PATTERN OF INHERITANCE OF A NEW AUTOSOMAL MUTANT "BLACK SCALE" IN *ANOPHELES STEPHENSI*

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ABSTRACT. A new mutant, "black scale" (*bs*), in the adults of the malaria vector mosquito *Anopheles stephensi* is described. The genetic analysis of the mutant suggested that the phenotype is determined by a single, recessive, autosomal gene. Linkage analysis between *bs* and "diamond palpus" *dp* showed that the two genes are linked at a distance of 21.03 ± 0.56 map units. The gene *bs* appears to exhibit complete penetrance and expressivity is constant.

INTRODUCTION

Anopheles stephensi Liston, an important vector of malaria in the Indo-Pakistan subcontinent and Middle East, is known to be resistant to DDT (W.H.O. 1980) and has recently developed resistance to malathion (Rathor and Toqir 1980). This increasing problem of insecticide resistance has generated renewed interest in research on the formal genetics of the species. A number of mutant markers have recently been described on chromosome 3, including short palp (Rathor et al. 1983a), and a sex-linked recessive, chestnut eye (Rathor et al. 1983b). This paper presents data on the inheritance and linkage of a new autosomal morphological mutant, black scale (*bs*), which can be distinguished from wild type adults with the naked eye.

MATERIALS AND METHODS

The following stocks were used in the genetic crosses:

1. Leti (*LT*): A strain colonized in 1975 from the village of Leti, 42 km west of Talagong Tehsil, Attock District, Punjab Province. The adults show normal scale patterns and banding on palpi, legs and wings (Fig. 1, A and B).

2. Diamong palpi (*dp*): The strain originated from the Khano-Harni colony and is homozygous for diamond palpi (Sakai et al. 1981).

3. Black scale (*bs*) (Fig. 1, C and D): The mutant was discovered during routine checking of another mutant stock, short palp (*st*) maintained in our laboratory (Rathor et al. 1983a). In the adults, brownish-black scales replace white scales on different body parts: palpi are black and devoid of the apical pale band. Characteristic spots formed by patches of white scales on dorsum of segment 3 of palps are absent. Femur and tibia are black and do not show any white speckling. Wings are black without any spotting.

4. Black scale and diamond palpi (*bs dp*). This strain is homozygous for both the mutant characters (Fig. 2, A and B).

Both larvae and adults were reared and scored for the expected phenotypes as described by Rathor et al. (1983a). Crosses were made in 0.95 liter paper carton cages. Chi-square tests were used for individual families to test for significant deviation from 1:1 sex ratio and expected phenotypic ratios. No family showed a statistically significant deviation ($P > .05$) from the expected ratio, therefore, the data from all these families were pooled for analysis.

RESULTS AND DISCUSSION

To elucidate the mode of inheritance of *bs*, reciprocal crosses were made between the mutant and normal strains (Table 1). The results of crosses 1 and 2 show that the parent strains used in these experiments were homozygous for the traits. When *bs* and normal adults were reciprocally crossed (crosses 3 and 4), their F_1 consisted of only normal females and males (Fig. 3), suggesting that *bs* was recessive to its normal allele. In *Anopheles stephensi* the XY mode of sex determination has been suggested (Aslamkhan and Baker 1969, Aslamkhan 1973). If the *bs* gene is located on the sex chromosome, the male being hemizygous (Aslamkhan 1973), *bs* males would be expected in cross 3. The absence of *bs* males from cross 3 suggests that the *bs* gene is located on an autosome. The phenotypically normal males and females obtained in the F_1 of the crosses 3 and 4 were sib-mated (crosses 7 and 8). The two crosses produced two phenotypes: normal scale and black scale in ratios not significantly different from 3:1 (Table 1). The Chi-square tests for 3:1:3:1 segregation of females and males were not significant. The F_1 heterozygous females from cross 3 and the F_1 heterozygous males from cross 4 were backcrossed to *bs* males and females respectively (crosses 5 and 6), normal and mutant adults, not differing significantly from 1:1 ratio, were obtained. These results suggest a monofactorial mode of inheritance. The results of cross 9 (Table 2) demonstrated that the double mutant strain *bs dp* was homozygous for the two genes. The reciprocal

