

## The Inheritance of Yellow-Larva and Ruby-Eye in *Culex pipiens*\*

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*It has been suggested that mosquito vectors of filariasis and other diseases might be controlled by genetic methods. This is not yet possible because of the lack of genetic information concerning the vector species. The present study describes the development of a marker strain which is being used to study genetic control.*

*Two spontaneous mutants of Culex pipiens are described, one for the first time. Ruby-eye (ru) is an autosomal recessive in linkage group 2. It is completely penetrant and completely expressed. It is evident in later instar larvae, pupae, and adults. Yellow-larva (y) is an autosomal recessive, sometimes behaving as a partial dominant, and is also in linkage group 2. It is evident in late fourth-instar larvae and pupae and is generally associated with lengthened larval development. Ruby-eye and yellow-larva are occasionally seen in collections from the field. The frequency of crossing over between ru and y differs significantly among progenies and also between the sexes, the median values being 17% in males and 24% in females.*

Insecticidal control of mosquitos is currently experiencing great difficulties owing to resistance, contamination problems, and the effect of insecticides on non-target organisms. This has stimulated the search for alternative control methods. One of these, genetic control, is still in a rudimentary state because of the paucity of genetic information on mosquitos. This study describes the development of a strain bearing two markers for chromosome 2 which should be useful for genetic analyses.

Most mutations described in *Culex pipiens* (*sensu lato*) have been isolated after X-irradiation (Laven, 1955, 1956, 1957, 1958; Kitzmiller, 1958). Among the few spontaneous mutants isolated in this species, the sex-linked white eye described by Gilchrist & Haldane (1947) is classic. The sex-linked, red-eyed mutant discovered after irradiation (Wild, 1963) may actually have been a spontaneous mutation

(Spinner, 1964). There have been several studies of larval colour mutants (Huff, 1929; Ghelelovitch, 1950; Spielman, 1957; Laven, 1957), and Laven has isolated at least two other spontaneous mutants. Kitzmiller (1958) suggested that mutations might occur less frequently in mosquitos than in many other groups, such as *Drosophila*. This point was disputed by Craig, VandeHey & Hickey (1961), who found a spontaneous mutation rate of 13.5% for structural mutations alone in *Aedes aegypti*, as compared with Kitzmiller's X-ray-induced rate of 9.2% in *C. pipiens*. A series of spontaneous scale colour mutations was later also described in *A. aegypti* (McClelland, 1960; Craig & VandeHey, 1962). VandeHey's recent description<sup>5</sup> of four spontaneous mutations in *C. pipiens* suggests that previous estimates of the frequency of mutation in this species may be too low. The present paper presents linkage and recombination data for two mutants, each of which occurred spontaneously in more than one Californian population of *C. pipiens*.

### MATERIAL

Larvae and pupae with ruby-eye (*ru*) have bright brick-red eyes. They are conspicuously different

\* This investigation was supported in part by US Public Health Research Fellowship 4-F1-GM-19, 526-03.

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<sup>5</sup> Unpublished working document WHO/Vector Control/73 (1964).

from wild-type larvae, which have black eyes. In adults the deep-red eyes darken shortly after emergence but they always lack the greenish lustre typical of the wild type and retain a rather glassy appearance. Ruby-eye was selected from an auto-genous population of *C. pipiens* collected near Dixon, Calif., which was referable to subspecies *pipiens*. Backcross replicates were all derived from auto-genous rafts.

The mutant yellow-larva (*y*) can be distinguished in late fourth-instar larvae and pupae. The mutant colour typically is orange, but it varies from pale yellow to deep orange. Wild-type late fourth-instar larvae are typically violet or green; typical pupae are blue or green with the anterior part of the abdomen violet. Wild-type pupae, however, may vary from light grey to deep green or blue. Occasional *y* mutants are of a salmon colour which may be difficult to distinguish from wild-type pupae; when this happened the results were discarded. The *y* mutant was isolated from a strain of *C. pipiens* which has been in culture since its colonization in Orange County, Calif., in 1959, and is referable to subspecies *quinquefasciatus*. Previous reports of yellow larval mutants all concerned subspecies *pipiens*. The two subspecies are completely inter-fertile.

#### METHODS

Some of the work was done in Davis and some in Fresno, Calif., Mosquitos in both Davis and Fresno were reared by customary techniques,

modified in minor ways. Larvae were reared in water which fluctuated in temperature from 20°C to 25°C. Adults were kept at a temperature of 22°C to 27°C. Pupae were sexed by the terminalia as described by Barr (1954).

#### RESULTS

Repeated replicate crosses indicate that ruby-eye is fully penetrant and recessive. Since sex seems to be determined by a single allele in *Culex* (Gilchrist & Haldane, 1947), male being dominant, sex-linkage should be apparent in the offspring of the two kinds of heterozygous males backcrossed to homozygously recessive females. The results of such crosses are shown in Table 1. No association between sex and ruby-eye was found.

