Journal of the American Mosquito Control Association, 11(3):354–357, 1995

OPERATIONAL AND SCIENTIFIC NOTES

FIELD OVERWINTER SURVIVORSHIP OF *AEDES ALBOPICTUS* EGGS IN JAPAN

SCOTT M. HANSON[1]

Laboratory of Applied Entomology, Faculty of Agriculture, University of Tokyo, 1-1-1 chome, Yayoi, Bunkyo-ku, Tokyo 113 Japan

ABSTRACT. The overwintering ability of eggs of 3 temperate and 2 tropical strains of *Aedes albopictus* was assessed at 5 field sites during winter 1991–92 and 4 field sites during winter 1992–93 in Japan. Nagasaki, the warmest site, is the only site at which tropical *Ae. albopictus* could possibly become established. The eggs of temperate *Ae. albopictus* can survive less than half of the winters in Sapporo. Temperate *Ae. albopictus* can survive at least some of the winters at Bijo Daira, Kitakami, Nagasaki, Tokyo, and Toyama.

Before its recent colonization of various sites worldwide, *Aedes albopictus* (Skuse) was confined to Asia. *Aedes albopictus* occurs in both tropical and temperate Asia. Its temperate Asian distribution includes China, South Korea, and Japan. Temperate *Ae. albopictus* overwinters as a diapausing egg (Hong et al. 1971). Tropical *Ae. albopictus* cannot diapause and is less cold hardy than temperate *Ae. albopictus* (Hawley et al. 1987).

Nawrocki and Hawley (1987) have suggested that, during the summer, *Ae. albopictus* in a temperate region may expand its range to include areas in which it may not be able to survive the winter. In Japan, the northernmost site where *Ae. albopictus* has been collected is Sendai (Fig. 1) (Kamimura 1968). After comparing temperature and distribution data of *Ae. albopictus* populations in China, South Korea, and Japan, Nawrocki and Hawley (1987) suggested that winter temperatures north of Sendai would not prevent the establishment of *Ae. albopictus.* However, the northernmost Japanese site at which *Ae. albopictus* is known to successfully overwinter is Ashikaga, a city ≈80 km northwest of Tokyo (Fig. 1). First instars were collected in tree holes in Ashikaga in March (Kurashige and Ogawa 1967). Because so little is known about the overwintering ability of *Ae. albopictus* in temperate Japan, I measured how well *Ae. albopictus* eggs survive the winter at different sites in Japan to better delineate the northern limits of *Ae. albopictus* overwintering ability in Japan. International commerce has made Japan vulnerable to invasion by *Ae. albopictus*; however, low winter temperatures may prevent tropical populations from becoming established. Thus, tropical strains were included in this study to determine whether tropical *Ae. albopictus* can become permanently established on the main islands of Japan.

Experiment 1, winter of 1991–92: The following temperate *Ae. albopictus* strains were used: 1) the F_9 generation of INDY, collected in Indianapolis, IN, in 1986; 2) the F_5 generation of KAGOSHIMA, collected in Japan in 1988 (Fig. 1); and, 3) the F_9 generation of ZAMA, collected in Japan in 1986 (Fig. 1). The tropical strains used were the F_3 generation of PENANG, collected in Malaysia in 1989, and the F_5 generation of SINGAPORE AMOY, collected in Singapore in 1988.

Larvae were reared at 27°C, 80% RH, and 16 h:8 h (L:D) as described by Munstermann and Wasmuth (1985) and Hanson and Craig (1994). Upon pupation, they were transferred to an environmental chamber maintained at 21°C, 80% RH, and 8 h:16 h (L:D) to induce the production of diapause eggs (Mori et al. 1981). Eggs in this experiment were produced by 100 females of each strain.

A subset of the eggs of each strain was placed in the field in Kitakami (altitude: 155 m; latitude: 39°20′), Nagasaki (altitude: 27 m; latitude: 32°44′), Sapporo (altitude: 17 m; latitude: 43°03′), Tokyo (altitude: 5 m; latitude: 35°41′), and Toyama (altitude: 9 m; latitude: 36°42′) in early October 1991 (Fig. 1). There were 75–331 eggs of each strain at each site. The eggs were placed at ground level away from direct sunlight in a sealed plastic bag. A small piece of moist cotton inside each bag maintained suitable hu-

[1] Present address: Medical Entomology Program, Illinois Natural History Survey, 607 E. Peabody Drive, Champaign, IL 61820-6970.

