THE GENETICS OF *DIAMOND PALPUS* IN *ANOPHELES STEPHENSI*

RICHARD K. SAKAL, RICHARD H. BAKER, CUSROW J. DUBASH AND KAUKAB RAANA

Pakistan Medical Research Center, 6, Birdwood Road, Lahore, Pakistan and The International Health Program, University of Maryland School of Medicine, 10 South Pine Street, Baltimore, MD 21201

ABSTRACT. Genetic analysis of a new-mutant, diamond palpus, in *Anopheles stephensi* indicated it is an autosomal recessive and linked to the dieldrin resistance locus. The observed frequency of recombination is 45.11 ± 1.29%.

INTRODUCTION

The resurgence of malaria has stimulated genetic studies on *Anopheles stephensi* Liston an important malaria vector in the Indo-Pakistan subcontinent and the Middle East. A number of morphological mutants has been studied (Mason and Davidson 1966, Aslamkhan 1973, Sakai et al. 1974, Sharma et al. 1977, Subbarao and Adak 1978, Sharma et al. 1979, Aslamkhan and Gul 1979) and a few biochemical variants subjected to genetic analyses (Bianchi 1968, Bullini et al. 1971, Iqbal et al. 1973a, b). The number of mapped loci in this species is still rather meager, restricting the range of genetic manipulations which can be used to synthesize strains with potential to suppress or replace vector populations. The search for genetic markers continues, and this paper reports the genetic analysis of a new morphological mutant, diamond palpus, *dp*.

The mutant was detected in a laboratory colony of *An. stephensi* originating from Karno Harni, a village approximately 33 km southeast of Lahore, Pakistan. The males are characterized by an enlargement of the distal third of the palpi which form approximate isosceles triangles so that when the palpi are apposed and are viewed from the ventral

aspect, they appear to form a diamond which is bisected by the proboscis (Fig. 1). The female palpi have enlarged bulbous tips and are frequently shortened (Fig. 2).

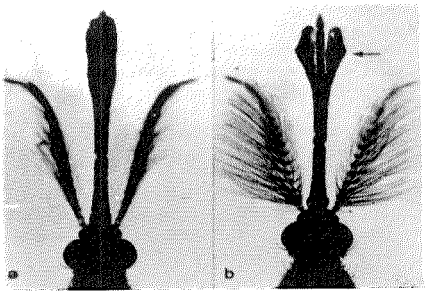

Fig. 1. Ventral view, whole head of male. a = wild type palpi, b = diamond palpi (arrow).

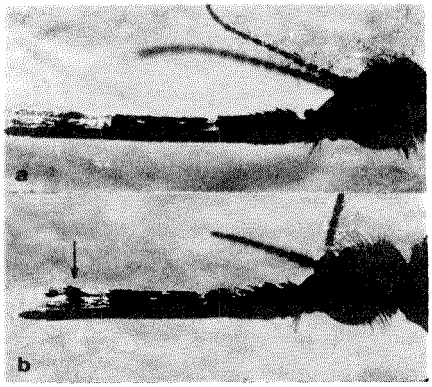

Fig. 2. Lateral view, whole head of female. a = wild type palpi, b = diamond palpi (arrow).

MATERIALS AND METHODS

The following strains were used for the experiment:

1) Diamond palpus *(dp)*—this strain originated from the Karno Harni colony and is homozygous for diamond palpus.
2) Karno Harni—a wild type laboratory colony.
3) Diamond palpus; dieldrin susceptible (*dp*; $D1^s$)—this strain is homozygous for diamond palpus and is dieldrin susceptible. Adults are killed by 1 hr. exposure to 0.4% dieldrin-impregnated papers.
4) Karno Harni, dieldrin resistant ($D1^r$)—this is a wild type dieldrin resistant strain selected from the Karno Harni colony. The adults survive 2 hr exposure to 4.0% dieldrin-impregnated papers.

