
Thinking Matters Symposium

2021 Thinking Matters Symposium

Apr 30th, 12:00 AM

Effect of temperature on questing activity of *Ixodes scapularis* in a laboratory setting

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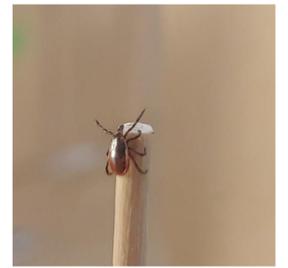
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Meagher, Molly, "Effect of temperature on questing activity of *Ixodes scapularis* in a laboratory setting" (2021). *Thinking Matters Symposium*. 28.

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ABSTRACT

Understanding deer tick (*Ixodes scapularis*) behavior is increasingly important due to the species' northerly range expansion, and consequently, increased human contact. Deer ticks are a major public health concern, as they are the primary vector of Lyme disease and other serious diseases. Differences in questing behavior are of considerable interest as fluctuating weather due to climate change effect the species' range expansion and time spent questing. Although previous research has examined temperature preferences of tick behavior, there are conflicting reports about direct effects on questing activity. Therefore, I addressed the relationship between temperature and questing behavior of adult deer ticks. I predicted that most ticks exhibit questing behavior when exposed to intermediate temperatures, which are closest to average temperatures when ticks are active in Maine. My experiment exposed deer ticks to cold (10°C), intermediate (20°C), and warm temperatures (30°C) in an enclosed arena with wooden rods placed into a leaf litter substrate. I observed the ticks determine the proportion that exhibit questing behavior, as well as location in the enclosure. For standardized observation, I counted the number of ticks crawling on the surface of the leaf litter, on arena walls, and on rods. The results revealed the highest questing activity occurred at the intermediate temperature. Overall, females quested more often than males. Females were much more likely to quest than males in the cold treatment. The results provide further understanding into tick questing behavior in three different temperatures.

INTRODUCTION

The Centers for Disease Control and Prevention have reported range expansion and an increase in reported tick-borne disease cases in recent decades (U.S. CDC, 2019). Thus, the study of tick behavior in relation to disease transmission is of significant public health interest. The deer tick (*Ixodes scapularis*), is known to transmit many disease-causing pathogens, including *Borrelia burgdorferi*, the cause of Lyme disease (U.S. DHHS, 2018). Temperature must be considered when studying tick behavior, because warming due to climate change has contributed to the species' expansion, and when considering the risk of human contact (Thomas et al., 2020). Questing occurs in each life-stage of a tick, when it seeks a host for a blood meal (U.S. CDC, 2020). When not actively seeking hosts, deer ticks live in leaf litter, protected from desiccation (Thomas et al., 2020). A questing tick leaves the leaf litter in which it normally rests, and climbs leaves and vegetation to a place where it may latch onto a passing host (U.S. CDC, 2020).

There is conflicting evidence in the literature as to whether environmental conditions such as temperature independently affect questing activity of deer ticks. Certain studies suggested that warmer temperatures lead to decreased questing activity among *Ixodes* spp. due to increased risk of desiccation (Gilbert et al., 2014) whereas others found no relationship (Thomas et al., 2020). I aimed to answer the question: Is *I. scapularis* questing behavior influenced by temperature? I hypothesized that adult ticks' questing behavior varies with temperature. I predicted that the highest percentage of ticks exhibits questing behavior when exposed to moderate temperatures, which are closest to average temperatures during summer in Maine (U.S. Climate Data, 2021).

METHODS

- I collected adult deer ticks in Wells (43.3370/70.5501) and Cape Elizabeth (43.5711/-70.2571), Maine using standard tick-flagging methods (Saloman et al., 2020) in March, 2021.
- I created an arena by filling a cylindrical glass container (22 cm tall, 25 cm diameter) with 750 mL leaf litter put through a Berlese funnel to remove insects. I placed wooden rods secured with Play-Doh in the leaf litter (Fig. 1).



Figure 1. Glass cylinder fashioned with a leaf litter substrate and wooden rods. Ticks were placed on leaf litter and exposed to each treatment.

