RECESSIVE LETHAL MUTATIONS IN *ANOPHELES ALBIMANUS*

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ABSTRACT. Six recessive lethal mutants of *Anopheles albimanus* are described. Homozygotes for three of the autosomal mutants, viz., bar eye, dot eye and hairy, die during the last larval or early pupal stages; complete linkage data are lacking for bar eye and hairy but dot eye is tightly linked to red eye on the right arm of chromosome 2. Larval and pupal mortality is high for homozygotes of the other two autosomal mutants, diseased larva and lunate. Survival to the adult stage of the lunate type is about 60% for both sexes, and for diseased larva the rates differed according to sex, 15% for males and 3% for females. A general lack of vitality of the surviving adults has prevented the establishment of homozygous stocks. From analyses of linkage data, diseased larva and lunate were assigned to chromosome 2, most probably on the left arm. The mutant, bubble head, is on the X chromosome, and therefore is expressed only in the hemizygous male, which usually dies during the early pupal stage.

INTRODUCTION

During the course of studying the formal genetics of *Anopheles albimanus* Wiedemann, we have isolated several recessive lethal traits in this species. Previously, we described the inheritance and linkage of four lethal mutants, viz., black larva (Rabbani et al. 1976), ebony (Benedict et al. 1979), curled (Seawright et al. 1982a) and sable (Seawright et al. 1985b). Homozygotes for these four mutants usually die when they are fourth stage larvae or pupae. Several other recessive lethals have been studied more recently, and a description of their inheritance and assignment to linkage groups are presented in this paper.

METHODS AND PROCEDURES

Anopheles albimanus were reared according to Benedict et al. (1979), except that the food for larvae was a 2% suspension of 1:2:1 of brewer's yeast: Tetramin® fish food: finely ground hog supplement.

Other mutants used to study the inheritance and linkage of the lethal traits are listed in Table 1, and the crosses employed to study the mutants are shown in Tables 2–5. Chi-square analyses of the data collected for the inheritance and linkage of the mutants were conducted in accordance with the methods outlined by Mather (1957).

The lethal traits described below were isolated from a variety of experimental situations; the details regarding their source will be included in the description of each mutant. In *An. albimanus,* crossing over occurs in both sexes (Kaiser et al. 1979), but at a higher rate in the female (Seawright et al. 1982b). Sex determination is an X–Y system, with the male having heteromorphic sex chromosomes, of which the Y is heterochromatic and lacks the genes found on the euchromatic part of the X chromosome (Seawright et al. 1985a).

RESULTS

For all of the mutants described in this paper, the lethal effects of the genes are usually manifest during the fourth larval or the pupal stages. All the traits are recessive, but the genetic analysis, in terms of the exact location of the mutant genes on the genetic map, is incomplete or lacking in accuracy because of the difficulty imposed by setting up dihybrid crosses with recessive lethal traits and other mutants in repulsion and the existence of unequal rates of crossing over in males and females of *An. albimanus.* During the earlier part of our work with this type of mutant, we did not have any marked balancer chromosomes (heterozygous inversions marked with dominant traits) for the maintenance of the recessive lethals, so there was a problem of crossing unknown genotypes where a mosquito heterozygous for the lethal trait could not be recognized phenotypically. However, balancer chromosomes are now available in *An. albimanus,* and these stocks

Table 1. List of mutants and inversion balancer stocks used to study the inheritance and linkage of recessive lethal traits.

Mutant	Linkage group	Reference
Propoxur resistance (pr^r)	2R	Kaiser et al. 1979
Ebony (*eb*)	2L	Benedict et al. 1979
Stripe (st^+)	3R	Rabbani and Seawright 1976
Red eye (*re*)	2R	Seawright et al. 1982b
In(3) D-310	3	Unpublished
In(2) D-318	2	Unpublished

