

References Cited

- Legner, E. F. 1977. Response of *Culex* spp. larvae and their natural predators to two inoculation rates with *Dugesia dorotocephala* (Woodworth) in shallow ponds. Mosquito News 37:435-40.
- Lenhoff, H. M. and R. D. Brown. 1970. Mass culture of hydra: an improved method and its application to other aquatic invertebrates. Lab. Anim. 4:139-154.
- Lenhoff, H. M. and W. F. Loomis. 1957. Environmental factors controlling respiration in hydra. J. Exper. Zool. 134:171-181.
- Loomis, W. F. and H. M. Lenhoff. 1956. Growth and sexual differentiation of hydra in mass culture. J. Exper. Zool. 132: 554-574.
- Qureshi, A. H. and E. C. Bay. 1969. Some observations on *Hydra americana* Hyman as a predator of *Culex peus* Speiser mosquito larvae. Mosquito News 29:465-471.
- Tsai, S. C. and E. F. Legner. 1977. Exponential growth in culture of the planarian mosquito predator *Dugesia dorotocephala* (Woodworth). Mosquito News 34: 474-478.
- Yu, H. S. and E. F. Legner, 1976. Regulation of aquatic Diptera by planaria. Entomophaga 21: 3-12.
- Yu, H. S., E. F. Legner, and R. D. Sjogren. 1974. Mass release effects of *Chlorohydra viridissima* [Coelenterata] on field populations of *Aedes nigromaculis* and *Culex tarsalis* in Kern County, California. Entomophaga 19:409-420.

GENETIC MAPPING OF A LARVAL COLOR MUTANT, GREENISH LARVA, WITH THE HELP OF MALE-LINKED TRANSLOCATIONS AND RUBY-EYE MARKER IN *CULEX QUINQUEFASCIATUS*

SARALA K. SUBBARAO AND T. ADAK

Malaria Research Centre, 22, Sham Nath Marg, Delhi-110054, India

ABSTRACT. A larval color mutant, *greenish larva*, was isolated from *Culex quinquefasciatus* Say (*fatigans* auct.) from a laboratory colony. Linkage studies with the help of male-linked

translocations and *ruby-eye* marker have shown that it is an autosomal recessive mutant on chromosome 3.

INTRODUCTION

In *Culex quinquefasciatus* Say (*fatigans* auct.) there is a striking lack of phenotypic markers which are essential in many genetic studies. A detailed description of most of the available phenotypic markers is given by Laven (1967). Larval color mutants viz., green (*g*)-dominant (Ghelelovitch 1950), melonotic (*mel*)-autosomal recessive and lethal (Laven and Chen 1956), green (*g*) and yellow (*y*)-autosomal recessives on chromosome 2 (Laven 1957), black (*B1*)-dominant and sex-linked (Vande Hey 1964, WHO, Unpublished) and green (*g*), brown (*br*), golden yellow (*go*) and greenish brown (*bg*)-

autosomal recessives (Shetty and Chowdaiah 1976) have so far been reported in the *Culex pipiens* complex. Recently green larvae were observed in one of our laboratory colonies, and linkage studies with the help of male-linked translocations and *ruby-eye* (*ru*) marker (Iltis et al. 1965) have shown that this mutant is located on chromosome 3. Since there is a green color larval mutant *green larva* on chromosome 2 described by Laven (1957) this mutant will be referred to as *greenish larva* (*grs*).

MATERIALS AND METHODS

Greenish larva was isolated from a laboratory strain (Pa) De/De (Paris cytoplasm

and Delhi genome). Green color expresses itself in the 3rd instar but is more prominent in the late 4th instar. It shows complete penetrance but has variable expression, and when cultured at lower temperatures, i.e., 16–20°C, color expression is better:

Stocks. The following stocks were used in test crosses:

(Pa)De/De: Paris cytoplasm with Delhi genome (Krishnamurthy and Laven 1976).

(De 19) Tr 31B *ru/ru*: A male-linked translocation with minority type Delhi cytoplasm (De 19). The male-linked translocation involves chromosomes 1 and 3 (Krishnamurthy et al. 1977, unpublished) and chromosome 2 is marked with a recessive mutant *ruby-eye* (*ru*) (Iltis et al. 1965). Synthesis of this stock is given in Figure 1.

(Ba) Tr 328: A male-linked translocation involving chromosomes 1 and 2 with Bangkok cytoplasm. (Krishnamurthy et al. 1977, unpublished.)