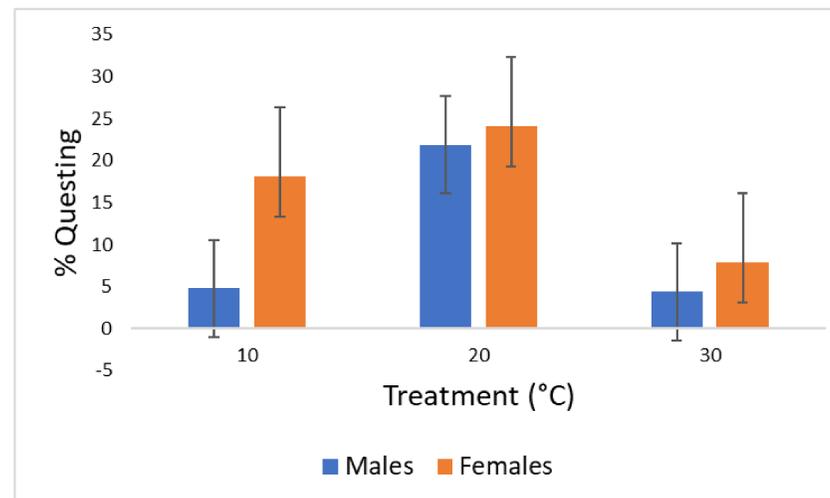


Figure 2. Mean (\pm SE) percentage of questing separated by sex at 3 temperatures.

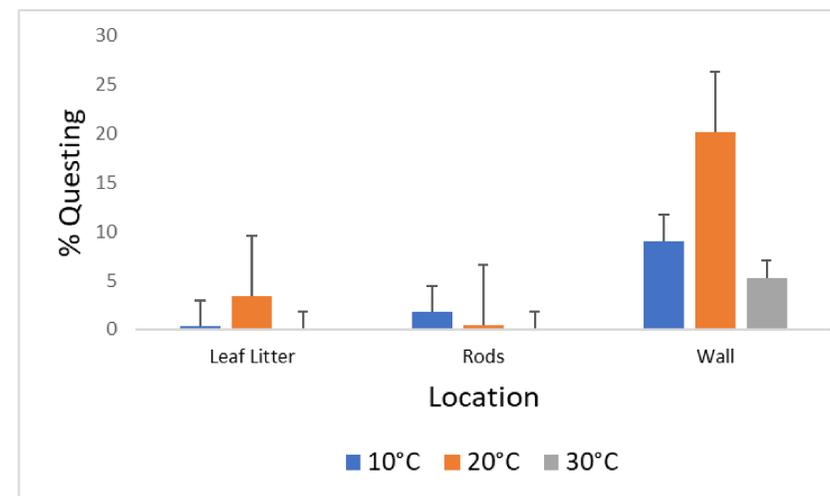


Figure 3. Mean (\pm SE) percentages of questing in each location for each temperature.

- I exposed ticks to a cold treatment (10°C), an intermediate treatment, (20°C), or a warm treatment (30°C). I repeated trials twice at each temperature.
- I created the cold treatment using a refrigerator supplemented with artificial light for consistency; the intermediate treatment used the light and temperature in the laboratory; and I produced the hot treatment using an overhead heat lamp.
- I placed 18-30 adults into the arena, both male and female. After 15 mins of acclimation, I observed their behavior every 15 min over 3 h. I recorded total number of questing ticks, sex, and location: rods, leaf litter, or arena walls.
- I analyzed the data using a Generalized Linear Model in JMP 14 (SAS Institute, Inc., 2018).

RESULTS

The analysis revealed a relationship between percentage of ticks questing and temperature ($\chi^2 = 15.3$, $df = 2$, $p = 0.0005$), sex ($\chi^2 = 15.52$, $df = 1$, $p < 0.0001$), and temperature*sex ($\chi^2 = 12.83$, $df = 2$, $p = 0.0016$). Ticks quested most often at the intermediate temperature. Female ticks were more likely to be questing in any given treatment. Although males were found to quest less in every treatment, there were significantly more females questing than males in the cold treatment than in any other treatment.

DISCUSSION

To find a host, a tick may quest for several months, and may encounter a wide range of temperatures (Arsonoe et al., 2020). The results supported my hypothesis that most ticks quest at the intermediate temperature of 20°C. Other studies also found that ticks are most active at 20-25°C (Arsonoe et al., 2020). As environments are warmed by climate change, average temperatures may be more suitable for longer periods of tick questing during the year (Gilbert et al., 2014).

Female ticks were more likely to quest than males were. Other studies did not compare questing activity between males and females, possibly because females' possibility of disease transmission (Arsonoe et al., 2020).

There was no relationship between the location of questing in among any of the treatments. The height that a tick climbs when questing may be associated with temperature (Arsonoe et al., 2020); therefore, future studies should also measure height. Other studies have temperature gradients in laboratory settings, which help to establish both warm and cold temperature thresholds for deer tick questing (Gilbert et al., 2014).

Despite the small scale of this study, the results provide insight into the unknowns of tick questing behavior in relation to temperature. Future studies should contain more replicates, and if possible, be conducted in a field setting to better test a natural environment.

ACKNOWLEDGEMENTS

I thank the Vector Borne Disease Lab at Maine Medical Center Research Institute for allowing me to use equipment and for assistance throughout this research. I also thank Dr. Maher for aiding in the research process and statistical analyses.

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