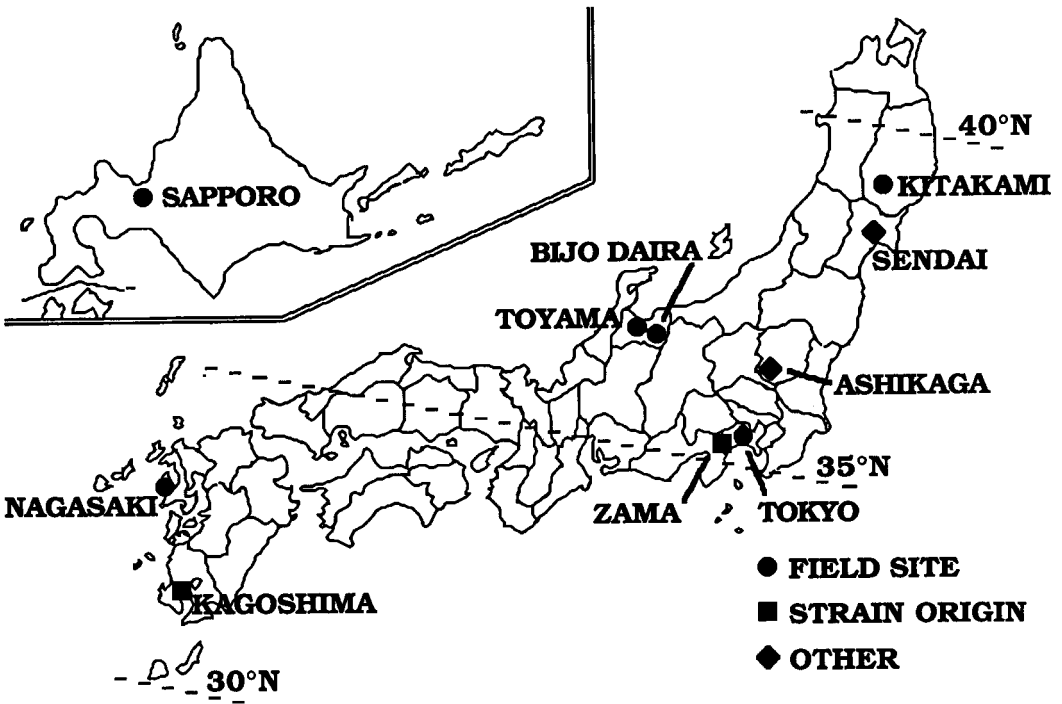

Fig. 1. Map of the main islands of Japan. Sendai is the northernmost site where *Aedes albopictus* has been found. Ashikaga is the northernmost site where *Ae. albopictus* is known to survive the winter. A subset of the eggs of each strain was placed in the field in Kitakami, Nagasaki, Tokyo, Toyama, and Sapporo in early October 1991. All subsets of eggs were taken from the field on April 1, 1992, and transported to the laboratory. A subset of the eggs of each strain was placed in the field in Kitakami, Bijo Daira, Tokyo, Toyama, and Sapporo in early October 1992. All subsets of eggs were taken from the field on April 1, 1993, and transported to the laboratory.

midity. Maximum/minimum thermometers were placed beside each bag in order to record the absolute minimum temperature (a winter's lowest temperature) experienced by the eggs at each site. All subsets of eggs were taken from the field on April 1, 1992, and transported to the laboratory. The minimum temperature reading on each maximum/minimum thermometer was recorded when each set of eggs was retrieved from each field site. They were hatched as in Novak and Shroyer (1978) and Hanson and Craig (1994). After hatching, the eggs were bleached to remove the chorion to determine the number of embryonated unhatched eggs. The bleach, consisting of 40 g $NaClO_2$ and 10 ml acetic acid added to 1 liter of water, was a modified version of the bleach described by Trpis (1970).

Temperature data were collected from the weather station nearest each field site. Absolute minimum temperatures of winter 1991–92 at weather stations near all field sites were compared to their 10-year mean absolute minimum temperatures in order to determine the relative severity of the winter in the vicinity of each field site. I did not determine the relative severity of the winter by comparing field site temperatures with weather station temperatures for 2 reasons: First, there may be microclimatic differences between the field sites and weather stations, which make such a comparison invalid. Second, the field site temperatures were measured at ground level, but the weather station temperatures were measured 1.5 m above ground level. There could be substantial temperature differences between ground level and 1.5 m above ground level.

Experiment 2, winter of 1992–93: This experiment was conducted using the methods of Experiment 1. Eggs were placed in the field at 4 sites: Bijo Daira (altitude: 980 m; latitude: 36°32′), Kitakami, Tokyo, and Sapporo (Fig. 1). There were 111–308 eggs of each strain at each site.