Mass matings were made in 3.8 liter, cylindrical, cardboard cartons fitted on 1 side with a cloth sleeve and with the top covered with nylon netting. A 3% sucrose solution was present at all times, and beginning with the 3rd night for 3 nights after the initiation of the crosses, a mouse was put into each cage as a blood source. Gravid females were individually isolated into filter paper-lined vials ⅓ filled with tap water and plugged with cotton. After ovipositing, females were removed from the vials and discarded. On the next day a small quantity of liver powder was placed into each vial, and on the following day the eggs were counted to determine hatch rate. Only egg batches showing nearly complete hatch were saved for the experiment. The larvae from each female were reared separately. Upon emergence, the adults were classified for the palpal phenotypes and sex.

For the dieldrin tests, adults were exposed to 0.4% dieldrin-impregnated papers for 1 hr in WHO insecticide test kits. The mortalities were recorded after 8 hr as it was found that no additional mortality occurred beyond a 6 hr holding period at 28°C.

RESULTS AND DISCUSSION

Table 1 summarizes the crosses to elucidate the mode of inheritance of *dp* and also gives the chi square analysis of the data. As no significant heterogeneity was detected among families within a cross, the data for each cross are pooled in the table. In reciprocal crosses (1 and 2) between the *dp* and wild type strains, all F_1

Table 1. Summary of crosses to elucidate the mode of inheritance of *dp*.

Cross No.	Parental Genotypes ♀		♂		f[a]	Progeny phenotypes ♀ +	♀ dp	♂ +	♂ dp	Chi square testing for: 1:1 ratio ♀:♂	1:1 ratio +:dp	1:1:1:1 ratio
1.	dp/dp	X/X	+/+	X/Y	9	603	0	650	0	1.76	—	—
2.	+/+	X/X	dp/dp	X/Y	7	287	0	272	0	0.40	—	—
3.	dp/+	X/X	dp/dp	X/Y	5	112	92	114	106	0.60	1.85	2.79
4.	+/dp	X/X	dp/dp	X/Y	6	243	240	264	231	0.15	1.32	2.39
5.	dp/dp	X/X	dp/+	X/Y	12	621	572	615	596	0.13	1.92	2.43
6.	dp/dp	X/X	+/dp	X/Y	8	200	183	192	182	0.11	0.96	1.13

[a] Number of families tested.

progeny were wild type indicating that *dp* is recessive and also not sex linked. Other sex linked mutants which have been studied in *An. stephensi* showed the mutant phenotype among the F_1 male progeny in one of the reciprocal parental crosses (Aslamkhan 1973, Sharma et al. 1979, Aslamkhan and Gul 1979). When the F_1 heterozygous females were mated to *dp* males (3 and 4) and also in the reciprocal crosses (5 and 6), there was no significant deviation from the 1:1 segregation of ♀:♂ or +:*dp*. The wild type and diamond palpi phenotypes and sexes segregated among all progeny in a 1:1:1:1 ratio also suggested that *dp* is recessive and autosomal.

Since dieldrin resistance has previously been reported to be controlled by a single, incompletely dominant, autosomal gene (Davidson and Mason 1963), a series of crosses was performed to investigate a possible linkage relationship. Diamond palpi, dieldrin susceptible females were crossed to Karno Harni dieldrin resistant males. All the F_1 progeny were wild type, and a subsample of them all survived 1 hr exposure to 0.4% dieldrin-impregnated

Table 2. Summary of crosses to investigate the linkage relationships among *dp*, *D1* and sex.

Cross No.	Parental genotypes ♀		♂		f[a]	Progeny phenotypes ♀ + A^b	♀ + D^b	♀ dp A	♀ dp D	♂ + A	♂ + D	♂ dp A	♂ dp D
7.	dp D1[s]/+ D1[r]	X/X	× dp D1[s]/dp D1[s]	X/Y	6	140	111	89	114	127	118	106	129
8.	dp D1[s]/dp D1[s]	X/X	× dp D1[s]/+ D1[r]	X/Y	4	75	60	55	83	72	68	62	74
	Combined data				10	215	171	144	197	199	186	168	203

[a] f = number of families tested.

[b] A = number alive after 1 hour exposure to 0,4% dieldrin, D = number dead after dieldrin treatment.

Table 3. Chi square analysis and observed recombination frequencies from data in Table 2.

