GENETIC ANALYSIS OF A LARVAL COLOR MUTANT, YELLOW LARVA, IN ANOPHELES SUNDAICUS

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ABSTRACT. One larval body color mutant, yellow larva (yl), was isolated from a newly established cyclic colony of Anopheles sundaicus. The inheritance pattern revealed that yellow larva was an autosomal recessive mutant.

Anopheles sundaicus (Rodenwaldt) was a primary vector of malaria throughout its range in coastal areas of Andhra Pradesh, Orissa, Tamil Nadu, West Bengal, and the Andaman and Nicobar group of islands (Christophers 1912, Iyengar 1931, Senior White 1947). At present this species is restricted to the Andaman and Nicobar group of islands, where it plays a major role in malaria transmission (Malaria Research Centre 1989). Although *An. sundaicus* has not been incriminated since 1927 (Covell 1927), it is believed that *An. sundaicus* is the only vector responsible for malaria transmission on these islands, as evidenced by epidemiological findings (Kalra 1980).

Until now colonization of *An. sundaicus* has not been attempted and, therefore, our knowledge of its biology and genetics has remained rudimentary. We have now successfully colonized *An. sundaicus*, and in this process we isolated a mutant, *yellow larva* (*yl*). This paper reports the mode of inheritance of this new larval mutant.

Anopheles sundaicus breeds mainly in creeks and other brackish water bodies. On Car Nicobar Island, breeding of this species has also been noticed in freshwater ponds, pools, wells, and puddles.

Nearly 500 An. sundaicus gravid females were collected with the help of a mouth aspirator and torch light between 0500 and 0800 h from keori (Pandanus larum) bush, one of the daytime outdoor resting sites of this species. Live mosquitoes were transported to the Malaria Research Centre laboratory located at Car Nicobar Island (Malacca, 6-10°N, 92–94°E, in the Bay of Bengal). Mosquitoes were held in a $60 \times 60 \times 60$ -cm cloth cage and kept in the insectary maintained at $28 \pm 2^{\circ}C$ and 70-80% RH. Eggs were collected in plastic bowls containing water and lined with filter paper. Larvae were reared in enamel trays and fed on a mixture of dog biscuit and yeast powder in a ratio of 3:2. After emergence adults were offered 1% glucosesoaked cotton pads and fresh water-soaked raisins. On the 5th day after emergence females were bloodfed on a pigeon. In the larval rearing trays of the wild colony 9 yellow larva mutants were observed. These were isolated and a pure homozygous colony of the mutant was established from 6 adult females and 3 adult males emerged from these 9 larvae. The mutant strain, yellow larva, and the wild type were inbred separately for 3 continuous generations before they were accepted as pure homozygous strains. The yellow larva mutant expresses its phenotype from the early 2nd instar through the pupal stage, but the adults are not distinguishable from the wild type. Series of genetic crosses were set up between yellow larva and the wild type. Individual gravid females from different crosses were held in small plastic ice cream cups (100-ml capacity) containing little water and lined with filter paper for single female oviposition. After oviposition each batch of eggs from one female was reared as a single isofemale progeny. Because mutant phenotypes were easily distinguished at an early instar, larvae were separated and the phenotype was recorded at the 2nd larval stage. This procedure provided accurate numbers in each category and helped in avoiding errors due to occasional mortality in the late instars.

Yellow larva and the wild type were crossed reciprocally and the results of crosses showing the mode of inheritance of yellow larva and its linkage relationship with sex are given in Table 1. Absence of the mutant phenotype in F_1 progeny of both the reciprocal crosses (crosses 1 and 2) indicated that yellow larva is a recessive mutant. This was further supported by a 3:1 ratio of wild type to yellow larva in F_2 progeny, when F_1 progeny of cross 1 were inbred (cross 3). When F₁ males and females obtained from cross 1 were backcrossed to mutant females and males, respectively (crosses 4 and 5), wild and mutant phenotypes were obtained in a ratio of 1:1. Absence of mutant phenotypes in F_1 males of cross 2 indicated that yellow larva is not a sex-linked mutant, assuming that the sex determination mechanism in An. sundaicus is XX and XY type as in Anopheles stephensi Liston (Aslamkhan 1973) and Anopheles culicifacies Giles (Sakai et al. 1977), where the females are homogametic (X/X) and the males are heterogametic (X/Y). This was further supported by free segregation of yellow larva with sex and nonsignificant chi-square values of 0.12, 0.58, and 1.78 observed in F₂ progeny of crosses 3, 4, and 5, respectively. The yellow larva is a recessive, autosomal mutant that showed complete penetrance and constant ex-

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Table 1.	Crosses elucidating t	he mode of inheritance	of yellow	larva in Anopheles sundaicus.