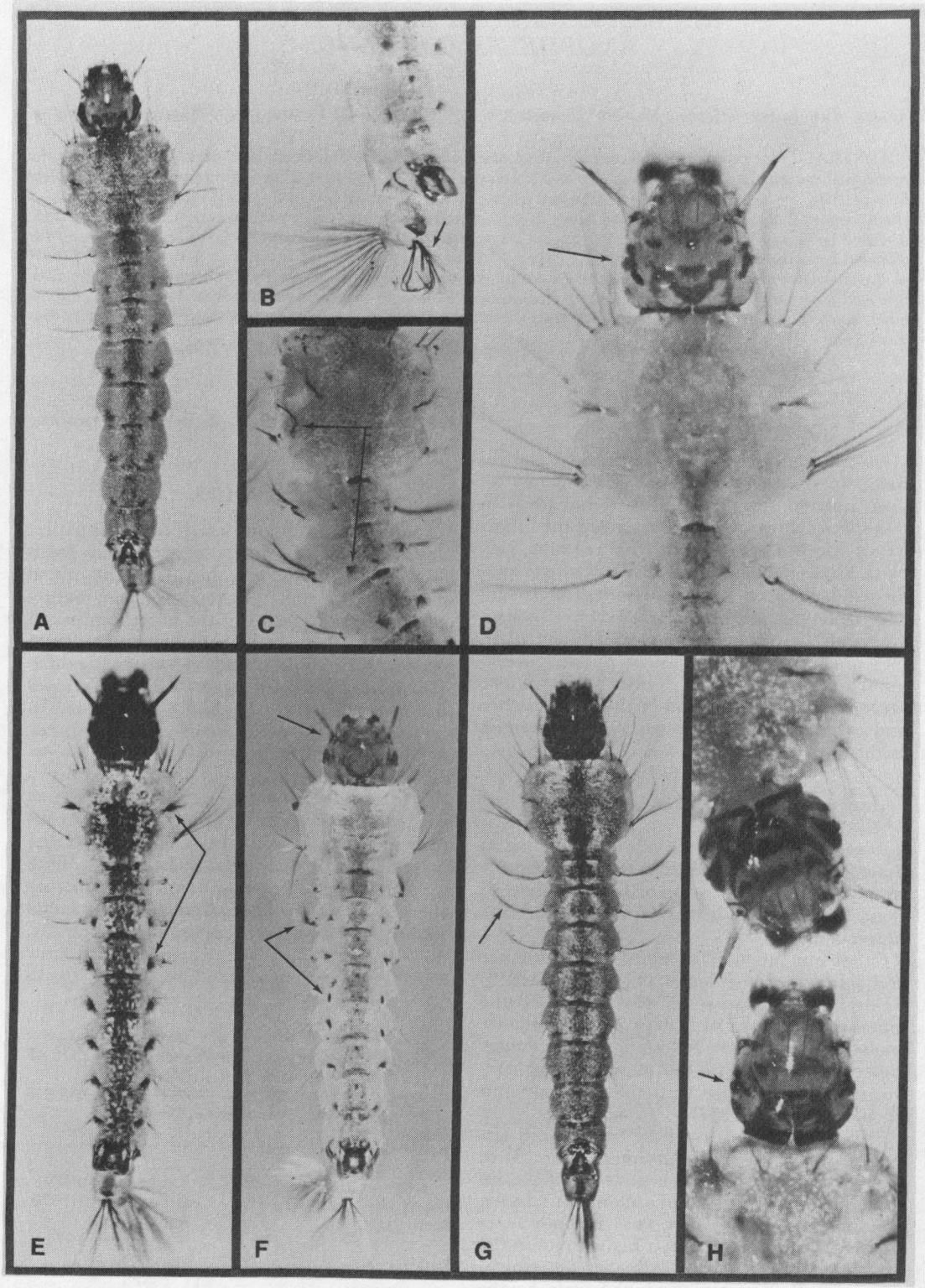

Fig. 1A–H. Phenotypes of normal (A) and six recessive lethals of *An. albimanus*. Arrows point to the phenotypic characters associated with the lethal genes: (B) diseased, (C) diseased, (D) bar eye, (E) hairy, (F) bubble head, (G) lunate, (H) dot eye.

make the maintenance of lethals an easier task and also facilitate crosses.

