survival and elucidate potentially important underlying factors affecting these patterns. The findings of this study allowed us to investigate differences in survival among local and transplanted ticks, northern and southern sites, and ticks of northern and southern sites of origin, while controlling for the seasonal timing of feeding (and associated temporally-varying temperatures and temperature-independent factors). We also investigate potential important site-specific and genetic factors affecting maximum and post-peak survival and discuss the implications of these factors with respect to the observed survival patterns.

Geographical patterns of tick survival

In summary, 1) We found evidence that nymphal survival may be higher for transplanted versus local nymphs. However, there was no difference in the survival patterns of transplanted and local larvae. 2) Larval survival may be higher at northern placement sites than at southern placement sites. However, for nymphs there was no difference in the survival patterns at northern and southern placement sites. 3) Nymphal survival may be higher for nymphs from northern versus southern sites of origin. However, there was no difference in the survival patterns of larvae from northern and southern sites of origin. The results of these tests demonstrated significant differences between individual sites with respect to post-peak larval and nymphal survival, and in some cases, general trends among pairwise comparisons emerged.

First, somewhat counterintuitively, the survival of transplanted nymphs was generally higher than local nymphs, suggesting that local adaptation to environmental conditions may not play a large role with respect to the survival of immature life stages at these sites. Rather, the higher survival observed in transplanted versus local nymphs suggests that perhaps the site of origin and placement site may play a greater role in nymphal survival. For all of the local and transplanted nymphal comparisons, the transplanted nymphs either belonged to northern sites of

origin or were placed at a northern placement site (Supplemental Table 3.1); therefore, perhaps the higher survival observed for transplanted nymphs were due to potentially more robust biological traits associated with northern *I. scapularis* genotypes or more congenial northern environmental conditions. A greater number of comparisons that include more southern transplants would be required to investigate the differences in survival between local and transplanted nymphs. Second, larval survival was higher at northern placement sites than at southern placement sites, which is a finding that is consistent with previous laboratory experiments (Ginsberg et al. 2014). This pattern was not maintained for nymphs, which may again be due to the fact that that nymphs may be less sensitive to ambient temperatures than larvae, due to their larger size and lower surface-area-to-volume ratios. However, the relatively lower survival of larvae at southern sites provides further empirical support of the hypothesis that the conditions at southern sites may be less conducive to *I. scapularis* survival than northern sites (Ginsberg et al. 2014). This pattern could potentially contribute to reduced Lyme disease incidence in the south in different ways. For example, harsher southern conditions, such as higher temperatures and a greater periods of low relative humidity, could mediate the questing behaviors of southern ticks, resulting shorter periods of time spent questing above the leaf litter, relative to northern ticks (Ginsberg et al. 2017); this pattern would reduce incidence in the south by driving down encounter rates between ticks and humans (Arsnoe et al. 2015; Arsnoe et al. 2019) as well as potential reservoir hosts. For example, increased host-seeking below the leaf litter layer or nidicolous ticks using underground lizard burrows may place immature ticks in the south in greater contact with less pathogen-competent lizard species, resulting in reduced transmission and maintenance of the Lyme disease pathogen. Such differences in habitat use among larvae placed at northern and southern sites may have contributed to reduced survival in

southern pottles if the these pottles did not provide access to a suitable range of microclimate conditions. Third, the survival of nymphs from northern sites of origin was generally higher than nymphs from southern sites of origin, suggesting that northern nymphs may be more robust than southern nymphs, and that perhaps these differences in nymphal survival may be related to geographical genetic differences; although the biological traits associated with these genetic differences have yet to be elucidated, the southward expansion of northern *I. scapularis* genotypes (Zee et al. 2015) provides a basis for such a hypothesis. These survival patterns merit further investigation with greater sample sizes and additional sites in order to determine whether these patterns vary by site or are representative of more broad regional patterns.

Factors affecting tick survival

In order to elucidate the potential roles of explanatory genetic and abiotic factors on tick survival, we investigated similarities and differences among the best-fitting models of larval and nymphal maximum and post-peak survival (Table 3.1).

