AMBER, A RECESSIVE MUTANT ON THE RIGHT ARM OF CHROMOSOME 2 IN *ANOPHELES ALBIMANUS*

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ABSTRACT. *Amber (am)* is a recessive, autosomal trait expressed as an amber-colored head capsule and saddle on abdominal segment X during the 4th larval stage in *Anopheles albimanus*. Male-linked translocations and previously mapped mutant markers were used in crosses to assign the *am* locus to the right arm of Chromosome 2.

Accumulation of information on the genetics and cytogenetics of the important malaria vector, *Anopheles albimanus* Wiedemann, has proceeded at a steady pace in recent years (Seawright et al. 1982c). Most of this work has been done in our laboratory for the specific purpose of developing chromosomal aberrations which can serve as genetic control mechanisms, e.g., reciprocal translocations (Curtis 1968a,b) or compound chromosomes (Foster et al. 1972). A significant effort in this basic work with *An. albimanus* involves the establishment of a genetic map of mutant and enzyme loci, and a constant effort is expended in screening for new mutants. In this paper, we describe the inheritance and map position for *amber*, a color variant expressed in fourth stage larvae.

MATERIALS AND METHODS

Routine procedures were employed for rearing and maintenance of the mosquitoes (Benedict et al. 1979). Appropriate crosses (Tables 1–3) were used to determine the mode of inheritance and linkage group of *amber*. Other mutants and Y-linked translocations used in the linkage study were: *propoxur resistance* (pr^r), a dominant trait on 2R (Kaiser et al. 1979); *red eye (re)*, a recessive mutant, on 2R (Seawright et al. 1982a); *T(Y;2R)3* and *T(Y;3L)1* with breakpoints at 15A and 37B, respectively (Kaiser et al. 1982). The breakpoints of the two translocations are very close to the centromeric regions as seen in preparations of salivary gland chromosomes (map by Keppler et al. 1973). Male-linked translocations are strictly holandric, but crossing over occurs on the autosomes of both sexes of *An. albimanus*, therefore, the backcrosses used in the linkage study (Table 3) were conducted with both hybrid males and females. Sex determination is an XY system in this species, and the male is the heteromorphic sex (Kaiser et al. 1979, Seawright et al. 1982b).

The statistical procedures of Mather (1957) were used in analyzing the data on mode of inheritance and for estimation of linkage distances.

RESULTS AND DISCUSSION

The *amber* mutant was found during the inbreeding of a stock that was originally collected in Panama. In 4th stage larvae that are homozygous for this mutant, the head capsule and the saddle on the last abdominal segment (X) of the abdomen have an amber color. Penetrance is complete and homozygotes are readily distinguished from normal larvae. The amber color is not visible during the earlier larval stages, pupae or adults.

After the initial detection of *amber*, a vigorous, homozygous stock was established in one generation and the crosses in Table 1 were conducted to establish the mode of inheritance.

Table 1. Summary of crosses showing that *amber (am)* is a recessive, autosomal trait.

Cross ♀ × ♂	No. of families	Phenotype of progeny		χ^2	
		am	am^+	Deviation	Heterogeneity
$F_1(am \times am^+) \times am$	5	522	551	0.784(P. = .37)	2.843(P = .58)
$am \times F_1(am \times am^+)$	6	492	471	0.229(P = .63)	3.660(P = .60)
$F_1(am^+ \times am) \times am$	6	434	452	0.366(P = .54)	4.324(P = .50)
$am \times F_1(am^+ \times am)$	5	367	361	0.494(P = .48)	1.549(P = .82)
$F_2(am \times am^+)$	5	221	760	3.197(P = .07)	2.896(P = .57)
$F_2(am^+ \times am)$	5	214	675	0.408(P = .52)	2.576(P = .63)

The progeny of reciprocal crosses of *am* homozygotes to wild type (am^+) did not express the mutant phenotype. Analysis of the data obtained from the backcrosses and monohybrid

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Table 2. Summary of crosses with male-linked translocations that show pseudolinkage between *sex* and *amber (am)* for *T(Y;2R)3* but not *T(Y;3L)1*.

Cross ♀ × ♂	No. of families	Phenotype of progeny				χ^2		
		am female	*am male*	am^+ *female*	am^+ *male*	Linkage	*sex*	*amber*
$am \times F_1(am \times T(Y;3L)1\ am^+$	7	92	93	96	98	0.002(P = .96)	0.024(P = .88)	0.214(P = .64)
$am \times F_1(am \times T(Y;2R)3\ am^+)$	7	148	59	56	126	64.98(P<.001)	0.928(P = .33)	1.607(P = .20)

crosses, shown in Table 1, clearly shows that *am* is a recessive, monofactorial trait. Since all of the χ^2 estimates for heterogeneity among families were acceptable (range of P:0.57–0.82), the data were pooled as shown in Table 1. Each backcross family was scored for *sex* and *am,* and sex linkage of *am* was excluded.

The crosses (Table 2) with *T(Y;2R)3* and *T(Y;3L)1* were done to assign *am* to a linkage group. Pseudolinkage between *sex* and *am* was detected in the cross with *T(Y;2R)3,* and there was independent assortment of *am* and *T(Y;3L)1.* Crossing over between *T(Y;2R)3* and *am* was 29%, a value close to the frequency of recombination that Kaiser et al. (1979) reported between pr^r and *T(Y;2R)3.* Hence, we suspected that *am* might show tight linkage with pr^r and *re* on the right arm of chromosome 2. The alternative was for *am* to be located on the other side of the centromere on 2L close to the *ebony (eb)* locus, for which Narang et al. (1984) reported a frequency of 22% crossing over between *eb* and *T(Y;2R)3.* Since the melanotic phenotype of *eb* obscures *amber,* crosses with that mutant marker were deferred until the results of crosses with *re* and pr^r were completed.

