GENETICS OF *GOLDEN-YELLOW LARVA* IN *ANOPHELES STEPHENSI*

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ABSTRACT. Two larval body color mutants, *golden-yellow larva* (*gy*) and *Black larva* (*Bl*) were isolated from laboratory strains of *Anopheles stephensi*. The inheritance pattern revealed that *golden-yellow larva* was an autosomal recessive and *Black larva* an autosomal semi-dominant mutant. Both of these mutants were found to be linked with a map distance of 3.75 ± 0.42 and have been placed in linkage group III.

INTRODUCTION

Anopheles stephensi Liston is an important vector of urban malaria in the Indian subcontinent and Middle East. Research on genetic aspects of *An. stephensi* started with the isolation and analysis of genetic markers that have been periodically reviewed by Kitzmiller (1976), Narang and Seawright (1982) and Subbarao and Sharma (1984). A few markers have been placed in their respective linkage groups using chromosome correlation studies (Sakai et al. 1983, Parvez et al. 1985).

This paper reports the mode of inheritance of 2 larval color mutants, *golden-yellow larva* and *Black larva*, and their linkage relationship with 2 other autosomal markers, *colorless-eye* (Sharma et al. 1977) and *Alcohol dehydrogenase* (*Adh*). The *Adh* locus was used in this study as a reference locus to place other markers in their respective linkage groups. Parvez et al. (1985) placed *Adh* in linkage group II.

MATERIALS AND METHODS

Mosquitoes were reared in an insectary maintained at 28 ± 1°C and 70–80% RH. All crossing experiments were carried out in 30 cm^3 cloth cages and eggs were collected en masse. Since mutant phenotypes could easily be distinguished at an early instar, larvae were separated and scored at the second larval stage. This procedure provided accurate numbers in each category and helped in avoiding errors due to occasional mortality in the late instars.

For the isolation of homozygous stocks for different enzyme electromorphs, gravid females from different stocks were held in small ice cream cups for single female oviposition. Soon after oviposition the females were numbered and immediately frozen. Each batch of eggs from one female was reared as a single isofemale progeny. When the progeny reached an appropriate stage, enzyme assays of a few individuals of F_1 progeny and their corresponding mother were carried out on the same gel. This permitted an examination of the maternal as well as the F_1 progeny enzyme phenotypes. Wherever identical enzyme phenotypes were observed in the maternal and in F_1 progeny, the cultures were continued as single female progenies. These selected lines were tested for 2 to 3 generations before they were accepted as pure homozygous lines of the desired enzyme phenotypes.

The following strains of *An. stephensi* were used in this study:

GOLDEN-YELLOW LARVA (gy): This new mutant was observed in a laboratory colony originating from Sonepat (Haryana). The mutant expresses its phenotype from early second instar through pupal stage, but the adults are not distinguishable from the wild type.

BLACK LARVA (Bl): Isolated from a laboratory colony that originated from Pondicherry. It expresses its black phenotype from late second instar through pupal stage.

WILD TYPE (+): A colony established with mosquitoes collected from Okhla, Delhi, in 1978.

COLORLESS-EYE (c): An autosomal recessive mutant (Sharma et al. 1977) expressed as white colored eyes in larvae, pupae and adults. This mutant was tentatively assigned to linkage group II (Subbarao and Adak 1981).

Bl Adh-S: Homozygous for the slow (S) electromorph of alcohol dehydrogenase and *Black larva* (*Bl*).

Adh-I: Homozygous for the intermediate (I) electromorph of alcohol dehydrogenase.

Adh-F: Homozygous for the fast (F) electromorph of alcohol dehydrogenase.

Adh-S: Homozygous for the slow (S) electromorph of alcohol dehydrogenase.

gy Adh-I: Homozygous for intermediate (I) electromorph of alcohol dehydrogenase and *golden-yellow larva* (*gy*).

The *alcohol dehydrogenase* locus under investigation exhibits similar phenotypic profiles starting from late 3rd-stage larvae to the pupal stage. In this study pupae were chosen to establish the electrophoretic phenotype of the progeny, and larvae can be used for photography because of the clarity of the bands.

