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RED EYE AND VERMILLION EYE, RECESSIVE MUTANTS ON THE RIGHT ARM OF CHROMOSOME 2 IN *ANOPHELES ALBIMANUS*[1]

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ABSTRACT. Two new eye color mutants, *red eye (re)* and *vermillion eye (ve)* arose spontaneously in laboratory stocks of *Anopheles albimanus*. Both mutants are recessive and expressed during the larval, pupal and adult stages. In individuals homozygous for *re*, the eyes darken with age in the adult stage, but in *ve* homozygotes there is no change in the bright red color. The loci for both mutants are on the right arm of chromosome 2, but the mutants are not allelic. *Vermillion eye* homozygotes are weak and a pure stock has been established and maintained with difficulty.

INTRODUCTION

For the past several years we have been involved in basic studies on the genetics of *Anopheles albimanus* Wiedemann. The linkage groups and mode of inheritance of several mutants have been reported for this species of neotropical mosquito which is an important vector of human malaria. A current list of genetic markers in *An. albimanus* was given in a recent report by Narang et al. (1981).

In this present report, we describe two new non-allelic, recessive, eye-color mutants, *red eye (re)* and *vermillion eye (ve)*.

METHODS AND MATERIALS

Established procedures were used for the rearing and maintenance of the mosquitoes (Rabbani and Seawright 1976, Benedict et al. 1979). Appropriate crosses (Tables 1–4) were used to determine the mode of inheritance and linkage group for the two mutants. Other mutant markers used during the linkage study

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were *propoxur resistance* (pr^r), a dominant trait on chromosome 2 (Kaiser et al. 1979), and *non-stripe (st)*, which is a recessive variant on chromosome 3 (Rabbani and Seawright 1976). Crossing over occurs on the autosomes of both sexes of *An. albimanus;* therefore, backcrosses were conducted with both hybrid males and females. Sex determination is an X-Y system, and the male is the heteromorphic sex (Seawright et al. 1982, Keppler et al. 1973).

RESULTS

Red eye was isolated from an inbred strain bearing a small, pericentric inversion, In(3)24, on chromosome 3 and a vigorous, homozygous *re* stock was established. The eyes of homozygotes are bright red during the larval and pupal stages and retain the distinctive red color in young adults. The red color darkens as the adult ages so that after 2–3 days the eyes of *re* homozygotes are barely distinguishable from the normal type. The F_1 progeny of crosses between *red eye* and *normal eye* invariably had normal-color eyes, and as shown by the results of the crosses in Table 1, *red eye* is a recessive monofactoral trait.

Two-point testcrosses with *propoxur resistance* on the right arm of chromosome 2 and *red eye* were performed, and a tight linkage was found between these loci

Table 1. Summary of crosses showing the recessive nature of *red eye (re)* in *Anopheles albimanus.*

Cross ♀ × ♂	No. of families	Phenotype of progeny re^+	re	X^2
F_1 ($re \times re^+$) $\times re$	19	596	597	0.001
$re \times F_1$ ($re \times re^+$)	10	370	395	0.817
F_1 ($re^+ \times re$) $\times re$	5	511	470	1.714
$re \times F_1$ ($re^+ \times re$)	5	392	378	0.255
F_2 ($re^+ \times re$)	26	1002	345	0.269
F_2 ($re \times re^+$)	13	900	309	0.201

(Table 2). *Non-stripe,* which is on the right arm of chromosome 3, was included in these crosses, and independent segregation was found for *re* and *st.*

Red eye is a fully-penetrant mutant and is an excellent marker for the right arm of chromosome 2. *Propoxur resistance* was assigned somewhere in regions 9 or 10 by Kaiser et al. (1979), who used translocations and inversions to calculate the location of the pr^r locus. However, the precise locations of the pr^r or *re* loci are not known at this time.

Vermillion eye was found at about the same time as *red eye* and arose spontaneously in inbred material from a recently colonized stock from El Salvador. The phenotypic similarity of *re* and *ve* was so remarkable that we thought the same mutant had been found from two different sources. However, the idea was discarded when the eyes of *ve* homozygotes did not darken with age and crosses between the *ve* and *re* types yielded F_1 progeny with normal-color eyes. *Vermillion eye* homozygotes are weak and show reduced viability after they reach the 4th (last) larval stage. The initial attempts to isolate a homozygous stock were unsuccessful because of high mortality during the pupal and adult stages. Since this mutant is visible in the larval stages, crosses to study its inheritance were possible and a few successful matings were obtained for evaluation of the inheritance and linkage of *ve.* The *ve* mutant was maintained until recently by inbreeding heterozygotes, but after outcrossing this mutant with reisolation in the F_2 followed by a second outcross and reisolation, a weak homozygous stock has been established. The lack of vigor of the *ve* homozygote precludes the usefulness of this mutant as a reliable marker in genetic crosses.