Experiment 1, winter of 1991–92: No eggs survived the winter in Sapporo (Fig. 2). At least 90% of the eggs of each strain survived in Nagasaki. At Kitakami, Tokyo, and Toyama, eggs of temperate strains survived at rates of at least 73%, and eggs of tropical strains survived at rates of less than 14%. Eggs at sites with colder

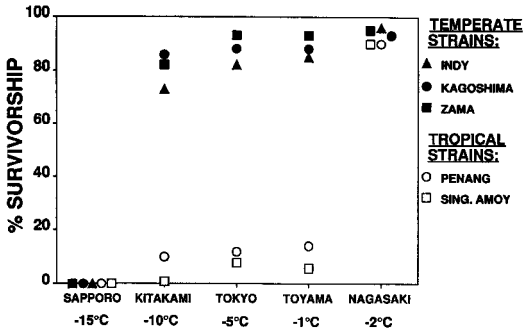

Fig. 2. Survivorship of *Aedes albopictus* eggs at different Japanese sites during winter 1991–92. Each sample had 75–331 eggs. The absolute minimum temperature recorded at each field site is written below the name of each field site.

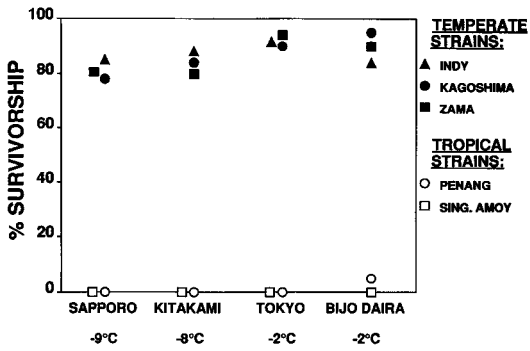

Fig. 3. Survivorship of *Aedes albopictus* eggs at different Japanese sites during winter 1992–93. Each sample had 111–308 eggs. The absolute minimum temperature recorded at each field site is written below the name of each field site.

absolute minimum temperatures tended to have lower survival rates, and eggs in sites with higher absolute minimum temperatures had higher survival rates. Absolute minimum temperatures at weather stations near all field sites were higher than 10-year mean absolute minimum temperatures (Table 1).

Experiment 2, winter of 1992–93: At least 78% of eggs of temperate strains survived at all sites (Fig. 3). Five percent of the PENANG eggs and none of the SINGAPORE AMOY eggs survived at Bijo Daira. No tropical eggs survived at any other sites. Absolute minimum temperatures at weather stations near all field sites were higher than 10-year mean absolute minimum temperatures (Table 1).

Because the absolute minimum temperatures at weather stations near all field sites during both winters were higher than their 10-year mean absolute minimum temperatures, I concluded that both winters were milder than usual at all field sites. The absolute minimum temperature recorded on the maximum/minimum thermometer at Bijo Daira was considerably lower than the absolute minimum temperature of the nearby weather station (Table 1). This probably was the result of the insulating effect of the considerable snow cover typical of Bijo Daira.

My results showed that the tropical strains were not as cold hardy as the temperate strains, which agrees with earlier studies (Hawley et al. 1987, Hanson and Craig 1994). The data show that Nagasaki is the only site at which tropical *Ae. albopictus* could possibly become established. Because there are no data that show tropical *Ae. albopictus* survivorship in Nagasaki during a winter that is colder than average, it is not

Table 1. Temperature data (°C) collected at or near the field sites. Absolute minimum *Aedes albopictus* egg temperatures were the lowest temperatures recorded by the maximum/minimum thermometers placed at each site with the eggs.

	Winter 1991–92					Winter 1992–93			
	Nagasaki	Tokyo	Toyama	Kitakami	Sapporo	Tokyo	Bijo Daira	Kitakami	Sapporo
Absolute minimum field-site temperature	−2	−5	−1	−10	−15	−2	−2	−8	−9
Absolute minimum temperature at nearest weather station (winter 1991–1992)	0.3	−0.5	−3.0	−9.7	−12.3	0.7	−8.0	−10.3	−11.6
Absolute minimum temperature at nearest weather station (10-year mean)	−1.3	−1.1	−5.4	−13.0	−14.1	−1.1	−9.0	−13.0	−14.1

certain whether tropical *Ae. albopictus* eggs could survive every winter in Nagasaki. Another factor that would discourage the permanent establishment of tropical *Ae. albopictus* in temperate Japan is the inability of tropical *Ae. albopictus* to diapause. Thus, any individuals hatching in the fall would probably not survive the winter in temperate Japan, because the larvae, pupae, and adults of *Ae. albopictus* are less cold hardy than the eggs (Ishii et al. 1954).

