WHITE EYE AND YELLOW LARVA: MUTANTS IN *ANOPHELES STEPHENSI* LISTON (DIPTERA: CULICIDAE)

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ABSTRACT

Two spontaneous mutants, white eye (*w*) and yellow larva (*y*), were isolated and characterized from the Bangalore (southern India) and Poona (western India) strains, respectively, of the malarial mosquito, *Anopheles stephensi* (Liston) (Diptera: Culicidae). The *w* mutation is a sex-linked recessive. The second mutant, *y*, is an autosomal recessive.

Key Words: Genetic mutants, *Anopheles stephenci*, malaria.

RESUMEN

Fueron aislados y caracterizados dos mutantes espontáneos, ojo blanco (*w*) y larva amarilla (*y*), a partir de las cepas de Bangalore (sur de India) y Poona (oeste de India), respectivamente, del mosquito de la malaria, *Anopheles stephensi* (Liston). La mutación *w* es recesiva y ligada al sexo. El segundo mutante, *y*, es autosomal recesivo.

Genetic studies of mosquitoes, especially of species and strains which are vectors, continue to be an essential component of genetic control strategies aimed at disrupting the transmission of diseases. These alternative strategies for control require genetic characterizations of geographically isolated strains because any one control mechanism must operate throughout the range of the target mosquito species. Therefore, genetic profiles of different geographical isolates can be used to predict the potential success of laboratory-altered strains intended for release into native populations of mosquito vectors. Our laboratories are currently performing genetic studies towards the eventual development of strains for genetic control of the vectors of infectious diseases. These studies include the following: genetic fingerprinting of laboratory and natural populations of malaria- and encephalitis-carrying mosquitoes; restriction mapping of mitochondrial genomes from diverse geographical isolates; characterization of disease-refractory strains; and the isolation and characterization of Mendelian mutants.

Considerable progress has been made on the genetics and cytogenetics of *Anopheles stephensi* Liston (Diptera: Culicdae). These studies have been reviewed by Kitzmiller (1976), Narang & Seawright (1982), and Parvez *et al*. (1985). We report here two morphological mutants of the mosquito, *An. stephensi*, one of the most important vectors of malaria throughout India and in the Middle East. Both mutants are easily distinguished from the wild-type phenotype, and the viability of the mutants is as good as that of the wild-type. Therefore, they are excellent genetic markers and can be used for the design of strains for genetic control. The first mutant, white eye (*w*), from the Bangalore (southern India) strain of *An. stephensi* is sex-linked and recessive in females and hemizygous in males, as revealed by our crossing experiments. It may or may not be related to white eye reported for other strains of *An. stephensi*. The second mutant, yellow larva (*y*), is autosomal and recessive and comes from the Poona (western India) strain. White eye contrasts readily with the brownish-black wild-type eye color in this mosquito, and yellow larva is easily distinguished from the wild-type straw-tan color.

Materials and Methods

Rearing

Both the Bangalore (southern India) and the Poona (western India) strains of *An. stephensi* were utilized in this study. All mosquito stages were maintained at 26° ± 1°C and at a relative humidity of 75% ± 5% with a photoperiod of 14:10 (L:D). Adults were kept in 20 cubic-inch cages containing 10% sucrose. Females were bloodfed on mice, and all eggs were deposited 70-80 hours after blood feeding in an enamel bowl with water and a lining of filter paper. Larvae were reared in 25 x 30 x 6 cm white enamel pans containing tap water and fed commercial bakers' yeast.

Isolation of Mutants

The white eye (*w*) mutant appeared spontaneously in the Bangalore colony of *An. stephensi*. Its phenotype is an entirely white eye in larvae, pupae, and adults that could be distinguished easily from the wild-type with the naked eye. The yellow larva (*y*) mutant appeared spontaneously in the Poona colony of *An. stephensi*. Its phenotype is an entirely yellow larva except for black eyes. It is manifested in early third instars and persists throughout the fourth instar and pupal stage with the fourth instar showing the most conspicuous color. Yellow larva (*y*) is also easily distinguishable from the wild-type with the naked eye, and both mutants are as viable and easy to maintain as their wild-type counterparts.

Design of Crosses

Pure colonies of *w* and *y* were obtained by crossing mutant adults *inter se*. Several generations of such crosses involving 25 adults of each sex in 8 cubic-inch cages were necessary to obtain sufficient numbers of pure-breeding mutant mosquitoes.

For the inheritance studies, mass matings were made between the pure-bred mutants and pure-bred wild-types. Portions of the F_1 males and females were backcrossed to stocks of the pure-bred mutants, and the F_2 backcross progeny were scored for phenotypes and ratios. The remaining F_1 males and females were inbred to produce the F_2 generation.

