PROBOSCIPEDIA, A HOMEOTIC MUTANT IN *AEDES AEGYPTI*[1]

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ABSTRACT. Descriptive and linkage studies have been conducted for a homeotic mutant, proboscipedia (*prb*) in *Aedes aegypti* (L.). The mutant was isolated from a strain of palp-extended (*pe*), a recessive, sex-linked, and sex-limited mutation, collected in Dar es Salaam, Tanzania. In addition to descriptions of various labellar and maxillary palp modifications produced by the homeotic condition, environmental effects on penetrance were also considered; cooler rearing temperatures producing an increase in the penetrance. Linkage studies, due to female sterility, are based on F_2 data and show the mutant *prb* to be a genetically controlled recessive located between red-eye (*re*) and sex (*m*) on linkage group I. Homeotic mutants provide excellent means of studying imaginal disc development in homometabolous insects but due to female sterility of the prb mutation its use as a genetic marker is severely limited.

INTRODUCTION

A great deal of interest has been generated in homeotic mutants. These mutants are useful in understanding morphogenesis, development, and the interplay of environment and genotype. The term homeosis was coined by Bateson (1894) to describe alternation in which one member of a homologous series assumed characteristics normally associated with another member of that series.

The mutant phenotype proboscipedia in mosquitoes was first isolated and briefly described by Bat-Miriam and Craig (1966) in *Aedes (Stegomyia) albopictus* (Skuse). They determined that the genetic basis of the mutant was a sex-linked recessive gene which they designated *prb*. Some of the data on linkage were presented, but female sterility made precise analysis difficult. Quinn and Craig (1971) studied the mutant in more detail and refined the linkage data. The mutation proboscipedia in *Ae. albopictus* provides an interesting example of homeosis. This mutation and intersex (Craig and Hickey 1967) were, until this time, the only homeotic mutants known in *Stegomyia* mosquitoes. In proboscipedia, the labella of the proboscis are modified into tarsi. Moreover, the maxillary palps contain elements of both tarsi and antennae. This mutation is female-sterile because they are unable to pierce the skin to obtain a blood meal needed for egg production.

Investigations by Hartberg (1975) have shown the proboscipedia phenotype to be present in a strain of *Ae. aegypti* homozygous for palp-extended (*pe*). This strain was established from a mutant female collected in Dar es Salaam, Tanzania in 1970 by W. K. Hartberg.

Hartberg (1975) reports the palp-extended gene on chromosome 1, near the red-eye locus. He believes approximately 10% of the palp-extended population show the proboscipedia phenotype. Interestingly, palp-extended is expressed only in females; whereas, the proboscipedia phenotype is expressed in both sexes.

MATERIALS AND METHODS

STRAINS. The strains of *Ae. aegypti* used in this investigation were from colonies maintained at Georgia Southern College and the University of Notre Dame (Table 1).

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Table 1. Strains of *Aedes aegypti* used in this investigation.

Strain	Strain composition
Palp-extended	Palp-extended, sex-linked and sex-limited expression. Established from a mutant female collected in Dar es Salaam, Tanzania in 1970 by W. K. Hartberg. Strain maintained at the Mosquito Genetics Laboratory, Georgia Southern College.
Red eye	Multiple marker strain constructed from a synthesis of several strains. Contains markers—I: sex red eye; II: spot abdomen, yellow larvae; III: black tarsi. Strain maintained at Vector Biology Laboratory, University of Notre Dame.
Red, Rust, Small Antennae	Mutants red, rust, small antennae. Linkage group I. (sex-linked) Reconstituted after outcrossing. Strain maintained at Vector Biology Laboratory, University of Notre Dame.

Rearing methods were generally similar to those described by Craig and VandeHey (1962). *Ae. aegypti* was reared at 26±3°C and ambient humidity. Larvae were fed on a suspension of Liver Powder NF (Nutritional Biochemicals Co.). Adults were provided with dry sugar cubes and an opportunity to take a blood meal from an anesthetized mouse.

To determine temperature effects on penetrance of the proboscipedia phenotype in the palp-extended population, temperature controlled rearings were conducted. Mosquitoes were reared from eggs to adults at the following temperatures: 17°C, 26°C, and 30°C. The mosquitoes were reared in similar fashion as those at room temperature except the temperatures were maintained at ± 1°C.

