PALP-EXTENDED, A SEX-LINKED AND SEX-LIMITED MUTANT OF *AEDES AEGYPTI*[1]

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INTRODUCTION

Since 1960, knowledge of the genetics of *Aedes aegypti* (L.) has been expanding rapidly. A major portion of the genetic research on this species has been concerned with the isolation and characterization of mutants. Over 80 of the more than 100 mutants known for *Aedes aegypti* are morphological in nature (Craig and Hickey, 1967; Rai and Hartberg, in press). These mutants provide essential tools for genetic analysis and the establishment of a formal genetics.

Nearly one-third of the mutants of *Aedes aegypti* have been assigned to one of 3 linkage groups, 2 autosomal and 1 sex-linked. McDonald and Rai (1970) have correlated the 3 linkage groups with the 3 pairs of homologous chromosomes.

At least 12 mutants affecting the maxillary palps have been previously isolated and described, but only 3 have been assigned to linkage groups (1 each to linkage group I, II, and III) (Craig and Hickey, 1967; Petersen et al., in press). In this paper an additional mutant affecting the palps, palp-extended (*pe*) is described and assigned to a linkage group.

MATERIALS AND METHODS

The mutant palp-extended (*pe*) was first isolated in 1970 by the author from the progeny of a female *Aedes aegypti* taken biting in Oyster Bay, Dar es Salaam, Tanzania. Eleven of the female progeny from the field-collected female exhibited the mutant trait. These females were crossed with their sibs and the resulting progeny inbred to establish a pure-breeding *pe* stock.

Strains used in this investigation were PALP-EXTENDED, G.S.C. (wild-type) and BRONZE-WHITE (genetic markers *bz* and *w* on linkage group I). The first two are maintained at the Mosquito Genetics Laboratory, Georgia Southern College, and the BRONZE-WHITE was obtained from Dr. G. B. Craig, Jr., Vector Biology Laboratory, University of Notre Dame.

Rearing methods used were generally similar to those described by Craig and VandeHey (1962) for genetic research with *A. aegypti*. Rearing was in an insectary room with a temperature of 27± 2° C and ambient RH. Larvae were fed on a suspension of Liver Powder NF (Nutritional Biochemicals Co.) and adults were provided with dry sugar cubes and an opportunity for the females to take a blood meal from an anesthetized mouse.

McClelland (1962a, b) has shown that sex is determined by a single gene (or a small block of chromosome) designated as *m* on linkage group I. Females were homogametic (*m*/*m*) and males heterogametic (*M*/*m*). The sex locus *m* can be used as a genetic marker for linkage studies.

In the present study, the linkage relationship of *pe* with sex was determined from the results of testcrosses and F_2 data. Males from the *pe* stock were crossed to wild-type females from the G.S.C. strain

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and F_1 males were backcrossed to *pe* females or F_1 females. PALP-EXTENDED females were crossed to BRONZE-WHITE males and the F_2 progeny scored to determine linkage relationships between *pe, bz, w* and between *bz, w,* and *m*. Due to the sex-limited characteristics of *pe* and the female sterility of *bz,* these crosses alone were possible.

F_2 data are not particularly satisfactory for calculation of linkage in mosquitoes (Craig and Hickey, 1967), but one has to resort to F_2 data when backcross experiments are impractical or difficult. Stevens (1939) has given formulae and tables to calculate linkage intensities and standard errors from F_2 data using the "product ratio method." This method was used for calculating recombination between *pe, bz,* and *w*.

The calculation of recombination between the sex locus and *pe, bz,* and *w* from F_2 data is not feasible by the above method because it is not possible to differentiate phenotypically between some of the crossover and non-crossover genotypes. Therefore, another method developed and described in detail by Bhalla and Craig (1967) was used. The method of Bhalla and Craig (1967) was selected over the maximum likelihood method (Kempthorne, 1969) owing to its simplicity, and it is felt that the results obtained are adequate for the present study.