Shared explanatory factors

Our results demonstrate that temperature and relative humidity significantly affect larval and nymphal survival. Larval and nymphal maximum and post-peak survival were affected by TAMEs (i.e., the number of tick adverse moisture events of >8 hours exposure to <82% RH from the first to final date that active ticks were observed) and mean temperatures of the season in which death occurred, perhaps due to the relationships between abiotic conditions and energy expenditures of ticks. Previous findings show a negative relationship between the number of TAMEs and abundances of questing nymphal I. scapularis later in the same year (Berger et al. 2014). These reduced numbers of questing nymphs may have been due to reductions in abundance caused by lower survivorship under low humidity conditions. Alternatively, under

low humidity conditions nymphs spend a greater amount of time under the leaf litter layer, where it is more humid and they are less prone to desiccation, but consequently where they are less likely to be sampled via dragging methods. TAMEs may reduce activity periods via negative impacts on survival, or alternatively may extend activity periods via temporary reductions in activity (as long as the tick survives). For example, with increasing TAMEs, larvae and nymphs may mitigate these low humidity conditions by retreating to the duff layer to rehydrate (Schulze et al. 2002), reducing expenditures of finite energy reserves and extending survival periods. Lower temperatures may contribute to lower metabolic rates, reduced energy expenditures, and longer survival periods as well. There was also an effect of diapause and the interaction between site of origin and placement site on the survival of larvae and nymphs suggesting that, in addition to abiotic factors of known importance (Gray et al. 2016), diapause, genetics, and plasticity also play a potentially important role in immature *I. scapularis* survival.

Differences among explanatory factors

There were some notable differences among stages and the measure of mortality in what factors explained observed survival patterns. First, the duration of time from placement to emergence and peak abundance were correlated with the maximum survival of nymphs and post-peak survival of larvae and nymphs, respectively, but not the maximum survival of larvae. This finding may be due in part to within-pottle variation in larval emergence, in which larvae that developed after the date of emergence (i.e., the date that molted, active ticks of a given life stage were first observed) and before peak abundance for a given pottle, may have made the relationship between days to emergence and larval maximum survival non-significant. Second, the mean temperature of the season in which emergence occurred affected maximum and post-peak larval survival, but not nymphal survival. A possible explanation for this finding is that

nymphs are larger in size with lower surface-area-to-volume ratios relative to larvae and are thus less sensitive to temperatures during the season of observed emergence.

In conclusion, abiotic factors, genetics, and plasticity, and diapause are important factors in the among-population differences in immature *I. scapularis* survival. The inclusion of temperature and relative humidity explanatory variables in our best-fitting survival models suggests that these factors may play a role in the observed survival pattern in which larval *I. scapularis* survival was higher at northern versus southern placement sites. Also, the inclusion of site of origin in our best-fitting survival models suggests that geographical genetic differences may play a role in the observed survival pattern in which *I. scapularis* survival was higher for nymphs from northern versus southern sites of origin. The results of this study differ from the findings of previous laboratory experiments demonstrating that between-population differences in survival do not follow broad north-south geographical genetic patterns (Ginsberg et al. 2014). These differences may be due in part to the fact that our survival patterns are based on relatively low sample sizes compared to the numbers of ticks used in these laboratory trials. Or, perhaps the more natural conditions used in this study, such as greater seasonal and diurnal fluctuations in temperature and relative humidity conditions, and especially access to microhabitat to mediate abiotic conditions, produced different phenotypic responses between nymphs of northern and southern sites of origin, resulting in observed differences in survival. For example, genetically-based behavioral differences between northern and southern genotypes (Arsnoe et al. 2015, Arsnoe et al. 2019) could have resulted in different utilization of the available microhabitat within pottles, scaling up to impacts on survival that were not observed in a laboratory setting. Our results support previous findings that diapause appears to delay the activity of immature *I. scapularis* populations at both northern and southern sites in the eastern U.S. (Ogden et al.

2018), but also suggest that the relationship between diapause and survival may be complex, especially when considering impacts on tick fitness. A limitation of this study was that pottles were not opened or destructively sampled, in order to prevent the escape of ticks and maintain high enough sample sizes to monitor changes in abundance over time. Future studies including the destructive sampling of pottles or a modified design providing greater visual access to developing ticks would elucidate distinctions between developmental and behavioral diapause, as well as preoviposition and egg diapause in the female to larval transition. Such measures are needed in order to gain a more complete understanding of the role of diapause in the life cycle of each population. A greater understanding of the relative roles of abiotic factors, genetics, and plasticity, and diapause on tick survival is a necessary component to investigate existing geographical patterns in Lyme disease incidence, as well as predict how changing environmental conditions may impact Lyme disease risk.