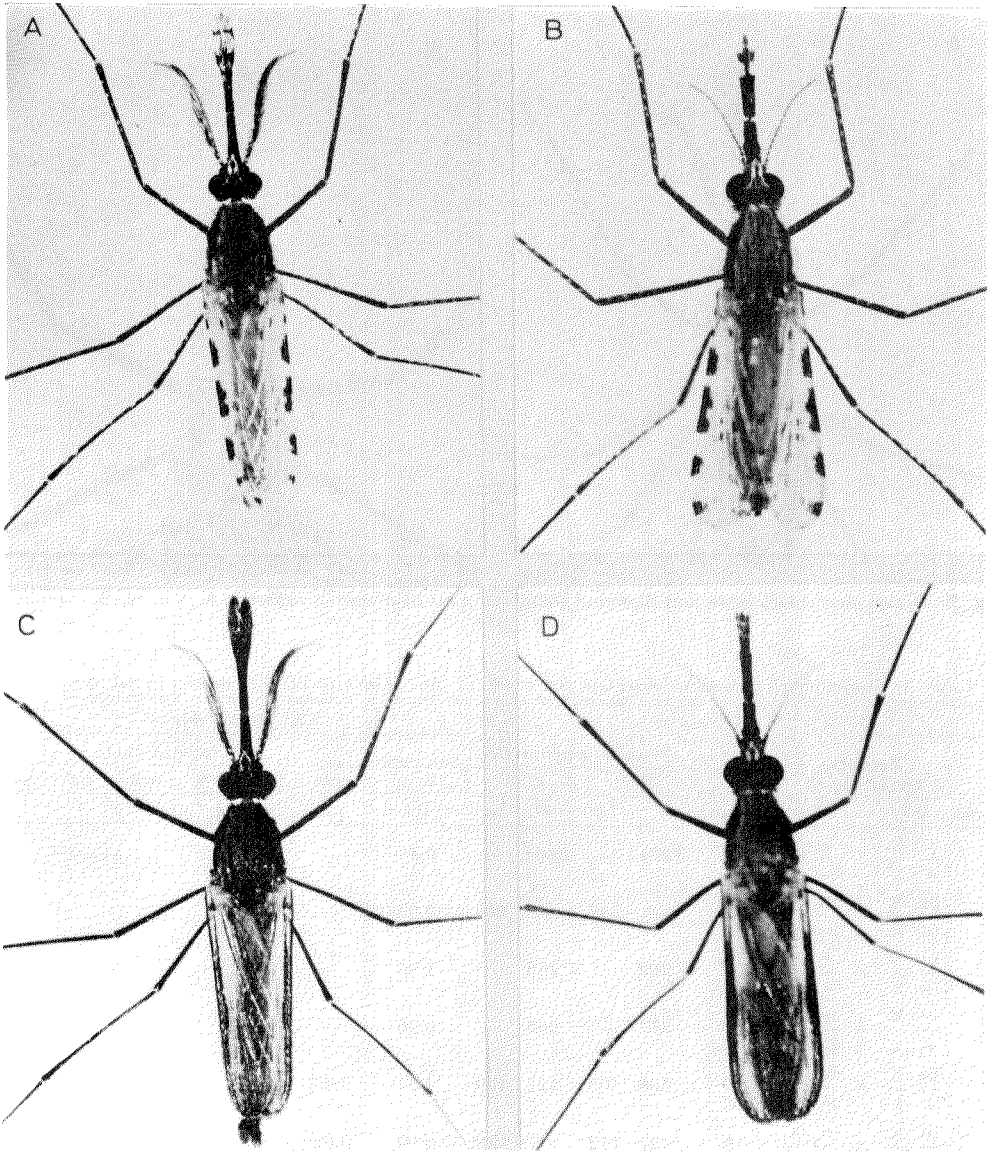

Fig. 1. Dorsal view, *Anopheles stephensi*. A = normal male, B = normal female, C = black scale male, D = black scale female.

