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# EXPERIMENTAL HYBRIDIZATION BETWEEN *AEDES SOLLICITANS* (WALKER) AND *AEDES MITCHELLAE* (DYAR)

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ABSTRACT. *Aedes sollicitans* and *Aedes mitchellae* were hybridized using induced mating techniques. Viable, fertile, $F_1$ hybrids were obtained from reciprocal crosses between the two species. These $F_1$ hybrids were successfully backcrossed to both parental species. In general, each backcross progeny had a normal sex ratio with minimal mortality in developmental stages. However, the hatch rate was less than 50% in most of the backcross progenies. Most of the non-hatching eggs did not contain embryos. The larval siphons of the $F_1$ hybrids were more like those of *A. sollicitans* than *A. mitchellae*. Hybrid females could be readily distinguished from parental types. No evidence was found for natural hybridization between field populations of *A. sollicitans* and *A. mitchellae*.

Hybridization studies with highly eurygamous mosquitoes must use some type of induced mating technique (McDaniel and Horsfall, 1957). This procedure can sometimes overcome the premating mechanisms that serve to isolate closely related species. The elimination of premating barriers has frequently uncovered additional isolating mechanisms (Davidson *et al.*, 1967; McClelland, 1967; Kitzmiller *et al.*, 1967), including hybrid inviability, hybrid sterility, and hybrid breakdown. Although most hybridization experiments have encountered at least one of these isolating mechanisms, there have been notable exceptions. For example, in the hybridization of *Aedes sollicitans* and *Aedes nigromaculis* by Fukuda and Woodard (1974), the $F_1$, $F_2$ and backcross progeny were fully viable and fertile.

In the present study, we examined the genetic affinity and potential for gene flow between *A. sollicitans* and *A. mitchellae*. Both *A. mitchellae* and *A. nigromaculis* have morphological similarities with *A. sollicitans*. There is a close relationship between larval, as well as adult, characteristics. All three species are highly eurygamous and they can be mated in the laboratory only by induced copulation.

MATERIALS AND METHODS. Field collections of *Aedes mitchellae* were made with a tractor aspirator (Bidlingmayer and Edman, 1967) in Indian River County, Florida. A few *A. sollicitans* were also obtained in these collections, but many more were obtained from human biting collections in both Florida and Louisiana. The $F_1$ offspring of these wild caught females were used in the mating experiments.

All mosquitoes were kept in a bioclimatic room with the temperature maintained at $26 \pm 1.5$ C and the relative humidity at $80 \pm 15\%$. The daily photoperiod was 14.5 hours. Larvae were reared in tap water on the standard diet of O'Meara and Evans (1973). Adults fed *ad libitum* on 10% sugar solution.

Induced mating techniques were modifications of Miura's (1969). The sexes were separated at emergence, even though neither species is known to mate under caged conditions. Females were blood-fed 2 days prior to induced copulation attempts. Mated females were isolated individually in glass shell vials which were lined with moist cheesecloth. Seven days after the eggs were laid, they were submerged in tap water containing a suspension of brewer's yeast (Nutritional Biochemicals Company). All eggs that failed to hatch during a 24-hour period of submergence were examined for embryos by bleaching the egg shell (Mortenson, 1950; Moore and Bickley, 1966).

RESULTS. Viable $F_1$ hybrids were obtained from reciprocal crosses between *A. mitchellae* and *A. sollicitans*. In each cross the sex ratio was about 1:1. Hybrid adults appeared normal. Practically no mortality occurred in the larval or pupal stages. The hatch rate was highly variable, but most embryonated eggs hatched when submerged for 24 hours (Table 1).

$F_1$ hybrids were fertile and they were successfully backcrossed to both parental species. A total of 31 progenies were produced by six different kinds of backcrosses. In general, for each backcross progeny the sex ratio was normal and the larval and pupal mortality was minimal. The hatch rate was less than 50% in most of the backcross progenies. Yet with the exception of a few progenies, most of the unhatched eggs did not contain embyros (Table 2). When the male reproductive system was examined mature, motile spermatozoa were found in nearly all the males from each backcross progeny.

The larvae of *A. sollicitans* and *A. mitchellae* can be best distinguished by morphological characteristics of their siphons. In *A. mitchellae* the siphonal index (ratio of total length to width at base) is usually greater than 3.0 and the pecten teeth extend less than half the length of the siphon, whereas in *A. sollicitans* the siphonal index is less than 2.5 and the pecten teeth extend one half or more of the length of the siphon. The $F_1$ hybrids had an average siphonal index of 2.4 and the pecten teeth nearly always extended more than half the length of the siphon.