Alcohol dehydrogenase enzyme separation was achieved by subjecting the samples to elec-

trophoresis on a 5% horizontal polyacrylamide gel following Munstermann (1979),[1] Steiner and Joslyn (1979) and Hilburn and Rai (1981). The buffers used were 0.016 M Tris, 0.002 M citric acid (pH 8.1) for gels, and 0.228 M Tris, 0.052 M citric acid (pH 8.1) for the tank. Larvae/pupae were homogenized in 20 μl of grinding buffer (10 μl of mercaptoethanol per 10 ml of 0.01 M Tris-HCl, 0.001 M EDTA, pH 7.0). The procedures of Adak et al. (1988) were followed in the analysis. After electrophoresis, the enzyme activity was visualized by incubating gels in 20 ml of Tris-HCl (pH 8.5) containing octanol, 1 ml; NAD^+, 30 mg; NBT, 15 mg; and PMS, 4 mg (added after 1 h) at 37°C in the dark for 20 min. After staining, gels were fixed in alcohol gel fixative, dried at room temperature and photographed.

RESULTS AND DISCUSSION

To test the mode of inheritance of *golden-yellow larva* (*gy*) and *Black larva* (*Bl*) of *Anopheles stephensi*, a series of crosses were made. When *golden-yellow larva* females (*gy/gy*) were crossed with wild males (+/+), F_1 progeny were wild type and when F_1 progeny were inbred, F_2 progeny consisted of 1,159 wild type and 347 golden-yellow individuals in a ratio of 3:1 ($\chi^2 = 5.98$, $P < 0.05$). The reciprocal cross produced 1,010 wild and 338 golden yellow individuals again in a ratio of 3:1 ($\chi^2 = 0.0$). A backcross of F_1 heterozygous females (*gy/+gy*) with *golden-yellow* males (*gy/gy*) produced 1,782 wild type and 1,756 golden-yellow individuals in a ratio of 1:1 ($\chi^2 = 2.37$, n.s.) and in the reciprocal backcross, wild type (418) and mutant (455) individuals were found in 1:1 ratio ($\chi^2 = 4.78$, $P < 0.05$). Absence of a mutant phenotype in F_1 progeny suggests that *golden-yellow larva* is a recessive mutant whereas its absence in F_1 males indicated that it is not a sex linked mutant, since *An. stephensi* mosquitoes have the "X" and "Y" sex determination mechanism (Aslamkhan 1973). Results from inbreeding and backcrosses of F_1 progeny suggested that *golden-yellow larva* is an autosomally inherited recessive mutant.

The F_1 progeny of the reciprocal crosses between *Black larva* and wild type strain were of a color intermediate between that of black and wild; thus, heterozygous F_1 larvae could easily be differentiated from wild type and homozygous black larvae. The intermediate color of F_1 larvae of the 2 parental reciprocal crosses suggested that *Black larva* was semi-dominant to wild type. This was further supported by the 1:2:1 ratio of black (143; 164), intermediate (287; 346) and wild phenotypes (148; 167) in the crosses where F_1 progeny were inbred. Non-significant chi-square values (0.01 and 0.39) obtained for linkage testing between sex and *Bl* indicated that *Bl* is not sex linked. Backcrossing of female and male F_1 progeny with wild type produced wild (120; 131) and intermediate phenotype (128; 145) in a ratio of 1:1, whereas backcrosses with *Black larva* resulted in intermediate (757; 125) and black phenotype (704; 134) in a ratio of 1:1. Thus, the results from inbreeding and backcrosses of F_1 progeny indicated that *Black larva* is an autosomally inherited semi-dominant mutant.

Since the above data showed that both the mutants were autosomal, the 2 mutants were reciprocally crossed and also crossed with another autosomal mutant, *colorless-eye* (*c*), to establish possible linkage.

Table 1 summarizes the results of crosses between *golden-yellow larva*, *Black larva* and *colorless-eye*. Inbreeding of F_1 progeny from crosses between *golden-yellow* and *colorless-eye larva* (crosses 1 and 2) resulted in 4 phenotypes in a 9:3:3:1 ratio: (9) wild, (3) white-eye, (3) golden-yellow and (1) a new category, white eyes with golden-yellow body color. Backcrossing of F_1 progeny with *colorless-eye* produced wild type larvae and white eyes in 1:1 ratio while with *gy* produced wild type and golden-yellow phenotypes in 1:1 ratio (data not presented in the table).