Similar results are shown in Table 2 except that the crosses involve subspecies hybrids and the comparison is between heterozygous males and females rather than between the two types of heterozygous males. These results, too, indicate no association between ruby-eye and sex. The pooled results of the backcrosses of heterozygous females (in Table 2) suggest an association between sex and eye-colour, but the individual progenies do not. The pooled results for heterozygous females also do not differ significantly from those for males.

Repeated replicate crosses indicate that yellow-larva is fully penetrant and recessive, with variable expressivity. Heterozygotes were therefore backcrossed to yellow homozygotes to test for sex-linkage (Table 3). The results indicate that the

TABLE 1  
BACKCROSSES OF RUBY-EYE IN *C. P. PIPPIENS*

Parents	Number of offspring				Total
	Ruby-eye		Black-eye		
	♂	♀	♂	♀	
$\frac{ru\ M}{+ m} \times \frac{ru\ m}{ru\ m}$ (10 replicates)	187	164	203	191	745
$\frac{+ M}{ru\ m} \times \frac{ru\ m}{ru\ m}$ (11 replicates)	139	124	150	121	534
$\frac{ru\ m}{+ m} \times \frac{ru\ M}{ru\ m}$ (3 replicates)	27	23	29	28	107
Total . . . . .	353	311	382	340	1386

TABLE 2  
BACKCROSSES OF RUBY-EYE IN HYBRIDS OF *C. P. PIPPIENS*  
AND *C. P. QUINQUEFASCIATUS*

Parents	Number of offspring				Total
	Ruby-eye		Black-eye		
	♂	♀	♂	♀	
$\frac{ru\ M}{+ m} \times \frac{ru\ m}{ru\ m}$ (15 progenies)	591	592	579	601	2 363
$\frac{ru\ m}{+ m} \times \frac{ru\ M}{ru\ m}$ (4 progenies)	194	170	157	189	710 <sup>a</sup>
Total . . . . .	785	762	736	790	3 073

<sup>a</sup>  $\chi^2 = 4.1; 0.05 > P > 0.02.$

progeny are similar irrespective of which parent in the backcross is heterozygous.

*Linkage of ruby-eye and yellow-larva*

Crosses were made between *ru* and *y* to produce heterozygotes in the repulsion phase. The proportion of individuals expressing both ruby-eye and yellow-larva in the F<sub>2</sub> was very much less than the 6% expected on the assumption of independent assortment, which indicated linkage. These double homozygotes were crossed with the wild type to give heterozygotes in coupling phase. Males and females of both phases of heterozygotes were backcrossed to double homozygotes. The results of these crosses are summarized in Table 4.

Laven (personal communication) has suggested that in mosquitos crossover rates may differ from

one individual to another. There was significant heterogeneity among the progenies in these crosses. A rank sum test (Dixon & Massey, 1957) showed no significant difference in the progeny crossover ratios between the repulsion and coupling phases for males. For females there were too few progenies in the repulsion phase to provide a meaningful test, but the four ratios were well within the range of those of the coupling phase. The combined progenies of the two phases yielded a rank difference between the sexes which was significant at the 0.026 level. The non-parametric rank test, which requires no assumptions about the distribution of crossover rates among the individuals of a sex, does not establish the magnitude of this difference. However, the median crossover ratios given in Table 4 provide estimates for each sex.

TABLE 3  
BACKCROSSES OF YELLOW-LARVA IN HYBRIDS OF *C. P. PIPPIENS*  
AND *C. P. QUINQUEFASCIATUS*

Parents	Number of offspring				Total
	Yellow-larva		Violet-larva		
	♂	♀	♂	♀	
$\frac{+ M}{y\ m} \times \frac{y\ m}{y\ m}$ (26 progenies)	1 002	1 046	1 070	1 012	4 130
$\frac{+ m}{y\ m} \times \frac{y\ M}{y\ m}$ (6 progenies)	390	379	398	372	1 539
Total . . . . .	1 392	1 425	1 468	1 384	5 669

TABLE 4  
CROSSING-OVER BETWEEN *RU* AND *Y* IN BACKCROSSES: COMPARISON  
OF PROGENIES OF HETEROZYGOUS MALES WITH THOSE OF HETEROZYGOUS  
FEMALES

Class of offspring	Numbers of offspring			
	Fathers heterozygous		Mothers heterozygous	
	Repulsion	Coupling	Repulsion	Coupling
$\frac{y}{y} \frac{ru}{ru}$	233	1 355	76	1 231
$\frac{y}{y} \frac{ru}{+}$	1 058	299	267	381
$\frac{y}{+} \frac{ru}{ru}$	1 060	334	288	370
$\frac{y}{+} \frac{ru}{+}$	230	1 344	79	1 325
Total . . . . .	2 581	3 332	710	3 307
Number of progenies . . . . .	15	20	4	20
Median crossover ratio . . . . .	0.168	0.190	0.225	0.241
Median crossover ratio of combined phases . . . . .	0.169		0.241	

## DISCUSSION

A mutation to red-eye (*r*) in autogenous *C. p. pipiens* has been described by Wild (1963). This mutation was closely linked with sex; Laven (unpublished data<sup>1</sup>) found it to be about 1.2 crossover units from the sex factor. The mutation described in this paper—ruby-eye (*ru*)—was found in the same form but is not sex-linked. Ruby-eye differs from red-eye not only in its linkage group but also in its phenotype, this being much more uniform and darker. Crosses of ruby-eye and red-eye produce heterozygotes with wild-type eye colour.

