Published in final edited form as:

J Am Mosq Control Assoc. 2011 December ; 27(4): 433–436.

RETENTION AND ADAPTIVENESS OF PHOTOPERIODIC EGG DIAPAUSE IN FLORIDA POPULATIONS OF INVASIVE *Aedes* *albopictus*

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Abstract

Female *Aedes albopictus*, F₂–F₃ descendents from individuals collected as immatures at 6 geographic sites in the USA during 2008, exposed to short daylengths (10 h of light and 14 h of darkness at 21°C) laid eggs in diapause, whose frequency depended upon population origin. Diapause responses in northern Florida and Illinois were strong, as had been reported approximately 10 years earlier for *Ae. albopictus* from these regions. For southern Florida, the diapause response was polymorphic, and its mean incidence decreased at 2 of 3 collection sites compared to 10 years earlier. Exposure in the field for 2- to 4-wk intervals in Vero Beach (lat 27°35'N) during January 2009 revealed that eggs laid by short-day females had significantly higher survivorship, even though <50% were estimated, from laboratory results, to be in diapause. Enhanced desiccation resistance may select for retention of diapause in southern Florida.

Keywords

Daylength; desiccation resistance; mosquito; polymorphism; survivorship

Phenological adaptations, such as diapause mediated by anticipatory photoperiodic cues, are known to be subject to rapid selection to accommodate insect populations to new or shifting environments (Shapiro 1980, Tauber et al. 1986, Bradshaw and Holzapfel 2006). Among mosquitoes, the North American pitcher plant inquiline *Wyeomyia smithii* (Coq.) has responded rapidly to recent global warming trends by terminating diapause earlier, cued by shorter daylengths (Bradshaw and Holzapfel 2001).

Shortly after *Aedes albopictus* (Skuse) established and spread in the USA during the 1980s, photoperiodic diapause traits of the founding populations were used to trace their origin to northern, temperate Japan (Hawley et al. 1987). Diapausing eggs of *Ae. albopictus* in Indiana were able to survive northern temperate winters better than nondiapausing counterparts (Hawley et al. 1989). As *Ae. albopictus* expanded its range in the USA to more southerly latitudes, the capacity for egg diapause was reportedly lost (Craig 1993, Focks et al. 1994). However, working with progeny of geographic collections from Florida during 1998–2000, Lounibos et al. (2003) discovered that diapause was retained, albeit with incidences inversely proportional to latitude of population origin.

To test the hypothesis that diapause incidence continues to evolve in Florida, we performed geographic collections in 2008 and tested offspring for photoperiodic responses for comparisons with results from 10 years earlier (Lounibos et al. 2003). Additionally, we compared winter survivorship at one South Florida site of eggs laid in the laboratory by long- versus short-day females.

Immatures of *Ae. albopictus* were collected during the summer of 2008 in ovitraps or by suctioning of aquatic stages from artificial containers at 6 locations in the USA (Table 1). *Aedes albopictus* from these 6 locations, and others, had been tested for diapause responses 10 years previously (Lounibos et al. 2003). Sample sizes for the 2008 field collections ranged from approximately 100 to 500 immatures per site.

Site-specific colonies were established for each of the geographic populations, using husbandry methods described previously (Lounibos et al. 2003). To minimize nongenetic maternal effects (Mousseau 2000), experimental populations exposed to short and long daylengths to test diapause incidence were F₂–F₃ of field collections.

Egg diapause in *Ae. albopictus* is known to be induced by exposure of females during the pupal and adult stages to short daylengths (Mori et al. 1981, Hawley 1988). Experiments were run in incubators maintained at $21 \pm 0.5^{\circ}\text{C}$, at which temperature the photoperiodic responses of this species are optimally expressed (Pumpuni et al. 1992). Daily illumination of 14- or 10-h light, which daylengths are within 30 min of the longest and shortest (sunrise–sunset) observed annually at 26–27° latitudes (Nautical Almanac Office 1977), was provided by 8-W fluorescent bulbs, controlled by timers.