Cross				Fami-	Progeny phenotype						
	Par	Parental genotype			Wild type		Yellow type			-	
	Ŷ	×	ð	_ lies _ tested	Ŷ	ð	Total	Ŷ	δ	Total	$-\chi^2$ (df 1)
1	$\frac{+yl}{+}$	×	$\frac{yl}{yl}$	13	457	434	891	_			_
2	$\frac{yl}{yl}$	×	$\frac{+yl}{+yl}$	17	502	491	993			_	_
3	$\frac{+yl}{yl}$	×	$\frac{+yl}{yl}$	13	355	341	696	108	118	226	0.12 (ns)
4	$\frac{yl}{yl}$	×	$\frac{+yl}{yl}$	8	188	201	389	176	192	368	0.58 (ns)
5	$\frac{+yl}{yl}$	×	$\frac{yl}{yl}$	15	296	275	571	322	295	617	1.78 (ns)

ns = Not significant.

pressivity. This is the first report of any mutant in *An. sundaicus*.

The mutant yellow larva is reported in Culex quinquefasciatus Say (Shetty and Chowdaiah 1976) as autosomal recessive and in Culex pipiens Linnaeus (Iltis et al. 1965) as partially dominant. Similar larval mutants yellow larva are also reported in Aedes togoi Theobold (Tadano 1977), Aedes mascarensis MacGregor (Hartberg and Craig 1974), Aedes aegypti Linnaeus (Craig and Gilham 1959), and Aedes albopictus Skuse (Bat-Miriam and Craig 1966).

The X-linked mutant golden body has been reported in An. culicifacies (Sakai et al. 1981). Another autosomal mutant, yellow, reported in An. culicifacies was recessive to the wild type but codominant with black allele at a single locus (Dubash et al. 1982). In An. stephensi, golden yellow larva has been reported as an autosomal and recessive marker (Adak et al. 1990).

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REFERENCES CITED

- Adak, T., S. K. Subbarao and V. P. Sharma. 1990. Genetics of golden yellow larva in Anopheles stephensi. J. Am. Mosq. Control Assoc. 6:672–676.
- Aslamkhan, M. 1973. Sex chromosome and sex determination in the malaria mosquito, *Anopheles stephensi*. Pak. J. Zool. 5:127–130.
- Bat-Miriam, M. and G. B. Craig, Jr. 1966. Mutants in Aedes albopictus (Diptera: Culicidae). Mosq. News 26: 13-22.
- Christophers, S. R. 1912. Malaria in Andamans. Sci. Mem. Med. Sanit. Dep. India 56:48.

Covell, G. 1927. Report of an enquiry into malaria con-

ditions in the Andaman. Government Press, Delhi, India.

- Craig, G. B., Jr. and N. W. Gilham. 1959. The inheritance of larval pigmentation in *Aedes aegypti*. J. Hered. 50: 115–123.
- Dubash, C. J., R. H. Sakai and R. H. Baker. 1981. The genetics of *yellow* and *green* in *Anopheles culicifacies*. J. Hered. 73:340-344.
- Hartberg, W. K. and G. B. Craig, Jr. 1974. Three new mutants in Aedes mascarensis: currant-eye, small-antenna and yellow. J. Med. Entomol. 11:447-454.
- Iltis, W. G., A. R. Barr, G. A. H. McClelland and C. M. Myers. 1965. The inheritance of *yellow larva* and *ruby-eye* in *Cx. pipiens*. Bull. W.H.O. 33:123–128.
- Iyenger, M. O. T. 1931. The distribution of An. ludlowi in Bengal and its importance in malaria epidemiology. Indian J. Med. Res. 19:499-524.
- Kalra, N. L. 1980. Emergence of malaria zoonosis of simian origin as natural phenomenon in greater Nicobars, Andaman & Nicobar islands—a preliminary note. J. Commun. Dis. 12:49–54.
- Malaria Research Centre. 1989. Science and technology project report on integrated vector control of malaria, filaria and other vector borne diseases, pp. 374–377. Annual report. Delhi, India.
- Sakai, R. K., R. W. Ainsley and R. H. Baker. 1977. The inheritance of *rose eye*, a sex linked mutant in the malaria vector, *Anopheles culicifacies*. Can. J. Genet. Cytol. 19:633-636.
- Sakai, R. K., R. H. Baker and C. J. Dubash. 1981. The inheritance of golden body, a sex linked mutant in the malaria vector, Anopheles culicifacies. Can. J. Genet. Cytol. 23:579–583.
- Senior White, R. 1947. On the anthropophilic indices of some Anopheles found in east central India. Indian J. Malariol. 1:111-222.
- Shetty, N. J. and B. N. Chowdaiah. 1976. Test for allelism among certain larval color mutants of *Culex quinquefasciatus*. Mosq. News 36:477–482.
- Tadano, T. 1977. The inheritance of a mutant yellow larva in the mosquito Aedes togoi. Ann. Trop. Med. Parasitol. 71:361-365.

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