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Summary. To test the association between the breeding density of the sand flies *Culicoides furens, C. barbosai* and *C. insignis* and the shadiness of the habitat, the overall density of breeding in a mangrove swamp was estimated by means of 28 emergence traps. A 12-yard-square area in the centre was then cleared of vegetation and observations continued for a further 6 weeks. No appreciable change in density was observed over this period. One year later, however, the density of *barbosai* had been reduced to about one tenth of that in an untouched control area, but the densities of *furens* and *insignis* were still unchanged. It is suggested that felling the mangroves might be a feasible abatement method for *barbosai* in areas where this species is dominant and the breeding area limited in extent.

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WHITE EYE, A FEMALE-STERILE AND SEX-LINKED MUTANT OF *CULEX TRITAENIORHYNCHUS*

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A number of interesting mutants have been found in *Culex tritaeniorhynchus*, an important vector of Japanese encephalitis virus and West Nile virus, from various colonies collected in East and West Pakistan (Baker and Aslamkhan, 1968). This paper describes one of these mutants, white eye (*w*). White eye appears to be a fairly common eye color mutant in most species of mosquitoes where genetic studies have been carried out. It has been found in *Anopheles pharoensis, Anopheles gambiae*, and *Anopheles quadrimaculatus* (Kitzmiller and Mason, 1967), the *Culex pipiens* complex (Gilchrist and Haldane, 1947 and Laven, 1967), *Culex tarsalis* (Barr and Myers, 1966), and in *Aedes aegypti* (Bhalla 1968). Thus far, white eye appears generally to be sex linked for all species except in *C. tarsalis* where it appears to be autosomal. The white-eye mutant in *C. tritaeniorhynchus* is also sex linked, but differs from these mutants in that homozygous white-eye females are generally sterile.

In *Culex tritaeniorhynchus*, another sex-linked mutant, golden (*go*), was previously reported (Baker 1968). In that case, sex appeared to be determined by a single pair of alleles *M* and *m*, or a segment of the chromosomes for which males are heterozygous, *M/m*, and females homozygous, *m/m*.

MATERIALS AND METHODS

The white-eye mutant was first isolated from the Dacca strain by inbreeding by means of brother-sister matings through the F_2 generation. This mutant has also been found and established from other colonies originally collected from Khulna and Chittagong (Baker and Aslamkhan 1968). It is interesting that these 3 colonies are all from East Pakistan. As yet no white-eye mutants have been found in colonies collected from West Pakistan.

Although the females are sterile, the males remain fertile. Therefore, this mutant is maintained by a continuous backcross mating system where heterozygous (+/*w*) females are crossed to homozygous (*w/w*) males for each generation. The white-eye females (*w/w*) from this backcross are saved and crossed separately to the wild-type males, but normally no fertilized eggs are produced. This breeding system is currently being carried out for the three white-eye mutant strains originally isolated from the Dacca, Khulna, and Chittagong colonies.

The wild-type strain used in the crosses reported here is from the Dacca strain (Baker and Aslamkhan 1968). The method of mass mating and rearing for the crosses reported below are similar to those reported for the mutant golden (Baker 1968).

RESULTS AND DISCUSSION

Description of the Mutant. White eye is an excellent marker mutant. The entire eye of this mutant appears to lack pigment in the first instar larvae and in all later stages. The eye remains white throughout the entire life of the adult.

White-eye females (*w/w*) generally appear to be sterile. Dissection of the spermatheca from these *w/w* females after they have had a chance to mate with any normal males (+/+) contain motile sperm. Some of these females do lay egg rafts, but these eggs give the appearance of being unfertilized as no embryonic development can be detected in these eggs. Thousands of the white-eye females have been mated to normal males since the discovery of this mutant.

Linkage. The results of scoring for white eye and sex are given in Table 1. Normally only one P_1 type of cross (Cross #1) can be made, namely, wild-type females (+*m*/+*m*) and white eye males (*wm/wM*). When this cross is made, all the F_1 offspring are normal, and the sex ratio approximates an expected 1:1 ratio (X^2_1=1.78), thus the gene *w* is recessive with no visible effect in the heterozygote. Since it was not possible to backcross the F_1 males to white-eye females because they are sterile, the F_1 progeny were crossed with each other (+*m/w m* x +*m/wM*) in cross #2. The segregation of both wild-type and white-eye mosquitoes did not depart significantly at the 5 percent level from a 3:1 ratio (X^2_1=.598) nor from an expected 1:1 sex ratio (X^2_1=.778). Estimation of sex linkage by the method of maximum likelihood (Gilchrist and Haldane, 1947) gave a recombination percentage of 6.03±1.50.

Exceptional White Eye Females. White-eye females normally appear to be

Table 1.—Showing the results of wild-type x white eye crosses.

Cross #	Parental Genotypes	Phenotype wild-type ♀	wild-type ♂	white eye ♀	white eye ♂
1	+*m*+*m* X *wm/wM*	453	494	0	0
2	+*m/wm* X +*m/wM*	510	308	14	244
3	*wm/wm* X +*m*/+*M*	46	54	0	0
4	*wm*/+*m* X *wm*/+*M*	131	341	182	7

sterile. Routinely, white-eye females are put into a cage containing normal males (+/+). Usually no apparent fertilized eggs are laid. A large number of white-eye females have been crossed in this manner. On August 8, 1968 the first raft was found with 44 eggs all of which hatched except one. Four more fertile egg rafts were laid, almost one per week. The last raft occurred on September 6, 1968. It is possible that all five rafts were laid by only one female as the opportunity for blood meals was given every other day. The total number of eggs from the five rafts was 352, with a 96.5 percent hatch. Larval mortality was high, and only 46 normal females and 54 normal males reached the adult stage (cross #3). An F_2 (cross #4) was made by crossing these F_1 males and F_1 females. The sex ratio approximates an expected 1:1 ratio (X^2_1= 1.78). However, the segregation of wild-type and white-eye mosquitoes did depart significantly at the 5 percent level from a 3:1 ratio (X^2_1=4.550). Estimation of sex linkage gave a recombination percentage of 2.99±1.1. The resulting white-eye females from the F_2 cross were then given the opportunity to mate with both heterozygous (+/*w*) males and homozygous (*w*/*w*) males. No fertile rafts were laid. Since the exceptional fertile rafts were laid, there have been no other fertilized eggs from white-eye females although we are continuously mating them to normal males. The reason for the occurrence of these exceptional fertile females is not presently known. Possibly the fertile eggs were the result of penetrance of the *w* gene. On the other hand, eye color and female sterility could be influenced by two separate genes which are closely linked or are involved in an inversion.

SUMMARY

A gene is described in the mosquito *Culex tritaeniorhynchus* for white eye color, *w*, which expresses itself in the egg, larval, pupal and adult stages. The gene is recessive and sex-linked, with uniform expression and complete penetrance. The recombination distance of *w* to the sex locus is 6.03±1.50. White eye females are generally sterile.

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