Forty to 50 male and female pupae exposed to these conditions were allowed to emerge in 0.1-m³ cages, in which adults mated and females were offered blood from restrained chickens (UF IACUC Approval VB-17). Five replicate cages were established in each photoperiod for each population. Oviposition on damp germination paper, which lined a black cup half-filled with water, was allowed to continue for 10 days in each incubator. Papers containing eggs were then removed, dried to dampness under insectary humidity (80–85% RH), and sealed in humidified (80% RH with a saturated salt solution) plastic containers at 21°C for 7–10 days before testing hatchability. Numbers of eggs on collected papers ranged from 127 to 1,362.

Hatchabilities for each population were examined simultaneously for long- and short-day eggs. Each egg paper was submerged for 24 h in deoxygenated water in a tray with 50 mg of larval food, after which period hatchlings were counted in a ceramic dropping plate under a dissecting microscope. Unhatched eggs were bleached by the method of Trpis (1970) and examined under a dissecting microscope for embryos. Unembryonated eggs were discarded from counts, and eggs containing fully formed embryos that satisfied Shroyer's (1979) morphological criteria for viability were scored as diapausing. Percentage of hatch was assessed for each treatment as the number of 1st instars eclosed divided by the total number of viable eggs.

Vero Beach females reared in either the short-day (SD) or long-day (LD) conditions were allowed to oviposit on moist paddles (2 × 10 cm) of Masonite in water-containing black cups. After eggs were allowed 7 days to embryonate in high humidity at 25°C in the laboratory, paddles with eggs were suspended in plastic cemetery vases, used previously to compare egg survivorship of *Ae. albopictus* and *Ae. aegypti* L. in South Florida cemeteries (Lounibos et al. 2010). Each SD, egg-laden paddle was suspended opposite an LD paddle in a vase, within which temperature and relative humidity were monitored with an Ibutton (Maxim Corp., Sunnyvale, CA) data logger. Eight such vases were set early in January, typically Florida's coldest month (Chen and Gerber 1990), of 2009. Each vase opening was covered with fine-mesh screen to deter entry of predators, and 2 drain holes were drilled on the bottom to prevent rainfall accumulation that might hatch eggs. Locations of experimentally exposed vases in a 50 × 75-m shaded field at the Florida Medical Entomology Lab were assigned by random number draws of a gridded, numbered template of the area.

Four vases were randomly selected for removal from the field after 2 wk of exposure, and the remaining 4 vases were withdrawn after 4 wk. Paddles with eggs were exposed for 24 h in trays to deoxygenated water and 50 mg of larval food to hatch larvae. After drying a paddle for 24 h, this stimulus was reapplied 2 successive times to hatch pharate larvae in diapause, and remaining unhatched eggs were bleached to distinguish viable from nonviable embryos, by the previously described method of Shroyer (1979). Survivorship per paddle was calculated as numbers of hatched larvae + viable unhatched eggs divided by total number of larvae + viable and dead embryos.

Differences between SD and LD diapause incidences were assessed for each population by 1-tailed *t*-tests, performed on arcsine-transformed proportions hatching for each replicate. Survivorship of SD versus LD eggs in the field was compared by paired *t*-tests on arcsine-transformed proportions.

For 5 of the 6 geographic samples, significantly fewer viable eggs from SD-exposed females hatched than from LD-exposed *Ae. albopictus* females ($t_8 \geq 8.2$, $P < 0.001$). For the Vero Beach sample, the difference in egg hatch from LD-versus SD-exposed females was not significant ($P = 0.137$, 1-tailed test) because of the unexpectedly high SE (= 14.5) (Table 1) caused by low hatchability (25.5%) of 1 LD replicate. The LD versus SD differences of 2 populations from North Florida were not appreciably different from the Illinois sample nor from collections made from these regions in 1998–2000 (Table 1). By contrast, >60% of eggs laid by SD females from Vero Beach and Miami hatched, compared to 13–30% tested 10 years previously (Table 1). However, the southernmost population sampled, Card Sound, did not follow the trend of Vero Beach and Miami, with a mean of only 20.7% of viable eggs from SD females hatching in response to 1 flooding stimulus, indicating a stronger diapause response.

