GENETIC ANALYSIS OF A NEW SEX-LINKED MUTANT "CHESTNUT EYE" AN ALLELE OF THE WHITE EYE LOCUS IN THE MALARIA VECTOR *ANOPHELES STEPHENSI*

HAMAYUN R. RATHOR, SHAHIDA RASHID AND GHAZALA TOQIR

Pakistan Medical Research Center, International Health Program, University of Maryland School of Medicine, 6, Birdwood Road, Lahore, Pakistan

ABSTRACT. A new eye color mutant "Chestnut" w^{ct} has been described in the malaria vector mosquito, *Anopheles stephensi*. The chestnut eye color is a recessive sex-linked monogenic trait and is allelic with the genes for rosy and white eye colors. The alleles are recessive to wild and codominant to each other.

INTRODUCTION

Chemical control is perhaps the only control method being employed against the malaria vector mosquitoes in the Indo-Pak subcontinent. Chemical control methods in spite of their effectiveness have some drawbacks such as pollution and development of resistance. Genetical control methods although difficult to develop and to employ, are free of problems such as pollution and resistance. Studies on the formal genetics of the vector species form the first step in the development of any genetical control method. Recently a renewed interest in the formal genetics of the vector species *Anopheles stephensi* Liston has resulted in elucidation of the genetics of a number of mutants. The genetics of the following eye color mutants have been reported: white eye, sex-linked recessive (Aslamkhan 1973); colorless eye, autosomal, recessive (Sharma et al. 1977); rosy eye, sex-linked, recessive (Aslamkhan and Gul 1979); red eye, sex-linked, recessive (Sharma et al. 1979).

This paper presents the studies on the genetics of a new sex-linked mutant "chestnut eye" (w^{ct}).

MATERIALS AND METHODS

1) Wild type: Colonized from the Khano-Harni village located 12.4 km southeast of Lahore. The eye color of the wild adults is blackish brown. It is dominant to all its alleles.

2) Chestnut eye: The mutant individuals were found during routine checking of a laboratory colony which was under malathion selection pressure for a number of generations. The eye color of the mutant adults is intense dark brown and the males are slightly lighter in color as compared to the females.

3) White eye (w): This recessive sex-linked mutant was obtained from a Karachi colony. The eyes are white (Aslamkhan and Gul 1979).

4) Rosy eye (w^{ro}): The mutant is a semidominant allele of w, but recessive to wild-type. It was obtained from a Karachi colony. The eyes are rosy when homozygous (Aslamkhan and Gul 1979).

Rearing was done in insectaries maintained at 28 ± 1°C and 75 ± 5% relative humidity and illuminated with fluorescent and incandescent lighting. An artificial dawn and dusk effect was produced at 0500 and 2100 hr for 80 min using an automatic voltage regulator switch. Larvae were reared in enameled pans measuring 45 × 22 cm, filled to 1 cm depth of water and were fed liver powder. Both female and male adults were provided with a 3% sugar solution, and females were offered restrained mice for blood meals. Mass matings were made for all genetic crosses. After blood meals, females were isolated singly in 35 ml glass vials lined with filter paper and flooded with 10 ml water to fill the vial to a 3 mm depth for oviposition. The resulting progeny from each female were reared as a family.

Scoring was done on 1 day old adults under a stereomicroscope. Chestnut eye and normal eye are easy to distinguish as newly emerged adults.

Families were subjected to heterogeneity chi-square tests for sex-ratio and for expected phenotypic ratios. No family showed statistically significant deviation from the expected ratio ($P > 0.05$), therefore, the data from all the families were pooled. A number of genetic crosses were made to elucidate the mode of inheritance of the mutant chestnut eye and its relationship with other eye color alleles w and w^{ro} in adults stage.

RESULTS AND DISCUSSION

Table 1 summarizes the crosses to elucidate the mode of inheritance of w^{ct}. When wild type females and males were crossed, their F_1 (cross 1) and F_2 (cross 9) produced only wild females and males. Similarly, when chestnut females and males were crossed, F_1 (cross 2) and F_2 (cross 10) produced only chestnut females and males. This proved that the parental strains used in this experiment were homozygous for the traits. When wild type females were crossed with chestnut males (cross 3), all F_1 females and

Table 1. Summary of crosses to elucidate the mode of inheritance of w^{ct}.