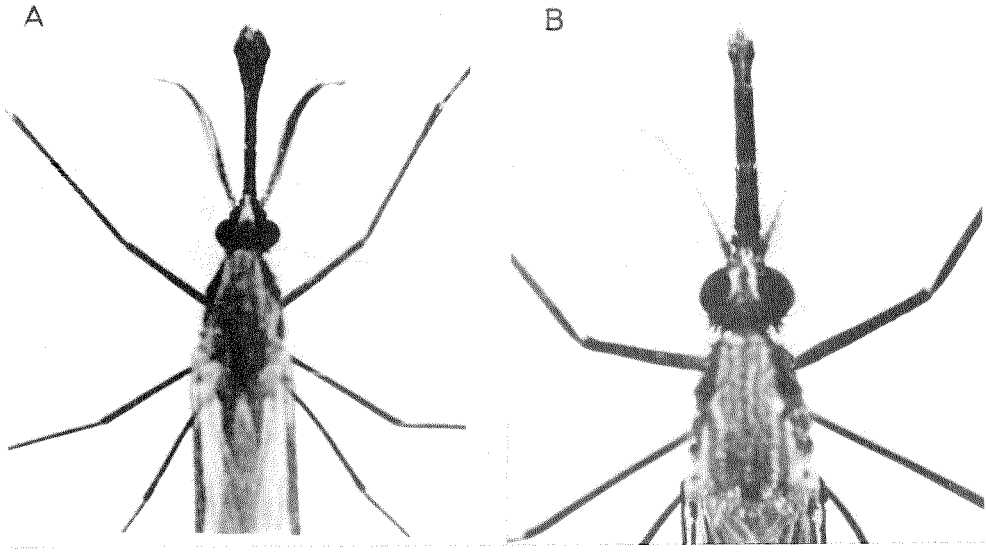

Fig. 2. Dorsal view, black scale and diamond palpus mutant of *Anopheles stephensi*. A = male, B = female.

Table 1. Summary of crosses to elucidate the mode of inheritance of black scale (*bs*) in *An. stephensi*.

Cross	Parental Genotypes** ♀♀		Parental Genotypes** ♂♂	No. of families tested	Progeny phenotype ♀♀ +	Progeny phenotype ♀♀ bs	Progeny phenotype ♂♂ +	Progeny phenotype ♂♂ bs	Sex ratio ♀ ♂	Chi-square values* Phenotypic ratio + bs	+ bs	+♀	bs♀	+♂	bs♂
									1:1	1:1	3:1	3 :	1 :	3 :	1
1	+ / +	X / X	× + / +	X / Y	25	1274	—	1240	—	0.46	—	—			—
2	bs / bs	X / X	× bs / bs	X / Y	8	—	347	—	328	0.53	—	—			—
3	bs / bs	X / X	× + / +	X / Y	5	252	—	283	—	1.80	—	—			—
4	+ / +	X / X	× bs / bs	X / Y	5	277	—	263	—	0.36	—	—			—
5	bs / +	X / X	× bs / bs	X / Y	17	568	505	521	499	1.34	3.45	—			—
6	bs / bs	X / X	× + / bs	X / Y	5	127	142	147	129	0.09	0.02	—			—
7	bs / +	X / X	× bs / +	X / Y	9	366	101	357	132	0.51	—	0.20		4.33	
8	+ / bs	X / X	× + / bs	X / Y	5	172	66	181	66	0.17	—	1.27		1.49	

♂ = male, ♀ = female, bs = black scale, + = normal.
* = $P > 0.05$ for all values.
** = Alleles above the lines in heterozygous genotypes are of maternal origin.

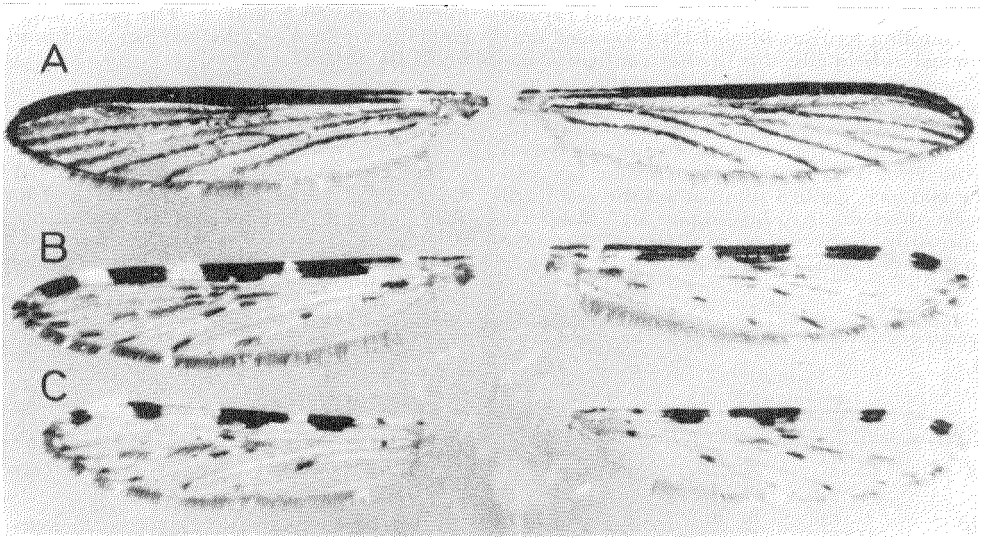

Fig. 3. Wings of *Anopheles stephensi*. A = black scale mutant. B = F_1 hybrid, C = normal.

