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INHERITANCE AND LINKAGE OF MALIC ENZYME IN *ANOPHELES STEPHENSI*

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ABSTRACT. Genetics and linkage analysis of malic enzyme (Me) have been worked out in *Anopheles stephensi*. Genetic analysis revealed the 2 variants to be codominant alleles at a locus *Me*, which is sex-linked. Linkage studies with another X-linked mutant *red-eye* (*r*), indicated that the map distance between 2 loci was 44.60 ± 1.07.

INTRODUCTION

Allozymes can serve as genetic markers for linkage map studies, and can be used to assess genetic diversity among populations. Although a large number of morphological and biochemical markers are known in *Anopheles stephensi* Liston (Narang and Seawright 1982, Subbarao and Sharma 1984), only a few enzyme markers have been mapped in this species (Parvez et al. 1985; Adak et al. 1991, 1992).

A survey of wild populations of *An. stephensi* has uncovered 2 electrophoretic variants of malic enzyme. Malic enzyme is autosomal in the *Culex pipiens* complex (Miles 1974[1]) and in *Anopheles darlingi* Root (Narang et al. 1979). In *Aedes aegypti* (Linn.) (Tabachnick and Lichtenfels 1978), *Anopheles quadrimaculatus* Say (Lanzaro et al. 1991) and *Anopheles culicifacies* species B (Adak et al. 1988) it has been reported as sex-linked.

We report the mode of inheritance and linkage analysis of malic enzyme (Me 1.1.1.40) in *An. stephensi*.

MATERIALS AND METHODS

Two homozygous strains Me-S/Me-S, $+^r/+^r$ and Me-F/Me-F, *r*/*r*, were used in crosses where S and F denote slow and fast electromorphs of malic enzyme and *r* refers to *red-eye*, a sex-linked marker (Sharma et al. 1979). Rearing and handling of adult mosquitoes, isolation of homozygous enzyme electromorphs and genetic crosses were as previously described (Adak et al. 1990).

Enzyme phenotypes were determined by subjecting samples to electrophoresis on 5% horizontal polyacrylamide gels following the technique of Munstermann (1979[2]) and Steiner and Joslyn (1979). 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 tank. Sample preparation and other electrophoretic conditions were as previously described (Adak et al. 1988).

Enzyme activity was visualized by incubating gels in 25 ml of 0.05 M Tris-HCl (pH 8.5) containing 1 M Na malate (pH 7.0), 2 ml; NADP, 10 mg; NBT, 15 mg; PMS, 4 mg; 0.1 M $MgCl_2$, 2.5 ml, at 37°C in the dark for 20 min. After straining, gels were fixed in alcohol gel fixative (acetic acid : distilled water : methanol; 1:5:6) and scored. The malic enzyme locus under investigation exhibits similar phenotypic profiles starting from late 3rd-instar larvae to adult stage. This enzyme remains stable at −70°C for more than a month.

RESULTS AND DISCUSSION

Mass homogenate examination of the malic enzyme profile for *An. stephensi* strains revealed that this species is polymorphic for slow and fast electromorphs. Individual females of polymorphic strains exhibited slow, fast and slow–fast (SF) phenotypes, whereas males exhibited either an S or F electromorph. Absence of the SF heterozygous phenotype in males suggested X-linkage as a probable mode of inheritance of malic enzyme. In *An. stephensi*, sex chromosomes are heteromorphic (XY) in males and are homomorphic (XX) in females (Aslamkhan 1973). Complete penetrance and uniform expression of *Me* genes were observed in all individuals tested, from egg through adult. Further, no variation was observed in either the qualitative activity or relative mobility of slow and fast alleles of the *Me* locus in adults of either sex studied up to the age of 1 month at different stages of their gonotrophic cycles.

To establish X-linked control of this polymorphism and its linkage with another sex-linked morphological marker, *red-eye* (*r*), a series of crosses were made (Table 1). In both reciprocal crosses (crosses 1 and 2) all F_1 males had a single electrophoretic band of the maternal type; F_1 fe-

[1] Miles, S. J. 1974. Biochemical polymorphisms and evolutionary relationships in the *Culex pipiens* complex (Diptera: Culicidae). Ph.D. thesis. University of Western Australia.

[2] 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.

Table 1. Inheritance of malic enzyme (Me) and its linkage relationship with *red-eye* (*r*) in *An. stephensi*.