Diseased larva (*ds*) was found in inbred material of the CAMPO (El Salvador) stock. The name, diseased, was given to this mutant because of the progressive darkening of the abdomen of fourth instar larvae. The palmate setae of the fourth and fifth abdominal segments of *ds* homozygotes are oriented backwards so that the tips of these fan-shaped setae are directed toward the midline of the abdomen, and the large setae on the metathorax have a deformed, hook-shaped appearance (Fig. 1B and 1C). The *ds* larvae grow more slowly than the wild type and most die during or just prior to pupation. A few males (10–15%) do survive and are fertile, so that backcrosses with *ds* males were possible. Three percent of the homozygous females emerge as adults, but they are not fertile enough to allow the establishment of a colony. The backcrosses shown in Table 2 and the monohybrid cross shown in Table 3 demonstrate the monofactorial inheritance of *ds* and the linkage of *ds* to ebony, a marker on the left arm of chromosome 2. There was no linkage between *ds* and propoxur resistance on the right arm of chromosome 2 or stripe on chromosome 3. With four exceptions, the chi-square values for backcross families were not significant, but in the overall totals for these crosses there were deficiencies for the *ds* type, even when the four exceptional families were not included in the analysis. In the analysis of the data for the monohybrid cross, significant chi-square values were calculated for 6 of 47 families. When these families were removed from the analysis, the chi-square value for the totals of the other 41 families was acceptable as shown in Table 3. We concluded that diseased larvae is a recessive lethal trait located on the left arm of chromosome 2. The diseased stock has been lost.

A second mutant found in the CAMPO strain was called bar eye (*bar*); the phenotype of which is shown in Fig. 1D. The imaginal eye is more rectangular, in comparison to the crescent shape of wild type, and the larvae die during the fourth larval stage. Complete data on the linkage of this mutant are lacking, but a monohybrid cross, obtained by inbreeding the wild type sibs of larvae with the *bar* phenotype, showed that *bar* is recessive (Table 3). Dihybrid crosses (coupling) involving stripe and propoxur resistance substantiated that *bar* is recessive and showed that it is not linked to either of these loci. There was no indication of sex linkage. The stock containing *bar* has been lost.

The autosomal trait, hairy (*h*), was found in a radiation-induced inversion stock. Homozygotes for hairy have abnormal setae on the body

Table 2. Summary of backcrosses showing that *diseased larva (ds)* is linked to the semi-dominant trait, *ebony (eb)* on the left arm of chromosome 2. *Stripe (st^+)* and *propoxur resistance (pr^r)* are dominant traits on 3R abd 2R, respectively.

Cross (female × male)	No. of families	Phenotype of progeny				χ^2 analyses		
						Marker[1]	*ds*	Linkage
		st^+ ds^+	st^+ *ds*	*st* ds^+	*st ds*			
F_1 (st^+ ds^+ × *st ds*) × *st ds*	7	132	100	136	94	0.009 (P=.92)	11.853 (P<.01)	0.216 (P=.88)
		pr^r ds^+	pr^r *ds*	pr^s ds^+	pr^s *ds*			
F_1 (pr^r ds^+ × pr^s *ds*) × pr^s *ds*	10	156	112	139	127	0.007 (P=.93)	5.873 (P=.02)	1.918 (P=.17)
		eb ds^+	*eb ds*	*eb*$^+$ ds^+	*eb*$^+$ *ds*			
F_1 (*eb* ds^+ × *eb*$^+$ *ds*) × *eb*$^+$ *ds*	12	579	125	150	509	1.486 (P=.22)	6.621 (P=.01)	484.94 (P<.01)

1—Other markers, *st*, pr^r, or *eb*.

Table 3. Monohybrid crosses showing the inheritance of four recessive lethal traits in *Anopheles albimanus*.

