Movement and Questing Activity of *Dermacentor variabilis* (Acarina: Ixodidae) in
Response to Host-Related Stimuli and Changing Environmental Gradients

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Abstract

Tick activity and questing behaviors are largely dependent on the biology of the tick as well as the stimuli provided by the host. Ticks quest to find a suitable host from which to take a blood meal that will allow them to further their development and increase their energy reserves. In addition, because a tick spends the majority of its life cycle off of a host, tick activity is affected by environmental factors. A tick works to maximize questing time while maintaining proper water balance and metabolic functions, and its activities reflect this balance. This study examined the effects of host-related and environmental stimuli on *Dermacentor variabilis* activity and questing behaviors. Ticks were attracted to a nearby heat source, but they were not responsive to light or odor cues. These responses to host-related stimuli indicated that *D. variabilis* uses an ambush strategy of locating and making contact with its host. In addition, ticks demonstrated a significant pattern of activity along an experimental temperature gradient, indicating that certain temperatures provide optimal conditions for tick activity and that temperatures outside of both upper and lower thresholds cause activity to decrease. These responses give implications for tick activity, distribution, and the spread of associated pathogens in the wake of global climate change.

Introduction

Ixodid ticks engage in a life cycle during which time is spent both on and off of a single host, and a tick cannot mature into each new life stage until it takes a blood meal (Randolph 2004). However, ticks spend the majority of their lives as free-living organisms, and their physiological processes and behaviors are thus susceptible to variable abiotic factors, such as temperature and humidity (Needham and Teel 1991). Perhaps the most vulnerable physiological

process is that of water balance within the tick; due to their large surface area to volume ratio, ticks are extremely susceptible to desiccation (Needham and Teel 1991). Abiotic factors that induce desiccation, such as high temperature and low humidity, cause a decrease in questing activities (Loye and Lane 1988; Perret *et al.* 2000). When persistent, these same conditions affect seasonal tick abundance (Oorebeek and Kleindorfer 2008), and the long-term effects of climate change have implications for potential range shifts of several Ixodid species (Gilbert 2010).

During their time as free-living organisms, ticks streamline their behaviors to conserve energy while maximizing the probability of finding a host; thus, ticks work to find a suitable host in the shortest possible amount of time. Ixodid ticks accomplish this by responding to host-specific cues such as heat (Oorebeek *et al.* 2009), CO_2 output (Anderson *et al.*1998), and host odor (Crooks and Randolph 2006). These cues are often interconnected, and ticks therefore respond along a gradient of the given stimuli (Beelitz and Gothe 1991). In addition, environmental factors often threaten various metabolic processes, and ticks must thus alter their questing behaviors to avoid these hazards. Such modified behaviors may be demonstrated in the form of questing strategies: some tick species are ambush hunters, whereas others actively pursue their hosts (Oorebeek *et al.* 2009). These strategies reflect those host-related cues to which a tick responds. A tick is considered an ambush hunter when it responds to close-range cues that indicate host proximity, and it is considered an active hunter if it responds to long-range, directional cues (Oorebeek *et al.* 2009). Overall, questing behaviors and sensitivity to specific stimuli vary among species, and they are also dependent on physiological properties of individual ticks, host availability, and abiotic factors in the given environment (Crooks and Randolph 2006).

Dermacentor variabilis (Acarina: Ixodidae), the American dog tick, is one of the most widely distributed tick species in North America, and it is a known vector of both Rocky Mountain spotted fever and tularemia (Burgdorfer 1969). Its range currently stretches from the Atlantic Coast to the western edge of the Great Plains region, and it is found along the California coast as well (Burgdorfer 1969). Peak abundance and questing activity of *D. variabilis* is variable: in temperate zones, adults are most abundant in June and July; in subtropical zones, adult ticks are most abundant in July and August (Burgdorfer 1969; Cilek and Olson 2000). These variations are largely attributed to abiotic factors in the surrounding environment: ticks are most abundant when environmental conditions do not threaten their physiological processes (Needham and Teel 1991). In addition, adult ticks are most active when exposed to higher levels of solar radiation (Atwood *et al.* 1967). Together, these studies indicate that *D. variabilis* is susceptible to similar effects of climate change that have been demonstrated in other Ixodid species (Burgdorfer 1969; Cilek and Olson 2000; Atwood *et al.* 1967).