EXPERIMENTAL PROCEDURE. As mentioned earlier, this mutant was observed in (Pa)De/De colony. Paris cytoplasm is bidirectionally incompatible with the majority of the Delhi population while it is unidirectionally compatible with minority of the Delhi population. These 2 types of populations were isolated as single raft isolates from the Delhi population: the majority type-De 2 and the minority type-De 19 (Subbarao et al. 1974, and 1977, unpublished). Since *ruby-eye* marker was available in De 2 cytoplasm, a cytoplasm which is compatible with both De 2 and Paris cytoplasm was needed so that *ruby-eye* marker could be transferred into that cytoplasm, to carry out linkage studies with *greenish larva*. As De 19 cytoplasm fulfills the above requirements, a new stock was synthesized which has De 19 cytoplasm, and *ruby-eye*. In addition to this another marker, a male-linked translocation was added by crossing males of a variant, ISB49, from Tr31B translocation colony (Subbarao et al. 1977, unpublished) with De 19 cytoplasmic type females carrying *ruby-eye*. Synthesis of this stock is given in Fig. 1, and it has been

designated as (De 19) Tr 31B *ru/ru*. Males of this stock are compatible with Paris type females and this stock was utilized in cross No.3.

It may be stated that Bangkok cytoplasmic type males are compatible with Paris type females, and this property was utilized in cross No.2.

In cross No.1 in Table 1 F₁ heterozygotes were back crossed while in crosses 2 and 3 they were inbred.

RESULTS AND DISCUSSION

Table 1 gives the data obtained from the crosses between *greenish larva* and marker stocks. Absence of a mutant phenotype was observed in F₁ progeny of all crosses which indicates that it is a recessive mutant. This was further supported by the 1:1 ratio of wild type to mutant type observed in cross No.1. In crosses No.2 and No.3 male-linked translocations involving chromosomes 1 and 2, and 1 and 3 respectively were used. These translocations were analyzed both genetically (Krishnamurthy et al. 1977, unpublished) and cytologically (Subbarao and Adak, unpublished). In cross No.2 *greenish larva* showed free segregation with sex. Since in this cross, a translocation involving chromosomes 1 and 2 was used, free segregation of *greenish larva* with sex suggests that it is located on chromosome 3. This was further supported by the pseudo-linkage of *greenish larva* with sex in cross No.3, where chromosome 1 and 3 were involved in the translocation. In addition to this, *greenish larva* showed independent assortment with *ruby-eye*. Percent recombination was 3.8 i.e., *greenish larva* can approximately be placed 3.8 map units from the translocation breakpoint of Tr 31B translocation. Tr 31B translocation breakpoint was mapped in the left arm i.e., the longer arm of chromosome 3 (Subbarao and Adak, unpublished), and thus it can be said that *greenish larva* is located on the left arm of chromosome 3. Ruby-eye and green category obtained from F₂ progeny of cross No.3 has been established as a stock and designated as *grs/grs; ru/ru* Tr 31B (1;3).

