DIFFERENCES IN THE LARVAL ALARM REACTION IN POPULATIONS OF *AEDES AEGYPTI* AND *AEDES ALBOPICTUS*

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ABSTRACT. The time spent submerged during the larval alarm reaction was measured for several strains of *Aedes aegypti* and *Ae. albopictus* from different locations. Differences between the 2 species were not significant. Differences in strains within the species were highly significant. Greater differences were seen among strains of *Ae. albopictus* than among strains of *Ae. aegypti.* These differences represent an additional indication of extensive local differentiation in *Ae. albopictus.*

Since the discovery of *Aedes albopictus* (Skuse) in Houston, Texas, in 1986 (Sprenger and Wuithiranyagool 1986) much work has been done to gain a better understanding of this potential vector. Since the first recognition, reports from as far north as Chicago and Delaware show that *Ae. albopictus* is well established (Moore et al. 1988). The apparently rapid spread of this mosquito has generated questions about the numbers of introductions. Craven et al. (1988) found evidence of the potential for multiple introductions in imported tires. Black et al. (1988a) demonstrated significant genetic differences among strains of *Ae. albopictus* collected from different locations and concluded that multiple introductions followed by local inbreeding were the most likely explanation for the genetic patterns. In another study, Black et al. (1988b) showed that patterns of local inbreeding are typical of populations of *Ae. albopictus.* They found significant differences among different local populations, even in locations in which *Ae. albopictus* has been well established for long periods of time. Hawley et al. (1989) showed significant differences in the abilities of different populations to withstand winter conditions, indicating not only genetic differences between strains, but subsequent local adaptation.

Another relatively poorly documented aspect of the spread of *Ae. albopictus* is the possible displacement of *Aedes aegypti* (Linn.) by *Ae. albopictus,* as the 2 species share the same larval habitats. Gilotra et al. (1967) reported exclusion of *Ae. aegypti* by *Ae. albopictus* in suburban and rural outdoor areas in India, while *Ae. aegypti* excluded *Ae. albopictus* in urban and indoor areas. Black et al. (1989) looked at various factors involved in competitive displacement in American populations of the 2 species and found no inherent factors to explain the spread of *Ae. albopictus.* As *Ae. albopictus* becomes established, it could displace populations of *Ae. aegypti.* Our collections indicate that *Ae. albopictus* is now more common than *Ae. aegypti* in locations where *Ae. aegypti* was the only artificial container breeding mosquito 5 years ago (Duhrkopf, unpublished data). Spielman and Feinsod (1979) reported that *Aedes bahamensis* Berlin appeared to exclude *Ae. aegypti* from habitats on Grand Bahama Island, and a similar phenomenon has been reported in Florida, where local populations of *Ae. bahamensis* have apparently displaced *Ae. aegypti* (O'Meara et al. 1989).

One aspect of interest in both species is the larval behavior. Mellanby (1958) described the larval alarm reaction in several different species of mosquitoes. When larvae are disturbed by a passing shadow or vibration, they leave the surface of the water and move to the darkest area available. They stay submerged for a period of time before returning to the surface. Duhrkopf and Young (1979) showed that the time spent submerged by *Ae. aegypti* larvae responded to positive and negative selection and is apparently controlled by a polygenic system. Thus, under controlled laboratory conditions, differences in this parameter can be taken as indications of genetic differences between populations. Behavioral differences could translate into survival differences. Differences in larval behavior may result in different abilities to exploit the habitat. Thus, such a difference may be one factor in displacement of one species by another.

MATERIALS AND METHODS

Aedes aegypti and *Ae. albopictus* were reared under laboratory conditions. Larvae and adults were maintained using standard procedures in an insectary at a temperature of 26° ± 0.5° C and RH 80% ± 2%. Upon hatching, larvae were reared in groups of 200 in 750 ml of tap water in 32- × 26- × 7-cm Nalgene™ pans covered with a 6.6-mm thick 34.5 × 28-cm sheet of plexiglass. Larvae were fed on a liver powder suspension (15-g liver powder in 1-liter tap water).