D. variabilis parasitizes a wide variety of hosts, including mice, cattle, and horses; however, adult ticks principally infect canids (Burgdorfer 1969). Previous studies have examined the distribution of *D. variabilis* in various habitats and suggested that ticks are located in areas of high host odor concentrations (Smith *et al.* 1946). However, the direct responses of *D. variabilis* to such host-specific stimuli have not been studied.

The purpose of this study is to examine movement and questing behaviors of adult *Dermacentor variabilis* in response to host-related stimuli and changing environmental gradients. The hypotheses that were tested were *(i)* ticks will be negatively phototactic, *(ii)* ticks will be attracted to host odor, *(iii)* ticks will be attracted to a source of heat, and *(iv)* tick movement will be negatively related to an increasing temperature gradient.

Materials and Methods

This experiment was conducted during June and July 2011 at the University of Notre Dame Environmental Research Center (46° 13' N, 89° 32' W) in Vilas County Wisconsin and Gogebic County, Michigan. Ticks were collected using a blanket drag method adapted from Falco and Fish (1992) and Gilbert (2010). A 2.06 x 2.40m white cotton sheet was dragged across four different fields, and those ticks that were found on the blanket after each drag were collected. Captured ticks were stored in a light, temperature, and humidity-controlled environment, and they were not fed during storage.

Experimental methods were adapted from Oorebeek *et al*. (2009). Experiments were carried out in temperature and light controlled areas, with ambient temperature at 18°C and no available light. The experimental arenas consisted of covered glass dishes 20cm in diameter. Each experiment was replicated three times, and five ticks were used for each replicate.

Phototactic Behavior

To produce a shadow on one half of the experimental container, half of each dish was covered with a piece of corrugated cardboard, and the sides of the same half were covered with duct tape. The containers were placed in a dark chamber underneath a growing lamp equipped with a non-heating light bulb. Ticks were monitored over a four-hour period, and their location was recorded every ten minutes for the first hour and every thirty minutes for the next three hours.

Host Odor Attraction

A cotton ball was saturated with 1ml of coyote urine and placed on one side of the experimental container. The container was then covered with parafilm to prevent odor from

escaping. Ticks were monitored over a four-hour period, and their location was recorded every ten minutes for the first hour and every thirty minutes for the next three hours.

Heat Attraction

Each container was divided using a 4.5cm high modeling clay (Play-Doh®, Hasbro) barrier, and a heat source was placed in one half of the dish. The heat source used was an activated hand warmer (The Coleman Company, Inc) that was able to heat up to 68°C in 15-30 minutes. At the beginning of the monitoring period, the temperatures of the three heat sources used were 43°C, 42°C, and 39°C, and at the end of this period, temperatures had fallen to 34°C, 33°C, and 32°C, respectively. Ticks were monitored for one hour, and their location was recorded every five minutes.

Movement

To examine ticks' responses to both cold and warm temperatures, ticks were tested along a gradient of three different temperatures: cold (18°C), room temperature (22°C), and warm temperature (29°C). Experimental containers were placed in controlled chambers at each of the specified temperatures. Ticks were monitored over a four-hour period, and their location was recorded every thirty minutes. Containers were marked with a grid, and tick movement was quantified by examining their location on the grid at each time interval. Three replicates were taken at each temperature, and a total of 45 ticks were tested.

Data Analysis

Heat attraction, phototactic behavior, and host odor attraction were analyzed using a replicated G-test of goodness-of-fit (Zar 2010). This test determined whether the data from individual experiments as well as pooled data differed from expected values. In addition, it analyzed whether additive observations differed from expected values. Ticks were tested against

the expectation that they would distribute themselves equally across control and experimental portions of the testing container.

Tick movement was not normally distributed, and it could not be transformed to a normal distribution. Thus, movement was examined using a non-parametric Kruskal-Wallis one-way analysis of variance. Temperature was used as a grouping variable, and tick movement was used as the dependent variable. If results were found significant, then a Dwass-Steel-Chritchlow-Fligner test for pairwise comparisons was conducted on tick movement between all possible temperature pairs. All results were considered significant if $p < 0.05$.

