SHORT COMMUNICATION

Cold-Hardiness of a Laboratory Colony of Lone Star Ticks (Acari: Ixodidae)

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ABSTRACT The cold-hardiness of a lone star tick, *Amblyomma americanum* (L.), laboratory colony was characterized. Fed and unfed larvae, fed and unfed nymphs, and unfed adults did not survive exposure to −17°C for 7 d. After an 8-d exposure to −10°C, adults tolerated cold better than immatures and unfed specimens fared better than fed ticks. Exposing unfed 6-wk-old (postmolt) adult males and females to −15°C for increasing intervals up to 2 h suggests that males were more tolerant to cold than were females. Half of all adults were alive 3 d after the 2-h low-temperature treatment. Males may have survived because of a significantly higher hemolymph osmotic pressure, although the solute concentration increased for both sexes after a 2-h exposure to 0°C. Acclimation to 5°C for 7 d had no influence on supercooling points for unfed males and females, engorged nymphs and larvae, and eggs. None of the life stages survived supercooling, which strongly suggests that this species is freeze intolerant. Intolerance of immature stages to chilling may be a limiting factor in the northern distribution of lone star ticks in North America.

KEY WORDS *Amblyomma americanum*, tick, cold-hardiness, survival, osmotic pressure, supercooling point

THE LONE STAR tick, *Amblyomma americanum* (L.), is one of the most important pest tick species in North America. This particular status has less to do with disease transmission problems and more to do with the tick's broad host range, a propensity to reach epidemic populations, a tenacious feeding behavior by all stages, and the resulting inflammations in host skin (Hair and Bowman 1986). All 3 postembryonic stages feed on a variety of avian and mammalian hosts in the south–central portion of the United States. The tick ranges west to the Great Plains and north to the Ohio River, and up the Atlantic coastal areas to Rhode Island (Sonenshine 1993). In general, *A. americanum* distribution is associated with ecotones of forested regions or scrubbrush habitat being used by host animals and where moist microhabitats are plentiful (Hair and Bowman 1986).

Amblyomma americanum is among the most intensively investigated ticks in the world, yet little is known about the effect of low temperature on its survival and distribution. In fact, there are few studies on the cold-hardiness of ticks (see Lee and Baust 1987; Burks et al. 1996a, b; Dautel and Knulle 1996; Strey et al. 1996). When one examines the geographic distribution of this species the northern limit may be defined by low winter temperatures, because other factors necessary to sustain the species seem to be present (e.g., hosts, moisture, habitat type). We characterized the cold-hardiness of a colony maintained for numerous generations in the laboratory as a beginning point for comparison to other ticks.

In our initial experiment, different stages (egg, fed and unfed immatures, and unfed adults) were exposed to a range of low temperatures to evaluate general sensitivity. Low temperature survival was further assessed by exposing groups of unfed males and females to −15°C for ≤120 min. We also measured the hemolymph osmotic pressure as a possible index for cold-hardening. In preparation for winter, many terrestrial arthropods accumulate low molecular mass polyhydric alcohols and sugars (e.g., glycerol, sorbitol, and trehalose) that enhance their cold-hardiness (Lee 1991). The concentrations of these or other compounds can reach multimolar levels causing an increase in the hemolymph osmotic pressure. The supercooling point is the temperature at which ice forms spontaneously within body fluids and is used as an index of cold-hardening. We compared the effect of acclimation to low temperature (5°C) on the supercooling of embryonic and postembryonic stages. This study

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describes the susceptibility of laboratory-reared *A. americanum* to low temperatures to increase our understanding of off-host survival for an economically and medically important ixodid.

Materials and Methods

Specimens used in these studies were from a laboratory colony maintained continuously at The Ohio State University, Columbus, for several years, but which originated from a colony at Oklahoma State University, Stillwater. Subadults were fed on chickens and adults on rabbits. Off-host ticks were held at 93% RH over supersaturated KNO_3 (Winston and Bates 1960) and 26°C, at a photoperiod of 14:10 (L:D) h.

Low-Temperature Survival. Mortality at low temperatures was determined for larvae (fed and unfed, n = 20 for each temperature/time treatment), nymphs (fed and unfed, n = 10 each temperature/time treatment), and unfed adult males and females (n = 5 each temperature/time treatment). Ticks were exposed to 93% RH (glycerol/water solutions, Newman 1968) in a desiccator in incubators as follows: 26°C for 13 d (control), −10°C for 8 and 12 d, and −17°C for 7 d.

