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RELATIONSHIP BETWEEN COLD HARDINESS AND SUPERCOOLING POINT IN *AEDES ALBOPICTUS* EGGS[1]

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ABSTRACT. For many insects, low temperatures are not lethal unless their tissues freeze. To determine whether freezing is the cause of low temperature mortality of *Aedes albopictus* eggs, we measured their lower lethal temperature, the temperature that causes 50% mortality in a population, and supercooling point, the temperature at which the tissues freeze. Tropical and temperate *Ae. albopictus* eggs were tested under different diapause and cold acclimation conditions. Temperate *Ae. albopictus* eggs had a lower lethal temperature than tropical *Ae. albopictus* eggs. Diapause and cold acclimation decreased the lower lethal temperatures of temperate eggs. However, neither diapause, cold acclimation, nor geographic origin affected *Ae. albopictus* egg supercooling points. All lower lethal temperatures were above −13°C and all supercooling points were below −26°C, indicating prefreeze mortality. Eggs of *Aedes triseriatus* and *Aedes aegypti* also died before they froze.

INTRODUCTION

Aedes albopictus (Skuse) is a mosquito with a comparatively wide geographic distribution, including both hemispheres and spanning both temperate and tropical climatic zones (Hawley 1988). Short-day photoperiods and moderate temperature during the pupal and adult stages induce production of diapause eggs in temperate *Ae. albopictus* populations (Hawley et al. 1987). Temperate *Ae. albopictus* overwinters as a pharate 1st instar inside the egg. Tropical *Ae. albopictus* populations cannot diapause and are much less cold hardy than temperate populations (Hanson et al. 1993). Both diapause and cold acclimation increase temperate *Ae. albopictus* cold hardiness in the laboratory and in the field (Hanson and Craig 1994). Nevertheless, this species can suffer considerable overwintering mortality in the colder regions of its range (Hawley et al. 1989). The specific cause of low temperature mortality in *Ae. albopictus* eggs has yet to be determined.

Most workers originally believed that the only cause of low temperature mortality in temperate and polar insects was freezing (Lee 1989). However, more recent studies have shown that some species from cold regions suffer mortality at temperatures above their supercooling point, the temperature at which their tissues freeze. In this paper, we report that diapausing, cold-acclimated *Ae. albopictus* eggs die at temperatures well above their supercooling points. *Aedes triseriatus* (Say) and *Aedes aegypti* (Linn.) eggs were included in this experiment for interspecific comparison.

MATERIALS AND METHODS

Strains: The following mosquito strains were used: 1) F_4 generation of *Ae. albopictus* INDY, a strain collected in Indianapolis, IN, in 1986, 2) F_5 generation of *Ae. albopictus* SABAH, a strain collected in Malaysia in 1986, 3) F_{30+} generation of *Ae. triseriatus* WALTON, a strain collected in St. Joseph County, IN, in 1969, and 4) F_{60+} generation of *Ae. aegypti* ROCK, which was obtained from the Rockefeller Institute in 1959.

Rearing procedures: To produce nondiapause eggs, all stages were reared at 27°C, 80% RH, and 16:8 h (L:D). Diapause eggs were obtained by rearing pupae and adults at 21°C, 80% RH, and 8:16 h (L:D). Details of the rearing procedure are described in Hanson and Craig (1994).

Experiment 1, lower lethal temperature: Our primary interest was to determine the lower lethal temperature of the temperate *Ae. albopictus* strain, INDY; SABAH, a tropical strain, was included for comparison. Lower lethal temperatures were determined for 3 groups: 1) nondiapause eggs that were not cold acclimated, 2) nondiapause eggs cold acclimated at 10°C for 60 days, and 3) diapause eggs cold acclimated at 10°C for 60 days. The latter cold acclimation regime has been shown to induce a level of cold hardiness equal to that achieved in the field (Hanson and Craig 1994). The eggs were chilled at various temperatures for 24 h. Following chilling, they were thawed and hatched as described

[1] In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association of Laboratory Animal Care.

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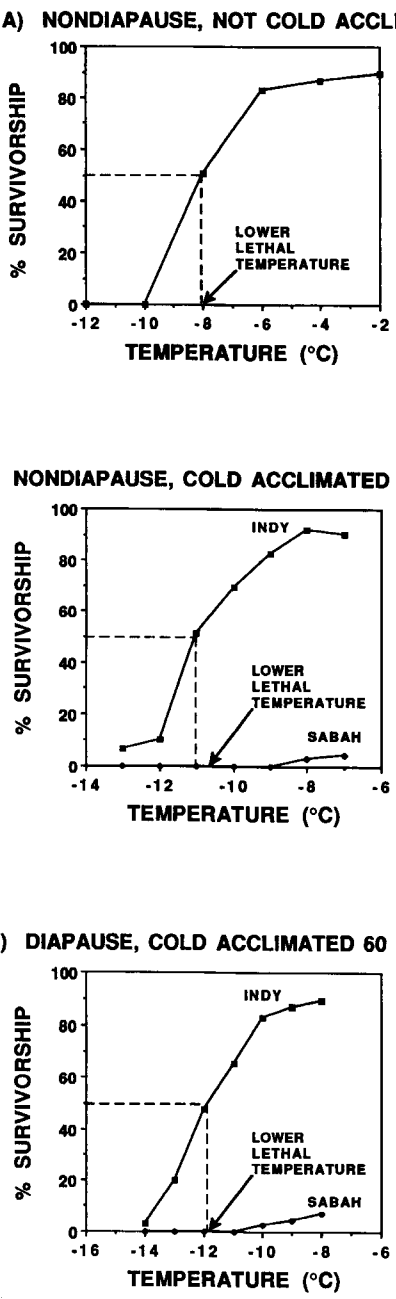