Four strains of *Ae. aegypti* and 4 strains of *Ae. albopictus* were used for the experiment. The strains of *Ae. aegypti* used were: ROCK (from George Craig, Jr., Department of Biology, Uni-

versity of Notre Dame), UTMB (from the University of Texas at Galveston), WACO (established from larvae collected in Hewitt, TX, in June 1986), and BU (established from larvae collected in Woodway, TX, in September 1987). The strains of *Ae. albopictus* tested were: HC (from Daniel Sprenger, Harris County Mosquito Control District, Houston, TX), ESL (collected in East St. Louis, IL), MIL-2 (collected in Milford, DE) and DOM (Collected in Chicago, IL). The last 3 strains were obtained from George Craig, Jr. For each strain, 100 larvae were tested as described in Duhrkopf and Young (1979). Individual fourth instar larvae of the same size were put into 12 × 75-mm culture tubes in 2 ml of a water/food suspension (10 ml of the liver powder suspension in 300 ml of tap water). Test tubes with larvae were kept in groups of 10 in wooden supports for at least 3 h before testing. During that time, all groups were separated by index cards to minimize stimulation caused by the diving of neighboring larvae. All testing was done in the controlled environment of the insectary at the same time of day to minimize any environmental (temperature or light intensity) effects.

Each larva was tested 3 times, with each test beginning with the larva at the surface. Larvae were stimulated by passing an index card along the side of the tube and gently tapping the wooden support at the bottom, thus causing the larva to experience a shadow and a vibration. Both stimuli consistently elicit the alarm reaction. The length of time taken to recover from the alarm reaction was recorded for each individual. Recovery was complete when the larva resumed surface contact with its respiratory siphon. The mean of the 3 tests for each larva was used as its score. The 100 means from each strain were used for the analyses. For those analyses, the data were transformed to a normal distribution using a $\log_{10}$ transformation. Comparisons of the strains were made via a nested analysis of variance (Snedecor and Cochran 1989) and means were grouped using Fisher's protected least significant difference (PLSD).

RESULTS

The mean times of submergence for the experimental strains are presented in Table 1. These data are also presented in the form of box plots in Fig. 1. The data allow various comparisons to be made. First, between the 2 species, the mean time spent submerged for the 4 populations of *Ae. aegypti* was 21.4 sec, compared with 29.0 sec for the 4 populations of *Ae. albopictus*. Although the difference appears large, the analysis of variance indicated that the submergence time was not significantly different between the 2 species ($P = 0.05$).

Between populations within species, the analysis of variance indicated the time spent submerged within the populations is significantly different ($P = 0.0001$). The means of the 4 populations of *Ae. albopictus* ranged from 22.4 to 38.5 sec. Comparisons of the means via Fisher's PLSD shows that 2 of the means (MIL-2 (23.1 sec) and ESL (22.4 sec.)) are not significantly different from each other. However, both HC and DOM are significantly different from MIL-2 and ESL and from each other. For the 4 populations of *Ae. aegypti*, the means ranged from 19.5 to 23.7 sec. Similar comparisons of the means show that the populations assort into 2 different groups. ROCK (22.6 sec) and BU (23.7 sec) were not significantly different, and UTMB (19.5 sec) and WACO (19.6 sec) were not significantly different.

DISCUSSION

Behavioral differences between species and between populations can be highly significant. In these populations of *Ae. aegypti* and *Ae. albopictus*, the behavioral differences indicate greater genetic differences between various pop-

Table 1. Mean times of submergence during the larval alarm reaction.

Species	Strain	Mean time (sec)	SE	Grand mean (sec)	SE
Aedes aegypti	ROCK	22.6	1.0		
	UTMB	19.5	0.7		
	WACO	19.6	0.6		
	BU	23.7	1.0		
				21.4	0.4
Aedes albopictus	HC	32.1	1.0		
	MIL-2	23.1	0.6		
	ESL	22.4	0.6		
	DOM	38.5	1.4		
				29.0	0.6