TABLE 1. The occurrence and viability of $F_1$ offspring from crosses between *Aedes sollicitans* (AS) and *Aedes mitchellae* (AM).

| Cross ♀ x ♂ | Female no. | Total no. eggs | Percent hatched | No. nonhatched embryonated eggs | No. 1st instar larvae | No. pupae | No. adults ♀ | No. adults ♂ |
|---|---|---|---|---|---|---|---|---|
| AM x AS | 1 | 96 | 82.3 | 0 | 79 | 78 | 30 | 48 |
| | 2 | 48 | 47.9 | 6 | 23 | 23 | 16 | 7 |
| | 3 | 94 | 50.0 | 6 | 47 | 47 | 27 | 20 |
| | 4 | 160 | 66.9 | 12 | 107 | 105 | 48 | 57 |
| | 5 | 87 | 87.4 | 6 | 76 | 76 | 40 | 36 |
| | 6 | 105 | 69.5 | 7 | 73 | 70 | 36 | 31 |
| AS x AM | 1 | 193 | 12.4 | 0 | 24 | 23 | 12 | 9 |

The siphons of the $F_1$ hybrid larvae are much more like those of *A. sollicitans* than *A. mitchellae.*

Adults of *A. sollicitans* and *A. mitchellae* differ in several of their scaling patterns. The $F_1$ hybrids possess some of the characteristics of both parental species. In *A. sollicitans* light and dark scales are scattered over the entire wing, whereas in *A. mitchellae* all wing scales are dark. The wings of the hybrid are covered with both light and dark scales, but the light scales are restricted to the costa and subcosta. The white scaling pattern of the pleural area of the hybrid is intermediate between the dense scaling on *A. sollicitans* and the rather sparse scaling of *A. mitchellae.* Unlike *A. sollicitans,* most hybrid specimens showed a separation between the scale patches of the sternopleuron and those of the prealar area, but the separation was not as distinct as that found on *A. mitchellae.* Although most hybrids have yellow scaling on the first segment of the hind tarsus, the middle of this segment is seldom completely ringed with yellow scales as it is in *A. sollicitans.* There is no yellow scaling on the legs of *A. mitchellae.* The first abdominal tergite in *A. sollicitans* has a medial patch of yellowish scales, while in both *A. mitchellae* and in the hybrid this medial patch is

Table 2. Results of backcrosses of $F_1$ hybrids to *A. sollicitans* and *A. mitchellae.*

| Cross ♀ x ♂ | Female no. | Total no. eggs | Percent hatched | No. non-hatched embryonated eggs | No. 1st instar larvae | No. pupae | No. adults ♀ | No. adults ♂ |
|---|---|---|---|---|---|---|---|---|
| AS x $F_1$AM/AS | 1 | 226 | 71.7 | 2 | 162 | 149 | 71 | 76 |
| | 2 | 208 | 81.3 | 5 | 169 | 77 | 41 | 34 |
| | 3 | 165 | 1.2 | 1 | 2 | 2 | 0 | 2 |
| | 4 | 192 | 7.3 | 0 | 14 | 14 | 7 | 6 |
| | 5 | 183 | 20.8 | 0 | 38 | 37 | 16 | 21 |
| | 6 | 124 | 37.1 | 0 | 46 | 42 | 14 | 27 |
| | 7 | 197 | 21.8 | 1 | 43 | 40 | 18 | 22 |
| | 8 | 256 | 89.1 | 4 | 228 | 178 | 91 | 69 |
| | 9 | 187 | 77.5 | 26 | 145 | 134 | 69 | 61 |
| | 10 | 221 | 79.6 | 31 | 176 | 154 | 84 | 60 |
| AS x $F_1$AS/AM | 1 | 215 | 2.3 | 0 | 5 | 5 | 3 | 2 |
| | 2 | 223 | 45.7 | 13 | 102 | 100 | 47 | 36 |
| | 3 | 167 | 27.5 | 0 | 46 | 45 | 19 | 26 |
| $F_1$AM/AS x AS | 1 | 89 | 66.3 | 3 | 59 | 56 | 28 | 28 |
| | 2 | 149 | 21.5 | 2 | 32 | 28 | 12 | 16 |
| | 3 | 135 | 70.4 | 10 | 95 | 85 | 43 | 40 |
| | 4 | 194 | 72.2 | 6 | 140 | 101 | 50 | 51 |
| | 5 | 70 | 70.0 | 0 | 49 | 45 | 26 | 19 |
| | 6 | 95 | 23.3 | 40 | 22 | 19 | 8 | 11 |
| | 7 | 218 | 50.5 | 25 | 110 | 90 | 44 | 46 |
| | 8 | 138 | 1.4 | 0 | 2 | 2 | 0 | 2 |
| | 9 | 67 | 3.0 | 0 | 2 | 2 | 0 | 2 |
| | 10 | 169 | 15.4 | 87 | 26 | 26 | 7 | 17 |
| | 11 | 181 | 3.3 | 116 | 6 | 6 | 1 | 5 |
| $F_1$AM/AS x AM | 1 | 149 | 77.9 | 4 | 116 | 83 | 44 | 39 |
| | 2 | 171 | 56.1 | 9 | 96 | 68 | 24 | 40 |
| | 3 | 158 | 23.4 | 11 | 37 | 33 | 15 | 18 |
| | 4 | 123 | 5.7 | 2 | 7 | 1 | 0 | 1 |
| AM x $F_1$AM/AS | 1 | 10 | 70.0 | 1 | 7 | 5 | 2 | 3 |
| | 2 | 103 | 30.1 | 3 | 31 | 24 | 9 | 14 |
| $F_1$AS/AM x AS | 1 | 153 | 26.1 | 30 | 40 | 38 | 19 | 18 |