Table 2. Results of crosses to find out the genetic relationship between *bs* and *dp* in *An. stephensi*.

Cross	Parental genotypes[A] ♀♀			♂♂		f	Progeny phenotype +	bs	dp	bs dp	Chi-square values* Segregation ♀ ♂ 1 : 1	Phenotype ratio + bs dp bs dp 9 : 3 : 3 : 1
9	bs dp / bs dp	×		bs dp / bs dp		16	—	—	—	1533	0.00	—
10	bs + / bs +	×		+ dp / + dp		6	682	—	—	—	0.15	—
11	+ dp / + dp	×		bs + / bs +		12	1467	—	—	—	1.77	—
12	bs + / + dp	×		bs + / + dp		8	629	329	356	34	0.11	117.18**
13	+ dp / bs +	×		+ dp / bs +		15	1246	564	633	74	0.05	137.42**
14	bs dp / bs dp	×		+ + / + +		11	1234	—	—	—	0.59	—
15	+ + / + +	×		bs dp / bs dp		16	1850	—	—	—	0.14	—
16	bs dp / + +	×		bs dp / + +		16	1180	230	213	229	1.69	220.64**
17	+ + / bs dp	×		+ + / bs dp		14	1467	282	158	242	1.21	326.68**

* = $P > 0.05$ for all values, ** = $P < 0.001$, f = Number of families included.
[A] = Alleles above the lines in heterozygous genotypes are of maternal origin.
+ = wild, *bs* = black scales, *dp* = diamond palpi, *bs dp* = black scales diamond palpi.

F_1 obtained in repulsion (crosses 10 and 11) and in coupling (crosses 14 and 15) produced only normal individuals; this further confirms complete dominance of wild type over the two mutants *bs dp*. The F_2 data obtained by crossing the F_1 heterozygous females and males (crosses 12, 13, 16 and 17) showed significant deviation from 9:3:3:1 ratio indicating linkage between the two loci. Table 3 summarizes the results of backcrosses made to investigate the linkage relationship between *bs* and *dp*. Chi-square analysis of these crosses is given in Table 4.

quency between *bs* and *dp*. The percentage recombination between the two loci was found to be 21.03 ± 0.56.

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Table 3. Results of crosses to elucidate the linkage relationship among *bs*, *dp* and sex in *An. stephensi*.

Cross	Parental genotypes** ♀♀		♂♂	n	Progeny phenotypes +	bs	dp	bs dp	χ^2 1:1 Segregation ♀ ♂
18	bs + / + dp	×	bs dp / bs dp	6	101	421	392	91	0.12*
19	+ dp / bs +	×	bs dp / bs dp	10	177	620	608	151	0.04*
20	bs dp / + +	×	bs dp / bs dp	9	523	136	120	507	0.00*
21	+ + / bs dp	×	bs dp / bs dp	10	576	195	156	584	2.79*

n = number of families tested, * = $P > 0.05$.
** = Alleles above the lines in heterozygous genotypes are of maternal origin.
bs = black scales, dp = diamond palpi, bs dp = black scales diamond palpi.

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

Cross	Chi-square 1:1 segregation +:bs	+:dp	Linkage bs–dp	Percentage recombination
18	0.36*	1.51*	383.72**	19.10 ± 1.04
19	0.13*	0.93*	520.57**	21.08 ± 0.93
20	0.00*	0.80*	465.84**	19.91 ± 0.77
21	1.46*	0.64*	433.14**	23.23 ± 0.82
Combined data	0.50*	3.66*	1798.21**	21.03 ± 0.56

* = $P > 0.05$, ** = $P < 0.001$.
bs = black scales, dp = diamond palpi.

The Chi-square values for 1:1 ratio between normal and *bs* and between normal and *dp* were non-significant (crosses 18–21). A highly significant chi-square value ($P < 0.001$) was obtained for the segregation between *bs* and *dp*, (Table 4, crosses 18–21) indicating linkage between the two loci. Since the heterogeneity chi-square between the crosses 18–21 were non-significant, the data from the four crosses were pooled to calculate the recombination fre-

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