Cross	Proposed parental genotypes[1,2] Female		Male	Total	Progeny phenotypes: Wild Female S	SF	F	Wild Male S	F	Red-eye Female SF	F
1	$\frac{\text{Me-S } +^r}{\text{Me-S } +^r}$	×	Me-F r	70	0	35	0	35	0	0	0
2	$\frac{\text{Me-F r}}{\text{Me-F r}}$	×	Me-S $+^r$	70	0	35	0	0	0	0	0
3	$\frac{\text{Me-S } +^r}{\text{Me-F r}}$	×	Me-S $+^r$	774	180	205	0	114	83	0	0
4	$\frac{\text{Me-F r}}{\text{Me-S } +^r}$	×	Me-F r	464	0	72	38	64	36	70	51
5	$\frac{\text{Me-S } +^r}{\text{Me-F r}}$	×	Me-S $+^r$	694	168	233	0	71	69	0	0
6	$\frac{\text{Me-F r}}{\text{Me-S } +^r}$	×	Me-S $+^r$	1,249	298	234	0	181	154	0	0
7	$\frac{\text{Me-S } +^r}{\text{Me-F r}}$	×	Me-F r	412	0	74	42	58	68	53	41

[1] Me-S = slow variant; Me-F = fast variant; S = slow homozygote; F = fast homozygote; SF = slow/fast heterozygote.
[2] In all test crosses (1–7) in Table 1 in heterozygous parents, the genes of maternal origin are printed above the line and those of paternal origin below the line.
[3] Expected values were calculated based on 44.60 + 1.07 crossover rate.

males, whose X chromosomes come from both parents, were invariably double banded, suggesting that slow and fast variants of malic enzyme are codominant alleles at the *Me* locus. In cross 1, all F_1 progeny were wild type for eye color, and in the reciprocal cross 2, F_1 females were wild type and males were red-eyed because of its recessive and sex-linked nature (Sharma et al. 1979). The F_1 progeny were inbred to obtain the F_2 (crosses 3 and 4). In cross 3, females were of S and SF phenotypes in a ratio of 1:1 whereas the majority of wild and red-eyed males exhibited the slow and fast phenotype, respectively. Presence of F-type individuals in wild and S-type in red-eyed categories provided the genetic evidence for crossing-over between X chromosomes in the females of *An. stephensi*. In cross 4, however, recombinant types were observed in both sexes because of the genotype of males and females used in the cross.

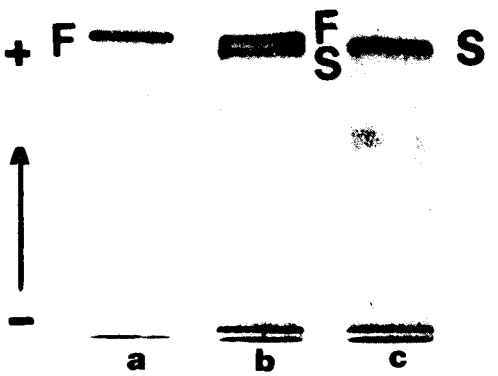

Fig. 1. Malic enzyme (Me) profile in *Anopheles stephensi*. a. Me-F/Me-F; b. Me-FS/Me-FS; c. Me-S/Me-S heterozygote.

Backcrossing of F_1 females from crosses 1 and 2 with wild males and S phenotype (crosses 5 and 6) produced progeny phenotypes similar to those observed in cross 3. Backcrossing of F_1 females from cross 1 with red-eyed males with F phenotype (cross 7) produced progeny similar to those observed in cross 4. As in cross 4, in cross 7 recombinants were observed both in males and females. From all the above mentioned crosses, it is evident that the slow and fast variants of malic enzyme are codominant alleles at the *Me* locus, which is sex-linked. In crosses 3, 5 and 6, where only male recombinants were observed, the recombination frequency was 44.19, whereas in crosses 4 and 7, where both

Table 1. Extended.

Red-eye Male S	Red-eye Male F	% recombination[3]	χ^2
0	0		
0	35		
76	116	40.87 ± 2.49	2.184
58	75	43.53 ± 2.30	0.213
70	83	47.44 ± 2.92	0.956
122	160	44.73 ± 2.00	0.004
31	45	47.09 ± 2.46	1.032

male and female recombinants were observed, recombination frequency was 45.21. As the recombination frequencies between *red-eye* and *Me* locus in the 5 crosses are in reasonable agreement with each other, an overall frequency of recombination was calculated as 44.6 ± 1.07 from the pooled data. Using the pooled recombination frequency of 44.60, the expected numbers of parental types and recombinant types were calculated for crosses 3–7. The chi-square values calculated based on expected values for these crosses with 1 degree of freedom (Table 1) were not significant and hence these 2 loci, *Me* and *r*, were assigned to the X chromosome at a distance of 44.60 ± 1.07. Among all the biochemical markers studied so far in *An. stephensi*, only malic enzyme was found to be sex-linked.

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