white scaled. In the hybrid the 5th segment of the mid-tarsus is either entirely dark or ringed with a very narrow band of white scales. In *A. sollicitans* this segment is broadly ringed in white or mostly white, but it is entirely dark in *A. mitchellae*.

DISCUSSION. The results of our crossing experiments indicate a great deal of genetic affinity between *A. sollicitans* and *A. mitchellae*. Numerous $F_1$ hybrids and backcross individuals were obtained using induced mating procedures (Tables 1 and 2). Moreover, the males of each backcross progeny appeared to develop normal, functional sperm. However, many of the backcrosses produced large numbers of non-embryonated eggs. This situation could have been the result of inadequate sperm transfer. Throughout our study, difficulties were encountered getting the mosquitoes to copulate, even for intraspecific matings. Obviously, other factors might also have precluded embryonic development.

In nature the opportunities for hybridization between *A. sollicitans* and *A. mitchellae* are very limited because the two species occur in different habitats. The larval habitat of *A. sollicitans* is usually the coastal salt marsh or occasionally other areas which contain brackish water, whereas *A. mitchellae* occurs in fresh water. Only in a few areas are both species found in relative close proximity. A light trap located near a salt marsh in Indian River County, Florida has on numerous occasions taken *A. sollicitans* and *A. mitchellae* in the same nightly collection. During the daytime, both species were frequently collected along the same roadside swale with a tractor aspirator. Yet even in these populations we found no evidence of interspecific hybridization such as the occurrence of $F_1$ hybrids which are distinguishable from either parental species. Apparently, there are other pre-mating isolating barriers in addition to geographical factors which prevent hybridization between *A. sollicitans* and *A. mitchellae*.

Besides providing a means for investigating phylogenetic relationships, experimental hybridization can be utilized to study the genetics of specific morphological, behavioral or physiological characters (Truman and Craig, 1968; Hartberg and Craig, 1973). Sufficient gene flow can be generated by forced mating *A. sollicitans* with either *A. mitchellae* or *A. nigromaculis* to evaluate the degree of dominance associated with certain traits. It is reasonable to assume that the same situation would exist if *A. mitchellae* were crossed with *A. nigromaculis*. In preliminary tests we have successfully hybridized *A. sollicitans* with a third species, *A. taeniorhynchus*. All four species (*A. sollicitans, A. taeniorhynchus, A. mitchellae,* and *A. nigromaculis*) are definitely closely related, and additional hybridization experiments would surely increase our understanding of the relationships among these mosquitoes.

ACKNOWLEDGMENTS. We thank D. B. Woodard of the Gulf Coast Mosquito Research Laboratory, USDA, for providing us with eggs of *Aedes sollicitans*. The research was supported in part by research grant No. AI-11583 from the National Institutes of Health.

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# XV INTERNATIONAL CONGRESS OF ENTOMOLOGY

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