mined by field evaluation against natural populations.

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INHERITANCE OF SHORT PALPI, A MORPHOLOGICAL MUTANT IN THE MALARIA VECTOR MOSQUITO *ANOPHELES STEPHENSI*

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ABSTRACT. Genetic studies suggest that the mutant phenotype "short palpi" (*st*) is determined by a single autosomal recessive gene. The gene is fully penetrant and its expressivity is constant. The *st* locus is linked with the diamond palpi locus at a distance of 38.55 ± 0.73 crossover units.

INTRODUCTION

Although *Anopheles stephensi* Liston is an important malaria vector of high genetic variability, the genetic information on the species is still very meagre. The genetics of 12 morphological and eight biochemical mutants have been published on this species (Davidson and Mason 1963; Mason and Davidson 1966; Bianchi 1968; Aslamkhan and Baker 1969; Bullini et al. 1971; Aslamkhan 1973; Iqbal et al. 1973a, b; Sakai et al. 1974, 1977, 1981; DiDeco et al. 1978; Sharma et al. 1977, 1979; Subbarao and Adak 1978; Rathor and Toqir 1981; Suguna 1981). This paper presents the genetic analysis of a new morphological mutant, short palpi (*st*).

MATERIALS AND METHODS

The following strains were used:

1. Wild type: Colonized in 1978 from the village of Khano-Harni, 12.4 kilometers southeast of Lahore.
2. Short palpi (*st*): Mutant strain obtained from KH stock colony. The palps in this mutant are shorter than proboscis, the mutant stock is homozygous for the character (Fig. 1).
3. White eye (*w*): The eyes are white in color, the mutant strain was obtained from the Karachi stock colony. The mutant is controlled by a recessive sex-linked gene (Aslamkhan 1973).
4. Diamond short palpi (*dp-st*): The distal end of each palpus is triangular-shaped and the palpi are shorter than the proboscis. (Fig. 2)

Both larvae and adults were reared in insectaries maintained at 28 ± 1°C and 75 ± 5% relative humidity. Insectaries were illuminated with fluorescent and incandescent lighting. An artificial dawn and dusk was produced at 0500 and 2100 hr for 80 min. Larvae were reared in 45 × 22 cm enameled pans filled to 1 cm depth of water and were fed on liver powder. Adult females and males were fed 3% sugar solution, and females were offered restrained mice for blood meals. Mass matings were made for all genetic crosses. After a blood meal females were isolated singly for oviposition in 35 ml glass vials lined with filter paper and flooded with 10 ml of water. The resulting progeny from these females were individually reared.

Scoring for short and normal palpi and sex was done on one day old adults under a binocular microscope. The families were subjected to heterogeneity chi-square test for sex-ratio and for expected phenotypic ratios. No family showed any significant deviation ($P > 0.05$) from the expected ratios. Therefore, the data from these individual families were pooled.