After only 2 wk of exposure in the field, a significantly higher proportion of SD eggs remained viable, compared to LD-derived counterparts in the same vase microenvironments ($t_3 = 3.90$, $P = 0.030$). Results were very similar after 4 wk of exposure, the SD eggs surviving significantly better ($t_3 = 3.92$, $P = 0.029$) (Fig. 1). Mean daily temperatures in vases during the exposure period were mild, between 12°C and 22°C, and mean daily RH ranged from 80% to 100% (Fig. 1B). The minimum vase temperature recorded during the exposure period was 10.1°C and minimum RH was 60.8%.

Contrary to previous claims almost 2 decades earlier that photoperiodic diapause had disappeared among *Ae. albopictus* in Florida (Craig 1993, Focks et al. 1994), this inducible trait remained prevalent, at site-specific frequencies, in all Florida populations sampled in 2008, approximately 27–30 years after this invasive species became established in the state (O'Meara et al. 1995). Diapause incidence was as high in Pensacola and Jacksonville as in East St. Louis, Illinois, and decreased in peninsular Florida (Table 1), confirming earlier observations (Lounibos et al. 2003) that North Florida seems to represent a latitudinal break point between total and variable diapause. The unexpectedly high diapause incidence in *Ae. albopictus* from Card Sound could be explained by recent establishments at a population margin, as was formerly invoked to explain a high diapause rate in a sample from the northern Caribbean (Lounibos et al. 2003).

Eggs from SD females had superior survivorship compared to eggs from LD females after only 2–4 wk of exposure to relatively mild winter conditions in Vero Beach, even though more than half of the SD-derived eggs were estimated to not be in diapause (Table 1). Given that temperatures within experimental vases were not low during the exposure period (Fig. 1B), one might conjecture that improved desiccation resistance, known to be conferred by

diapause in *Ae. albopictus* (Sota and Mogi 1992, Urbanski et al. 2010), might have been a factor improving the survivorship of SD cohorts.

Mosquito species with drought-resistant eggs have enhanced capacities to disperse outside their native ranges (Juliano and Lounibos 2005), and low humidities have been shown to increase mortality of nondiapause eggs of *Ae. albopictus* in the laboratory (Juliano et al. 2002) and in the field (Lounibos et al. 2010). Because the diapause period for *Ae. albopictus* is restricted to early winter in peninsular Florida, diapause would not protect eggs of this species in the spring, when seasonal drought is most likely to inflict differential egg mortality on *Ae. albopictus* compared to its competitor *Ae. aegypti* (Lounibos et al. 2010).

Acknowledgments

We thank Steve Juliano and Don Yee for providing field-collected *Ae. albopictus* from East St. Louis and Pensacola, respectively, and Barry Alto and George O'Meara for critical readings of a draft of this Note. Research was supported by NIH grant R01 AI-044793.

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Fig. 1.

(A) Mean ($n = 4$) proportions of eggs surviving after exposures in cemetery vases for 2- and 4-wk intervals; (B) mean ($n = 8$) temperatures and relative humidities within experimental cemetery vases during the exposure periods.

Collection sites and differential responses of eggs from mothers exposed to 2 daylengths of geographic populations of *Aedes albopictus* separated by 8–10 years.

Table 1

Site	Latitude (°N)	% hatch (no. eggs) 1998–2000 ¹		% hatch (SE) 2008	
		14L:10D ²	10L:14D	14L:10D	10L:14D
East St. Louis	38°38'	92.3 (1,359)	0.6 (1,511)	91.0 (4.9)	6.9 (2.6)
Pensacola	30°25'			98.6 (0.5)	3.2 (0.6)
Cottondale	30°48'	98.2 (979)	1.2 (1,241)		
Jacksonville	30°19'	89.4 (1,390)	8.9 (1,267)	97.7 (0.8)	6.6 (1.9)
Vero Beach	27°35'	91.3 (403)	13.1 (381)	83.6 (14.5)	66.1 (3.3)
Miami	25°45'	91.0 (600)	30.3 (892)	95.5 (0.8)	69.4 (3.9)
Card Sound	25°25'	99.4 (325)	23.3 (365)	96.5 (1.4)	20.7 (4.5)

¹ Hatchabilities of 1998–2000 colonies were from unreplicated experiments (reported in Lounibos et al. 2003); hatchabilities of 2008 populations are mean values from 5 replicates.

² Daylengths: long = 14 h of light and 10 h of darkness (14L:10D); short = 10 h of light and 14 h of darkness (10L:14D).