RESULTS AND DISCUSSION

DESCRIPTION. The normal 5-jointed female palp of *A. aegypti* consists of 2 small segments at the base, then 2 longer segments forming the greater part of the length of the structure, and a minute globular apical segment (Fig. 1). Palp-extended (*pe*) is a recessive, sex-limited mutation affecting only females with no visible manifestation in the heterozygote. The minute globular apical segment of the palp is enlarged and extended to a length one-third or more of that of the 4th palpal segment. Some variation in expression was noted and the 4th segment also appears to be somewhat swollen (Figs. 2, 3). The results of scoring the adults reared from a random sample of eggs from the homozygous *pe* stock for the penetrance of *pe* are given in Table 1. Over 83% of the females of the stock exhibit the *pe* trait and no males are affected. Interestingly, another phenotype, proboscipedia (Figs. 4, 5, 6) manifests itself in the homozygous *pe* stock (approximately 10% of the ♀ ♀ and 2% of the ♂ ♂). In the proboscipedia phenotype the labella are transformed into the 2 apical tarsal segments including tarsal claws (Figs. 4, 5), and the palps often show features of a 2nd set of antennae (Fig. 6). The expression is the same as proboscipedia (*prb*) in *Aedes albopictus* (Bat-Miriam and Craig, 1966; Quinn and Craig, 1971). Roberts (1973) has shown that the proboscipedia phenotype reported here in *A. aegypti* is due to a recessive gene (*prb*) closely linked to *pe*. His data also indicate that *pe* and *prb* may interact in a dosage system to produce the proboscipedia phenotype.

When females from the *pe* stock exhibiting the proboscipedia (*prb*) phenotype are mated to males from the *pe* stock, most of the resulting female progeny exhibit the *pe* phenotype. Therefore, all *prb* females were counted as *pe* in the linkage studies as it was assumed they were homozygous for *pe*.

LINKAGE. Table 2 gives the data ob-

Table 1. Penetrance of mutant palp-extended.

♀ phenotypes				♂ phenotypes			
pe	prb	+	Total ♀ ♀	pe	prb	+	Total ♂ ♂
687 (83.1%)	82 (9.9%)	58 (7.0%)	827 (51.9%)	0 ..	16 (2.1%)	750 (97.9%)	766 (48.1%)

