HYBRIDIZATION OF *AEDES SOLLICITANS* (WALKER) AND *AEDES NIGROMACULIS* (LUDLOW) BY INDUCED COPULATION [1, 2]

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ABSTRACT. The possibility of genetic incompatibility of two closely related species, *Aedes sollicitans* (Walker) and *Aedes nigromaculis* (Ludlow), was studied through hybridization by using induced copulation. Egg fertility, mortality, size, and rearing time of larvae resulting from 12 combinations of F_1 and F_2 crosses and F_1 backcrosses did not appear to differ from those of the parent types. Also, no dominance of one parent species over the other was apparent in counts of pecten teeth or comb scales in F_1 and F_2 larvae. In larvae of F_1 backcrosses, the counts tended toward the parent with which the F_1 adults were crossed. The distal pecten teeth of hybrid larvae were generally barbed, which is characteristic of *A. sollicitans*, and displaced, which is characteristic of *A. nigromaculis*. In general, characteristics of *A. sollicitans* rather than *A. nigromaculis* were more emphasized in the hybrids though none of the adult females from the various crosses were identical with *A. sollicitans* females. There was no genetic incompatibility of the two species when hybridized by induced copulation, but in nature behavioral differences probably prevent crossmating.

Hybridization between closely related species has been studied extensively in the genus *Aedes*, particularly in the subgenus *Stegomyia*, the *maculipennis* complex of the genus *Anopheles*, and the *Culex pipiens* complex (Wright and Pal, 1967). The results of these studies have varied greatly: in some crosses, fertilization of the eggs failed to occur; in others fertilization occurred but the resulting larvae died before or within a few hours of hatching; in still others, eggs hatched, and the progeny developed to vigorous adults but with either one or both sexes sterile. Only a few fully fertile hybrid adults that resemble one or both parents have been produced.

More recently, the possibility of using genetic incompatibility to control mosquito populations has stimulated new interest in hybridization studies. Since *Aedes sollicitans* (Walker) and *Aedes nigromaculis* (Ludlow) are phylogenetically closely related and were being maintained in our laboratory by induced copulation, the two species were cross-mated, so we could see whether genetic incompatibility would occur. These studies were carried out at the Gulf Coast Mosquito Research Laboratory, Lake Charles, Louisiana.

MATERIALS AND METHODS. The colony of *A. sollicitans* maintained in our laboratory was started from field collections in southwestern Louisiana. The colony of *A. nigromaculis* was obtained from the University of California Mosquito Control Research Laboratory, Fresno, California. All matings were accomplished by induced copulation by using a modification of the method of insemination for *A. nigromaculis* described by Miura (1967). Males did not require decapitation to enhance copulation. Also, since the females were reluctant to take a blood meal after anesthetization, they were given a blood meal before mating (this early engorgement also tended to extend the genitalia which facilitated copulation). The mated females were placed in 10-dram shell vials and allowed to oviposit on moist compressed cotton. Oviposition usually occurred in about 5 (3–7) days at 27° C. The eggs were matured 7 or more days before they were flooded with distilled water containing nutrient broth as a hatching stimulus. Hatching commenced almost immediately and was usually completed in less than 1

[1] Mention of a commercial or proprietary product in this paper does not constitute an endorsement of this product by the U. S. Department of Agriculture.

[2] In cooperation with McNeese State University, Lake Charles, Louisiana.

hour. The hatched larvae were then reared in the manner described by Chapman and Barr (1969). As the larvae pupated, they were transferred to holding cages and allowed to emerge. Raisins placed in the cage provided the adult mosquitoes with a carbohydrate source. One to three days after emergence, the adult females were allowed to take a blood meal from a guinea pig and were then mated. All populations of mosquitoes that were to be cross-mated were hatched and reared synchronously.