Cross No.	Chi square testing for 1:1 segregation ♀:♂	+:dp	A:D	linkage dp-sex	D1-sex	D1-dp	% recombination D1 – dp
7.	0.72	3.60	0.11	—	—	7.92**	45.40 ± 1.63
8.	0.02	0	0.80	0.09	0.04	6.34*	44.63 ± 2.12
Combined data	0.57	2.35	0.65	—	—	14.18**	45.11 ± 1.29

* $P < 0.05$.
** $P < 0.01$.

papers. Reciprocal backcrosses of the heterozygous F_1 females and males to the *dp*, susceptible strain were made. Table 2 summarizes these results, and Table 3 gives the chi square analysis and the observed recombination frequencies between the 2 loci. There were no significant deviations from the 1:1 ratio for ♀:♂, + : *dp* or alive : dead in either cross; and in the male backcrosses, there was no evidence of sex linkage for either *dp* or *D1* confirming the results obtained above and those of Davidson and Mason (1963), respectively. Chi squares were significant for the segregation between *dp* and *D1* indicating linkage between these two loci. As no significant heterogeneity was observed between the data from either cross, the data from both crosses were pooled and the recombination frequency between *dp* and *D1* was calculated as 45.11 ± 1.29%.

ACKNOWLEDGMENT

This study was supported by Grant No. AI-10049 from the National Institute of Allergy and Infectious Diseases, NIH. We appreciate the technical assistance of Messrs. M. Saghir, N. Hussain, T. Mahmood, L. A. Chowdhry, Z. Ahmad, M. Ali, R. Yamin and M. R. Sindhoo and the assistance of the U.S. Agency for International Development in Pakistan. We thank Dr. W. K. Reisen for the Karno Harni strain of *An. stephensi*.

References Cited

Aslamkhan, M. 1973. Sex chromosomes and sex-determination in the malaria mosquito, *Anopheles stephensi*. Pak. J. Zool. 5:111–115.

Aslamkhan, M. and Gul, R. 1979. Inheritance of the sex-linked mutant rosy, an allele of white, in the malaria mosquito, *Anopheles stephensi*. Pak. J. Sci. 31:245–249.

Bianchi, U. 1968. Genetica formale di una proteina dotata di attivita catalitica esterasica in *Anopheles stephensi*. Accad. Naz. dei. Lincei, Rend. Sc. Fis. Mat. Nat. 45:60–62.

Bullini, L., Coluzzi, M., Cancrini, G. and Santolamazza, C. 1971. Multiple phosphoglucomutase alleles in *Anopheles stephensi*. Heredity 26:475–478.

Davidson, G. and Mason, G. F. 1963. Genetics of mosquitoes. Ann. Rev. Entomol. 8:177–196.

Iqbal, M. P., Sakai, R. K. and Baker, R. H. 1973a. The genetics of an alcohol dehydrogenase in the mosquito *Anopheles stephensi*. J. Med. Ent. 10:309–311.

Iqbal, M. P., Tahir, M. K., Sakai, R. K. and Baker, R. H. 1973b. Linkage groups and recombination in the malaria mosquito. J. Hered. 64:133–136.

Mason, G. F. and Davidson, G. 1966. Morphological mutants in anopheline mosquitoes. Trans. Roy. Soc. Trop. Med. Hyg. 6:20.

Sakai, R. K., Iqbal, M. P. and Baker, R. H. 1974. The genetics of *Stripe*, a new morphological mutant in the malaria mosquito, *Anopheles stephensi*. Can. J. Genet. Cytol. 16:669–675.

Sharma, V. P., Mani, T. R., Adak, T. and Ansari, M. A. 1977. Colorlesseye, a recessive autosomal mutant of *Anopheles stephensi*. Mosquito News 37:667–669.

Sharma, V. P., Subbarao, S. K., Ansari, M. A. and Razdan, R. K. 1979. Inheritance pattern of two new mutants, red-eye and greenish brown-larva in *Anopheles stephensi*. Mosquito News 39:655–658.

Subbarao, S. K. and Adak, T. 1978. Genetic analysis of a larval color mutant, greenish larva, in *Anopheles stephensi*. Mosquito News 38:51–53.