Cross	Parental genotype** ♀♀	♂♂	f	Progeny genotype ♀♀ w^+	♀♀ w^{ct}	♂♂ w^+	♂♂ w^{ct}	Chi-square* ♀ ♂ 1:1	w^+♀ w^{ct}♀ w^+♂ w^{ct}♂ 1 : 1 : 1 : 1	w^+♀ w^+♂ w^{ct}♂ 2 : 1 : 1
1.	$\frac{w^+ X}{w^+ X}$	$\times \frac{w^+ X}{— Y}$	5	293	—	287	—	0.06	—	—
2.	$\frac{w^{ct} X}{w^{ct} X}$	$\times \frac{w^{ct} X}{— Y}$	8	—	413	—	403	0.12	—	—
3.	$\frac{w^+ X}{w^+ X}$	$\times \frac{w^{ct} X}{— Y}$	28	1521	—	1548	—	0.24	—	—
4.	$\frac{w^{ct} X}{w^{ct} X}$	$\times \frac{w^+ X}{— Y}$	21	1346	—	—	1325	0.17	0.17	—
5.	$\frac{w^+ X}{w^{ct} X}$	$\times \frac{w^{ct} X}{— Y}$	41	972	920	960	922	0.03	2.22	—
6.	$\frac{w^{ct} X}{w^+ X}$	$\times \frac{w^{ct} X}{— Y}$	30	650	680	630	679	0.17	2.76	—
7.	$\frac{w^+ X}{w^{ct} X}$	$\times \frac{w^+ X}{— Y}$	58	3261	—	1677	1646	0.58	—	0.88
8.	$\frac{w^{ct} X}{w^+ X}$	$\times \frac{w^+ X}{— Y}$	20	1120	—	550	540	0.41	—	0.50
9.	$\frac{w^+ X}{w^+ X}$	$\times \frac{w^+ X}{— Y}$	7	415	—	441	—	0.79	—	—
10.	$\frac{w^{ct} X}{w^{ct} X}$	$\times \frac{w^{ct} X}{— Y}$	13	—	889	—	876	0.10	—	—

* = All values $P > 0.05$. f = Number of families checked.
** = Alleles above the lines in heterozygous parental genotypes are of maternal origin.
w^+ = wild, w^{ct} = chestnut.

males were wild type but in reciprocal crosses when chestnut females were crossed with wild type males (cross 4), females were wild type but males were chestnut, suggesting that the mutant is recessive and sex-linked (Aslamkhan 1973). When the F_1 heterozygous females from the above reciprocal crosses were mated with w^{ct} males (crosses 5 and 6), no significant deviation ($P > .05$) was observed from the expected (1 female wild type: 1 female chestnut: 1 male wild type: 1 male chestnut) but when these F_1 heterozygous females were crossed with wild type males (crosses 7 and 8), the mutant phenotype was absent among female progeny and both wild and chestnut males were present in a 1:1 ratio. In crosses 7 and 8 the typical Mendelian ratio 3:1 was modified to 2:1:1 ($\chi^2 = 0.88$ and 0.50, $p > .05$) because the parental males were hemizygous. Thus the chestnut gene has a typical sex-linked Mendelian inheritance and is recessive, sex-linked and monofactorial.

