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SMALL-ANTENNA AND BROKEN-APPENDAGE - TWO NEW SEX-LINKED MUTANTS OF CULEX PIPIENS FATIGANS

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During recent years considerable research in the genetics of mosquitoes has been directed towards the isolation and characterization of mutants. Several mutants that are naturally occurring as well as radiation-induced have been described in the Culex pipiens complex (Laven, 1967) and Culex tritaeniorhynchus (Baker and Sakai, 1974). Most of these are autosomal but a few are sex-linked. Two new spontaneous sex-linked genes in Culex p. fatigans, the chief vector of Bancroftian filariasis in Asian countries, are described in this paper. These mutants, designated 'small-antenna' (sma) and 'broken-appendage' (brap), were isolated during a continuing program on the genetics of this important disease vector.

MATERIALS AND METHODS

The mutants described here were isolated from the Kolar strain, one of the seven strains of Culex p. fatigans being maintained, in our laboratory. Gravid females were collected in the field, then allowed to deposit egg rafts in the laboratory. Virgin adults of both sexes were anaesthetized with ethyl ether and examined in detail at 20 X magnification under a stero microscope. Any mosquito showing other than the normal pattern with regard to colour or structure was isolated for further studies.

Mutants discovered were crossed with their normal sibs for a few generations in order to build up large cage populations of mutant stocks. All males and females used in the experiments were originally isolated as single pupae in vials and were sexed before introduction into the cages. Individual egg rafts were collected, reared and scored for normal and mutant forms in each cross. The mutants reappearing in subsequent generations were considered to be inherited and were tested further to determine the exact mode of inheritance. These mutants have thus far bred true. In all crosses 5 females and 5 males were placed in a cage 8 x 8 x 8 inches, made of an iron frame covered with nylon net. A water-soaked sponge and a 10 per cent sugar pad provided humidity and food.

RESULTS

Pheno type

Small-antenna (sma) : The normal antennae of both sexes contain 13 segments in the flagellum. The mutant small-antenna contains anywhere from 8 to 12 segments and reveals a somewhat fused appearance. The phenotype of sma homozygotes thus appears to result from a shortening and fusion of flagellar segments. In small-antenna heterozygotes, the segmental fusions are fewer and less severe than in most homozygotes. The female small-antenna homozygote has an antenna about half as long as the wild-type. Heterozygous males and females may show segmental fusions and shortenings, but in general, the effects are less severe than those shown by the great majority homozygous females.

Broken-appendage (brap) : The hind leg in normal individual of each sex has 5 tarsal segments. In the mutant of both sexes several tarsal segments of the metathoracic legs are broken off upon emergence. The broken segments often remain in the pupal case. Usually the break occurs on the 3rd or the 4th tarsal segment. As a result, either the last or the last two tarsal segments are missing. The expression of this mutant is occasionally asymmetrical, but both penetrance and viability are good.

MODE OF INHERITANCE

The results of the crosses involving small-antenna and broken-appendage with normal mosquitoes are given in Tables I and II respectively. The F_1 adults were then backcrossed in both directions with the respective mutants. The results of crosses 3, 4, 5 and 6 fit the expected 1:1 ratio of normal to mutant. F_1 mosquitoes in each case were crossed to get the F_2 generation. The results of crosses 7 and 8 in each case fit the expected 3:1 ratio of normal to mutants.

However, among the mutants of the F_2 generation of a cross between mutant females and normal males, there were far more females than males. Conversely, in the F_2 generation of a cross between mutant males and normal females, the resulting mutants were all males. These crossing experiments clearly indicate that both sma and brap are recessive and sex-linked.

In both Culex and Aedes mosquitoes, there are no heteromorphic sex chromosomes (Breland, 1961; Akstein, 1962; Rai, 1963) and sex appears to be determined by a single pair of alleles or chromosome segments, M and m, for which males are heterozygous, M/m, and females homozygous, m/m (Gilchrist and Haldane, 1947; Laven, 1957; Wild, 1963; Macdonald and Sheppard, 1965; McClelland, 1966; VandeHey, 1967; Baker, 1968). The data on the inheritance of mutants sma and brap indicate that these two loci are very closely linked to the locus determining sex.

