# GENETICS OF *GREEN LARVA*, A RECESSIVE MUTANT ON CHROMOSOME 2 IN *ANOPHELES ALBIMANUS* WIEDEMANN[1]

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ABSTRACT. Genetic crosses were made with the mutant marker, *green larva (gl)*, obtained from the APASTEPEQUE strain of *Anopheles albimanus* Wiedemann. *Green larva* is characterized by the presence of green pigmentation in the fat body of late 4th-stage larvae and pupae. It is inherited as an autosomal, recessive gene located on chromosome 2.

As noted by Rabbani et al. (1976b), genetic information on *Anopheles albimanus* Wiedemann has been somewhat limited; however, we recently initiated an investigation into the feasibility of developing genetic-control mechanisms for this important malaria vector. A necessary phase in our effort includes the isolation and characterization of mutants. We have isolated several useful mutants, and in the present report, evidence is given to show that *green larva (gl)* is a recessive trait located on the right arm of chromosome 2. Warren et al. (1975) reported a description of a trait designated *green pupa,* but they did not show inheritance data.

## MATERIALS AND METHODS

The stocks used in crosses were:

(1) APASTEPEQUE—The *green larva* phenotype constitutes 0.5% of this strain from which pure-breeding stocks of *tan-stripe ($gl^+st^+$), tan-nonstripe ($gl^+st$), green-stripe ($gl st^+$),* and *green-nonstripe (gl st)* were selected.

(2) FEST—This propoxur resistant strain was obtained from C. Curtis and G. Davidson, Ross Institute of Hygiene, London. Larvae of this strain were tan.

(3) *T(Y;2R)1*—This Y-autosome translocation was obtained from M. Rabbani and J. B. Kitzmiller, University of Illinois. Larvae of this strain were tan.

(4) *T(Y;2R)6*—This stock originated in our laboratory and is homozygous for *tan (gl+),* and the males are heterozygous for $pr^r$.

Larvae were reared as described by Rabbani et al. (1976a) and Rabbani and Seawright (1976) at a temperature of 28±1°C. The adults were sustained on 10% sugar water and held at 85±5% relative humidity and 25±1°C. Guinea pigs were used to provide a blood meal for females.

Appropriate crosses (Table 1) were done with $gl^+$ and *gl* stocks to determine the mode of inheritance. Assignment of *gl* to a linkage group was accomplished through crosses that used the *T(Y;2R)1* and *T(Y;2R)6* translocations and the dominant markers *stripe ($st^+$)* on chromosome 3 (Rabbani and Seawright 1976) (Table 2) and *propoxur resistance ($pr^r$)* on chromosome 2 (Kaiser et al. 1979) (Table 3). Crossingover occurs in both sexes of *An. albimanus* (Rabbani and Seawright 1976), which necessitated backcrosses with both hybrid males and females for map distance estimates from the known markers. Sex determination is an XY system in this species, with the male being heteromorphic (Keppler et al. 1973; Kaiser et al. 1979). Based on studies with male-linked translocations (Rabbani and Kitzmiller 1972; Kaiser et al. 1979), the X and Y chromosome do not homologize; thus, crossingover is limited to the autosomes in males.

[1] The research reported in this manuscript was conducted in part with contract funds transferred from the Medical Research and Development Command, Office of the Surgeon General, U.S. Army.

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Table 1. Summary of crosses with *green larva (gl)* showing the trait is recessive.

| Crosses | Phenotypes | | | |
|---|---|---|---|---|
| Female X Male | $gl^+$ | *gl* | Total | $x^2$ |
| $F_1(gl^+ \times gl) \times gl$ | 1002 | 960 | 1962 | 0.899 |
| $F_1(gl \times gl^+) \times gl$ | 1137 | 1120 | 2257 | 0.128 |
| $gl \times F_1(gl^+ \times gl)$ | 623 | 572 | 1195 | 2.176 |
| $gl \times F_1(gl \times gl^+)$ | 715 | 746 | 1461 | 0.658 |
| $F_2(gl \times gl^+)$ | 519 | 185 | 704 | 0.614 |
| $F_2(gl^+ \times gl)$ | 561 | 195 | 756 | 0.254 |

## RESULTS AND DISCUSSION

The *g1* variant was observed in the APASTEPEQUE strain, and a *g1* strain was established by inbreeding 50 of these mosquitoes. Close examination with a stereomicroscope revealed green pigmentation in the fat body of late 4th-stage larvae. The green color is visible in younger larvae, but the intensity of the green color is much more pronounced in the older larvae and in pupae. Male larvae are usually a lighter shade of green than are females, as is generally the case for the tan color of wild-type.