RESULTS

The mode of inheritance of white eye (*w*) and yellow larva (*y*) in *An. stephensi* was determined using classical Mendelian crosses and analyses. White eye was determined to be a sex-linked recessive in the homogametic female and hemizygous in the heterogametic male. Yellow larva was found to be an autosomal recessive trait with full penetrance and uniform expression in both sexes, but the specific autosome involved (2N = 6) remains undetermined.

The data in Table 1 summarizes the crosses between the white-eyed strain and the wild-type strain. In cross 1 in which white-eyed males were crossed with wild-type females, the F_1 progeny consisted of all wild type individuals. In the reciprocal crosses (cross 2) in which white-eyed females were crossed with wild-type males, F_1 progeny consisted of wild-type females and males of the mutant phenotype. Backcrosses of the F_1 females from both crosses 1 and 2 mated with white males resulted in wild-type and white-eyed females and males in a 1:1:1:1 ratio (crosses 4 and 5). When F_1 males from cross 1 were mated to white females, the progeny consisted of wild-type females and white-eyed males (cross 3), whereas white-eyed females crossed to F_1 males from cross 2 resulted in all white-eyed progeny (cross 6). In both crosses F_1 progeny were inbred to obtain F_2 individuals. In the first cross among the F_2 progeny all females were wild-type while in males both wild-type and white-eyed phenotypes were found (cross 7). In the second cross among the F_2 progeny both wild type and white-eyed phenotypes were found in a 1:1:1:1 ratio (cross 8).

The results of the crosses between yellow larva and wild type are given in Table 2 which indicates all appropriate Chi-square values supporting our interpretation of

TABLE 1. MODE OF INHERITANCE OF MUTATION WHITE-EYE (*w*) IN *ANOPHELES STEPHENSI*. X, Y = X AND Y CHROMOSOMES, RESPECTIVELY; *w* = WHITE EYE; + = WILD TYPE.

	Presumptive Parental Genotypes			Progeny Phenotypes			
Cross	♀		♂	♀ +	♀ w	♂ +	♂ w
1	X+ X+ (Wild type)	×	Xw Y- (White)	543	0	538	0
2	Xw Xw (White)	×	X+ Y- (Wild type)	612	0	0	668
3	Xw Xw (White)	×	X+ Y- (Wild type)	506	0	0	473
4	Xw X+ (Wild type)	×	Xw Y- (White)	43	51	41	51
5	Xw X+ (Wild type)	×	Xw Y- (White)	668	600	609	659
6	Xw Xw (White)	×	Xw Y- (White)	0	404	0	372
7	Xw X+ (Wild type)	×	X+ Y- (Wild type)	229	0	150	155
8	Xw X+ (Wild type)	×	Xw Y- (White)	651	634	632	586

TABLE 2. MODE OF INHERITANCE OF MUTATION YELLOW LARVA (y) IN *ANOPHELES STEPHENSI*. *NOT SIGNIFICANT.

						Number of Larvae						
	Crosses					Wild Type			Yellow			χ^2
Cross No.	♀			♂		♂	♀	Total	♂	♀	Total	
1	$\frac{+}{+}$	wild type	×	$\frac{y}{y}$	yellow	320	339	659	0	0	0	0
2	$\frac{y}{y}$	yellow	×	$\frac{+}{+}$	wild type	202	177	379	0	0	0	0
3	$\frac{y}{y}$	yellow	×	$\frac{y}{y}$	wild type	311	356	667	325	337	662	.0188*
4	$\frac{y}{+}$	wild type	×	$\frac{y}{y}$	yellow	272	256	528	249	244	493	1.199*
5	$\frac{y}{+}$	wild type	×	$\frac{y}{y}$	yellow	324	331	655	316	324	640	.1737*
6	$\frac{y}{y}$	yellow	×	$\frac{+}{+}$	wild type	198	201	399	190	187	377	.6237*
7	$\frac{y}{+}$	wild type	×	$\frac{y}{+}$	wild type	460	489	949	148	160	308	.1657*
8	$\frac{y}{+}$	wild type	×	$\frac{y}{+}$	wild type	518	526	1044	168	178	343	.0541*

the mode of inheritance of *y* as an autosomal recessive. None of the resulting F_1 mosquitoes in crosses 1 and 2 could be distinguished from the wild-type parents. The dominance of the wild-type was complete. The F_1 heterozygotes were then backcrossed with the mutants. The results of the crosses (3, 4, 5, and 6) fit the expected 1:1 ratio of wild-type to mutants. The F_1 adults were inbred to yield F_2 generations. These crosses (7 and 8) also yielded the expected 3:1 ratio of wild-type to mutants.