Fig. 1. Effect of chilling for 24 h at various temperatures on survivorship of temperate (INDY) and tropical (SABAH) *Aedes albopictus* eggs. A. Eggs produced under nondiapause-inducing conditions (27°C, 80% RH, and 16:8 h L:D) and not cold acclimated. Each data point represents 83–344 eggs. B. Eggs produced under nondiapause-inducing conditions (27°C, 80% RH, and 16:8 h L:D) and cold acclimated at 10°C for 60 days. Each data point represents 94–563 eggs. C. Eggs produced under diapause-inducing conditions (pupae and adults were incubated at 20°C, 80% RH, and 8:16 h L:D) and cold acclimated at 10°C for 60 days. Each data point represents 182–385 eggs.

in Hanson and Craig (1994) and Novak and Shroyer (1978). Larvae that displayed a negative phototaxis and characteristic larval movements were considered alive.

Experiment 2, supercooling points: Two to 4 eggs were attached to a BC32L1 thermistor (Fenwal Electronics) using petroleum jelly. The thermistor was attached, by copper wire, to a YSI Model 42SC Tele-Thermometer, which was connected to a Fisher Recordall Series 5000 recorder. The thermistor and attached eggs were passed through a hole in a rubber stopper in the mouth of a 250-ml Erlenmeyer flask and suspended about 1 cm from the bottom of the flask. The flask then was placed in an RK 20 Brinkmann temperature bath. The eggs were allowed to equilibrate for 1 h at 0°C. The temperature then was decreased continuously at the rate of 1°C every 1–2 min. The supercooling point was represented on the recorder by a sudden spike in the temperature of the thermistor.

Supercooling points were measured for *Ae. albopictus* INDY and SABAH eggs collected under both nondiapause- and diapause-inducing conditions for the following different groups: 1) not cold acclimated, 2) cold acclimated at 10°C for 10 days, and 3) cold acclimated at 10°C for 60 days. The supercooling points were also determined for *Ae. albopictus* INDY and *Ae. triseriatus* WALTON eggs that had been cold acclimated at 10°C for 10 days and placed in the field in northern Indiana from December 8, 1988, through January 9, 1989. In addition, supercooling points were determined for *Ae. triseriatus* WALTON and *Ae. aegypti* ROCK eggs oviposited under diapause-inducing conditions and not cold acclimated. The supercooling points of 3–7 eggs were measured for each treatment group.

Experiment 3, influence of duration of chilling on the supercooling point: When we measured supercooling points in Experiment 2, the eggs were exposed to low temperature for less than 1 h. But an insect's supercooling point may increase with the duration of exposure to low temperature (Salt 1966). Therefore, the eggs in Experiment 1 (chilled for 24 h) may have frozen at a much higher temperature than the eggs in Experiment 2 (chilled less than 1 h). For that reason, we conducted this experiment to determine whether *Ae. albopictus* INDY eggs freeze when held for 24 h at a temperature higher than their supercooling point as measured in Experiment 2 but lower than their lower lethal temperature.

Six *Ae. albopictus* INDY eggs were attached to a thermistor and treated according to the pro-

Table 1. Comparison of supercooling points of different *Aedes* species.

Species	Strain	Acclimation[1]	Mean supercooling point ± SD (°C)	
			Nondiapause[2]	Diapause[3]
Ae. albopictus	INDY	None	−27.2 ± 1.0	−26.0 ± 1.1
Ae. albopictus	INDY	10 days	−27.7 ± 1.8	−29.8 ± 1.9
Ae. albopictus	INDY	60 days	−27.7 ± 2.0	−30.2 ± 1.5
Ae. albopictus	SABAH	None	−26.4 ± 2.3	−27.1 ± 1.2
Ae. albopictus	SABAH	10 days	−26.7 ± 1.7	−27.4 ± 0.7
Ae. albopictus	SABAH	60 days	−27.2 ± 2.0	−26.9 ± 3.6
Ae. aegypti	ROCK	None	−24.1 ± 2.2	—
Ae. triseriatus	WALTON	None	−36.9 ± 0.6	—
Ae. albopictus	INDY	10 days[4]	—	−28.4 ± 3.4
Ae. triseriatus	WALTON	10 days[4]	—	−36.4 ± 0.8