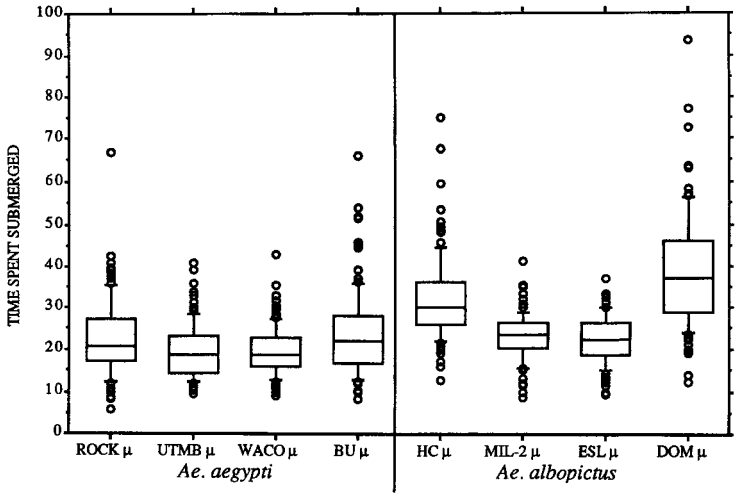

Fig. 1. Distributions of the means of the submergence times in the experimental strains.

ulations than between the 2 species. The estimates of the variance components indicate the variation between species ($\sigma^2_{Species}$ = 0.0198) is about half of the variance between populations within the species (σ^2_{Strain} = 0.0323). These genetic differences can have 2 sources; natural genetic differences between the populations sampled, and genetic differences induced by establishing the strains in the laboratory.

This study shows that, generally, larvae of the 2 species stay submerged for similar periods of time. However, a closer inspection of the strain means shows an interesting aspect of those differences. The mean of all *Ae. aegypti* larvae tested was 21.4 sec, and the mean of all *Ae. albopictus* larvae tested was 29.0 sec. There were, however, 2 strains of *Ae. albopictus* (MIL-2 and ESL) with means comparable to those of the *Ae. aegypti* strains. The other 2 *Ae. albopictus* strains (HC and DOM) had means much greater than those of all the *Ae. aegypti*. The 2 *Ae. albopictus* strains whose means were greater also had slower developmental times in the laboratory. For both the HC and DOM strains, larval development took about 24 h longer than for the other 2 strains of *Ae. albopictus* or the 4 strains of *Ae. aegypti*.

Although the differences between the species were not significant, there were large differences in the levels of variation. The nested analysis of variance produced a sum of squares for strains within species of 20.048 (MS = 3.341, 6 df). If that sum of squares is further partitioned into components due to strains within the 2 species, the sum of squares for strains of *Ae. aegypti* is 2.008, and the sum of squares for strains within *Ae. albopictus* is 18.040. Of the variation of strains within species, 90% is due to variation within strains of *Ae. albopictus*. Black et al. (1988b) argued that such differences were a result of the natural breeding structure of *Ae. albopictus*.

A second explanation for some of these differences involves a combination of drift induced by sampling and inbreeding as a result of establishing populations as laboratory colonies. In sampling, drift can be overcome by making sure that the sample is sufficiently diverse. However, in establishing a laboratory colony, there is the potential for inbreeding. In the present study, the *Ae. albopictus* strain designated as DOM differed significantly from ESL. It is possible, but unlikely, that these differences were a result of laboratory colonization and do not relate to the original populations.

Whether the observed differences can be translated into differences in survival under natural conditions is doubtful. Earlier, we proposed that such differences in larval behavior could contribute to displacement of one species by another. This is based upon the assumption that time spent submerged during the alarm reaction is correlated with time spent submerged during feeding. We assume the recovery stimulus for both behaviors is the same. If that were the case, larvae that stay submerged longer could have an advantage by improved avoidance of predators and enhanced feeding during each diving episode. If more food is obtained per diving episode, fewer diving episodes are necessary, and more resources could be channeled into growth. On the other hand, longer episodes of diving might indicate slower metabolic processes which might be translated into differences in fitness. Our data do not support the idea that *Ae. albopictus* and *Ae. aegypti* larvae differ enough for this to be likely. Local populations have enough genetic variation for selection to result in optimal diving

times. All populations tested were well within the limits of selection seen in previous work (Duhrkopf and Young 1979). At present, we are reluctant to postulate that the differences between the 2 species are an explanation for the apparent displacement of *Ae. aegypti* by *Ae. albopictus*.

We are confident that the differences indicate real differences between local populations of *Ae. albopictus*, and we see these behavioral differences as another indication that the breeding structure of natural populations of *Ae. albopictus* involves both inbreeding and genetic drift. The result of this is the establishment of local populations which are significantly different from each other both genetically and behaviorally.

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