When the F_1 progeny of a cross between *Bl* and *c* were inbred, 6 phenotypes were observed (cross 3) among the F_2 progeny in a ratio of 6:3:3:2:1:1; (6) intermediate-black, (3) black, (3) wild, (2) white eyes with intermediate-black body color, (1) white eyes with black body color and (1) white eyes. When F_1 progeny were backcrossed with *Black larva*, black and intermediate-black progeny were produced (cross 4) in a ratio of 1:1 ($\chi^2 = 0.0$), whereas backcrossing with *colorless-eye*, produced wild, white eye, intermediate-black, and white eye with intermediate-black body color in a ratio of 1:1:1:1, respectively (cross 5).

The appearance of 4 phenotypic categories among the F_2 progeny in a ratio of 9:3:3:1 (crosses 1 and 2 with χ^2 values of 4.71 and 0.77, respectively) indicated that *gy* and *c* were not linked. The presence of 6 phenotypic categories (because *Black larva* is semi-dominant) among the F_2 progeny (cross 3), though not in a perfect ratio of 6:3:3:2:1:1 ($\chi^2 = 87.13$, $P < 0.001$), and 4 phenotypic categories in a ratio of 1:1:1:1 (χ^2

[1] Munstermann, L. E. 1979. Isoenzymes of *Aedes aegypti*: phenotypes, linkage and use in the genetic analysis of sympatric subspecies populations in East Africa. Ph.D. thesis. University of Notre Dame, Notre Dame, IN.

Table 1. Results elucidating the linkage relationship between autosomal mutants *colorless-eye(c)*, *Black larva (Bl)* and *golden-yellow larva (gy)* in *Anopheles stephensi*.

Cross no.	Presumptive parental genotype: Female		Male	Total	Progeny phenotype: Eye: Body:	Wild Int.	Wild Wild	Wild Black	White Wild	Wild Yellow	White Yellowish	White Blackish	White Int.	Wild Blackish yellow	Wild Yellowish	Testing for: Seg.	Linkage	% recombination
1	$\frac{gy\ +^{c}}{+^{gy}\ c}$	×	$\frac{gy\ +^{c}}{+^{gy}\ c}$	1,109			624 (623.8)	—	211 (207.9)	221 (207.9)	53 (69.3)	—	—	—	—	4.71 (ns)	—	
2	$\frac{+^{gy}\ c}{gy\ +^{c}}$	×	$\frac{+^{gy}\ c}{gy\ +^{c}}$	1,196		—	681 (672.8)	—	215 (224.3)	229 (224.3)	71 (74.8)	—	—	—	—	0.77 (ns)	—	
3	$\frac{c\ +^{Bl}}{c\ Bl}$	×	$\frac{c\ +^{Bl}}{+^{c}\ Bl}$	1,363		445 (511.2)	285 (255.6)	308 (255.6)	71 (85.2)	—	—	23 (85.2)	231 (170.4)	—	—	87.13 ($P < 0.001$)	—	
4	$\frac{c\ +^{Bl}}{+^{c}\ Bl}$	×	$\frac{c\ +^{Bl}}{+^{c}\ Bl}$	752		376 (376)	376 (376)	—	—	—	—	—	—	—	—	0.0	—	
5	$\frac{c\ +^{Bl}}{+^{c}\ Bl}$	×	$\frac{c\ +^{Bl}}{c\ Bl}$	191		49 (47.8)	41 (47.8)	—	47 (47.8)	—	—	—	54 (47.8)	—	—	1.82 (ns)	—	
6	$\frac{+^{Bl}\ gy}{Bl\ +^{gy}}$	×	$\frac{+^{Bl}\ gy}{Bl\ +^{gy}}$	931		465 (465.5)	—	227 (232.8)	—	239 (232.8)	—	—	—	22	—	0.31 (ns)	—	
7	$\frac{Bl\ +^{gy}}{+^{Bl}\ gy}$	×	$\frac{Bl\ +^{gy}}{+^{Bl}\ gy}$	3,927		2,038 (1,963.5)	—	939 (981.8)	—	950 (981.8)	—	—	—	37	—	4.72 (ns)	—	
8	$\frac{gy\ Bl}{+^{gy}\ +^{Bl}}$	×	$\frac{gy\ Bl}{gy\ Bl}$	1,008		482	—	37	—	—	—	—	—	475	14	—	814.3	5.06 ± 0.69 ($P < 0.001$) $\bar{x}$ 3.75 ± 0.42
9	$\frac{gy\ Bl}{gy\ Bl}$	×	$\frac{gy\ Bl}{+^{gy}\ +^{Bl}}$	1,047		526	—	11	—	—	—	—	—	495	15	—	945.6	2.48 ± 0.48 ($P < 0.001$)

Int = intermediate; Seg = segregation.