In a separate experiment, the effect of low temperature on adult males and females was evaluated by determining mortality and injury/damage to provide greater resolution for our observations following low temperature exposure. A tick was scored a 0 if it was moving without stimulation or moved in response to human breath. A score of 1 was given if the legs moved in response to being touched, but was otherwise inactive. A score of 2 was given when the specimen failed to respond to touch and was apparently moribund. Numbers for each treatment were added together and multiplied by 2 to base the score on 100 (see Table 1 for sample). Twenty-five (1–2 mo postmolt) individual specimens were exposed to −15°C (water bath containing water and ethylene glycol at 1:1) for various periods of time (0, 10, 30, 60, 100, and 120 min) at 93% RH (using tubes containing a glycerol/water solution, Newman 1968). Following the low temperature exposure, ticks were held in an incubator at 93% RH and 26°C (14:10 h, L:D), then evaluated at 1 and 3 d posttreatment for damage and mortality.

Hemolymph Osmotic Pressure. Groups of 5 ticks were exposed to either 0, 26 (controls), or 40°C for 2 h before taking hemolymph samples. Osmotic pressure is a colligative property and values can be expressed as melting points or vapor pressures (see Arlian and Veselica 1979). A nanoliter osmometer (Clifton Technical Physics, Hartford, NY) was used essentially as described by Frick and Sauer (1973) to determine hemolymph osmotic pressure for adult males and females. Finely drawn capillaries were used to collect samples from the distal ends of legs that had been removed under hydrated immersion oil. Hydrated oil (water/oil mixture) was used to reduce water loss from minute fluid samples.

Table 1. Effect of −15°C exposure on morbidity and mortality of unfed adult *A. americanum* ticks

Min at −15°C	% alive at 24 h	Damage score	% alive at 72 h	Damage score
		Females		
0	100	0	100	0
10	100	0	84 ± 7.3	14 ± 6.9
30	92 ± 5.4	22 ± 8.3	60 ± 9.8	44 ± 9.9
60	80 ± 8.0	56 ± 9.9	60 ± 9.8	66 ± 9.5
80	68 ± 9.3	66 ± 9.5	60 ± 9.8	70 ± 9.2
100	44 ± 9.9	78 ± 8.3	42 ± 9.9	74 ± 8.8
120	56 ± 9.9	72 ± 9.0	48 ± 10	72 ± 9.2
		Males		
0	100	0	100	0
10	92 ± 5.4	30 ± 9.2	88 ± 6.5	34 ± 9.5
30	92 ± 5.4	34 ± 9.5	92 ± 5.4	40 ± 9.8
60	80 ± 8.0	60 ± 9.8	76 ± 8.5	62 ± 9.7
80	72 ± 9.0	64 ± 9.6	68 ± 9.3	66 ± 9.5
100	84 ± 7.4	58 ± 9.8	80 ± 8.0	60 ± 9.8
120	68 ± 9.3	64 ± 9.6	60 ± 9.8	70 ± 9.2

Damage scores: 0, a tick moved without stimulation or moved in response to human breath; 1, legs moved in response to being touched with a probe; 2, tick did not move in response to being touched and appeared to be dead. Numbers for each treatment were added and multiplied by 2 to base the score on 100. Damage score if all 25 died: 25 × 2 = 50 × 2 = 100. Ticks were 1–2 mo postmolt at the time of evaluation; 25 specimens were tested per time treatment.

Supercooling Point Determinations. The supercooling point is the lowest body temperature recorded before the latent heat of fusion release as a specimen freezes. Insects that are intolerant of freezing frequently exploit their capacity to supercool as an overwintering strategy (Lee 1991). Supercooling points for unfed males, unfed females, engorged nymphs, engorged larvae, and eggs were determined. The acclimation effect on supercooling points for these stages were also determined by exposing specimens to 5°C for 7 d at 93% RH. A 30-gauge copper-constantan thermocouple was positioned on the individual tick integument or egg surface being tested. Multiple specimens were analyzed simultaneously using separate leads for each. A cooling rate of 1°C/min was maintained using a low-temperature bath (Neslab RE-8DD, Newington, NH) and measured by a multichannel recorder (Leeds and Northrup, Stamford, CT).

Results and Discussion

In the initial low temperature experiment (Fig. 1), all immature and adult ticks died when exposed to −17°C for 7 d (fed larvae [n = 20], fed and [n = 10] unfed nymphs [n = 10], and unfed adult males [n = 5] and females [n = 5]). A single lone star male lived for 12 d at −10°C. For a shorter exposure of 8 d to −10°C, unfed immatures (larvae, 50%, n = 20; nymphs, 20%, n = 10), unfed adult males (80%, n = 5), and females (100%, n = 5) survived, while all of the fed larvae (n = 20)

