Journal of the American Mosquito Control Association, 18(2):126–127, 2002

SCIENTIFIC NOTE

A PRELIMINARY CHARACTERIZATION OF THE PHYSIOLOGICAL ECOLOGY OF OVERWINTERING *ANOPHELES* MOSQUITOES IN THE MIDWESTERN USA

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ABSTRACT. Field-collected *Anopheles quadrimaculatus* and *An. punctipennis* adult mosquitoes from various types of overwintering hibernacula in southwestern Michigan and northern Indiana were examined for cold tolerance. Adult mosquitoes were collected on a monthly basis from October 1997 to April 1998. The mean supercooling point for adult for *An. punctipennis* was significantly lower than that for *An. quadrimaculatus*. The lower lethal temperatures for these species were −17°C and −15°C for *An. punctipennis* and *An. quadrimaculatus*, respectively.

KEY WORDS *Anopheles* mosquitoes, overwintering, cold-hardiness

In the Midwest, *Anopheles quadrimaculatus* (Say) (sensu lato) and *Anopheles punctipennis* (Say) potentially may play a significant role as the principal vectors of Cache Valley virus (CV). In this region, both species exhibit overwintering diapause in the adult stage. The location of these overwintering sites (hibernacula) is geographically variable, for example, tree hollows and buildings in North Carolina (Balfour 1928) to bridges and culverts in California (Washino and Bailey 1970). Overwintering insects are confronted with a different set of challenges at low temperatures compared to those at high temperatures (Denlinger and Lee 1998).

In temperate zone insects, cold-hardiness pertains to the capacity of an organism to survive low temperatures. Winter survival usually involves 1 of 2 strategies: freeze avoidance via depression of the temperature at which the insect body freezes (termed the supercooling point or crystallization temperature); or freeze tolerance, which is often associated with an increase in the temperature at which spontaneous ice nucleation occurs, sometimes caused by ice-nucleating proteins, lipoproteins, or both (Lee 1989). Freeze-intolerant species that naturally experience subzero temperatures generally rely on supercooling for survival (Costanzo et al. 1998). The supercooling point is defined as the temperature at which ice crystals begin to form (Lee 1989). The supercooling capacity of insects may be influenced by geographic origin, season, thermal acclimation, life stage, feeding status, endogenous nucleators, and cryoprotectants, such as glycerol or trehalose (Lee et al. 1993, Duman et al. 1995). In general, the capacity to supercool is more than adequate to ensure that freezing of the animal does not occur in its usual winter hibernaculum (Costanzo et al. 1998). In the case of mosquitoes, little information is available for assigning potential disease vectors such as mosquito species to either a freeze-avoidance or freeze-tolerant strategy. The purpose of this preliminary study was to characterize the physiological ecology in terms of cold-hardiness.

To investigate the cold-hardiness of adult *An. quadrimaculatus* and *An. punctipennis,* mosquitoes were subsampled from anthropogenic overwintering hibernacula in northern Indiana and southern Michigan in February 1998. For this analysis, subsamples were collected from 1 date in February because of the low number of adults present in these hibernacula. Adult mosquitoes were collected with an aspirator from inside the hibernacula, maintained on ice, transported to a cold room (4°C), and kept there until analysis. Adults were placed inside a flask suspended in an ethanol cooling bath. A thermister probe was lightly adhered to each mosquito with petroleum jelly to record the supercooling point. The supercooling point was identified and marked by the recorder as the exotherm caused by the release of latent heat of each mosquito or the point at which ice crystals formed. Once the supercooling point for each species was determined, additional collections of adult anophelines were used to determine the median lower lethal temperature (LL_{50}). Lower lethal temperatures were estimated by pooling 10 mosquitoes from each species, lowering the temperature of the cooling bath to −1°C below the supercooling point, and maintaining the mosquitoes inside a glass flask for 24 h. After the 24-h time period, mosquitoes were allowed to thaw and the number of dead vs. live mosquitoes was recorded. Multiple trials were conducted, lowering the temperature until 50% of the individuals were dead (LL_{50}).

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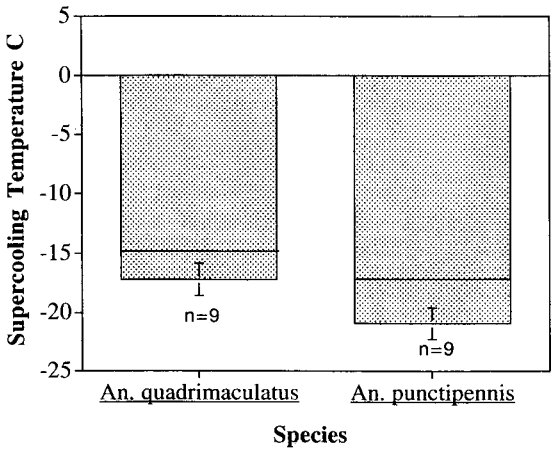

Fig. 1. Mean supercooling for *Anopheles quadrimaculatus* and *Anopheles punctipennis* in northern Indiana (February 1998). Mann-Whitney *U*-test analysis: $U = 18.0$; $P < 0.05$. Solid line across each bar indicates median lower lethal temperature.

The mean supercooling temperature for adult *An. punctipennis* (mean ± SE = −20.9 ± 1.33°C) was significantly lower than *An. quadrimaculatus* (mean ± SE = −17.2 ± 1.36°C) (Fig. 1). We determined the lower lethal temperatures for these species to be −17°C (*An. punctipennis*) and −15°C (*An. quadrimaculatus*).

We observed the LL_{50} and the supercooling point to be significantly lower in overwintering females of *An. punctipennis* compared to *An. quadrimaculatus*. The ability to withstand colder temperatures may allow *An. punctipennis* additional hibernaculum choices, including natural types, for example, animal burrows. Contrary to Hitchcock's (1968) conclusions that females die of exposure, our results indicate that female anophelines may be capable of reducing mortality through supercooling activities. Because the lower lethal temperatures approximate the supercooling points, *Anopheles* mosquitoes most likely utilize a freeze-avoidance strategy. Future work will address the mechanism these mosquitoes employ to survive such cold temperatures as well as the difference in microclimates found in selected hibernacula.

As shown by Washino and Bailey (1970), anopheline mosquitoes will take a midwinter bloodmeal. However, from our preliminary study, a midwinter bloodmeal(s) is contradictive of the overwintering strategy of these mosquitoes. In preparation for overwintering, potentially freeze-susceptible species (such as these anophelines) can lower their supercooling point by evacuating the gut, thereby removing potential ice-nucleating agents (Lee 1989). A midwinter bloodmeal might lead to extracellular ice formation and early death. It is unknown if adult mosquitoes change or shift from 1 hibernaculum to another during winter warming periods. However, until we understand the microclimates of these hibernacula, we can only speculate whether a hibernaculum-shifting hypothesis or natural mortality explains the variation in numbers of mosquitoes observed at different times of the winter.

This project was supported by the University of Notre Dame and by National Institutes of Health grant AI 02753 to P.J.G. We would like to acknowledge Jack Duman, Sandy Sass, Marcy Kern, Jen Meece, Mary Garvin, and Diane Lovin for their invaluable field and laboratory assistance. J.R.W. would like to thank Adriana Costero and Rick Lee for comments on earlier versions of the manuscript.

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