Results

Phototactic Behavior

When data from each replicate at each time interval were pooled, more ticks were found in the shaded half of the experimental container (95) than in the lighted half (85; Figure 1); however, the difference between the pooled numbers of ticks in each section was not significant (Pooled $G_1 = 0.556$, $p = 0.228$; Table 1). In addition, tick preference of lighted and shaded portions of the arena did not significantly change over time (Heterogeneity $G_{11} = 9.506$, $p = 0.287$; Table 1), and ticks did not exhibit a significant additive preference of either half of the container (Total $G_{12} = 10.063$, $p = 0.305$; Table 1). These results reject the first hypothesis and suggest that *D. variabilis* does not exhibit any phototactic behaviors.

Host Odor Attraction

Ticks were not attracted to host odor cues. When data were pooled, 95 ticks were found in the unscented part of the arena, and 85 ticks remained in the scented half (Figure 2). There was no significant difference between the pooled numbers of ticks found in each half of the arena (Pooled $G_1 = 0.556$, $p = 0.228$; Table 2), and ticks did not alter their response to odor over the

given period of time (Heterogeneity $G_{11} = 16.745$, $p = 0.058$; Table 2). Moreover, there was no significant additive preference for either half of the experimental dish (Total $G_{12} = 17.301$, $p = 0.069$; Table 2). Overall, these results reject the second hypothesis.

Heat Attraction

There was a significant difference between the number of ticks found in hot and cold environments (Pooled $G_1 = 5.023$, $p = 0.012$; Table 3). When pooled together, 105 ticks were found in the portion of the experimental container that was exposed to heat, and 75 ticks were found in the unheated half (Figure 3). However, ticks' preference for heat did not differ significantly over the experimental period (Heterogeneity $G_{11} = 5.531$, $p = 0.451$; Table 3). In addition, additive preferences for hot or cold portions of the container were not significant (Total $G_{12} = 10.554$, $p = 0.284$; Table 3). Overall, these results indicate that ticks are attracted to heat and thus support the third hypothesis.

Movement

There was a significant difference in tick movement across the experimental temperature gradient (K = 6.081, df = 2, $p = 0.047$; Figure 4). Ticks exhibited the greatest amount of movement at 22°C (15.51 ± 0.132cm) and the least amount of movement at 18°C (12.49 ± 0.142cm; movement at 29°C: 13.73 ± 0.114). Moreover, ticks moved significantly more at 22°C than at 18°C (Dwass-Steel-Chritchlow-Fligner statistic = 11.145, $p < 0.001$), and they also moved significantly more at 29°C than at 18°C (Dwass-Steel-Chritchlow-Fligner statistic = 11.990, $p < 0.001$). However, there was no significant difference in movement between 29°C and 22°C (Dwass-Steel-Chritchlow-Fligner statistic = 2.905, $p = 0.099$). These results fail to reject the initial hypothesis and suggest that *D. variabilis* exhibits the most activity at a median temperature.

Discussion

This study examined the effects of host-related and environmental stimuli on *Dermacentor variabilis* activity and questing behaviors. Ticks were attracted to a nearby heat source, but they were not responsive to light or odor cues. In addition, ticks demonstrated a significant pattern of activity along the experimental temperature gradient.

D. variabilis did not demonstrate any preference for either lighted or shaded portions of the experimental arena. A lack of phototactic behavior could indicate a predominant host-locating strategy. Although light intensity may indicate the presence of a nearby host, it may not be a reliable indicator of such an organism; differing light intensities may also be related to weather events. Thus, rather than using light intensity as an indicator of host proximity and a stimulus for questing activities, *D. variabilis* may pursue their hosts based on other, close-range cues.

Previous studies have found that other Ixodid species are negatively phototactic and suggest that this behavior is a mechanism with which to avoid desiccation and increase questing duration (Oorebeek *et al.* 2009). Other studies have shown that high levels of solar radiation in conjunction with light increase *D. variabilis* questing behaviors (Atwood *et al.* 1967). However, *D. variabilis* ticks did not exhibit any phototactic behaviors, indicating that light alone is neither a host-related nor an environmental stimulus. Such inconsistencies may signify differences between Ixodid species. Alternately, discrepancies may demonstrate that responses to light stimuli may not reflect behavioral preferences; ticks rarely encounter light alone in their natural environment, and they likely respond to other environmental factors that are very closely correlated with light.