Ruby-eye phenotypes were found in two widely separated natural populations. One female was taken near Dixon, Calif., in May 1962, but no offspring were obtained. In March 1963 five wild-type females were taken in this area. Their progeny were pooled to start a colony. In the second generation there were 47 autogenous rafts which gave rise to 2952 larvae, of which 34 were ruby-eyed. Red-eyed mutants, presumably identical, were also reared from larvae collected near Gridley, Calif.

The yellow mutant studied here has been observed in laboratory colonies of *C. p. quinquefasciatus* from Bakersfield and from Orange County, Calif. It has also been observed in collections from Fresno and Hanford. Crosses with the yellow-larva strain of Laven (1957) show that it is identical, although his mutant derives from a strain of autogenous *C. p. pipiens*. It is likely that this mutant is also identical with those studied by Ghelelovitch (1950) and Spielman (1957). Kitzmiller<sup>2</sup> has designated the linkage group which includes this factor as 2; by definition, chromosome 1 is the sex chromosome.

Yellow-larva is an excellent marker in that it is fully penetrant and subject to relatively little variability of expression. Laven (1957) has noted that in most cases it is possible to recognize heterozygotes which have factors for both yellow and green. In the present study heterozygotes for yellow and violet were recognizable in many, but not in all, cases. Such individuals were initially classified as "rose-violet" and all which were bred proved to be

<sup>1</sup> Summarized in WHO Information Circular on Insecticide Resistance, No. 43, January 1964.

<sup>2</sup> Unpublished working document WHO/VC/Sem./WP/23.64.

heterozygotes. The colour of larvae is due to pigments not in the integument but in underlying tissues, probably those of the fat body (Huff, 1929). Heterozygotes seem to have not only the dark pigment due to the dominant allele but also the lighter, yellowish pigment produced by the mutant allele. This makes it possible to recognize heterozygotes provided that the lighter pigment is not obscured by the darker, as seems frequently to be the case. It appears, therefore, that the mutant is actually not recessive but could always be distinguished if pigment composition were analysed.

In most progenies which included both yellow and wild-type larvae, the latter tended to pupate a day or so earlier than the former. This slower development of yellow mutants could result in

distortion of ratios of wild-type to yellow pupae in cultures with excessive mortality.

Variation in the frequency of crossing-over of *y* and *ru* from one progeny to another was found in all test crosses in which sufficient numbers of progenies were studied. The source of this variation was not apparent although it could possibly have been caused by variations in temperature or age.

There is little information on the effect of sex on crossing-over in mosquitos. Klassen & Brown (1964), studying *A. aegypti*, found less crossing-over in males than in females among a group of alleles on chromosome 2; the difference was small but significant. The presently reported sex difference in the frequency of crossing-over is in the same direction, higher in females, and of the same order of magnitude.

#### ACKNOWLEDGEMENTS

The writers wish to thank Mr Melvin M. Boreham and Miss Carolyn S. Shewcraft for technical assistance in this study. Statistical assistance was supplied by Dr Kathleen E. White. Mr Embree G. Mezger and Mr Richard

F. Frolli of the Solano and Kings County Mosquito Abatement Districts, respectively, assisted in collections. Various members of the Bureau of Vector Control, too numerous to name, also contributed in diverse ways.

#### RÉSUMÉ

On attache actuellement une grande importance aux études effectuées dans le cadre d'une lutte génétique éventuelle contre les moustiques vecteurs de maladies, et spécialement les vecteurs de la filariose. Ces recherches cependant ne sont encore qu'ébauchées, en raison de l'insuffisance des données sur la génétique de ces espèces.

Les auteurs décrivent un mutant de *Culex pipiens*, d'apparition spontanée, caractérisé par des yeux de couleur rubis (*ruby-eye* = *ru*). Ils montrent que le caractère *ru* a le même support chromosomal que le caractère *yellow-larva* (*y*), déjà connu, donnant aux larves d'un autre mutant une teinte jaunâtre. *ru* est autosomique, récessif, complètement pénétrant et d'expression uni-

forme. Il permet la distinction entre le mutant et le type sauvage de la larve aux derniers stades, de la pupa et chez l'adulte. *y* est autosomique, récessif, ou semi-dominant, et complètement pénétrant. La fréquence des passages de *ru* à *y* est d'un ordre de grandeur significativement différent suivant les générations et également suivant le sexe, les valeurs moyennes étant de 17% chez les mâles et de 24% chez les femelles.

Ces deux mutants ont été observés dans des populations de moustiques à l'état naturel. Les auteurs pensent que, au sein de l'espèce étudiée, comme d'ailleurs au sein d'autres espèces de moustiques, la fréquence des mutations spontanées a généralement été sous-estimée.

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