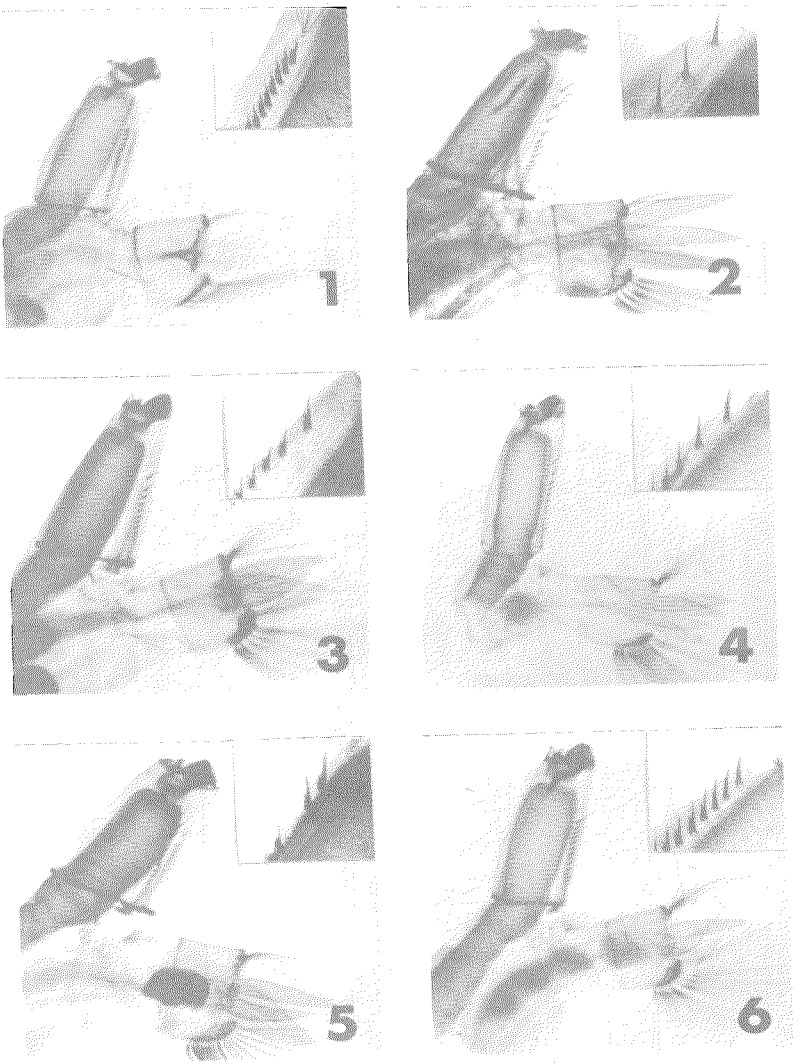
The parent *A. sollicitans* and *A. nigromaculis* were cross-mated in both directions, and the two F_1 hybrids were backcrossed (BC) in both directions to each parent. The F_1 hybrids were also interbred to produce F_2 progeny. As the larvae of the parent types and the types of all the crosses reached the fourth instar, representative samples were removed and preserved in 70 percent ethanol. Later 12–22 specimens of each type were dehydrated in ethanol, cleared in xylene, and permanently mounted in Pro-Texx^R on glass glides. All measurements (obtained by using a Vickers-AEI image splitting micrometer^R at 100X) and all counts were taken from larvae mounted on slides. Also, as 24-hour-old adults of each type were available, representative samples were mounted for future observations. The remaining F_1 adults were used for backcrossing or held in cages and given a blood meal to check for natural mating. The presence of only nonviable eggs served as a check against the possibility that some mating may have occurred within a given population as the young adults emerged.

RESULTS AND DISCUSSION. In general, no difference in egg hatchability, mortality, size, or rearing time could be detected in any of the hybrid populations compared with the parental types. The efficiency of the forced-mating procedure was measured by the percentage of egg batches producing viable larvae; it ranged from 70 to 80 percent in the two parent colonies and from 63 to 100 percent in the hybrid crosses.

The major differences between larvae of *A. sollicitans* and *A. nigromaculis* are the number, shape, and arrangement of the pecten teeth and the number of comb scales. These therefore were the characters most closely examined in the hybrids. *Aedes sollicitans* characteristically has 20–33 comb scales and 17 to 23 evenly spaced, barbed pecten teeth (Fig. 1). *A. nigromaculis* has 5–15 comb scales and 12–20 pecten teeth with the distal 1–3 teeth more widely separated and smooth (Carpenter and LaCasse 1955) (Fig. 2). Table 1 summarizes the variations in these characteristics in the 14 parental, F_1, F_2, and backcross combinations. Figures 3, 4, 5, and 6 show some of the individual variations in comb scales and pecten teeth patterns. Table 2 gives the significance of the differences. Of course, highly significant (0.001 level) differences existed between parent *A. sollicitans* and *A. nigromaculis* in the number of pecten teeth and comb scales. However, when the parent male was *A. sollicitans* and the female was *A. nigromaculis,* the F_1 larvae had significantly fewer pecten teeth than *A. sollicitans* but not significantly more than *A. nigromaculis* though the displacement was much less; and most (75 percent) also possessed the barbed distal pecten teeth characteristic of *A. sollicitans.* The comb scale counts of the F_1 larvae in both P crosses were about midway between and significantly different from those of either parent.