Mutant	No. of families	Phenotype of progeny		χ^2 analyses
		Wild	Mutant	
Diseased larva	41	2373	760	0.920 (P=.34)
Bar eye	15	1186	404	0.142 (P=.71)
Hairy	18	641	109	43.820 (P<.01)
Dot eye	12	419	109	—

and most of the setae are thick and dark at the base (Fig. 1E). Homozygous larvae die before pupation. Analysis of the cross shown in Table 3 indicated a significant deficiency (χ^2=43.8) of the *h* phenotype, but the analyses of families were not so clear. For 12 of the families, the chi-square values were not significant, but deficiencies of the hairy phenotype were noted for the other 6 families. Although these data indicate that hairy could be due to a single gene with perhaps lowered viability of the early instars, this certainly is not conclusive and more than one gene could be involved. The normal larvae in 10 families were allowed to complete development and the sex of the ensuing adults was scored in order to check for sex linkage of *h*. If *h* were on the X chromosome, there would be a distorted (twice as many females) sex ratio, but equal numbers of males and females were observed (χ^2=0.028) as would be expected for an autosomal trait. The hairy trait was lost before any additional genetic analysis could be attempted.

An X-linked lethal, bubble head (*b*), was found in the progeny of irradiated males of the Apastepeque strain. Since this trait is on the X chromosome and lethal, it has been observed only in the hemizygous males, and the presence of *b* in a family is accompanied by a distorted sex ratio of 2 females: 1 male amongst the adults (Table 4). Male larvae with the bubble head phenotype have a large head capsule and the setae usually have a "singed" appearance (Fig. 1F). This trait is lethal during the early pupal stage or during the transition of the pupal stage, so that the sex can be determined, by examination of the pupal terminalia, for most of the mosquitoes with the *b* phenotype. Linkage experiments, previously reported (Seawright et al. 1985a), were conducted to map bubble head relative to the loci for white eye (*we*) and snow (*sn*). Estimates for the map distances for the three loci were: *we* - 12.8 - *b* - 18.3 - *sn* - centromere.

The mutant, lunate (*ln*), was found in families homozygous for *T(X;3R)3*, a sex-linked translocation with chromosomal breakpoints on the heterochromatic, short arm of the X chromosome and at Region 33 on the right arm of chromosome 3. The setae of lunate homozygotes are curved or curled (Fig. 1G), usually forward, and *ln* is phenotypically similar to curled, a recessive lethal on the X chromosome (Seawright et al. 1982). It has been impossible to establish a lunate stock, for although the homozygous type survives well until the pupal stage, only 60% emerge as adults and these do not mate at a level sufficient enough to establish a colony. We were able to conduct two backcrosses and a monohybrid cross (Table 5). The results of these crosses were quite clear in showing that lunate is a recessive, autosomal trait. To assign lunate to a linkage group, a cross of mosquitoes which were heterozygous (in repulsion) for lunate and two heterozygous, pericentric inversions, In(2) D–318 and In(3) D–310, was used. The two inversions were marked with the genes, ebony (*eb*) (semidominant on 2L) and stripe (st^+) (dominant on 3R), so that lunate could be assigned to a linkage group if linkage to either of the two markers was detected. The results (summary of three families) of this cross were: 38 ln^+ st^+ *eb*; 6 ln^+ *st eb*; 6 *ln* st^+; 3 *ln st* eb^+. Since none of the lunate type were ebony, these results indicated that lunate is on chromosome 2.

Recently, the complex inversion, In(2) D–318, was used in a cross scheme to assay the

Table 4. Summary of cross of normal males to females that were sibs of larvae exhibiting the *bubble head (b)* phenotype. A similar cross of males that were sibs yielded only wild type progeny.

Presumed cross female × male	Phenotype of larvae of pupae		Sex of live pupae or adults		χ^2 analyses	
	wild	bubble head	male	female	bubble head[1]	sex[2]
b^+/b × b^+	1305	439	442	825	0.026 (P=.87)	1.374 (P=.25)

[1] Calculated for a 3 wild:1 bubble head ratio.
[2] Calculated for a 2 female:1 male ratio.

Table 5. Summary of crosses showing that lunate is a recessive, autosomal trait.

Cross (female × male)	No. of families	Phenotype of progeny		χ^2 Analyses
		wild	mutant[1]	
ln/ln^+ × ln/ln^+	12	1241	378	2.357 (P=.12)
ln × F_1(ln^+ × *ln*)	2	93	90	0.049 (P=.82)
F_1 (ln^+ × *ln*) × *ln*	7	508	503	0.025 (P=.87)

[1] Equal numbers of both sexes were observed; χ^2 values not significant.