D. variabilis did not demonstrate a significant response to host odor cues within the experimental arena. Ixodid ticks have been found capable of sensing host odor, and certain species respond to these cues by increasing questing behavior (Crooks and Randolph 2006). Although *D. variabilis* are likely able to sense such a cue, host odor is not a stimulus that induces questing behavior. Odor is a long-range locating cue that indicates direction, rather than proximity, of a given host, and it is used by those species of ticks that actively seek their host.

A lack of response to the given odor cues could also be a consequence of the conditions under which ticks were tested. Previous studies have suggested that odors are more volatile at higher temperatures (Howell 1975; Oorebeek *et al.* 2009); thus, because ticks were tested under cool temperatures, they may have exhibited low sensitivity to the host odor.

 In addition, the lack of response may indicate the ticks' host preferences. Different species of ticks have different host preferences, and although studies have found that adult *D. variabilis* generally parasitize canids (Burgdorfer *et al*. 1969), these results suggest that ticks may prefer certain genera or species. Alternatively, these results may indicate that ticks are sensitive to specific host odors; rather than responding to urine, a substance that a host uses to mark its location before moving away, ticks may respond to other odorous stimuli that give a stronger indication of the host's location, such as oil secretions on the host's skin. Overall, further study is needed to determine the mechanisms behind *D. variabilis'* response to host odors.

D. variabilis showed a preference for the portion of the experimental container in which a heat source was located. This is a host-seeking behavior: as ticks become able sense nearby body heat, the heat becomes a stimulus for ticks to climb onto their hosts. Moreover, because *D. variabilis* elicit a significant response only to a close-range stimulus, this species likely locates

and contacts its host using an ambush strategy. These findings are consistent with previous research on other Ixodid species that use ambush methods of locating their host (Oorebeek *et al.* 2009).

D. *variabilis* exhibited a significant pattern of activity along a changing temperature gradient, the greatest amount being at a median temperature, and the least amount being at cooler temperatures. This is consistent with similar studies of other Ixodid ticks and suggests that there is an upper and a lower threshold for tick activity (Needham and Teel 1991). Between these threshold temperatures, ticks are able to maximize questing activities while maintaining optimal water balance, and they do not have to limit their movement to conserve energy for water or heat retention.

Previous studies have suggested that, as global climate change causes shifts in current weather patterns, ticks are likely to move to different ranges, and the timing of their peak seasonal abundance and questing activities may change as well (Gilbert 2010; Oorebeek and Kleindorfer 2008; Perret *et al*. 2004). The current study suggests that D. *variabilis* is capable of following this trend. As temperatures rise, D. *variabilis* has the potential to increase its range into previously cooler areas, such as the Rocky Mountains and northern Canada, as well as the Pacific coast. Moreover, as their tick vectors have potential to expand their range, related diseases are likely to spread as well.

Movement and questing activities of Ixodid ticks are largely dependent on several factors, including individual tick biology, host-produced stimuli, and abiotic factors. *Dermacentor variabilis* exhibited host-seeking patterns consistent with sit-and-wait hunters that respond to certain close-range stimuli and ambush their host. In addition, D. *variabilis* maximizes its movements within a certain ambient temperature range, and as several regions in

North America experience increases in temperature, there is increasing potential for *Dermacentor variabilis* to extend its distribution.

Acknowledgements

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Tables

Table 1. Replicated G-test of goodness of fit on tick preferences of lighted and shaded portions of experimental containers.

	G value	Degrees of Freedom	*p*
Total G	10.063	12	0.305
Pooled G	0.556	1	0.228
Heterogeneity G	9.506	11	0.287

Table 2. Replicated G-test of goodness of fit on tick preference of scented and unscented portions of experimental containers.

	G value	Degrees of Freedom	*p*
Total G	17.301	12	0.069
Pooled G	0.556	1	0.228
Heterogeneity G	16.745	11	0.058

Table 3. Replicated G-test of goodness of fit on tick preference of heated and unheated portions of experimental containers.