A cross was set up with *red eye;* dihybrids (in repulsion) were inbred and the *re am* homozygotes were used to start the stock used in crosses shown in Table 3. A sample of the $F_2(am^+\ re \times am\ re^+)$ was scored as follows: 897 $am^+\ re^+$, 459 $am^+\ re$, 491 *am* re^+, and 2 *am re.* The very low frequency of the double recessive type, *am re,* indicated tight linkage between *am* and *re.* A tight linkage distance of 4 map units between *am* and *re* was measured from the results of the crosses shown in Table 3. The sequence and map distances measured for the three loci were: *amber*—2.1±0.31 - *propoxur resistance* - 1.9±0.30 - *red eye.* Previously, the distance between *re* and pr^r was estimated at 1.5±0.26 map units (Seawright et al. 1982a), but more recently additional data were collected in three-point testcrosses (shown in Table 4) with *re,* pr^r and *green larva (gl).* More crossing-over was observed for females than in the males as follows: female—*re* - 3.13 - pr^r - 8.31 - *gl;* male - *re* - 1.02 - pr^r - 4.30 - *gl.* For the crosses shown in Table 2, this was not true of the overall map distance between *re* to *am.* However, it is advisable to use the estimates of map distance from only one sex, and for this purpose we decided to use the hybrid female for two reasons, viz., single pair matings are not as successful as mass mating in a cage population, and hybrid females are usually more fecund than females of multiple-mutant stocks. The data in this report and from our previous work (Seawright et al. 1979, Seawright et al. 1982a) were pooled for the estimates in the following sequence of the map of the visible

Table 3. Three-point test crosses showing tight linkage between *amber (am), propoxur resistance (pr^r)* and *amber (am)*. The sequence and map distances (calculated for hybrid female) for these loci were: *am*—1.87±0.45—*pr^r*—2.31±0.50—*re*.

Cross ♀ × ♂	No. of families analyzed	Phenotype of progeny				χ^2		
		Parental	Crossovers					
			re:pr^r	*pr^r:am*	*re:pr^r:am*	*re*	*pr^r*	*am*
F_1(*re^+ pr^r am^+ × re pr^s am*) × *re pr^s am*	8	870	21	17	0	0.533(P=.46)	0.282(P=.40)	0.004(P=.95)
re pr^s am × F_1(*re^+ pr^r am^+ × re pr^s am*)	9	1126	17	26	1	1.370(P=.24)	1.510(P=.22)	1.510(P=.22)

Table 4. Three-point test crosses showing linkage relationships of *green larva (gl), propoxur resistance (pr^r)* and *red eye (re)*. The sequence and map distances (calculated with data from hybrid females) for these loci were *gl*—8.71±0.56—*pr^r*—2.82±0.41—*re*.

Cross ♀ × ♂	No. of families analyzed	Phenotype of progeny				χ^2		
		Parental	Crossovers					
			re:pr^r	*pr^r:gl*	*re:pr^r:gl*	*re*	*pr^r*	*gl*
F_1(*gl pr^s re* × *gl^+ pr^r re^+*) × *gl pr^s re*	9	977	26	74	9	0.004(P=.95)	0.236(P=.63)	0.033(P=.86)
gl pr^s re × F_1(*gl pr^s re* × *gl^+ pr^r re^+*)	3	207	2	8	0	1.332(P=.25)	0.779(P=.38)	0.115(P=.73)
F_1(*gl^+ pr^r re^+* × *gl pr^s* re) × *gl pr^s re*	5	527	12	42	0	0.759(P=.38)	0.291(P=.60)	1.076(P=.30)
gl pr^s re × F_1(*gl^+ pr^r re^+* × *gl pr^s re*)	6	679	5	28	2	0.358(P=.55)	0.275(P=.60)	2.022(P=.16)

markers on a segment from *gl* to *re* on 2R: *re* - 2.36±0.24 - *pr*^{r} - 1.87±0.45 - *am* - 6.84 - *gl*. The last value for the distance between *am* and *gl* was obtained by subtraction from the estimate, *pr*^{r} - 8.71±0.56 - *gl*, because the distance between *am* and *gl* has not been measured experimentally.

Ten loci (shown in Table 5) have been assigned to positions on the linkage map of Chromosome 2 of *An. albimanus*. The sequence for these loci is: *bw* - *eb* - centromere - *Got* - *Go* - *bp* - *gl* - *am* - *pr*^{r} - *re* - *6-Pgd*. The sequence from *green larva* to *red eye* covers a map distance of only 11 units. In a separate report, Seawright et al. (1984), assigned the *red eye* locus to Region 10C by using deficiencies. This means that, except for *6-Pgd*, the loci for the other markers are between Region 10C and the centromere on 2R. A few additional mutants and allozymes have been assigned to Chromosome 2 and linkage estimates are being developed. Therefore, the sequence shown above is tentative and will be revised with the addition of new information.

Table 5. Mutant and enzyme loci located on Chromosome 2 in *Anopheles albimanus*.

Locus	Symbol	Inheritance	Reference
brown larva	*bw*	recessive	unpublished
ebony	*eb*	codominant	Benedict et al. 1979
Glutamate oxaloacetate transaminase	*Got*	codominant	unpublished
Glucose oxidase	*Go*	codominant	Narang et al. 1984
bald palpi	*bp*	recessive	Seawright et al. 1981
green larva	*gl*	recessive	Seawright et al. 1979
amber larva	*am*	recessive	present paper
propoxur resistance	*pr*^{r}	dominant	Kaiser et al. 1979
red eye	*re*	recessive	Seawright et al. 1982
6-Phosphogluconate dehydrogenase	*6-Pgd*	codominant	Narang et al. 1984

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