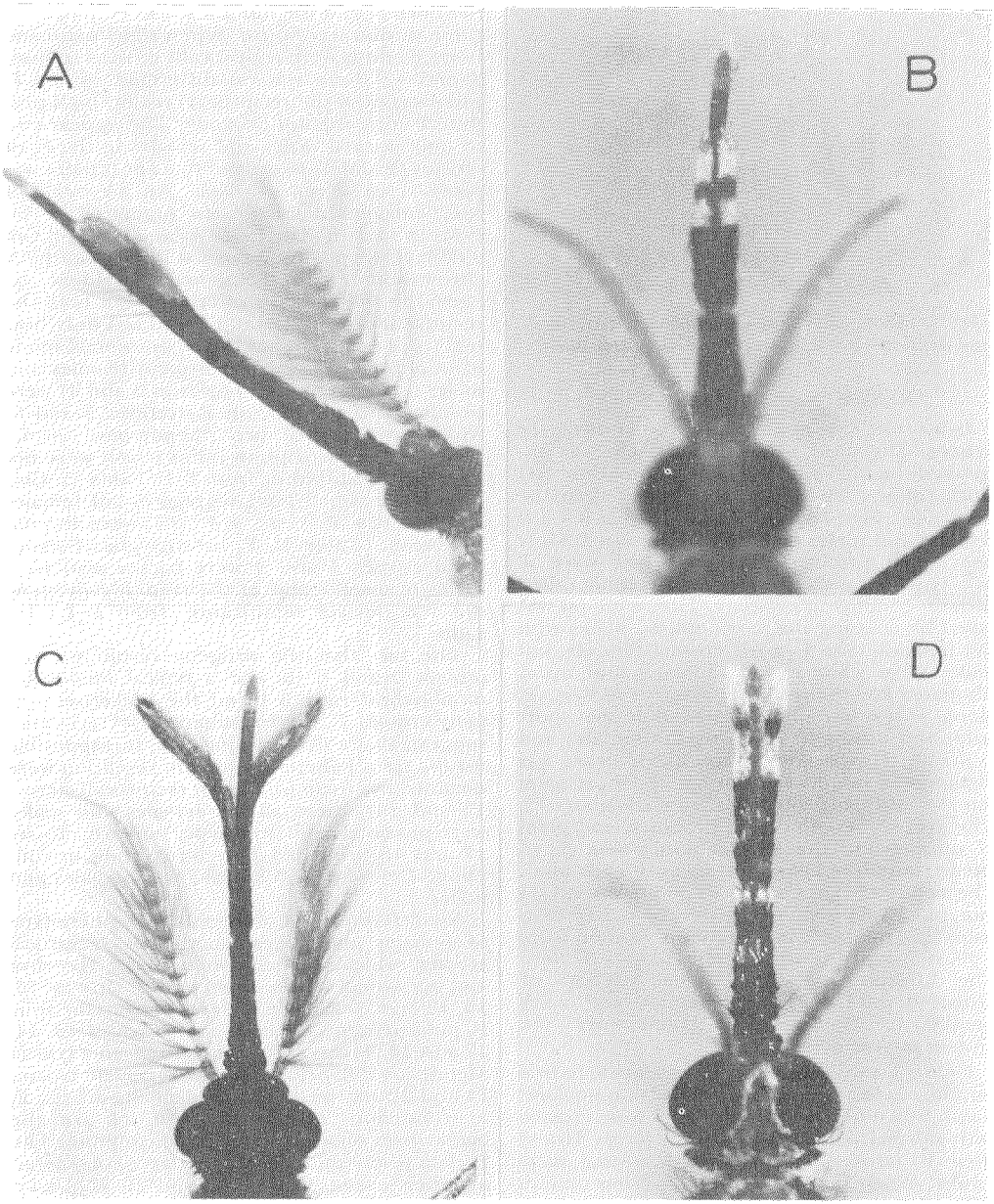

Fig. 1. Short palpi male (A), female (B), normal palpi male (C) and female (D); in *Anopheles stephensi*.

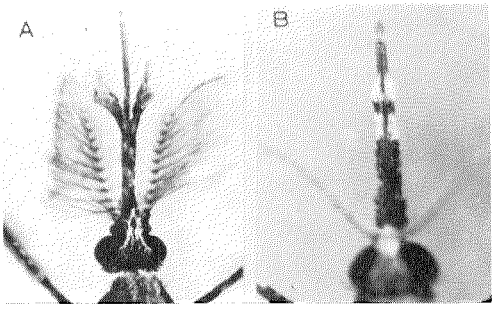

Fig. 2. Short and diamond palpi male (A), Short and diamond palpi female (B); in *Anopheles stephensi*.

RESULTS AND DISCUSSION

ISOLATION AND PURIFICATION OF SHORT PALP STRAIN. The short palp mutant strain was discovered during routine examination of the KH stock colony. The palpi are shorter than the proboscis: Fig. 1A and B. Initially a few (*st*) females and males obtained were inbred. Only one female produced progeny which were all (*st*). The family was inbred but all females died without producing progeny. The mutation was saved by crossing the week old (*st*) males with (w) females. The F_1 gave normal females, but males were white eye. F_1 females and males sib-mated and the resulting progeny in F_2 gave, along with other phenotypes, white eye short palpi and normal eye short palpi females and males. A true-breeding short palpi strain was obtained by inbreeding normal eye short palpi females and males obtained from the above F_2. The average length of the proboscis and palpi of normal and the mutant adults was calculated from 20 females and males of each kind. The average lengths were: + ♀ = proboscis 1.84 mm, palpi 1.84 mm, + ♂ = proboscis 2.18 mm, palpi 2.20 mm, st ♀ = proboscis 1.80 mm, palpi 1.40, st ♂ = proboscis 1.97 mm, palpi 1.57 mm. The difference between the mean length of proboscis and palpi in short females and males was 0.40 mm. Both male and female express the mutant character.