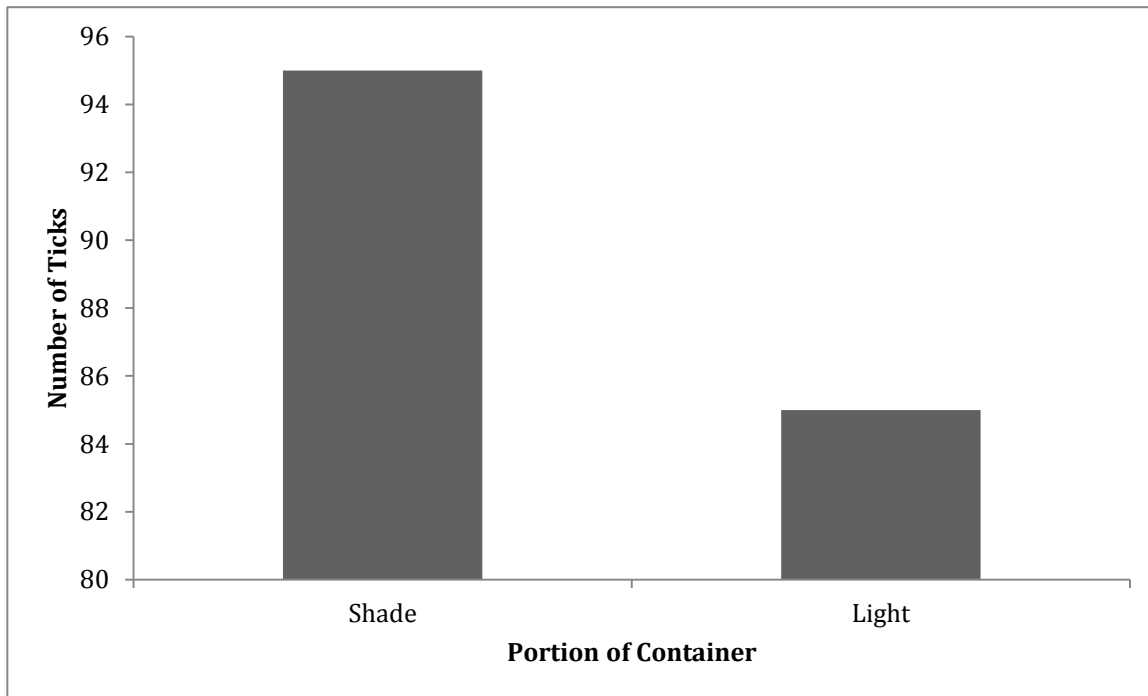|	G value	Degrees of Freedom	*p*
Total G	10.554	12	0.284
Pooled G	5.023	1	0.013
Heterogeneity G	5.531	11	0.451