When the F_1 hybrids were interbred, the F_2 larvae had pecten tooth counts that varied widely and were usually not significantly different from those of *A. nigromaculis;* however, in one cross, they were significantly lower than those for *A. sollicitans.* Displacement of the distal pecten teeth was evident but less than in the F_1 generation. Barbed distal pecten teeth occurred in 83 percent of the F_2 larvae. The F_2 larvae had comb scale counts similar to those of their F_1 parents.

In the four combinations resulting when the two F_1 hybrids were backcrossed to both male and female *A. sollicitans,* all larvae exhibited pecten tooth counts near those of *A. sollicitans* and significantly different from those of *A. nigromaculis.*



FIGS. 1–6.—*Aedes* larvae terminal segments (distal pecten teeth upper right inset). 1. *Aedes sollicitans*; 2. *A. nigromaculis*; 3 and 5. *A. sollicitans* male x *A. nigromaculis* female; 4 and 6. *A. nigromaculis* male x *A. sollicitans* female.

TABLE 1.—Pecten tooth and comb scale characteristics of *A. sollicitans*, *A. nigromaculis*, their hybrids and back crosses.

Crosses		Number of specimens	Generation	Number of pecten teeth			Distance between last two pecten teeth in microns			Percentage of specimens with barbed pecten teeth	Number of comb scales		
Male	Female			Mean	(S.D.)[a]	Range	Mean	(S.D.)	Range		Mean	(S.D.)	Range
S[b]	S	15	P[c]	20.1	(1.7)	17–23	40	(7)	26–43	100	23.1	(4.1)	20–33
N	N	13	P	16.6	(2.3)	12–20	143	(38)	66–168	0	8.2	(2.7)	5–15
S	N	15	F_1	17.9	(2.)	14–21	70	(20)	32–97	73	15.2	(3.5)	10–22
N	S	15	F_1	19.1	(2.4)	14–23	74	(18)	43–110	73	16.1	(3.5)	10–20
SN	SN	12	F_2	18.6	(2.9)	16–23	60	(21)	31–100	83	15.0	(6.4)	5–27
NS	NS	12	F_2	18.3	(2.3)	15–22	61	(22)	34–122	83	15.9	(4.5)	8–23
S	SN	12	BC	19.4	(1.2)	17–20	50	(15)	31–82	100	20.9	(3.5)	17–29
S	NS	12	BC	19.3	(1.9)	16–22	56	(18)	26–85	100	21.3	(2.2)	19–26
SN	S	23	BC	23.0	(1.7)	18–23	58	(24)	28–119	100	23.0	(3.2)	12–24
NS	S	13	BC	21.2	(2.0)	17–23	49	(16)	36–82	100	18.3	(4.2)	10–24
N	SN	12	BC	18.3	(1.6)	15–21	77	(27)	52–139	58	13.2	(3.7)	8–20
N	NS	22	BC	16.2	(2.5)	12–20	79	(30)	40–123	50	12.6	(4.8)	8–24
SN	N	12	BC	16.4	(1.7)	15–18	87	(22)	36–119	58	9.4	(3.6)	5–16
NS	N	12	BC	17.0	(3.0)	12–22	62	(27)	24–97	92	13.1	(7.1)	6–30

[a] (S.D.)=standard deviation.
[b] S=*A. sollicitans*, N=*A. nigromaculis*, SN and NS=hybrids (first letter denoting species of parent males).
[c] P=parent types, F_1=hybrids, F_2=progeny of hybrids, BC=backcross of parent types to hybrids.

TABLE 2.—Significance of differences in numbers of pecten teeth and comb scales of progeny in crosses of *A. sollicitans*, *A. nigromaculis*, their hybrids and backcrosses compared with the parent types.[a, b]

Crosses			*A. sollicitans*		*A. nigromaculis*	
Male	Female	Generation	Number of pecten teeth	Number of comb scales	Number of pecten teeth	Number of comb scales
S	S	P	.....	.....	0.001	0.001
N	N	P	0.001	0.001	.....	.....
S	N	F_1	0.01	0.001	0.2	0.001
N	S	F_1	0.3	0.001	0.02	0.001
SN	SN	F_2	0.1	0.01	0.1	0.01
NS	NS	F_2	0.05	0.001	0.1	0.001
S	SN	BC	0.3	0.2	0.01	0.001
S	NS	BC	0.3	0.2	0.01	0.001
SN	S	NC	0.4	0.9	0.001	0.001
NS	S	BC	0.2	0.01	0.001	0.001
N	SN	BC	0.02	0.001	0.05	0.01
N	NS	BC	0.001	0.001	0.7	0.01
SN	N	BC	0.001	0.001	0.9	0.3
NS	N	BC	0.01	0.001	0.7	0.05

[a] Analyzed by group comparison and standard "t" test.
[b] See Table 1 for key to symbols.