DISCUSSION

An. stephensi is an important vector which has developed resistance to insecticides. Therefore, it is mandatory that alternative strategies for its control be developed. Genetic control is one such strategy which requires basic genetic characterizations. We have recently reported several studies in these areas of our ongoing characterizations of *An. stephensi* (Gayathri & Shetty, 1989, 1992a, 1992b; Shetty & Gayathri, 1989; Bhaskar & Shetty, 1992; Rao & Shetty, 1992).

The two mutants, *w* and *y*, described in this study represent excellent markers for the extension of these kinds of studies. Traditionally, such morphological mutants have been used to construct special genetic load strains containing chromosomal translocations or inversions (Pal & Whitten, 1974; Joslyn, 1980). In such strains, genetic markers indicate the presence of the chromosomal aberrations through either altered linkage relationships or position effects of genes located close to chromosomal breakpoints. Genetic markers such as *w* and *y* also can be used in monitoring the pro-

duction and maintenance of genetic sexing systems (Curtis *et al*., 1976; Kaiser *et al*., 1978; Shetty, 1987; Baker *et al*., 1981; Weller & Foster, 1993). More fundamentally, such genetic markers are necessary for expanding the linkage maps being established for *An. stephensi* (Parvez *et al*., 1985).

Although the relatedness of *w* in the Bangalore strain to the *w* previously reported (Aslamkhan, 1973) in the Pakistani strain is unclear, the occurrence of the same phenotypic mutant in such widely separated geographical isolates is, nevertheless, significant. The extent of genetic homogeneity among native strains of *An. stephensi* will affect the ability of strains designed for genetic control to reduce natural populations of this vector.

Previous studies of mutations in eye and larval colors in *An. stephensi* indicate considerable genetic variability in this species. For example, in addition to the sex-linked white eye mutant reported for the Pakistani strain (Aslamkhan, 1973), an autosomal, colorless mutant was reported by Sharma *et al*. (1977). These and other authors also described rosy eye (Aslamkhan & Gul, 1979) and red eye (Sharma *et al*., 1979). Chestnut eye (Rather *et al*., 1983), scarlet, pigmentless, and red-spotted mutants (Parvez *et al*., 1985) have also been reported in *A. stephensi*.

Larval color mutants of *An. stephensi* include green (Subbarao & Adak, 1981; Suguna, 1981; Gayathri & Shetty, 1993), golden-yellow (Adak *et al*., 1990), black (Adak *et al*., 1990; Suguna, 1981; Shetty & Gayathri, unpublished data), stripe (Sakai *et al*., 1981; Shetty & Gayathri, unpublished data), greenish brown (Sharma *et al*., 1979), and brown (Shetty *et al*., unpublished data).

Of particular interest is the occurrence of the white eye mutation in the Bangalore strain as a sex-linked locus. This was reported previously for the Pakistani strain in the north (Aslamkhan, 1973) as well. Tests for allelism will be needed to verify whether the same locus is present in these two widely separated populations. If the *w* locus is the same in both strains, then some degree of genetic homogeneity in *An. stephensi* may exist throughout its range, and this would be useful for future genetic control efforts. If *w* involves different loci between strains, then the taxonomic status of such strains needs to be clarified in greater detail as suggested by Subbarao *et al*. (1987) and Shetty *et al*., (unpublished). In the study by Subbarao *et al*. (1987) the authors refer to different races, historically, of *An. stephensi* and relate these forms to vectorial capacity. Nevertheless, they considered their data as consistent with *An. stephensi* having "ecological variants."

Characterizations by classical cytogenetics along with the molecular approaches of DNA fingerprint analyses and characterizations of mitochondrial genomes are in progress in our laboratories. These integrated approaches could provide much information about the taxonomic status of geographical isolates and strains of *An. stephensi*.

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References Cited

Adak, T., S.K. Subbarao, and V.P. Sharma. 1990. Genetics of golden-yellow larva in *Anopheles stephensi*. Mosq. News. 6: 672-676.

Aslamkhan, M. 1973. Sex chromosomes and sex determination in the malaria mosquito, *Anopheles stephensi*. Pak. J. Zool. 5: 127-130.

ASLAMKHAN, M., AND R. GUL. 1979. Inheritance of the sex-linked mutant rosy, an allele of white in the malaria mosquito, *Anopheles stephensi*. Pakistan J. Sci. 31: 245-249.

BAKER, R.H., R.K. SAKAI, AND K. RAANA. 1981. Genetic sexing for a mosquito sterile-male release. J. Hered. 72: 216-218.

BHASKAR, P., AND N. J. SHETTY. 1992. Susceptibility status of *Anopheles stephensi* Liston to insecticides. J. Com. Dis. 26: 188-190.

CURTIS, C.F., J. AKIYAMA, AND G. DAVIDSON. 1976. A genetic sexing system in *Anopheles gambiae* species A. Mosq. News 36: 493-498.