GENETIC ANALYSIS. Crosses were made to find out the mode of inheritance of the mutant short palpi. When normal palpi females were crossed with normal palpi males, the F_1 (cross 1) and their F_2 (cross 9) all produced normal palpi adults. When females and males from mutant stock short palpi were mated the F_1 (cross 2) and F_2 (cross 10), all produced short palpi adults. When the double mutant strain (*dp-st*) was crossed to get their F_1 and F_2 (crosses 11 and 12), the resulting progeny in both the generations were *dp-st*. These crosses showed that the normal and the mutant stocks employed in these studies were pure. When short palpi and normal adults were reciprocally crossed (crosses 3 and 4), there was no difference in the F_1 phenotypes of the reciprocal crosses; both produced normal palpi progeny. The appearance of only normal males and females in the F_1 of crosses (3 and 4) suggested that short palpi was recessive to its normal allele. An XY mode of sex determination has been suggested in *An. stephensi* (Aslamkhan 1973, Aslamkhan and Gul 1979). If the mutant gene was located on the X chromosome, short palpi males could be expected in the progeny of cross 4, but both the reciprocal crosses 3 and 4 produced only normal palpi males suggesting an autosomal mode of inheritance. The normal palpi females and males obtained in the F_1 (crosses 3 and 4) were sib-mated to produce an F_2 (crosses 5 and 6) which consisted of two phenotypes, normal palpi and short palpi, in ratios which were not significantly different from 3:1 (Table 1). Chi-square tests for 3:1:3:1 segregation of females and males (+ ♀ : *st* ♀ : + ♂ : *st*♂) were also not significant (Table 1). F_1 heterozygous females from crosses 3 and 4 were backcrossed to *st* males (crosses 7 and 8) the resulting progeny did not deviate significantly from a 1:1:1:1 ratio.

The fact that the progeny resulting from crosses 5 and 6 showed a typical Mendelian monohybrid ratio 3:1 and the backcross progeny crosses 7 and 8 resulted in 1:1:1:1 ratio, suggests that a single major gene is responsible for the trait. F_1 heterozygotes in repulsion were obtained by crossing *dp* and *st* reciprocally (cross 13 and 14) where all the females and males were phenotypically wild type, similarly crosses 17 and 18 produced F_1 heterozygotes in coupling, they were also all wild type females and males.

No difference was observed in the phenotype of females and males among the reciprocal crosses, which further confirms the recessive and autosomal nature of *dp* and *st*. Crosses 15, 16, 19 and 20 are the F_2 derived from the four F_1 heterozygotes obtained from crosses 13, 14, 17 and 18. These F_2 families produced a typical Mendelian dihybrid ratio (9:3:3:1). The crosses 21 and 22 are the backcrosses obtained from F_1 in repulsion, crosses 23 and 24 are the backcrosses obtained from F_1 in coupling. The F_2 data where the two mutants were considered together (crosses 15, 16, 19 and 20) showed a typical Mendelian dihybrid ratio (9:3:3:1) and the backcross progeny (crosses 21, 22, 23 and 24) resulted in a 1:1:1:1 ratio, further support the inference that both mutants are monofactorial and autosomal.

Chi-square analysis for 1:1 segregation of

Table 1. Summary of crosses to elucidate the mode of inheritance of short palpi.

Cross	Parental genotypes** ♀♀		♂♂		No. of families tested	Progeny phenotypes ♀ ♀ +	♀ st	♂♂ +	♂♂ st	Chi-square values* +♀ st♀ +♂ st♂ 1 : 1 : 1 : 1	+ st 3 : 1	+♀ st♀ +♂ st♂ 3 : 1 : 3 : 1
1.	$\frac{+}{+}$	$\frac{X}{X}$	$\frac{+}{+}$	$\frac{X}{Y}$	13	535	—	529	—	—	—	—
2.	$\frac{st}{st}$	$\frac{X}{X}$	$\frac{st}{st}$	$\frac{X}{Y}$	13	—	352	—	326	—	—	—
3.	$\frac{st}{st}$	$\frac{X}{X}$	$\frac{+}{+}$	$\frac{X}{Y}$	7	316	—	303	—	—	—	—
4.	$\frac{+}{+}$	$\frac{X}{X}$	$\frac{st}{st}$	$\frac{X}{Y}$	7	242	—	244	—	—	—	—
5.	$\frac{st}{+}$	$\frac{X}{X}$	$\frac{st}{+}$	$\frac{X}{Y}$	12	515	188	500	160	—	0.205	2.726
6.	$\frac{+}{st}$	$\frac{X}{X}$	$\frac{+}{st}$	$\frac{X}{Y}$	15	653	211	701	199	—	2.905	4.973
7.	$\frac{st}{+}$	$\frac{X}{X}$	$\frac{st}{st}$	$\frac{X}{Y}$	7	220	205	231	179	7.285	—	—
8.	$\frac{+}{st}$	$\frac{X}{X}$	$\frac{st}{st}$	$\frac{X}{Y}$	7	185	198	200	203	0.961	—	—
9.	$\frac{+}{+}$	$\frac{X}{X}$	$\frac{+}{+}$	$\frac{X}{Y}$	15	779	—	751	—	—	—	—
10.	$\frac{st}{st}$	$\frac{X}{X}$	$\frac{st}{st}$	$\frac{X}{Y}$	12	—	453	—	413	—	—	—