Distal pecten teeth were only slightly displaced and were barbed in all larvae. Larvae with significantly fewer comb scales than *A. sollicitans* were produced only when male progeny of the *A. nigromaculis* male x *A. sollicitans* female F_1 was backcrossed with the *A. sollicitans* female. All four backcrosses produced larvae with significantly more comb scales than *A. nigromaculis*.

In contrast, four similar backcrosses to *A. nigromaculis* produced larvae with significantly fewer pecten teeth and comb scales than *A. sollicitans*. Also, the mean detachment of the distal pecten tooth was greater in three of the four backcrosses than in the F_1 larvae. The barbed condition of the distal pecten teeth ranged from 50 to 90 percent in the four backcrosses. In three of the four backcrosses, the pecten tooth count of the progeny was not significantly different from that of *A. nigromaculis*. Only the backcross to a female *A. nigromaculis* with a male from the *A. sollicitans* male x *A. nigromaculis* female cross produced progeny with comb scale counts not significantly different from those of *A. nigromaculis;* the other crosses produced significantly higher comb scale counts.

Phenotypic comparisons of adults resulting from the crosses were much more difficult than the comparisons of larvae because of the closeness of the two species in the adult stage. The major differences between *A. sollicitans* and *A. nigromaculis* are the coloration of the abdominal scales, yellow in *A. nigromaculis* and mixed yellow and white in *A. sollicitans,* and the banding and color patterns on the first and fifth tarsal segments of the metathoracic legs. The first tarsal segment has a median band of yellow scales in *A. sollicitans;* the presence of the median band is variable (but white when present) in *A. nigromaculis*. The last tarsal segment of *A. sollicitans* is completely white scaled; *A. nigromaculis* has black and white scaling (Carpenter and LaCasse 1955).

An examination of the parent populations revealed that only *A. sollicitans* was true for the three characters; about 27 percent of the *A. nigromaculis* had completely white scaled distal tarsal segments. In general, adults from all crosses looked more like *A. sollicitans* (Table 3); however, none of the hybrid crosses produced adult female mosquito populations that all had the three *A. sollicitans* characters. The median banding of the first tarsal segment

TABLE 3.—Phenotypes of adult females from crosses between *A. sollicitans*, *A. nigromaculis*, their hybrids, and back crosses.[a]

Crosses		Number of specimens	Generation	Percentage possessing *A. sollicitans* characters		
					Metathoracic leg	
Males	Females			Abdomen	First tarsal segment	Last tarsal segment
S	S	15	P	100	100	100
N	N	15	P	0	0	27
S	N	6	F_1	100	33	83
N	S	6	F_1	83	50	83
SN	SN	15	F_2	93	60	100
NS	NS	15	F_2	67	53	93
S	SN	15	BC	93	67	93
S	NS	15	BC	87	53	100
SN	S	15	BC	100	93	100
NS	S	15	BC	67	70	53
N	SN	15	BC	93	33	70
N	NS	15	BC	30	53	70
SN	N	15	BC	93	70	100
NS	N	15	BC	20	60	60

[a] See Table 1 for key to symbols.

was the most variable of the three characteristics, but most adult females in all but two crosses had banding typical of *A. sollicitans*.

The results show that *A. sollicitans* and *A. nigromaculis* are completely compatible genetically because all F_1, F_2, and backcross combinations produced uniformly fertile eggs, surviving and developing larvae, and fertile adults that demonstrated no alteration of the normal sex ratios. Little or no dominance of one parent species over the other was apparent since comb scale counts were generally between those of the two parent species. The distal pecten teeth were more likely to be barbed as in *A. sollicitans*, but the distal 2 or 3 pecten teeth tended to be displaced as in *A. nigromaculis*.

Aedes sollicitans and *A. nigromaculis* are therefore very close phylogenetically, even though they are easily separated, especially in the larval stage. However, they probably do not hybridize in nature since they are generally allopatric; and when they may come in contact, habitat preference or other behavioral differences probably prevent cross-mating. Even if hybridization occurred on occasion the hybrids would probably not be recognized.

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