[1] Duration of cold acclimation at 10°C prior to determining supercooling points.
[2] Oviposited under nondiapause-inducing conditions (27°C, 80% RH, and (16:8 h, L:D).
[3] Oviposited under diapause-inducing conditions (pupae and adults were incubated at 20°C, 80% RH, and (8:16 h, L:D).
[4] After cold acclimation at 10°C for 10 days, eggs were placed in a tire in the field on December 8, 1988. They were collected on January 9, 1989, for supercooling point determination.

cedure of Experiment 2 with the exception that their temperature was decreased to −20°C and held at that temperature for 24 h. We chose −20°C because it was substantially higher than the supercooling point as measured in Experiment 2 yet much lower than the lower lethal temperature of *Ae. albopictus* eggs. Because no temperature spikes were observed when the eggs were at −20°C for 24 h, their temperature was decreased below −20°C at the rate of 1°C every 1–2 min until temperature spikes were observed. This final procedure confirmed that no temperature spikes occurred when the eggs were at −20°C for 24 h.

RESULTS

Experiment 1, lower lethal temperature (Fig. 1): Cold acclimation and diapause decreased the lower lethal temperature of *Ae. albopictus* INDY eggs. The INDY lower lethal temperatures were about −8, −11 and −12°C, for the groups that were: 1) nondiapause and not cold acclimated, 2) nondiapause and cold acclimated at 10°C for 60 days, and 3) diapause and cold acclimated at 10°C for 60 days, respectively. SABAH egg survivorship was below 10% in all treatments.

Experiment 2, supercooling points (Table 1): Cold acclimation and diapause had no effect on the supercooling points of either *Ae. albopictus* INDY or SABAH. The supercooling points of INDY and SABAH were similar. The supercooling points of *Ae. albopictus* and *Ae. triseriatus* eggs that had been placed in the field were similar to those of eggs kept in the laboratory. The supercooling points of both *Ae. albopictus* strains were higher than those of *Ae. triseriatus* WALTON and lower than those of *Ae. aegypti* ROCK.

Experiment 3, influence of duration of chilling on supercooling point: The *Ae. albopictus* INDY eggs held at −20°C for 24 h did not freeze, indicating that the low supercooling points of Experiment 2 were not simply an artifact of the relatively short time they were chilled. All of the eggs froze after their temperature was subsequently reduced below −20°C.

DISCUSSION

Cold acclimation and diapause increased the ability of temperate (INDY) *Ae. albopictus* eggs to resist cold temperatures. Other studies had similar results in the laboratory (Hanson and Craig 1994) and in the field (Hawley et al. 1989).

Our data showed that *Ae. albopictus* eggs acclimated in the laboratory and *Ae. albopictus* eggs acclimated in the field died at temperatures much higher than their supercooling points. Moreover, field studies (Hanson et al. 1993; Hanson and Craig, unpublished data) demonstrated that *Ae. albopictus* eggs died at temperatures higher than the supercooling points recorded in this study. Because the supercooling point is the temperature at which the tissues freeze, we concluded that low temperature mortality of *Ae. albopictus* eggs is not caused by freezing. Because freezing is not the cause of the overwintering mortality of *Ae. albopictus* eggs, it is not surprising that *Ae. albopictus* eggs do not decrease their supercooling points in preparation for winter, as do insects that do not die until their tissues freeze (Zachariassen 1985).

Like *Ae. albopictus, Ae. aegypti* and *Ae. triseriatus* also die at temperatures higher than their supercooling points. *Aedes aegypti* eggs did not survive temperatures lower than 7°C in the field

nor −3°C for 24 h in the laboratory (Hatchett 1946), though they had a supercooling point of −24°C. Similarly, *Ae. triseriatus* eggs had a 24-h lower lethal temperature of −30°C in the laboratory (Hanson and Craig, unpublished data), though they had a supercooling point of about −37°C in the present study.

In addition to the mosquitoes in our study, many other temperate insect species die at temperatures higher than their supercooling points. For example, such prefreeze mortality has been demonstrated in diapausing, cold-acclimated collembolan (*Orchesella cincta* and *Tomocerus minor*) nymphs and adults (van der Woude and Verhoef 1986), dipteran pupae (*Delia radicum*) (Turnock et al. 1990), and lepidopteran pupae (*Mamestra configurata*) (Turnock and Bracken 1989). This is the first report of prefreeze mortality in eggs of temperate insects.

If the low supercooling points of the mosquito eggs in this study are not the result of a biochemical cold hardiness mechanism, as in freeze-avoiding insects, why do the eggs have such low supercooling points? Eggs usually have lower supercooling points than other stages (Sømme 1982). Low body volume (Salt 1966) and the absence of food particles in the gut to act as ice nucleators (Sømme 1982) are the most likely explanations. Even eggs of a species from a warm climate, *Locusta migratoria*, supercool to −30°C (Lozina-Lozinskii 1974). Consequently, it is not surprising that the eggs in our study had low supercooling points.

There are many postulated causes of prefreeze mortality in insects, such as membrane damage (Quinn 1985), protein inactivation (Franks and Hatley 1985), disruption of metabolic processes (Salt 1961), and membrane condensation (McGrath 1984). Although some progress has been made (Pullin et al. 1990), evidence for a specific cause of lethal effects in any insect remains elusive.

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