= 1.82) in the backcross with *colorless-eye* (cross 5), indicated that *Bl* and *c* were not linked. The significant chi-square value 87.13, observed in cross 3 was probably due to the failure to distinguish between white black (*c/c, Bl/Bl*) and white intermediate (*c/c Bl/+Bl*) phenotypes. The misclassification was probably due to an interaction between *c/c* and *Bl/Bl* genotypes (it was reported earlier by Subbarao and Adak (1978) that the *c* gene in homozygous condition reduced the intensity of body pigmentation). The fact that *gy* and *Bl* were not linked with *colorless-eye*, suggested linkage between *gy* and *Bl*. Inbreeding of F_1 progeny of reciprocal crosses between *gy* and *Bl* (crosses 6 and 7) supported this, where intermediate, black and golden-yellow phenotypes were observed in a ratio of 2:1:1 ($\chi^2 = 0.31$ and 4.72).

In crosses 6 and 7, few larvae with body color distinctly different from the black, intermediate-black and golden-yellow phenotypes were found. These larvae were suspected to be double homozygotes, e.g., *Bl/Bl, gy/gy*, resulting from crossing over between *gy* and *Bl*. To establish the genotype of the suspected double homozygotes, these were inbred and backcrossed to *gy* and *Bl* parental types separately. The inbred crossbred true to its phenotype, whereas the progeny from the *Bl* cross were identical to the black larval phenotype and those from the *gy* cross were identical to golden-yellow phenotype. This suggests that *gy* in a homozygous condition probably suppressed the *+Bl/Bl* phenotype.

To estimate the recombination frequency between *gy* and *Bl*, the double homozygous stock was crossed with the wild type. The F_1 progeny of this cross were backcrossed reciprocally to the double homozygous stock (crosses 8 and 9). The highly significant chi-square values, 814.3 and 945.6 for independent assortment between *gy* and *Bl*, indicated linkage between the two. The frequency of recombination calculated was 5.06 ± 0.69 in the heterozygous cross (cross 8) and 2.48 ± 0.48 in the heterozygous male cross (cross 9) and overall frequency of recombination was calculated as 3.75 ± 0.42 between the *gy* and *Bl* loci.

In alcohol dehydrogenase, three electrophoretic variants, slow (S), intermediate (I) and fast (F) were observed. All the F_1 offspring from crosses between S and I; I and F; and between F and S were characterized by 2 parental bands and a hybrid band between the 2 parental bands staining about twice as intensely as the 2 parental bands. This suggested that the active enzyme is a dimer and the 3 variants, S, I and F, were controlled by co-dominant alleles at a single locus. The enzyme patterns and their distribution among the crosses suggested that *Adh* locus is autosomal. This *Adh* locus probably is the same locus described by Iqbal et al. (1973).

Table 2 summarizes the results of the backcrosses to elucidate the linkage relationship of *Adh* and *Black larva* (*Bl*). Crosses 10 to 14 are backcrosses of heterozygous males and females to the parental strain. The non-significant chi-square values (0.22–1.32) for independent assortment between *Adh* and *Bl* indicated that the *Bl* and *Adh* loci are not linked.

Table 3 summarizes the results of backcrosses to elucidate the linkage between *Adh* and *golden-yellow larva* (*gy*). Crosses 15–19 are the backcrosses of heterozygous females to the parental strain. The non-significant chi-square values (0.32–5.36) for independent assortment between *gy* and *Adh* in crosses 15–19 suggested that *Adh* and *gy* loci are not linked.

Since both *gy* and *Bl* showed linkage with

Table 2. Linkage relationship between *Alcohol dehydrogenase* (*Adh*) and *Black larva* (*Bl*) in *Anopheles stephensi*.