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APPENDICES

APPENDIX C: Supplemental Material

Supplemental Table 3.1. Placement sites and sites of origin of active nymphal *I. scapularis* used in comparisons of local and transplanted nymphal survival.

Local Vs. Transplanted Ticks				
	Placement 1		Placement 2	
Life Stage	Placement Site	Site of Origin	Placement Site	Site of Origin
Nymph	TN	TN	TN	RI
Nymph	TN	TN	TN	WI
Nymph	TN	TN	RI	TN
Nymph	TN	TN	TN	FL
Nymph	TN	TN	TN	FL
Nymph	FL	FL	WI	FL

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CONCLUSION

We explored the among-site variation of the life cycle of *I. scapularis* (Chapter 1) and investigated the role of key factors contributing to this variation, via their effects on *I. scapularis* emergence (Chapter 2) timing and survival (Chapter 3). The key findings of each chapter are shown below.

Summary of Chapter 1 (Among site variation of the life cycle).

The objective of this chapter was to explore how critical life cycle processes (i.e., emergence and survival) varied throughout the geographical range of *I. scapularis*. We conducted a microcosm experiment monitoring the emergence and survival of larval, nymphal, and adult life stages of *I. scapularis*, and collected abiotic data to investigate the relationship between temperature and emergence timing among sites. We present the key findings of this chapter below:

- There is significant variation among sites with respect to both emergence timing and detection period.
- Bimodal emergence (i.e., pre-winter and post-winter emergence of ticks belonging to the same placement) was observed for all life stages in WI but not at other sites, perhaps due to reduced links between photoperiod and future conditions are responsible for bifurcating patterns of emergence.
- There is a significant negative relationship between temperature and days to emergence for all life stages.

To our knowledge, this chapter is the first experimental study characterizing both the emergence timing and survival of *I. scapularis* populations in the north central and the southern United States (US), and the first documentation of bimodal emergence for this species. This study provides a unique collection of standardized, longitudinal data that allow us to explore existing

variation in the life cycle processes of *I. scapularis* across its broad geographical range.

Summary of Chapter 2 (Key factors contributing to variation in emergence activity of different life stages).

The objective of this chapter is was to provide insights into the underlying factors contributing to among-site variation in *I. scapularis* emergence. We monitored the emergence of local and transplanted larval, nymphal, and adult *I. scapularis*. Using temperature data collected from field sites and previously established temperature-development models, we generated a range of predicted emergence dates and compared these estimates with observed emergence dates. We compared subsets of placements that 1) shared the same site of origin but were transplanted to different sites or 2) were from different sites of origin but were placed at the same placement site, to investigate the potential roles of plasticity and genetics on emergence. We also identified potentially important factors contributing to the accuracy of temperature-development models using regression models and the corrected Akaike's information criterion (AICc) to evaluate these models. We present the key findings of this chapter below:

- The temperature-development relationships developed for an Ontario population of *I. scapularis* under laboratory conditions (Ogden et al. 2004) tended to under-predict emergence timing, suggesting a role of diapause in emergence timing at all sites.
- We observed empirical evidence of genetic and plastic effects on emergence timing.
- There were fewer instances where we observed an effect of genetics, relative to plasticity, on emergence.
- There was no clear north-south gradient regarding the effect of placement site on observed emergence.
- Genetics (i.e., site of origin), plasticity (i.e., placement site), and abiotic conditions (i.e., temperature and relative humidity) are important explanatory factors in the accuracy of

temperature-development relationships in predicting immature *I. scapularis* emergence. This chapter provides a novel collection of standardized, longitudinal emergence data from broadly-distributed sites within this species' geographical range. These allowed us to evaluate the generalizability of existing temperature-development models, which are key tools in predicting the future establishment of *I. scapularis* populations, and also determine the roles of temperature-independent factors as well.

Summary of Chapter 3 (Key factors contributing to variation in survivorship of different life stages).