Figures

Figure 1. Pooled numbers of ticks in shaded and lighted portions of experimental containers. More ticks were found in the shaded half of the container (95) than in the lighted half (85). The difference in pooled numbers of ticks in each portion was not significant (Pooled G = 0.556, p = 0.228).
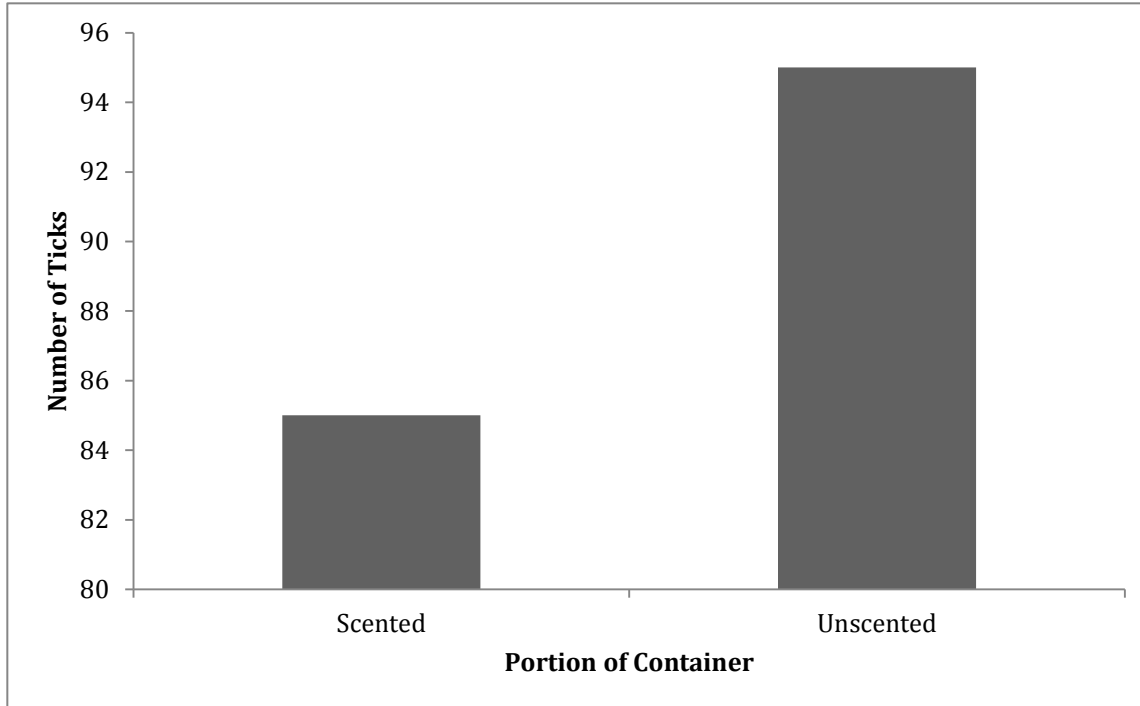
Figure 2. Pooled numbers of ticks in scented and unscented halves of experimental containers. More ticks were present in the unscented half of the arena (95) than in the scented half (85). There was no significant difference between the pooled numbers of ticks found in each half of the arena (Pooled G = 0.556 p = 0.228).
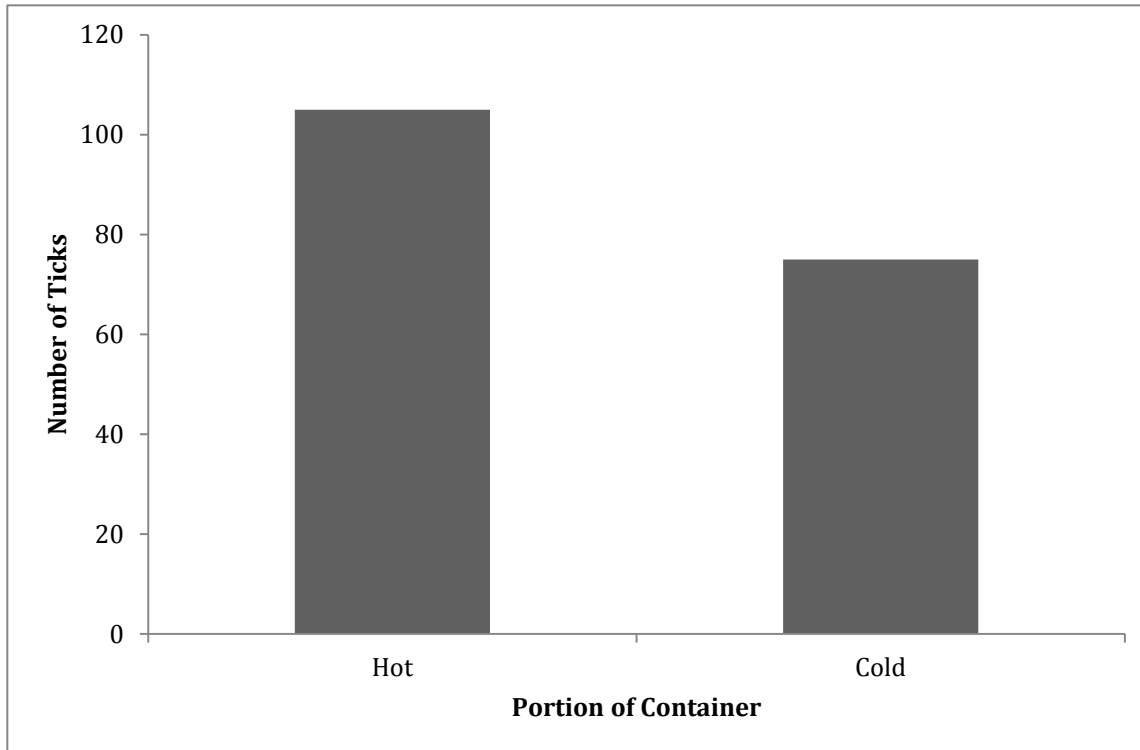
Figure 3. Pooled numbers of ticks in halves of experimental containers with and without a heat source. More ticks were found in the half of the arena containing the heat source (105) than in the other half (75). There was a significant difference between the pooled numbers of ticks in each half (Pooled G = 5.023, $p = 0.013$).