When *g1* homozygotes were crossed to wild type ($g1^+$), all of the $F_1$ progeny had a tan body color. The results of the crosses in Table 1 show the monofactoral, recessive nature of *g1*. Samples of 50 of each of the green and tan larvae from each cross were held until adult emergence and scored for sex, and since a 1:1 sex ratio was obtained for both of the green and tan classes, *g1* was assigned to an autosome. In the backcrosses the *g1/g1* type was noted to vary in expression of the green pigmentation, but they were quite easily distinguished from the tan wild-type. To obtain the best expression of green pigmentation in *g1/g1* homozygotes, care should be taken to avoid overcrowded conditions during the larval stage.

The results of the crosses summarized in Table 2 showed no linkage between *gl* and *stripe* ($st^+$), a dominant marker on linkage group 3. Significant chi square values observed in crosses 1 and 3 were caused by a marked deficiency of $st^+$ type, rather than by linkage between *gl* and $st^+$. Since $st^+$ is located at about the middle of

Table 2. Data from crosses with *green larva (gl)* showing independent assortment from $st^+$.

| Crosses | Phenotypes | | | | $x^2$ | | Linkage |
|---|---|---|---|---|---|---|---|
| Female X Male | $gl^+$ $st^+$ | $gl^+$ *st* | *gl* $st^+$ | *gl st* | *gl* | *st* | *gl st* |
| *gl st* X $F_1(gl\ st \times gl^+\ st^+$ | 199 | 248 | 209 | 243 | 0.028 | 7.661[a] | 0.25 |
| *gl st* X $F_1(gl^+\ st^+ \times gl\ st)$ | 149 | 154 | 132 | 130 | 2.975 | 0.016 | 0.08 |
| $F_1(gl\ st \times gl^+\ st^+) \times gl\ st$ | 297 | 396 | 372 | 384 | 3.739 | 8.503[a] | 5.22 |
| $F_1(gl^+\ st^+ \times gl\ st) \times gl\ st$ | 299 | 290 | 280 | 266 | 1.629 | 0.466 | 0.02 |
| $F_2(gl\ st \times gl^+\ st^+)$ | 220 | 89 | 79 | 29 | 0.180 | 2.418 | 0.14 |
| $F_2(gl^+\ st^+ \times gl\ st)$ | 264 | 87 | 88 | 29 | 0.000 | 0.011 | 0.00 |

[a] $p<0.05$; $x^2$ test.

Table 3. Summary of crosses showing linkage between $pr^r$ and *gl*.

| Crosses | Phenotype of progeny | | | | $x^2$ | | Linkage |
|---|---|---|---|---|---|---|---|
| Female X Male | $pr^r$ $gl^+$ | $pr^r$ *gl* | $pr^s$ $gl^+$ | $pr^s$ *gl* | $pr^r$ | *gl* | $pr^r$ *gl* |
| $F_1$♀ ($pr^s$ *gl*♀ X $pr^r$ $gl^+$♂) X $pr^s$ *gl*♂ | 388 | 52 | 40 | 354 | 2.53 | 0.58 | 506.59[a] |
| $pr^s$ *gl*♀ X $F_1$♂($pr^s$ *gl*♀ X $pr^r$ $gl^+$♂) | 383 | 25 | 14 | 366 | 0.99 | 0.04 | 639.73[a] |

[a] $p<.01$.

3R (Rabbani and Seawright 1976), *gl* was tentatively assigned to chromosome 2, but additional crosses were required to define the location of *gl*.