The objective of this chapter is was to provide insights into the underlying factors contributing to among-site variation in *I. scapularis* survival. We monitored the survival of local and transplanted larval, nymphal, and adult *I. scapularis*. We compared subsets of placements that 1) shared the same site of origin but were transplanted to different sites or 2) were from different sites of origin but were placed at the same placement site, to investigate survival patterns among ticks that were local versus transplanted, placed at northern versus southern sites, and from northern versus southern sites of origin. We identified potentially important abiotic and genetic factors contributing to *I. scapularis* survival using regression models and AIC to evaluate these models. We present the key findings of this chapter below:

- We observed empirical evidence of genetic and plastic effects on survival.
- Southern sites may be less conducive to *I. scapularis* survival than northern sites, based on post-peak larval survival patterns.
- Ticks of northern genotypes may have higher survival than southern genotypes, based on post-peak nymphal survival patterns.
- The interaction between genetics (i.e., site of origin) and plasticity (i.e., placement site), abiotic conditions (i.e., temperature and relative humidity), and diapause are important

explanatory factors in immature *I. scapularis* survival.

This chapter provides a novel collection of standardized, longitudinal survival from broadly-distributed sites within this species' geographical range. These allowed us to explore north-south differences in survival, which are comparisons that are rooted in observed geographical patterns of *I. scapularis* invasion (Zee et al. 2015), and also investigate the important factors contributing to the survival of this species.

Conclusions

Life cycles may be shorter at southern sites. The combination of observed median emergence and detection periods indicate that, in both WI and RI, the life cycle can likely be completed in as little as 2 years but can take 4 or more years. In TN, the life cycle can likely be completed in as little as 1 year but can take 3 or more years. In FL, the life cycle also can likely be completed in as little as 1 year but can take up to 2 or more years. While these life cycle lengths are plausible, they do not track the same individual ticks through the entire cycle. Such experiments are needed to make definitive statements about the tick life cycles at these sites. However, relative comparisons can still be made using these data, and suggest that among-site differences exist, with potentially shorter life cycles at our southern sites. The potential contributing factors to these observed life cycle differences are discussed below.

The effect of abiotic conditions on tick emergence and survival. Temperature-development models underpredicted development, which may be attributable to site-specific temperature-development rates (that may vary based on coastal-inland effects or factors at smaller spatial scale such elevation and aspect), or the influence of diapause. These findings are generally consistent with the findings of Ogden et al. 2018, which used the same sites as this study to explore the fit of temperature-development models with various modifications (e.g.

different diapause scenarios, temperature-development rates, and questing timing), and fitted these models using dragged and on-host ticks. Their study found that the inclusion of a diapause component to the temperature-development models generally improved the model fit at most sites, with the exception of TN larvae and FL nymphs. In contrast, our findings suggest that diapause does occur for TN larvae and FL nymphs. Such discrepancies may be due to annual variation in activity, as these studies were conducted in different years. Also, differences in experimental design may also play a role; for example, the emergence dates estimated from field observations could include re-emerging ticks that had molted the previous year, whereas emergence dates estimated in this study are based on single cohorts. Such a difference could thus result in dragged ticks earlier in the season, relative to ticks observed in pottles. Therefore, future experiments likely require pottle experiments with concordant drag sampling efforts to elucidate the role of diapause at these sites.

Temperature and relative humidity play an important role in survival. Our case studies (i.e., comparisons of the post-peak survival of placements sharing the same feeding and placement dates) showed that larval survival at northern sites was higher than at southern sites. This finding corroborates the results of previous laboratory experiments (Ginsberg et al. 2014) and supports the hypothesis that the conditions at southern sites may be less conducive to *I. scapularis* survival than northern sites. However, nymphal survival appeared to be more robust to abiotic conditions. The results of our case studies indicate that nymphal survival was not different at northern and southern sites. In addition, one of the temperature estimates used to model tick survival (i.e., the mean temperature during the season of emergence) was included in the best-fitting larval model, but not included in the best-fitting nymphal model. These findings suggest that nymphs may be less affected by abiotic conditions than larvae, potentially due to the