The lower limit of survival of temperate strains in the present study was an absolute minimum temperature between −10 and −15°C. This contrasts with Hanson and Craig (1995), who found that 78% of INDY eggs survived an absolute minimum temperature of −19°C in Indiana. A plausible explanation for this difference is the observation that both temperature and duration of exposure affect *Ae. albopictus* egg cold hardiness (Hanson and Craig 1994). Thus, the survivorship difference between the 2 studies is most likely the result of differing durations of exposure to extreme low temperature.

It seems unlikely that *Aedes albopictus* could become permanently established in Sapporo, because none of the eggs survived the winter of 1991–92 in Sapporo, even though that winter was milder than normal. On the other hand, the results suggest that temperate *Ae. albopictus* can survive winters in Toyama, Kitakami, and Bijo Daira that are warmer than normal. However, because there are no data indicating whether *Ae. albopictus* can survive winters that are colder than average at these sites, it is not clear whether temperate *Ae. albopictus* can survive every winter at these sites. Because temperate *Ae. albopictus* survived well in Tokyo and Nagasaki in this study, and because average temperatures at these 2 sites are quite mild, it is likely that *Ae. albopictus* can survive every winter at these 2 sites.

I gratefully acknowledge R. Arakawa of Toyama Medical and Pharmaceutical University in Toyama, K. Tadano in Kitakami, M. Kaneko of the Hokkaido Agricultural Institute in Sapporo, and M. Takagi of the Institute of Tropical Medicine in Nagasaki for their help in placing the eggs in various field sites. This research was supported by Postdoctoral Fellowship #190080 from the U.S. National Service Foundation and the Japanese Research and Development Corp. to S. Hanson and the Illinois Department of Energy and Natural Resources Waste Tire Grant SENR TM2 to R. Novak.

REFERENCES CITED

Hanson, S. M. and G. B. Craig, Jr. 1994. Cold acclimation, diapause, and geographic origin affect cold hardiness in eggs of *Aedes albopictus* (Diptera: Culicidae). J. Med. Entomol. 31:192–201.

Hanson, S. M. and G. B. Craig, Jr. 1995. *Aedes albopictus* eggs: field survivorship during northern Indiana winters. J. Med. Entomol. 32 (in press).

Hawley, W. A., P. Reiter, R. S. Copeland, C. B. Pumpuni and G. B. Craig, Jr. 1987. *Aedes albopictus* in North America: probable introduction in used tires from northern Asia. Science 236:1114–1116.

Hong, H. K., J. C. Shim, H. K. Shin and H. Y. Young. 1971. Hibernation studies of forest mosquitoes in Korea, 1971. Korean J. Entomol. 1:13–16.

Ishii, N., A. Nakayma and Y. Ishii. 1954. Biological observations on the mosquito, *Aedes albopictus*. Yokohama Med. Bull. 5:275–281.

Kamimura, K. 1968. The distribution and habit of medically important mosquitoes of Japan. Jpn. J. Sanit. Zool. 19:15–34. [In Japanese with English summary.]

Kurashige, Y. and T. Ogawa. 1967. Annual succession of the mosquito larvae breeding in the tree holes in Ashikaga City. Jpn. J. Sanit. Zool. 18:241–247. [In Japanese with English summary.]

Mori, A., T. Oda and Y. Wada. 1981. Studies on the egg diapause and overwintering of *Aedes albopictus* in Nagasaki. Trop. Med. 23:79–90.

Munstermann, L. E. and L. M. Wasmuth. 1985. *Aedes aegypti*, pp. 7–14. *In:* P. Singh and R. F. Moore (eds.). Handbook of insect rearing, Volume 2. Elsevier Science Publ., Amsterdam.

Nawrocki, S. J. and W. A. Hawley. 1987. Estimation of the northern limits of distribution of *Aedes albopictus* in North America. J. Am. Mosq. Control Assoc. 3:314–317.

Novak, R. J. and D. A. Shroyer. 1978. Eggs of *Aedes triseriatus* and *Ae. hendersoni*: a method to stimulate optimal hatch. Mosq. News 38:515–521.

Trpis, M. 1970. A new bleaching and decalcifying method for general use in zoology. Can. J. Zool. 48: 892–893.