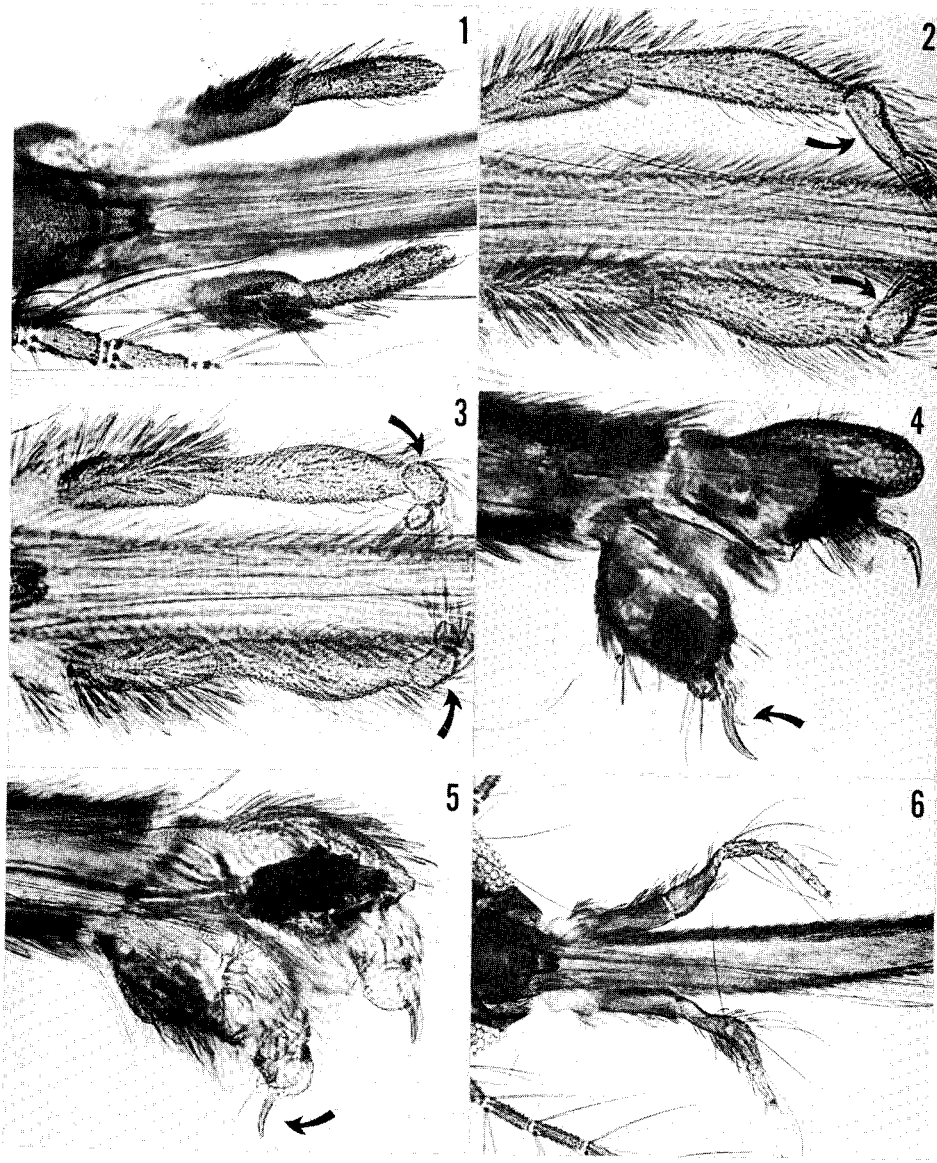

Fig. 1. Palps of normal female. Figs. 2, 3. Variations in abnormal palps of palp-extended females. Arrows point to enlarged apical segment.
Fig. 4. Distal portion of the proboscis of a proboscipedia female. Note serrated claw (arrow).
Fig. 5. Distal portion of the proboscis of a proboscipedia male. Note unserrated claw (arrow).
Fig. 6. Palps of a proboscipedia female showing features of a second set of antennae.

Table 2. Recombination between palp-extended and sex.

Cross ♀	Cross ♂	♀ progeny pe	♀ progeny +	♂ progeny pe	♂ progeny +	% recombination and S.E.[1]
pe m / pe m	x pe M / + m	5	38	0	30	11.6±4.9
pe m / pe m	x pe M / + m	10	191	0	187	5.0±1.5
pe m / + m	x pe M / + m	4	297	0	193	2.7±1.0[2]
				Average % recombination:		6.4±1.0

[1] Standard error calculated using following formula (Serra, 1965):

$$S.E. = \sqrt{\frac{P(1-P)}{n}}$$ where P = crossover value (as fraction of 1). n = number of individuals.

[2] Recombination based on method of Bhalla and Craig (1967).

tained from crosses between *pe* males and G.S.C. (wild-type) females carried to the F_2, and F_1 males back-crossed to *pe* females. The data indicate that *pe* is sex-limited and sex-linked, with an average recombination value of 6.40±1.0 (range 2.7±1.0 to 11.6±4.9). Due to the sex-limited characteristic of *pe*, only the female progeny can be used to calculate recombination values between *pe* and other markers.

Tables 3 thru 7 give data obtained from F_2 progeny from 3 trials of the cross PALP-EXTENDED females x BRONZE-WHITE males (NOTE: only the female progeny are given in Tables 3 and 4; both male and female progeny are included in Tables 5, 6, 7). Tables 3 and 4 give the recombination values obtained between palp-extended (*pe*) and the genetic markers bronze (*bz*) and white (*w*) based on the 3 trials involving the 5543 F_2 female progeny. The recombination value between *pe* and *w* based on the totals was 33.7± 1.2 (range 29.6±1.5 to 41±3.0), and the recombination value between *pe* and *bz* was 16±1.6 in Trial II, which was the only one in which all possible phenotypic classes were recovered. When based on the totals, recombination between *pe* and *bz* was 12.5±1.3 units.

Recombination values between white

Table 3. Recombination between white and palp-extended in F_2 progeny of the cross palp-extended ♀ x bronze, white ♂.

$$\left[\frac{\text{pe m + +}}{\text{pe m + +}} \times \frac{\text{+ M bz w}}{\text{+ m bz w}}\right]$$

Trial no.	F_2 ♀ phenotypes ++	w +	+ pe	w pe	Total	% recombination and S.E.[1]
I	455	29	268	9	761	41.0±3.0
II	1846	334	1306	52	3538	29.6±1.5
III	670	112	425	37	1244	40.8±2.3
Totals	2971	475	1999	98	5543	33.7±1.2

[1] Based on Stevens (1939).