Apastepeque stock for the presence of visible or lethal mutants on chromosome 2. In(2) D–318 covers about 65% of chromosome 2 and is marked with ebony (a semi-dominant gene on 2L) and propoxur resistance (pr^r) (a dominant gene on 2R), and is not viable when homozygous. A mutant, which we designated dot eye (*de*), was found on 4 of 38 chromosomes. Larvae of *An. albimanus* have two visual organs, a cresent-shaped imaginal eye which lies above and is much larger than the dot-shaped larval eye. In the mutant either the pigmented part of the imaginal eye is reduced in size or only the dot-shaped larval eye is pigmented (Fig. 1H). The *de* homozygotes require 9 days, compared to 5 days for normal larvae, to reach the fourth larval stage, at which time they invariably die. The data shown in Table 3 could not be used for chi-square analysis of the Mendelian inheritance of *de,* because there was no accurate method to use for the calculation of an expected ratio of *de* to wild eye. Since the parents were both heterozygous for In(2) D–318, a certain frequency (related to the amount of crossing-over within the inversion) of de^+ inversion heterozygotes arising from the union of complementary, unbalanced gametes would be expected. Therefore, a dihybrid cross (de^+ *re* / *de* re^+) was set up with red eye and dot eye in repulsion in order to determine the mode of inheritance and to measure the degree of linkage between the two mutants. The mosquitoes which were heterozygous for both recessive genes had a normal eye color. The progeny from this cross were: 418 de^+ re^+; 225 de^+ *re*; 235 *de* re^+ and 0 *de re*. Chi-square analyses for a 3:1 ratio were not significant for red eye (χ^2-0.184; P=0.67) or dot eye (χ^2=1.459; P=0.23). The complete lack of a double recessive class indicated that *de* and *re* are very closely linked.

DISCUSSION

Genetically, *An. albimanus* is one of the better known mosquitoes. Loci for 14 enzyme-genes and 15 morphological mutants, 13 of which are visible in the larvae, have been mapped. With the addition of the six lethals described in this paper, we have now studied a total of 10 visible, recessive lethal traits, all of which cause mortality during the larval and/or pupal stages. Three of these mutants are melanotic, and the remainder involve some structural abnormality, e.g., a malformation of the setae or imaginal eye. Whenever a new lethal mutant is found, we rear the mutant at three temperatures, 24°, 27° and 30°C, to determine whether the expression of the mutant phenotype or the lethal effects are temperature sensitive, in a manner similar to reduced palmate which is fully expressed only at a rearing temperature <27C (Seawright et al. 1979). None of the mutants described in this paper were sensitive to temperature. Although the lethals, curled (Seawright et al. 1982a) and bubble (Seawright et al 1985a), can be used to map other loci on the X chromosome, autosomal lethals are not very useful as genetic markers, because the differential rate of crossing over in males and females precludes an accurate assignment of lethal loci to the genetic map. However, they can be useful for the maintenance of heterozygous inversions in balanced lethal stocks. For example, dot eye has been used for the last year to maintain the In(2) D–318 inversion complex. It is unfortunate that the stocks containing diseased larva, bar eye and hairy were lost, but these mutants were studied prior to the isolation of 3 balancer (complex inversion) chromosome stocks. These or similar mutants are occasionally seen in our laboratory stocks, and probably they could be reisolated.

Other recessive lethals (without a visible phenotype) which cause mortality during the 4th larval and pupal stages are commonly observed during our work on the isolation and characterization of mutants of *An. albimanus.* The physiological basis has not been established for any of these recessive lethals, but some of the genes described in this paper could possibly be of value in studies on the genetics of development. In comparison to higher dipterans, the large number of visible mutants (viable and lethal) expressed during the larval stage in *An. albimanus* is unusual. Undoubtedly, this stems partly from the presence of more morphological features, subject to mutation, in anopheline larvae.

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