GAYATHRI, D.K., AND N. J. SHETTY. 1989. Polytene chromosomes of *Anopheles stephensi* Liston - a malaria vector. Vigna Bharathi 12: 1-8.

GAYATHRI, D.K., AND N.J. SHETTY. 1992a. Chromosomal translocations and inherited semisterility in the malaria vector *Anopheles stephensi* Liston. J. Com. Dis. 24: 70-74.

GAYATHRI, D.K., AND N.J. SHETTY. 1992b. Chromosomal inversions in *Anopheles stephensi* Liston - a malaria mosquito. J. Cytol. Genet. 27: 153-161.

GAYATHRI, D.K., AND N.J. SHETTY. 1993. Genetics of a larval colour mutant in *Anopheles stephensi*, a malaria vector. J. Cytol. Genet. 28: 105-106.

JOSLYN, D. 1980. The state of the art of genetic control of mosquitoes. Proc. New Jersey Mosq. Contr. Assoc. 67: 64-71.

KAISER, P.E., J A. SEAWRIGHT, D.A. DAME, AND D.J. JOSLYN. 1978. Development of a genetic sexing system for *Anopheles albimanus*. J. Econ. Entomol. 71: 766-771.

KITZMILLER, J.B. 1976. Genetics, cytogenetics and evolution of mosquitoes. Adv. Genet. 18: 315-433.

NARANG, S., AND J.A. SEAWRIGHT. 1982. Linkage relationship and genetic mapping in *Culex* and *Anopheles*, pp. 231-289 *in* W.W.M. Steiner, N.J. Tabachnick, K.S. Rai and S. Narang [eds.] . Recent developments in the genetics of insect disease vectors. Stipes Publ. Co., Champaign, IL.

PAL, R., AND M.J. WHITTEN. [eds.]. 1974. The use of genetics in insect control. Elsevier/ North Holland, Amsterdam, Holland. pp. 241.

PARVEZ, S.D., K. AKHTAR, AND R.K. SAKAI. 1985. Two new mutations and a linkage map of *Anopheles stephensi*. J. Hered. 76: 205-207.

RAO, G.D.E., AND N.J. SHETTY. 1992. Effect of insecticide resistance on reproductive potential in *Anopheles stephensi* Liston, a malaria mosquito. Int. J. Toxicol. Occup. Environ. Health 1: 48-52.

RATHOR, H.R., S. RASHID, AND TOQIR. 1983. Genetic analysis of a new sex-linked mutant - "chestnut eye" an allele of the white locus in the malaria vector *Anopheles stephensi*. Mosq. News. 43: 209-212.

SAKAI, R.K., M.P. IQBAL, AND R.H. BAKER. 1976. The genetics of stripe, a new morphological mutant in the malaria mosquito, *Anopheles stephensi*. Canadian J. Genet. Cytol. 16: 669-675.

SHARMA, V.P., T.R. MANI, T. ADAK, AND M.A. ANSARI. 1977. Colorless-eye, a recessive autosomal mutant of *Anopheles stephensi*. Mosq. News 37: 667- 669.

SHARMA, V.P., S.K. SUBBARAO, M.A. ANSARI, AND R.K. RAZDAN. 1979. Inheritance pattern of two new mutants, red-eye and greenish brown larvae, in *Anopheles stephensi*. Mosq. News. 39: 655-658.

SHETTY, N.J. 1987. Genetic sexing system for preferential elimination of females in *Culex quinquefasciatus*. J. Am. Mosq. Contr. Assoc. 3: 84-86.

SHETTY, N.J., AND D.K. GAYATHRI. 1989. Genetic control of mosquitoes: mating competitiveness of translocation heterozygote males of *Anopheles stephensi* Liston - a malaria vector in laboratory cage trials, pp. 41-45 *in* P.P. Reddy, [ed.], "Chromosome Damage by Environmental Agents".

SUBBARAO, S.K , AND T. ADAK. 1981. Linkage relationship between three autosomal mutants and functional relationship beween two eye colour mutants in *Anopheles stephensi*. Indian J. Malar. 18: 98-102.

SUBBARAO, S.K., K. VASANTHA, T. ADAK, V.P. SHARMA, AND C.F. CURTIS. 1987. Egg-float ridge number in *Anopheles stephensi*: ecological variation and genetic analysis. Med. Vet. Entomol. 1: 265-271.

SUGUNA, S.G. 1981. The genetics of three larval mutants in *Anopheles stephensi*. Indian J. Med. Res. 73: 120-123.

WELLER, G.L., AND G.G. FOSTER. 1993. Genetic maps of the sheep blowfly *Lucilia cuprina*: Linkage-group correlations with other dipteran genera. Genome 36: 495-506.