Table 4. Recombination between bronze and palp-extended in F_2 progeny of the cross palp-extended ♀ x bronze, white ♂.

$$\left[\frac{\text{pe m + +}}{\text{pe m + +}} \times \frac{\text{+ M bz w}}{\text{+ m bz w}}\right]$$

Trial no.	F_2 ♀ phenotypes					% recombination and S.E.[1]
	+ +	bz +	+ pe	bz pe	Total	
I	483	1	277	0	761	*
II	2151	29	1357	1	3538	16.0±1.6
III	762	20	462	0	1244	*
Totals	3396	50	2096	1	5543	12.5±1.3

[1] Based on Stevens (1939).
* Not possible to determine % recombination by product ratio due to lack of *bz pe* class.

(*w*) and sex (*m*) and between bronze (*bz*) and sex (*m*) are shown in Tables 5 and 6. The value between *w* and *m* based on the totals is 20.7±0.6 (range 10±1.2 to 24±1.4). Between *bz* and *m* it is 1.8±0.2 (range 0.3±0.2 to 3.2±0.5) based on the totals. These values obtained for recombination between *w* and *m* and between *bz* and *m* are near the values obtained in other work reported by Bhalla and Craig (1970).

Table 7 gives the recombination values obtained between *bz* and *w* based on the 3 trials. The recombination values range from 16.8±0.5 to 20.1±1.2. Based on the totals the value is 17.4±0.4. Back-cross data presented by Bhalla and Craig (1970) would give a value of over 19.2 for *bz–w*. Due to a miscalculation they reported the value as 11.8±0.9 units. They obtained a value of 9.7±1.6 from F_2 data. The value for *bz–w* in the present studies is comparable to theirs.

Figure 7 gives the linkage relationships of *pe* to *m*, *bz* and *w* based on the averages of the recombination values obtained in the 3 trials run in this investigation, and compares them to the linkage map presented by Bhalla and Craig (1970). The values obtained in the present study agree closely with those of Bhalla and Craig (1970). It appears that palp-extended (*pe*)

Table 5. Recombination between white and sex in F_2 progeny of the cross palp-extended ♀ x bronze, white ♂.

$$\left[\frac{\text{pe m + +}}{\text{pe m + +}} \times \frac{\text{+ M bz w}}{\text{+ m bz w}}\right]$$

Trial no.	No. adults					% recombination and S.E.[1]
	♀♀		♂♂			
	+	w	+	w	Total	
I	723	38	529	99	1389	10.0±1.2
II	3152	386	1836	674	6048	21.8±0.8
III	1095	149	827	329	2400	24.0±1.4
Totals	4970	573	3192	1102	9837	20.7±0.6

[1] Recombination based on method of Bhalla and Craig (1967).
Standard error calculated using following formula (Serra, 1965):

$$\text{S.E.} = \sqrt{\frac{P(1-P)}{n}}$$

where P = crossover value (as fraction of 1),
n = no. of individuals.