Table 2 summarizes the results of crosses between chestnut, white and rosy to show their linkage relationships. When white females were crossed with white males (cross 11), the F_1 were all white females and males. When rosy females were crossed with rosy males (cross 17), the F_1 were all rosy females and males. The above crosses showed that the two strains white and rosy employed in these crosses were true breeding. When chestnut and white individuals were reciprocally mated (crosses 12, 13) all females in both the crosses developed a new cherry-red phenotype, while males in cross 12 were all chestnut and in cross 13 were all white. These results suggest that the cherry-red phenotype in females is due to heterozygosity between w^{ct} and w, while the males are hemizygous (Aslamkhan 1973, Aslamkhan and Gul 1979), show only the maternal eye color. Cherry-red females were also crossed to white males (cross 14), chestnut males (cross 15) and rosy males (cross 16). The males in these crosses showed a 1:1 segregation of chestnut and white. Females showed a 1:1 segregation of cherry-red and white in cross 14, cherry-red and chestnut in cross 15. Cross 16 produced females of a new phenotype, "carmine eye" and rosy-pink eye in equal numbers. Females from this cross showed a 1:1 segregation of carmen eye and rosy-pink eye. When chestnut individuals were recipro-

Table 2. Results of crosses to find the genetic relationship between w^{ct}, w^{ro}, w and Chi-square analysis of the data.

Cross	Parental genotype** ♀♀	♂♂	f	Progeny genotype*** ♀♀ w^{ct}	w^{ro}	w	w^{ct}/w^{ro}	w^{ct}/w	w^{ro}/w	♂♂ w^{ct}	w^{ro}	w	χ^{2*} ♀ ♂ 1:1	Phenotype ratio 1 : 1 : 1 : 1
11.	$\frac{w\ X}{w\ X}$	$\times \frac{w\ X}{—\ Y}$	5	—	—	167	—	—	—	—	—	186	1.02	—
12.	$\frac{w^{ct}\ X}{w^{ct}\ X}$	$\times \frac{w\ X}{—\ Y}$	4	—	—	—	—	197	—	189	—	—	0.17	—
13.	$\frac{w\ X}{w\ X}$	$\times \frac{w^{ct}\ X}{—\ Y}$	10	—	—	—	—	654	—	—	—	613	1.32	—
14.	$\frac{w^{ct}\ X}{w\ X}$	$\times \frac{w\ X}{—\ Y}$	12	—	—	829	—	832	—	821	—	819	0.13	0.14
15.	$\frac{w^{ct}\ X}{w\ X}$	$\times \frac{w^{ct}\ X}{—\ Y}$	8	240	—	—	—	232	—	248	—	231	0.05	0.79
16.	$\frac{w^{ct}\ X}{w\ X}$	$\times \frac{w^{ro}\ X}{—\ Y}$	8	—	—	—	266	—	252	259	—	249	0.09	0.67
17.	$\frac{w^{ro}\ X}{w^{ro}\ X}$	$\times \frac{w^{ro}\ X}{—\ Y}$	5	—	218	—	—	—	—	—	223	—	0.06	—
18.	$\frac{w^{ct}\ X}{w^{ct}\ X}$	$\times \frac{w^{ro}\ X}{—\ Y}$	5	—	—	—	200	—	—	206	—	—	0.09	—
19.	$\frac{w^{ro}\ X}{w^{ro}\ X}$	$\times \frac{w^{ct}\ X}{—\ Y}$	6	—	—	—	227	—	—	—	232	—	0.05	—
20.	$\frac{w^{ct}\ X}{w^{ro}\ X}$	$\times \frac{w\ X}{—\ Y}$	5	—	—	—	—	148	150	160	149	—	0.20	0.61
21.	$\frac{w^{ct}\ X}{w^{ro}\ X}$	$\times \frac{w^{ct}\ X}{—\ Y}$	6	175	—	—	169	—	—	180	160	—	0.02	1.30
22.	$\frac{w^{ct}\ X}{w^{ro}\ X}$	$\times \frac{w^{ro}\ X}{—\ Y}$	5	—	156	—	168	—	—	160	152	—	0.23	0.88

* = All values $P > 0.05$. f = Number of families checked.

** = Alleles above the lines in heterozygous parental genotypes are of maternal origin.

*** = w^{ct}/w^{ro} = Carmine phenotype; w^{ct}/w = Cherry-red phenotype; w^{ro} = Rosy-pink phenotype. These phenotypes were found in females only.

cally crossed with rosy (crosses 18, 19), only carmine females were produced in both the crosses. In cross 18 (the females being homozygous for chestnut), F_1 progeny produced only chestnut males; similarly in cross 19, the females were homozygous for rosy; in F_1 only rosy males were produced. F_1 females in both the crosses (18, 19), being heterozygous between chestnut and rosy, had a carmine eye color.