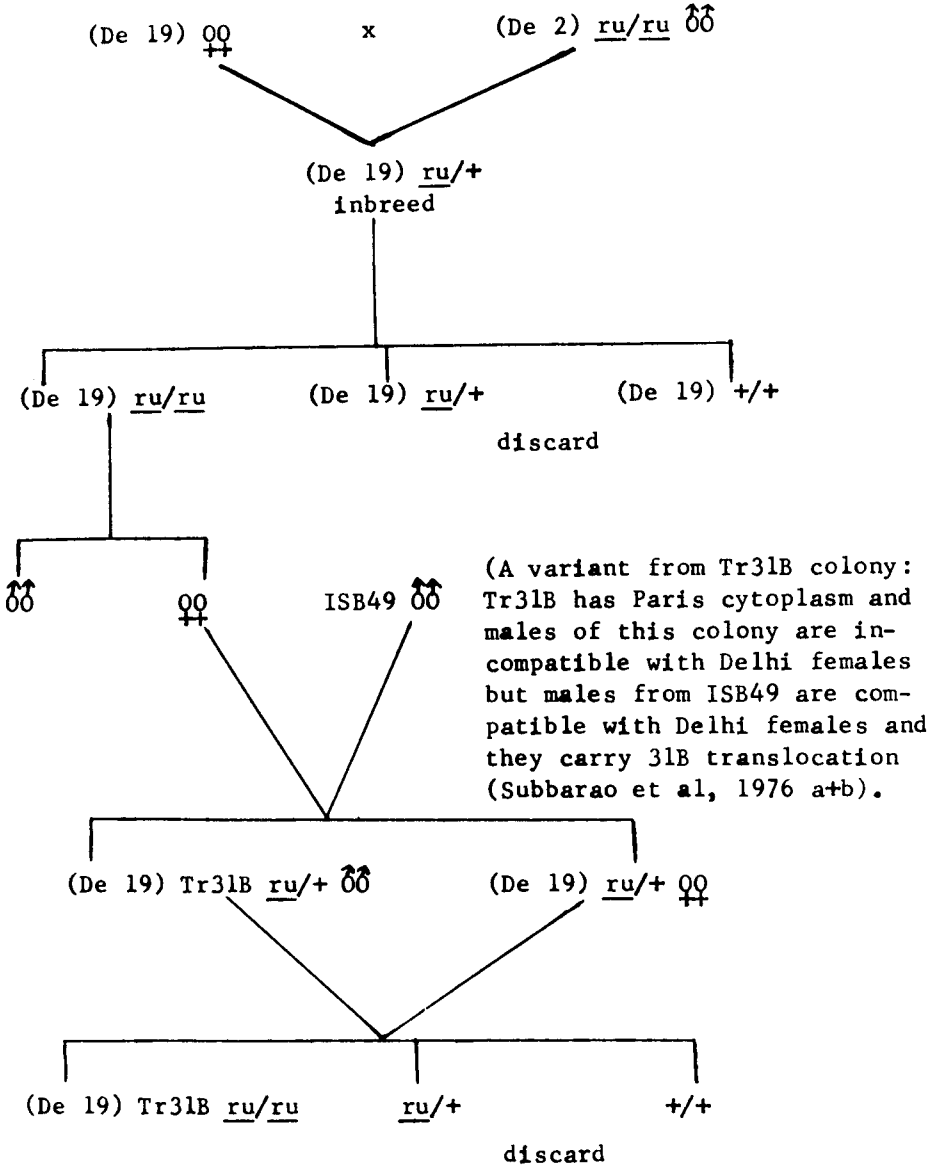


Fig. 1. Synthesis of (De 19) Tr 31B *ru/ru* stock.

Table 1. Data showing the mode of inheritance of *greenish larva* and its linkage relationship with *ruby-eye* and *sex*.

Cross No.	Parental genotypes	Phenotypes of the Progeny										χ^2 Value P=0.05	
		Wild type		Ruby eye				Green		Ruby & green			Total
		♀	♂	♀	♂	♀	♂	♀	♂	♀	♂		
1.	<i>grs/+</i> x <i>grs/grs</i>	592 (613.5)						685 (613.5)				1227	*1.5 n.s.
2.	<i>grs/+</i> x <i>grs/+Tr 328 (1;2)</i>	150 (150)	160 (150)			56 (50)	34 (50)					400	**6.51 n.s.
3.	<i>grs/+ +/ru x grs/+ +/ru Tr 31B (1;3)</i>												
	a)	86 (260.3)	419 (260.3)			176 (86.8)	13 (86.8)					694	**368.03 h.s.
	b)	385 (387)		120 (129)		129 (129)				60 (43)		694	***7.36 n.s.

* Recessive.

** Green is segregating with sex.

*** Green and ruby are segregating.

() Expected number.

n.s. Not significant.

h.s. Highly significant.

This study demonstrates that male-linked translocations are good genetic tools in mapping mutants in the absence of phenotypic markers.

ACKNOWLEDGMENTS. We thank Mr. R. C. Juyal for isolating the mutant and to Mr. K. B. Masiwal, Mr. Pritam Singh, Mr. Y. P. Chawla and Mr. A. R. Kotnala for their technical assistance.

References Cited

- Ghelelovitch, S. 1950. Etude genetique de deus caracteres de pigmentation chez *Culex autogenicus* Roubaud. Bull. Biol. 84. 217-224.
- Iltis, W. G., A. R. Barr, G. A. H. McClelland and C. M. Meyers. 1965. The inheritance of yellow larva and ruby-eye in *Culex pipiens*. Bull. Wld. Hlth. Org. 33: 123-128.
- Krishnamurthy, B. S. and H. Laven. 1976. Development of cytoplasmically incompatible and integrated (translocated incompatible) strains of *Culex pipiens fatigans* for use in genetic control. J. Genet., 62:117-129.
- Laven, H. 1957. Vererbung durch Kerngene un das Problem der ausserkaryotischen Vererbung bei *Culex pipiens*, I. Kernvererbung, Z. Vererbungal. 88, 443-477.
- Laven, H. 1967. Formal genetics of *Culex pipiens* In: *Genetics of insect vectors of disease* (J. W. Wright and R. Pal eds.), P. 17-65. Amsterdam, London, New York.
- Laven, H. and P. S. Chen. 1956. Genetische und papierchromatographische Untersuchungen an einer letalen Mutante Von *Culex pipiens*, Z. Naturforsch 11b:273-276.
- Shetty, N. J. and B. N. Chowdaiah. 1976. Tests for allelism among certain larval color mutants of *Culex quinquefasciatus*. Mosquito News 36(4): 477-482.
- Subbarao, Sarala K., C. F. Curtis, K. R. P. Singh and B. S. Krishnamurthy. 1974. Variation in cytoplasmic crossing type in population of *Culex pipiens fatigans* Wied. from the Delhi area. J. Com. Dis. 6(2): 80-82.