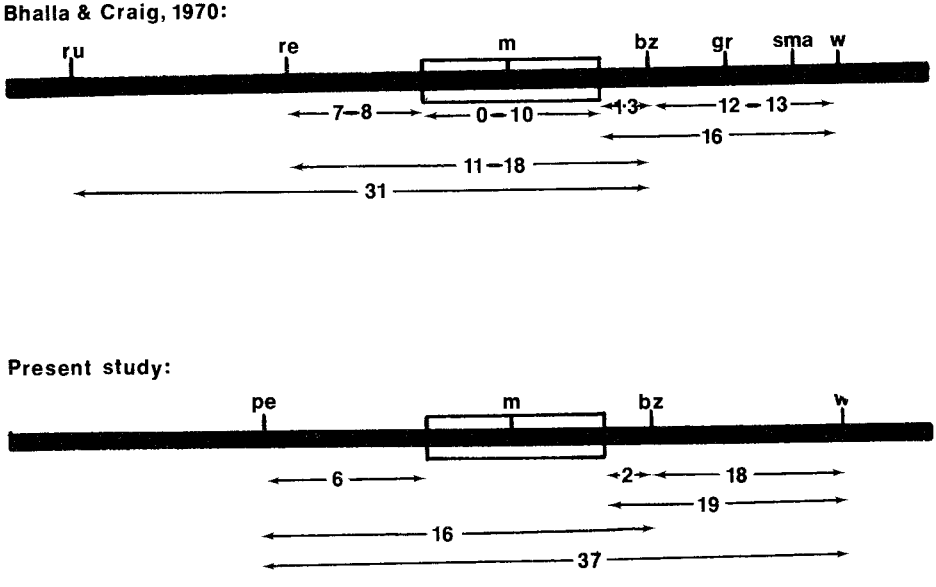

Fig. 7. Tentative map of linkage group 1 of *Aedes aegypti*.

is located near the red-eye (*re*) locus. It is not clear as to which side of red-eye the gene *pe* is located. Bhalla and Craig (1970) gave the values for *re* to *w* as between 23–31, *re–m* as 7–8, *re–bz* as 11–18. The present study gives a value of 37 for *pe–w*, and 6 for *re–m*, and 16 for *pe–bz*. Further linkage studies using the markers rust-eye (*ru*), small-antenna (*sma*), and red-eye (*re*) could determine the precise location of *pe* in relation to red-eye.

Palp-extended (*pe*) should prove to be a useful genetic marker on linkage group

Table 6. Recombination between bronze and sex in F_2 progeny of the cross palp-extended ♀ x bronze, white ♂.

$$\left[\frac{\text{pe m + +}}{\text{pe m + +}} \times \frac{\text{+ M bz w}}{\text{+ m bz w}}\right]$$

	No. adults					
	♀♀		♂♂			% recombination
Trial no.	+	bz	+	bz	Total	and S.E.[1]
I	760	1	509	119	1389	0.3±0.2
II	3508	30	1882	628	6048	1.7±0.2
III	1224	20	808	348	2400	3.2±0.5
Totals	5492	51	3199	1095	9837	1.8±0.2

[1] Recombination based on method of Bhalla and Craig (1967).
Standard error calculated using following formula (Serra, 1965):

$$\text{S.E.} = \sqrt{\frac{P(1-P)}{n}}$$

where P = crossover value (as fraction of 1),
n = no. of individuals.

Table 7. Recombination between bronze and white in F_2 progeny of the cross palp-extended ♀ x bronze, white ♂.

$$\left[\frac{\text{pe m + +}}{\text{pe m + +}} \times \frac{\text{+ M bz w}}{\text{+ m bz w}}\right]$$

Trial no.	F_2 phenotypes					% recombination S.E.[1]
	+ +	bz +	+ w	bz w	Total	
I	1188	64	81	56	1389	20.1±1.2
II	4796	192	594	466	6048	16.8±0.5
III	1807	115	225	253	2400	17.6±0.8
Totals	7791	371	900	775	9837	17.4±0.4

[1] Based on Stevens (1939).

I of *A. aegypti*. Also, since *pe* is sex-limited it is of interest developmentally.

A mutant in *A. aegypti* which is phenotypically similar to palp-extended (*pe*) was described by VandeHey and Craig (1962). The mutant, 5-jointed (*5-j*), was expressed only in females. The apical segment of the female palp was enlarged to a length more than 4 times the wild-type, and with a circumference similar to the preceding segments. The mutant had low penetrance and was unsuitable for use as a marker gene, so it was discarded. Interestingly, Baker and Aslamkhan (1968 unpublished) described a mutant in *Culex tritaeniorhynchus,* designated four-jointed (*fj*), which is similar to the *5-j* in *A. aegypti*. Just as with the *5-j* in *A. aegypti, fj* was discarded because its low penetrance and variable expressivity made it unsuitable as a marker. No information in regard to linkage group was given for *5-j* or *fj*.

SUMMARY

A recessive, sex-linked, sex-limited mutation affecting only females is described in *Aedes aegypti*. The mutant, palp-extended (*pe*), causes the minute globular apical segment of the female palp to be enlarged and extended to a length one-third or more of that of the 4th palpal segment. Some variation in expression was noted; penetrance was 83%; and viability was excellent. The mutant *pe* is located near the red-eye (*re*) locus on linkage group I. Palp-extended (*pe*) should prove to be a useful genetic marker.

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