When carmine females were crossed with white, chestnut and rosy males (crosses 20, 21, 22) the F_1 males produced a 1:1 segregation of chestnut and rosy eye color. The females in F_1 (cross No. 20) produced cherry-red and rosy-pink because of heterozygosity of white with chestnut and rosy respectively. When carmine females were crossed with chestnut males (cross 21), the F_1 females were chestnut and carmine. When carmine females were mated with rosy males (cross 22) the F_1 females were carmine and rosy.

The above results show that chestnut eye color is recessive to wild type, sex-linked and a monogenic trait. The chestnut, white, rosy and wild-type form a multiple allelic series for eye color in this mosquito. The alleles are codominant to each other but each is recessive to wild type.

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MOSQUITO CONTROL AND SALT MARSH MANAGEMENT: FACTORS INFLUENCING THE PRESENCE OF *AEDES* LARVAE

STEVEN S. BALLING AND VINCENT H. RESH

Division of Entomology and Parasitology, University of California, Berkeley, CA 94720

ABSTRACT. The addition of ditches to increase tidal circulation in salt marshes provides effective mosquito control, but economic and environmental factors require accurate determination of potential mosquito-breeding habitats to maximize the efficiency of this process. Stepwise multiple regressions were used to determine which of several physical and biological variables were related to the occurrence and abundance of *Aedes dorsalis* and *Ae. squamiger* larvae that were dip-sampled monthly from 44 ponds in eight San Francisco Bay, CA, salt marshes from August 1981 through June 1982. Most of the variance in the dependent variables (45% of occurrence and 22% of abundance) was accounted for by pond inundation height, i.e. the minimum tidal height required to flood a marsh pond; additional variance was accounted for by pond area (5% of occurrence and 4% of abundance), abundance of the water boatman *Trichocorixa reticulata* (12% of occurrence), and percent cover of emergent vegetation (6% of abundance). Based on these physical and biological characteristics, a flowchart of decision rules can be used to determine whether a given marsh pond should be ditched.

INTRODUCTION

Ditching has been a successful mosquito control technique in salt marsh management since the turn of this century (Smith 1904, Headlee 1936, Ferrigno et al. 1975). Recently, the use of ditches has increased because they provide an inexpensive alternative to insecticides (Telford and Rucker 1973, Provost 1977, Shisler et al. 1979, Resh and Balling 1979, De Bord et al. 1975). By connecting mosquito-breeding ponds to natural tidal channels, ditches alter the hydrological characteristics of the ponds in a manner that eliminates mosquito production. In San Francisco Bay salt marshes, ditches have no adverse impact on many terrestrial components of the marsh, such as arthropod community diversity (Balling and Resh 1982), the density of some arthropod populations (Barnby and Resh 1980), or plant composition and production (Balling and Resh 1983a), but they significantly lower the invertebrate diversity of marsh potholes (Resh and Balling 1983). In addition, Atlantic coast studies have shown that when larger ponds are ditched, waterfowl and shorebird composition and patterns of use are altered (Burger et al. 1977).

To minimize the impact of ditching on aquatic habitats, it is important to restrict ditching to only those ponds that produce significant numbers of mosquitoes. Ferrigno et al. (1975) suggest that this is best done by monitoring potential mosquito-breeding sites and ditching only those that produce mosquitoes. Unfortunately, the number of potential sites often makes monitoring prohibitively expensive. For example, if a monitoring program was undertaken in the 1,145 ha Petaluma Marsh in northern San Francisco Bay (Fig. 1), over 15,000 ponds and potholes would require regular sampling and evaluation.

Mosquito control efforts in San Francisco Bay salt marshes have been directed toward two species, *Aedes dorsalis* (Meigen) and *Ae. squamiger* (Coquillett). The multivoltine *Ae. dorsalis* emerges from tidal salt marshes along the California coast from January through October (Telford 1958). Coastal populations occur almost exclusively in saline water, whereas inland