lower surface-area-to-volume ratio that nymphs have relative to larvae. Relative humidity conditions, such as tick adverse moisture events (TAMEs [Berger et al. 2014]), may contribute to longer life cycles via extended activity periods. During TAMEs, ticks are able to modify their activity, likely seeking refuge in the duff layer and in the process increasing survival by reducing metabolic activity and the use of limited energy reserves. While such periods of quiescence may be correlated with a longer life cycle, they likely also confer a negative cost to reproductive fitness because during these time periods ticks are not able to quest for hosts. In contrast, warmer seasonal temperatures during the activity period may be more difficult to mitigate than periods of low humidity and are associated with reduced survival of both life stages, presumably due to higher metabolic rates and the exhaustion of energy reserves. The shorter survival of larvae placed at southern sites, relative to northern sites, suggests that the warmer average temperatures in the south may be contributing to shorter observed activity periods and life cycles at our TN and FL sites relative to our RI and WI sites.

The effect of diapause on tick emergence and survival. Broadly, our findings indicate that diapause affects emergence timing at both northern and southern sites, extending the total length of the life cycle at all sites. These delays in the life cycle are induced to mitigate adverse conditions but tend to reduce reproductive fitness (Gray et al. 2016). At northern sites, the diapause of overwintering ticks appears to play a similar role in the life cycle that has been observed at other temperate sites (Gray et al. 2016), allowing ticks to mitigate adverse cold winter conditions. Although we observed diapause at our southern sites, compared with the synchronous emergence patterns observed at our northern sites, its effects on emergence timing appeared to be more idiosyncratic. Perhaps at southern sites diapause may play a different role under different selection pressures, resulting in delayed but asynchronous emergence of different

placements, with no clear seasonal patterns. For example, rather than allowing ticks to mitigate cold winter conditions (Yuval Spielman 1990), perhaps diapause in the south allows ticks to avoid other adverse conditions such as hot summer temperatures (Ogden et al. 2018), periods of limited host availability, or periods of high TAMEs. For example, previous field experiments conducted for *I. pacificus*, the primary vector of the Lyme disease pathogen in the western US, indicate that tick populations that are adapted to warmer winter temperatures also exhibit delayed emergence (although these periods are shorter relative to populations with colder winters), but that the role of diapause may allow ticks to avoid periods of summer drought (Padgett and Lane 2001). Therefore, although winter conditions may predictably be the main drivers of diapause and emergence patterns for northern populations (Gray et al. 2016), site or regionally-specific conditions in the south may produce different diapause strategies, resulting in idiosyncratic patterns in emergence.

According to our best-fitting models of *I. scapularis* maximum and post-peak survival, diapause also plays an important role in the survival of immature *I. scapularis*. Although the role of diapause is generally considered to be adaptive (Gray et al. 2016), this may not always be the case with respect to tick survival. For example, diapause could negatively contribute to larval survival at sites where pre-ovipositional photoperiod and temperature cues are poor predictors of future abiotic conditions. Or, perhaps at southern sites with warmer winter and spring temperatures, larvae produced from non-diapausing replete females may to suitable abiotic conditions earlier in the season, resulting in longer survival periods compared to larvae from females with longer diapause periods. The role of diapause on the survival of different life stages of *I. scapularis*, and how these effects vary geographically, are topics that may have important implications for *I. scapularis* establishment and merit further study.

The effect of genetics and plasticity on tick emergence and survival. Although both genetics and plasticity affect emergence, based on case studies (i.e., comparisons of the emergence timing of placements sharing the same feeding and placement dates), plasticity appears to be correlated with differences in emergence timing more frequently for larvae and nymphs. However, there was no clear north-south gradient regarding emergence, suggesting that emergence timing may depend on site-specific factors. Both genetics and plasticity were important factors in the accuracy of temperature-dependent models on predicting emergence, highlighting the need for site-specific genetic, abiotic, and ecological data to accurately predict the emergence patterns of a specific population. Therefore, future models likely require the inclusion of both site-specific temperature-development rates as well as temperature-independent diapause in order to accurately predict emergence.