Cross no.	Proposed parental genotypes: Female		Male	Total	Progeny phenotypes: Black S	Black IS	Intermediate S	Intermediate IS	Intermediate FS	Wild IF	χ^2 (df)
10	$\frac{Bl\ Adh\text{-}S}{+^{Bl}\ Adh\text{-}I}$	×	$\frac{Bl\ Adh\text{-}S}{Bl\ Adh\text{-}S}$	78	17	23	18	20	0	0	1.08 (3)
11	$\frac{Bl\ Adh\text{-}S}{Bl\ Adh\text{-}S}$	×	$\frac{Bl\ Adh\text{-}S}{+^{Bl}\ Adh\text{-}I}$	151	38	39	33	41	0	0	0.92 (3)
12	$\frac{+^{Bl}\ Adh\text{-}I}{Bl\ Adh\text{-}S}$	×	$\frac{Bl\ Adh\text{-}S}{Bl\ Adh\text{-}S}$	263	72	65	59	67	0	0	1.32 (3)
13	$\frac{Bl\ Adh\text{-}S}{Bl\ Adh\text{-}S}$	×	$\frac{+^{Bl}\ Adh\text{-}I}{Bl\ Adh\text{-}S}$	146	37	33	36	40	0	0	0.70 (3)
14	$\frac{+^{Bl}\ Adh\text{-}F}{+^{Bl}\ Adh\text{-}F}$	×	$\frac{Bl\ Adh\text{-}S}{+^{Bl}\ Adh\text{-}I}$	158	0	0	0	0	76	82	0.22 (1)

Adh-S = slow variant; Adh-I = intermediate variant; Adh-F = fast variant; S = slow homozygote; IS = intermediate/slow heterozygote; FS = fast/slow heterozygote; IF = intermediate/fast heterozygote.

Table 3. Linkage relationship between *Alcohol dehydrogenase* (*Adh*) and *golden-yellow larva* (*gy*) in *Anopheles stephensi*.

Cross no.	Proposed parental genotypes: Female		Proposed parental genotypes: Male	Total	Progeny phenotypes: Wild I	Wild IF	Wild IS	Wild FS	Golden yellow I	Golden yellow IF	Golden yellow IS	χ^2 (df)
15	$\frac{gy\ Adh\text{-}I}{+^{gy}\ Adh\text{-}F}$	×	$\frac{gy\ Adh\text{-}I}{gy\ Adh\text{-}I}$	678	190	177	0	0	160	151	0	5.36 (3)
16	$\frac{+^{gy}\ Adh\text{-}S}{gy\ Adh\text{-}I}$	×	$\frac{gy\ Adh\text{-}I}{gy\ Adh\text{-}I}$	338	94	0	83	0	79	0	82	1.53 (3)
17	$\frac{+^{gy}\ Adh\text{-}F}{gy\ Adh\text{-}I}$	×	$\frac{gy\ Adh\text{-}I}{gy\ Adh\text{-}I}$	406	112	97	0	0	106	91	0	2.58 (3)
18	$\frac{gy\ Adh\text{-}I}{+^{gy}\ Adh\text{-}F}$	×	$\frac{gy\ Adh\text{-}I}{gy\ Adh\text{-}I}$	177	38	52	0	0	44	43	0	2.28 (3)
19	$\frac{+^{gy}\ Adh\text{-}F}{gy\ Adh\text{-}I}$	×	$\frac{+^{gy}\ Adh\text{-}S}{+^{gy}\ Adh\text{-}S}$	151	0	0	72	79	0	0	0	0.32 (1)

Adh-S = slow variant; Adh-I = intermediate variant; Adh-F = fast variant; I = intermediate homozygote; IF = intermediate/fast heterozygote; IS = intermediate/slow heterozygote; FS = fast/slow heterozygote.

each other and showed independent assortment with *Adh* and *c*, the *golden-yellow larva* (*gy*) and *Black larva* (*Bl*) could be placed in a different linkage group than that of *colorless eye* and *Adh*.

Parvez et al. (1985) have placed the *Adh* locus in linkage group II based on the chromosomal correlation studies of Sakai et al. (1983). Assuming that the *Adh* locus reported in this paper is same as that reported by Iqbal et al. (1973), we are placing *gy* and *Bl* in linkage group III and *c* in linkage group II. The *Bl* larva reported by Akhtar et al. (1982) and Suguna (1981) have also been placed in linkage group III by Parvez et al. (1985).

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