At this time in our study of *gl*, the only marker available on chromosome 2 was the *T(Y;2R)l* translocation. Females homozygous for *gl* were crossed to *gl+(T;2R)l* males, and the resultant $F_1$ males were backcrossed to *gl/gl* females. If *gl* were on chromosome 2, pseudolinkage between *sex* and *gl* was expected, thus causing unequal numbers of males and females in the $gl^+$ and *gl* classes. The results did show pseudolinkage between *gl* and *sex* as follows: 192 *gl* females:41 $gl^+$ females:65 *gl* males:192 $gl^+$ males. Crossing over between *gl* and the *T(Y;2R)l* breakpoint was estimated at 21.6±1.8%. Therefore, we knew that *gl* was on chromosome 2, but additional crosses were required to establish a more precise location. This information was acquired by crosses involving *propoxur resistance* ($pr^r$), a dominant trait located near the free end of the right arm of chromosome 2 (unpublished data). A cross of *gl* $pr^s$ females to *gl+* $pr^rT(Y;2R)6$ males was made, and the $F_1$ males were backcrossed to *gl* $pr^s$ females. Scoring of the backcross progeny yielded: 57 *gl* $pr^s$ females:1 *gl* $pr^r$ female:0 $gl^+$ $pr^s$ females:9 *gl+* $pr^r$ females:13 *gl* $pr^s$ males:2 *gl* $pr^s$ males:0 $gl^+$ $pr^s$ males:66 $gl^+$ $pr^r$ males. While the sample size was inadequate for estimation of the map distance between *gl* and $pr^r$, the results did establish tight linkage between the two loci. Kaiser et al. (1979) used crossover data from six *T(Y;2R)* translocations to map the $pr^r$ locus in the 9A-9B regions of the standard salivary chromosome map published by Keppler et al. (1973).

Interpretation of the crosses summarized in Table 3 lend further evidence to the tight linkage between *gl* and $pr^r$. Genetic recombination between $pr^r$ and *gl* differed in males (4.9%) and females (11.0%). From past studies of crossing over in other Diptera, an effect of sex on recombination was anticipated, but further evaluations with other linked loci are needed to assess the effect of sex, and to determine whether the situation with *gl* and $pr^r$ is unique or is typical for *An. albimanus*.

As mentioned earlier, our research effort with *An. albimanus* is directed toward the development of chromosome aberrations, e.g., reciprocal translocations, for use in genetic control schemes. Color mutants that are expressed during the larval stage are very useful in crosses for identifying aberrations, because cytological preparation of salivary gland chromosomes can be done immediately. The use of adult markers requires a 1-generation delay before cytological examination of males. Several color mutants of mosquito larvae have been described in other species of mosquitoes (Wright and Pal 1967, Kitzmiller 1976). *Green larvae* were reported in *An. pharoensis* (Mason and Davidson 1966), *Culex pipiens* L. (Laven 1957) and *Culex fatigans* Wiedemann (Consoli 1972).

There are now 7 known mutants in *An. albimanus*, of which 4 are particularly useful as genetic markers. In addition to *gl*, $pr^r$ is on 2R, and *ebony (eb)* (Benedict et al. 1979) is located close to the free end of 2L. Markers on chromosome 3 include *stripst* ($se^+$) on 3R (Rabbani and Seawright 1976), *black larva (bl)* (Rabbani et al. 1976b), *dieldrin resistance (Dl)* (Georghiou et al. 1967), and *red stripe (Rd)* (Nakashima et al. 1975). *Black larva* is a recessive lethal, and *red stripe* is not fully penetrant. As noted by Davidson (1963) the utility of *Dl* is limited because a dose that kills the homozygous-susceptible type also kills part of the heterozygotes.

The existence of 4 excellent mutants is encouraging. After the addition of a few more markers and concurrent cytological studies, enough basic information will be available to manipulate chromosomes for production of useful aberrations.

ACKNOWLEDGMENT. Excellent technical assistance was rendered by G. E. Jordan.

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# INSECTICIDES FOR THE CONTROL OF MOSQUITOES AND OTHER DIPTERA

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ABSTRACT. A compilation of insecticides and formulations labeled in the U. S. A. for use for control of mosquitoes and some other Diptera is provided.

The following tables contain a compilation of insecticides and formulations currently labeled for use for the control of mosquitoes and some other Diptera of public health importance and are presented as an aid to persons presently engaged in their control.

Some insecticides and formulations given in the following tables are labeled for use only in certain states. Some states may have 24C (special local need) or state labels for the use of additional insecticides or formulations which are not given here. It should also be pointed out that not all labeled insecticides or formulations are recommended for use by all state agencies. Therefore, appropriate state agencies or manufacturer's representatives should be contacted by potential users for further recommendations.