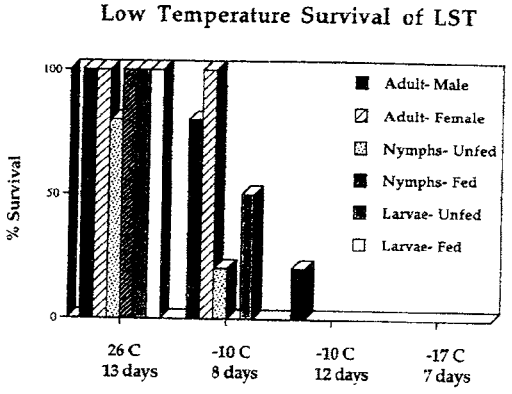

Fig. 1. Low-temperature survival of *A. americanum* larvae (fed, unfed, $n = 20$ each), nymphs (fed, unfed, $n = 10$ each), and unfed males and females ($n = 5$, each) at 93% RH.

and fed nymphs perished ($n = 20$). There was no mortality at 26°C (93% RH) after 13 d for unfed males, unfed females, engorged nymphs, and engorged larvae, while 20% (2) of unfed nymphs died. Overall for this 1st experiment, adults tolerated cold better than immatures and unfed specimens fared better than fed ticks.

For the next low-temperature survival experiment we held the temperature constant for 6-wk-old unfed lone star adult males and females while subjecting each of 25 specimens to −15°C for various times periods (10, 30, 60, 80, 100, and 120 min, Table 1). Adults were chosen for this experiment because this is the stage at which the lone star tick usually overwinters in nature (Hair and Bowman 1986). Males tolerated extreme short-term chilling better than females. The greatest difference between the sexes was observed for the 100-min exposure when only 44 ± 9.9% of the females were alive after 24 h, whereas 84 ± 7.4% of the males remained viable. This same trend was obvious 72 h after the exposure as well. Interestingly, damage scores were not reflective of this difference between sexes at 100 min. Females were apparently harmed because of chilling as reflected by the lower survival rates and higher damage scores at 72 h. Survival and damage scores were fairly consistent between 24 and 72 h for the males. Overall, damage and survival data indicate that males were more tolerant of low temperature than females under these laboratory exposures, although we performed only a single replication of 25 individuals. In the midst of these apparent differences between sexes, it should be noted that half or more of males and females survived a 2 h exposure to −15°C. When comparing *A. americanum* and *A. cajennense* for chilling injury, adult lone stars were significantly more tolerant to −12.5°C after 90 and 120 min, which may explain the more northern distribution for lone stars compared with the Cayenne tick, *Amblyomma cajennense* (F.), (Strey et al. 1996).

Table 2. Hemolymph osmolarity in milliosmoles ± SEM of adult male and female *A. americanum* exposed to chilling or heat shock for 2 h

Stage tested	25°C Control	0°C Chilling	40°C Heat shock
Unfed females ($n = 5$)	348.6 ± 2.3a	368.4 ± 4.5b	345.2 ± 4.0a
Unfed males ($n = 5$)	364.8 ± 4.5c	394.6 ± 7.0d	368.2 ± 4.1c

Specimens were 6 wk postmolt at the time of the experiment; ticks were exposed to temperature extremes or control (25°C) for 2 h before the hemolymph sample was taken; 5 hemolymph (1 per tick for 5 ticks) samples per experimental treatment. Means followed by the same letter are not significantly different ($P \geq 0.05$) as determined by 2-way ANOVA (Abacus Concepts 1986).

Our hypothesis was that the hemolymph osmotic pressure would increase for both the chilling and heat shock treatments. In the 1st case, low temperature would mobilize cryoprotectants and in the later treatment a loss of water by transpiration would concentrate solutes in the hemolymph. Hemolymph osmotic pressures for both 6-wk-old adult males and females increased significantly in response to chilling at 0°C for 2 h (Table 2). Female controls were 348.6 ± 2.3 mOsm (25°C), and 0°C treated specimens were 368.4 ± 4.5, a 5.7% increase ($n = 5$; 2-way analysis of variance [ANOVA], $P < 0.0001$). Males increased from 364.8 ± 4.4 to 394.6 ± 7 mOsm, an 8.2% increase ($n = 5$, ANOVA, $P < 0.0001$). On average, osmotic pressure values were higher for males than females in this small group comparison. Certainly, a larger sample size is needed to discriminate between sexes. The osmotic pressure difference between sexes may partially explain the advantage males had in the studies on low-temperature survival and damage (Tables 1 and 2). Males may also have mobilized more cryoprotectants in response to cold temperature. Mobilization of additional cryoprotectants into the hemolymph would offer protection against low temperatures to both sexes as has been described for insects (Lee et al. 1987, Lee 1991). We have not analyzed hemolymph for the identity of solute(s) that might account for increased osmotic pressures and potential protection from low temperature. *Ixodes uriae* White has a small amount of hemolymph glycerol (Lee and Baust 1987).