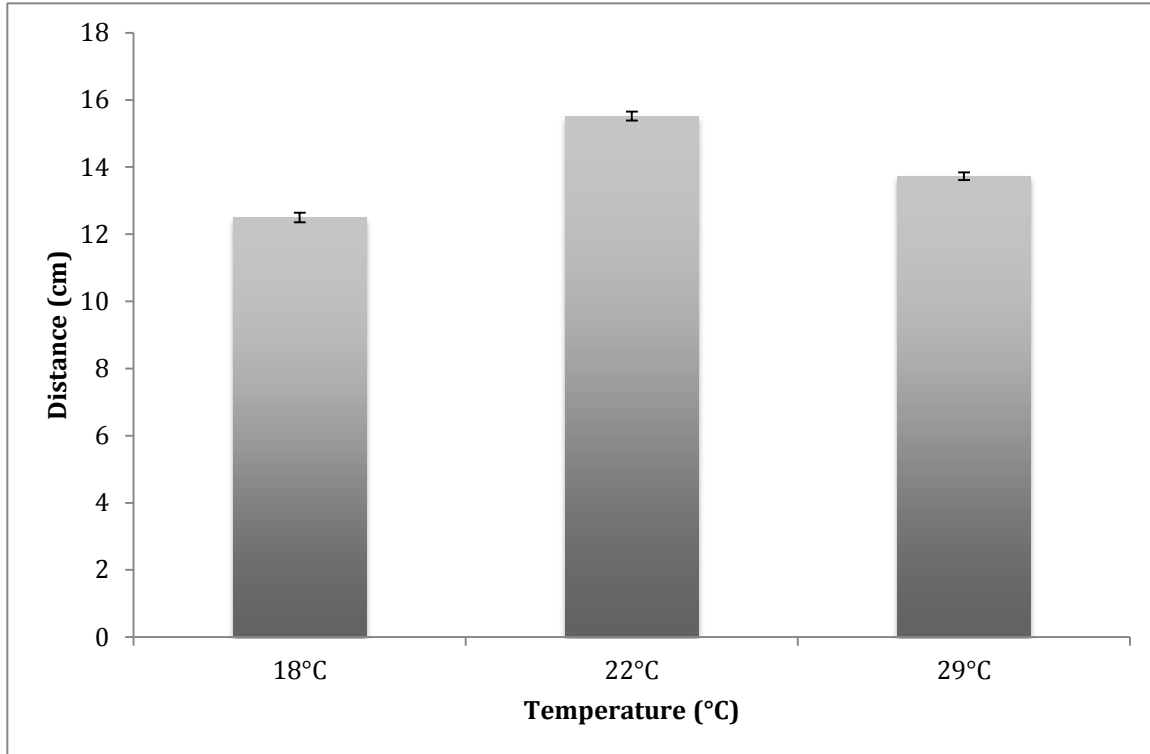
Figure 4. Mean tick movement across the experimental temperature gradient. There was a significant difference in tick movement between the three tested temperatures ($K = 6.081$, $df = 2$, $p = 0.047$). Ticks exhibited the greatest amount of movement at 22°C (15.51 ± 0.132cm) and the least amount of movement at 18°C (12.49 ± 0.142cm; movement at 29°C: 13.73 ± 0.114). Ticks moved significantly more at 22°C than at 18°C (Dwass-Steel-Chritchlow-Fligner statistic = 11.145, $p < 0.001$), and they moved significantly more at 29°C than at 18°C (Dwass-Steel-Chritchlow-Fligner statistic = 11.990, $p < 0.001$). However, there was no significant difference in movement between 29°C and 22°C (Dwass-Steel-Chritchlow-Fligner statistic = 2.905, $p = 0.099$).