Dihybrid crosses were used to determine that *ve* is linked to pr^r on chromosome 2 at a map distance of 6.5 units (Table 3). At this time the relationship of *ve* to other markers on chromosome 2 is not known. *Vermillion eye* is epistatic to the expression of *stripe* (st^+)

(Table 4), which is a dominant allele of *st* on chromosome 3 (Rabbani and Seawright 1976). Amongst the progeny of the dihybrid crosses shown in Table 4, there were no mosquitoes in the expected *ve* st^+ class. Progeny testing of the *ve st* phenotype was accomplished by crosses with ve^+ *st*. As expected for epistasis, part of the *ve st* phenotypic class actually had a *ve/ve* st^+/st genotype as evidenced by the presence of larvae with the dominant marker, *stripe*.

DISCUSSION

Red-eye color is a common mutant in mosquitoes. A literature search divulged a total of 52 eye color mutants which have been described in 19 species of mosquitoes. A complete listing of all of these mutants is impractical in this present paper, but a list of references is available upon request. Generally, the eye color mutants in mosquitoes are fully penetrant and are visible in three life stages, i.e., larvae, pupae, and adults. Red and white eyes are the most common forms which are observed. Of the eye color mutants in mosquitoes, 18 are variations of red eye color and 16 are some variation of white or colorless. Rosy (or pink), which is usually allelic with white was noted 6 times. Twenty-four of the eye-color mutants are sex-linked, but the linkage is not known for several mutants which have been reported.

Four eye-color mutants have been isolated in *An. albimanus*. In addition to *red eye* and *vermillion eye*, two different white-eye mutants are located on the X chromosome. *White eye (we)* is similar to *ve* in being epistatic to the expression of *stripe*

Table 2. Two-point testcrosses showing tight linkage between *propoxur resistance* (pr^r) and *red eye* (*re*). The linkage distance between these two genes was 1.59 ± 0.26 map units.

Cross ♀ × ♂	No. of families	Phenotype of progeny $re^+ pr^r$	$re^+ pr^s$	$re\ pr^r$	$re\ pr^s$	X^2 *re*	pr^r
F_1 ($re\ pr^s \times re^+ pr^r$) × $re\ pr^s$	5	324	1	5	323	0.014	0.038
$re\ pr^s$ × F_1 ($re\ pr^s \times re^+ pr^r$)	5	213	2	6	229	0.889	0.320
F_1 ($re^+ pr^r \times re\ pr^s$) × $re\ pr^s$	5	302	8	9	305	0.026	0.006
$re\ pr^s$ × F_1 ($re^+ pr^r \times re\ pr^s$)	5	248	3	1	217	2.322	1.793

Table 3. Summary of dihybrid crosses showing the recessive nature of *vermillion eye (ve)* and the linkage between *ve* and *propoxur resistance* (pr^r). The map distance as estimated by the maximum liklihood method was 6.51 ± 0.63.

Cross (presumed genotype) ♀ × ♂	No. of families	Phenotype of progeny $ve^+ pr^r$	$ve^+ pr^s$	$ve\ pr^r$	$ve\ pr^s$	X^2 *ve*	pr^r
F_2 ($ve\ pr^s \times ve^+ pr^r$)	5	466	13	20	150	0.494	0.005
F_2 ($ve^+ pr^r \times ve\ pr^s$)	7	743	36	35	204	1.259	1.102

Table 4. Summary of dihybrid crosses showing the epistatic relationship between *vermillion eye* (*ve*) and *stripe* (st^+).

Cross (presumed genotypes) ♀ × ♂	No. of families	Phenotype of progeny $ve^+ st^+$	$ve^+ st$	$ve\ st^+$	$ve\ st$	X^2 *ve*	*st*
F_2 ($ve\ st \times ve^+ st^+$)	7	434	190	0	176	3.840	1.300
F_2 ($ve^+ st^+ \times ve\ st$)	4	261	103	0	113	1.310	0.455

(Seawright et al. 1982). No research has been done on the biochemical pathways involved in the synthesis of eye pigments in *An. albimanus*, but the inhibition of the expression of the st^+ allele indicates that the mutants, *ve* and *we*, probably block the synthesis of pigment in closely related reactions. *Red eye* is not epistatic to *stripe* and also the red color darkens in the adult stage; hence, *re* should logically be thought of as a result of blocking the synthesis of pigment at a later stage.

Nine loci, seven visible mutants and two enzymes have been assigned to chromosome 2 in *An. albimanus*. Although some of the map distances are tentative, the order of seven of the loci is: *6-Phosphogluconate dehydrogenase (6-Pgd)* (unpublished data), *re* pr^r, *green larvae (gl)* (Seawright et al. 1979), *bald palpi (bp)* (Seawright et al. 1981), *ebony (eb)* (Benedict et al. 1979), and *brown larva (bw)* (unpublished data).

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