When exposure to low temperature occurs at a relative humidity above the water vapor uptake pump threshold, then ticks could theoretically reclaim salts that had been deposited on the mouthparts as a form of storage excretion (Sigal et al. 1991). For our experiment, this would have been possible because the relative humidity was 93% for the 2-h exposure, a relative humidity that is above the critical equilibrium humidity for our laboratory colony of lone stars (Sigal 1990). It would be interesting to repeat this experiment at a relative hu-

Table 3. Comparison of supercooling ponts ± SEM for *A. americanum* at 26°C or cold-acclimated at 5°C for 7d

Tick stage tested	Control (26°C)	Cold-acclimated (5°C)
Adult males, unfed	−13.1 ± 0.9 (n = 5)	−14.5 ± 0.7 (n = 5)
Adult females, unfed	−13.3 ± 0.8 (n = 6)	−12.8 ± 0.7 (n = 6)
Nymphs, engorged, and immobile	−19.5 ± 0.6 (n = 7)	−16.9 ± 0.8 (n = 7)
Nymphs, engorged, and mobile	−16.0 ± 1.2 (n = 9)	−18.7 ± 1.5 (n = 6)
Larvae, engorged	−22.2 ± 0.4 (n = 5)	−22.2 ± 0.5 (n = 8)
Eggs	−25.8 ± 0.3 (n = 6)	−27.2 ± 0.2 (n = 7)

Cold acclimation had no influence on the supercooling points (t-test, $P \geq 0.05$); all specimens perished following exposure to the supercooling regimen showing that they were freeze-intolerant; adult ticks were 1 mo postmolt at the outset of the experiment. Supercooling points lower than −28°C may have been missed, and therefore the difference between warm-and cold-acclimated eggs may not be accurate.

midity below the water uptake threshold to see whether an increase in osmotic pressure was still possible. Further experimentation is also required to determine whether mobilization of solutes between the hemolymph and mouthparts (by way of the salivary glands) accounts for the increase in hemolymph osmotic pressure we observed with low-temperature exposure.

At the other extreme, exposure to 40°C for 2 h resulted in no significant hemolymph osmotic pressure changes for either sex (Table 2). This result suggests they were regulating hemolymph osmotic pressure while losing water by transpiration through the integument (Needham and Teel 1986, 1991). *A. americanum* excretes salt via the salivary glands to the mouthparts when exposed to high temperature or severe desiccating conditions (Sigal et al. 1991). We conclude from the osmotic pressure experiment that these ticks regulated hemolymph solutes at both temperature extremes. For heat shock, unfed adult lone stars most likely removed hemolymph solute(s) or added water, and for chilling they mobilized solute(s) into the hemolymph from some storage site.

Unlike in the Antarctic tick *I. uriae* (Lee and Baust 1987), cold-acclimation of *A. americanum* for 7 d at 5°C had no influence on the supercooling point for unfed adult males and females, engorged nymphs and larvae, and eggs (Table 3, $P < 0.05$, paired t-test). For the soft tick *Argas reflexus* (Acari: Argasidae) there was no influence of seasonal fluctuations on supercooling points that ranged between −20 and 26.5°C for larvae, nymphs and adults, which were fed and unfed (Dautel and Knulle 1996). In the same study, these investigators found distinct differences between the mean supercooling points of field-collected *Ixodes ricinus* (L.) and unfed *I. ricinus* from an outdoor experimental site in Berlin, Germany. Supercooling points ranged from −1°C for unfed adult male and female *A. americanum* to a low of −26°C for the egg stage. With respect to size, smaller arthropods supercool to lower temperatures than larger ones (Lee 1991), and that this trend holds interspecifically is confirmed by our findings. Within comparable life stages, supercooling values for *I. uriae* and *A. americanum* were quite similar despite the very different habitats used by these species. No individual in any life stage survived the supercooling point, indicating that a laboratory colony of *A. americanum* is freeze intolerant.

The scientific value of supercooling determinations for ticks may have little predictive value in an ecological context (e.g., where ticks can live geographically) because specimens experienced damage and mortality before these low temperatures were reached. In the past, supercooling points were used as a criterion for characterizing the potential cold tolerance for an organism, but cryobiologists are relying less on this parameter (Denlinger 1991). For one reason, these extremely low temperatures are seldom reached in nature near the normal distribution for *A. americanum* (Burks et al. 1996b).

In an ecological context, these laboratory experiments represent only a beginning toward understanding the effect of low temperatures on *A. americanum*. Future studies are required on the egg stage and immatures because they may be the weak links in limiting the tick's northern distribution. The effect of the feeding state (unfed versus fed) needs to be examined thoroughly. Low temperature also influences the lower temperature limit at which water vapor can be absorbed by off-host stages. Sustained low temperature could result in tick desiccation if the active vapor uptake mechanism cannot replenish lost water. Comparative cold tolerance studies on field-collected ticks from both northern and southern distribution ranges should help discern the plasticity for a particular species to survive in colder climates if they are carried there by humans or through the movements of natural hosts.

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