DISCUSSION

Several important mutants have been recorded for Culex pipiens (Laven, 1967). Naturally occurring mutants isolated in this species include the sex-linked

TABLE I

Mode of inheritance of Mutation Small-antenna (sma)

Cross No.	Cross	ADULT PHENOTYPE Wild type ♂	♀	Total No. of wild type	Small antenna ♂	♀	Total No. of mutant	x^2
1	Antenna ♂ x wild type ♀	216	201	417	-	-	-	-
2	Antenna ♀ x wild type ♂	339	333	672	-	-	-	-
3	Antenna ♀ x F_1 ♂ (Antenna ♂ x wild ♀)	5	143	148	126	1	127	1.604^{+}
4	Antenna ♂ x F_1 ♀ (Antenna ♂ x wild ♀)	105	91	196	63	86	149	6.403^{++}
5	Antenna ♂ x F_1 ♀ (Antenna ♀ x wild ♂)	61	60	121	51	59	110	0.524^{+}
6	Antenna ♀ x F_1 ♂ (Antenna ♀ x wild ♂)	142	35	177	2	91	93	26.133^{++}
7	F_1 ♀ (Ant ♂ x wild ♀) x F_1 ♀ (Ant ♂ x wild ♀)	202	404	606	198	-	198	0.060^{+}
8	F_1 ♂ (Ant ♀ x wild ♂) x F_1 ♀ (Ant ♀ x wild ♂)	409	261	670	37	147	184	5.435^{++}

\+ not significant
++ significant ; x^2 3.84

TABLE II

Mode of inheritance of Mutation Broken-appendage (brap)

Cross No.	Cross	ADULT PHENOTYPE: Wild type ♂	Wild type ♀	Total No. of wild type	Broken appendage ♂	Broken appendage ♀	Total No. of mutant	x^2
1	Broken appendage ♂ x wild type ♀	198	186	384	-	-	-	-
2	Broken appendage ♀ x wild type ♂	216	194	410	-	-	-	-
3	Broken appendage ♀ x F_1♂ (Broken appendage ♂ x wild type ♀)	15	198	213	192	0	192	1.089^{+}
4	Broken appendage ♂ x F_1♀ (Broken appendage ♂ x wild type ♀)	112	119	231	120	81	201	2.083^{+}
5	Broken appendage ♂ x F_1♀ (Broken appendage ♀ x wild type ♂)	189	96	285	123	138	261	1.055^{+}
6	Broken appendage ♀ x F_1♂ (Broken appendage ♀ x wild type ♂)	154	19	173	3	165	168	0.073^{+}
7	F_1♂ (Broken appendage ♂ x wild ♀) x F_1♀ (Broken appendage ♂ x wild ♀)	118	202	320	140	-	140	7.247^{++}
8	F_1♂ (Broken appendage ♀ x wild ♂) x F_1♀ (Broken appendage ♀ x wild ♂)	357	189	546	42	135	177	0.104^{+}

+ not significant
++ significant ; P = 3.84

white-eye discovered by Gilchrist and Haldane (1947) and red-eye discovered by Wild (1963). Very few naturally occurring mutants have so far been recorded in *Culex p. fatigans*.

The mutant small-antenna is an excellent marker gene which might prove useful for genetic experiments because of its penetrance and expression. Small-antenna homozygotes, especially when female, show the most marked effects on the flagellum. The degree of expression may be scored rapidly and quantitatively in both the heterozygotes and homozygotes by a count of the number of distinct flagellar segments present. A similar mutant has also been described in *Aedes aegypti* (Dunn and Craig, 1968).

The mutant broken-appendage shows variability in the heterozygous condition. This mutant gene, expressed uniformly in both sexes in the homozygous condition, closely resembles a corresponding gene in *Aedes aegypti* (VandeHey and Craig, 1962). These two mutants, which are easily reared in the laboratory, are useful markers in *Culex p. fatigans*.

SUMMARY

This paper describes the inheritance of two new sex-linked mutants designated as 'small-antenna' (sma) and 'broken-appendage' (brap) isolated from laboratory strains of *Culex p. fatigans*. Both are recessive with partial penetrance and reduced expression in heterozygous condition.

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