Based on our case studies, the survival of nymphs from northern sites of origin was generally higher than nymphs from southern sites of origin; this difference may be related to broad geographical genetic, and potentially phenotypic, differences between northern and southern ticks, allowing for the previously documented southward expansion of northern *I. scapularis* genotypes (Zee et al. 2015). This finding is not consistent with previous findings of laboratory experiments (Ginsberg et al. 2014), which may due to the natural abiotic and microhabitat conditions provided in this study, or relatively low sample sizes compared to the numbers of ticks used in these laboratory trials. However, these case studies were corroborated by best-fitting models of larval and nymphal survival, which indicated that the interaction between genetics and plasticity was an important explanatory factor in models of the maximum and post-peak survivorship of immature stages of *I. scapularis*, albeit with potentially idiosyncratic effects. Thus, the effect of genetics on survival merits further investigation, with

greater sample sizes and project sites to better explore whether this relationship is significant, and how this relationship varies among populations.

Implications

The emergence and survival patterns of immature *I. scapularis* play a potentially important role in Lyme disease risk, considering their effects on vector tick activity and abundance, in the context of *Borrelia burgdorferi* (i.e., the Lyme disease pathogen) transmission. Our data generally corroborate established phenologies for these sites (Ogden et al. 2018) which indicate that seasonal nymphal activity precedes larval activity in RI and TN, and that nymphal and larval seasonal activity is highly synchronous in WI and FL. Although both of these scenarios are conducive to pathogen maintenance (which requires the infection of naïve larvae by infected nymphs via shared vertebrate hosts), each has potentially different implications for the diversity of *B. burgdorferi* strains, which vary in clinical presentations and severity (Wormser et al. 1999). Highly synchronous immature tick activity periods may promote a greater diversity of long-lived and short-lived *B. burgdorferi* strain types, whereas a longer temporal gap between nymphal and larval activity may confer higher fitness to long-lived, more virulent strain types (Gatewood et al. 2009). Thus, these findings suggest that the immature tick activity patterns in RI and TN may be more conducive to more virulent pathogen strains than those in WI and FL. Survival patterns may also play a role in Lyme disease risk, via their effects on vector tick abundance and the duration of the activity periods of different tick life stages. Considering relationships that we observed between temperature, relative humidity, and survival, these abiotic factors likely play a major role in the establishment of vector tick populations. Abiotic conditions may play a particularly critical role in the establishment of populations located at the distributional edge of the species' range where thermal conditions are limiting (Randolph 2013), as evidenced by *I.*

scapularis populations at its northern limits (Ogden et al. 2006, Leighton et al. 2012). Perhaps as we approach the southern edge this species' distributional range, where survival at southern sites is observed to be lower relative to northern sites, future increases in temperature due to climate change may reduce *I. scapularis* abundance in the southeastern US.

Future Directions

The results of this study highlight the important roles that abiotic conditions, diapause, and genetics and plasticity have on *I. scapularis* emergence and survival, and the consequences these relationships may have in the context of Lyme disease. There are some key limitations of the study design that could be improved upon in future experiments. First, in order to prevent the escape of ticks and to maintain the sample size needed to characterize the survival of ticks after emergence, we did not destructively sample our pottles throughout this study. Doing so would have allowed us to determine precisely when hatching and molting occurred for each life stage, and potentially distinguish between developmental and behavioral diapause. We also did not carry the same individual ticks through the entire life cycle which is necessary to create definitive life cycles (Yuval and Spielman 1990, Padgett and Lane 2001). Future experiments including these components would provide valuable additions to our understanding of the *I. scapularis* life cycle at these sites.

The role of diapause in different populations, and its effect on tick fitness, is an intriguing question that merits further investigation in future studies. Our findings show that diapause likely plays a role in tick emergence in the southern US, indicating that other factors besides cold winter conditions may contribute to the occurrence of diapause. In future studies, investigating how observed tick emergence patterns correlate with seasonal host activity patterns and periods of low relative humidity could elucidate potential explanations for the role of diapause in the

south. In future experiments, one could take individual cohorts through the entire life cycle, monitoring a subset of unfed ticks from each life stage; the relationship between the emergence patterns combined with the subsequent mortality rates of these ticks would provide a greater understanding of how diapause affects the mortality of subsequent stages. Using the data from studies such as this to explore this question, one could calculate mean mortality rates of diapausing and non-diapausing ticks for different life stages and apply population models to determine whether diapause positively or negatively contributes to the rate of population growth at our sites. These models would provide a potentially more comprehensive was to investigate how diapause may affect tick populations, and how this relationship may vary geographically.

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