* = $p > 0.05$ for all values.
** = Alleles above the lines in heterozygous genotypes are of maternal origin.
+ = wild st = short palpi.

females and males is given in Tables 2 and 3. No statistically significant deviation from 1:1 segregation was observed for females and males. Table 4 gives chi-square values for 1:1 distribution of both the mutants and their normal alleles in the backcross progeny, the chi-square values for their linkage with sex and the linkage between the two mutant loci. The chi-square values for the linkage of the two loci with sex were nonsignificant. Thus the two mutants were not linked with sex. The chi-square values for linkage between *dp* and *st* was highly significant ($P < .001$). Thus the *dp* and *st* were linked. The percentage crossing over between *dp* and *st* was 38.55 ± 0.73.

Thus the gene for short palpi is tentatively placed on one of the autosomes at the distance of 38.55 ± 0.73 crossover units.

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Table 2. Results of crosses to find the genetic relationship between *dp* and *st*.

Cross	Parental genotype** ♀♀	Parental genotype** ♂♂	f	Progeny phenotype ♀♀ +	dp	st	dp-st	Progeny phenotype ♂♂ +	dp	st	dp-st	Chi-square values* Segregation ♀ ♂ 1 : 1	Phenotype ratio + dp st dp-st 9 : 3 : 3 : 1
11.	dp st X / dp st X	dp st X / dp st Y	9	—	—	—	440	—	—	—	433	0.06	—
12.	dp st X / dp st X	dp st X / dp st Y	7	—	—	—	502	—	—	—	513	0.12	—
13.	dp + X / dp + X	+ st X / + st Y	7	304	—	—	—	266	—	—	—	2.53	—
14.	+ st X / + st X	dp + X / dp + Y	6	447	—	—	—	450	—	—	—	0.01	
15.	dp + X / + st X	dp + X / + st Y	3	112	43	47	13	113	35	30	17	0.98	0.90
16.	+ st X / dp + X	+ st X / dp + Y	4	160	60	60	17	172	66	60	19	0.63	1.90
17.	dp st X / dp st X	+ + X / + + Y	6	300	—	—	—	265	—	—	—	2.17	—
18.	+ + X / + + X	dp st X / dp st Y	4	281	—	—	—	268	—	—	—	0.31	—
19.	dp st X / + + X	dp st X / + + Y	3	113	28	38	13	118	31	41	14	0.36	3.95
20.	+ + X / dp st X	+ + X / dp st Y	4	121	32	38	15	128	30	36	17	0.06	5.82

* = P >0.05 for all values. f = Number of families included.
** = Alleles above the lines in heterozygous genotypes are of maternal origin.
+ = wild, *st* = short palpi, *dp* = diamond palpi, *dp-st* = diamond short palpi.

Table 3. Results of crosses to elucidate the linkage relationship among *st*, *dp* and sex.

Cross	Parental genotypes** ♀♀	Parental genotypes** ♂♂	n	Progeny phenotypes ♀♀ +	dp	st	dp-st	Progeny phenotypes ♂♂ +	dp	st	dp-st	χ^2 1:1 segregation ♀:♂
21.	dp + X / + st X	dp st X / dp st Y	7	120	158	185	112	126	198	165	108	0.41*
22.	dp st X / dp st X	dp + X / + st Y	7	113	146	146	84	88	183	155	90	0.73*
23.	dp st X / + + X	dp st Y / dp st Y	10	268	150	151	237	239	151	156	200	2.32*
24.	dp st X / dp st X	dp st X / + + Y	8	158	89	100	137	141	84	79	154	0.72*

n = number of families tested.
* = P >0.05.
** = Alleles above the lines in heterozygous genotypes are of maternal origin.
dp = diamond palpi.
st = short palpi.

Table 4. Chi-square analysis and observed recombination frequencies from the data in Table 3.

Cross	Chi-square: 1:1 segregation +:st	1:1 segregation +:dp	Linkage st-sex	Linkage dp-sex	Linkage st-dp	Percentage recombination
21.	0.87*	0.34*	—	—	49.15**	39.76 ±0.98
22.	3.01*	0.01*	1.09*	3.46*	64.70**	37.31 ±0.84
23.	2.64*	3.72*	—	—	72.74**	39.17 ±0.80
24.	0.004*	0.21*	0.34*	2.65*	60.13**	37.37 ±1.16
Combined data	6.52*	4.28*	1.43*	6.11*	246.72**	38.55 ±0.73

* = $p > 0.05$.
** = $p < 0.05$.
dp = diamond palpi.
st = short palpi.

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