Crosses were made to determine the genetic basis of the mutation proboscipedia, and to determine the position of the gene on the proper linkage group. All crosses listed were made with virgin mosquitoes with the female parent designated first:

A. RED-EYE × proboscipedia; F_2 progeny used for analysis.
B. Red, rust, small-antenna (*re, ru, sma*) × proboscipedia; F_2 progeny used for analysis.
C. Palp-extended × proboscipedia; F_1 progeny used for analysis.
D. Wild-type from palp-extended population × proboscipedia; F_1 progeny used for analysis.
E. F_1 palp-extended from "D" above × proboscipedia; F_1 progeny used for analysis.
F. F_1 palp-extended from "D" above × F_1 wild-type from "D" above; F_1 progeny used for analysis.
G. F_1 wild-type from "D" above × F_1 wild-type from "D" above; F_1 progeny used for analysis.

F_2 data are not particularly satisfactory in the calculation of linkage intensities in mosquitoes (Craig and Hickey 1967), but often must be resorted to when test cross data are impractical or impossible. Immer (1930) has given formulae and tables for calculating linkage intensities and probable errors from F_2 data using the "product ratio" method. This method was used for calculating recombination between *re, ru, sma,* and proboscipedia. Standard error was calculated by dividing Immer's probable error by .6745.

The calculation of recombination between the sex locus and *re, ru, sma,* and proboscipedia is not feasible by the above method; therefore, another method was used (Bhalla and Craig 1967). Since sex in culicine mosquitoes is determined by a single gene (or block of chromosome) designated as *m*, with the females homogametic (*m/m*) and males

heterogametic (*M/m*) (Gilchrist and Haldane 1947, McClelland 1962 a,b), the sex locus may be used as a genetic marker for linkage studies. Standard errors were calculated by a method developed by Serra (1965).

To eliminate distorted ratios, pharate adults which died during emergence were dissected and scored on all crosses dealing with the proboscipedia mutation. Many proboscipedia individuals were recovered with their proboscis trapped in their pupal exuviae.

RESULTS

The mouth parts of the wild-type *A. aegypti* consist of a pair of maxillary palps and the labium, a relatively stout organ containing six stylets: the labrum, the hypopharynx, a pair of mandibles, and a pair of maxillae. Distally, the labium terminates in a pair of small lobed structures, the labella (Fig. 1). The female on finding a suitable host uses the labella as a tactile and chemosensory structure for probing. Upon finding a proper location the labella then serve as a guide for the fasicle of stylets as they cut and puncture the skin while the labium bends backwards. The labella never enter the wound. Of the above mentioned structures, the length of the 5 segmented maxillary palps is one of the morphological characters distinguishing male from female. Those of the male are typically as long as the proboscis while those of the female are usually one-fifth of its length (Figs. 2,3).

In proboscipedia, the labella are modified into tarsal segments complete with tarsal claws. Sexual dimorphism in claws present on the labella is as evident as it is on the prothoracic and mesothoracic legs of adults (Figs. 4,5). In some individuals the distal portion of the labium is a twisted amorphous mass (Fig. 6) which prevents the pharate adult from emerging and may also trap the stylets in their sheath.

The labella appear to be the only structures of the labium which have gross morphological modifications. In some individuals the laciniae of the maxillae are often twisted, preventing the stylets from forming a compact fasicle.

Typically, male and female proboscipedia exhibit no sexual dimorphism in respect to maxillary palp length (Figs. 7,8). They are the same length in both sexes, although the females exhibit greater variation in expression. In all, three phenotypic classes of palpal variation are distinguishable: (1) the majority have maxillary palps reduced in the number of segments (from 5 to 3 or 4) terminating in a heavily scaled club-like mass of tissue perpendicular and lateral to the proboscis, instead of parallel and dorsal as found in the wild-type (Fig. 9); (2) in many the palps have tarsal-like segmentation complete with tarsal claws which exhibit sexual dimorphism similar to that present in the claws found on the modified labella (Fig. 10); (3) another modification produces antenna-like segmentation of the palps complete with nearly plumose setae (Fig. 11). The mutation proboscipedia in *Ae. aegypti* produces as severe an expression as the similar mutation in *Ae. albopictus*.

Mouth parts of mutant and wild-type larvae are similar. Differences become apparent only in the pharate adult just before emergence.

The proboscipedia mutation prevented mutant females from feeding; although many methods were tried (Quinn and Craig 1971 and others).

The labella modifications are nearly symmetrical while those of the palps are not. In some females two different palp forms are present (Fig. 12).

The 17°C and 30°C rearing temperatures were chosen as they are near the lethal limits for rearing this species. The 26°C temperature was chosen as it is near optimal for *Ae. aegypti*. The optimal rearings were used as expected values in determining the temperature effects on the penetrance of proboscipedia in the palp-extended population. Chi-square analysis of the data showed no significant difference at the .05 level between room

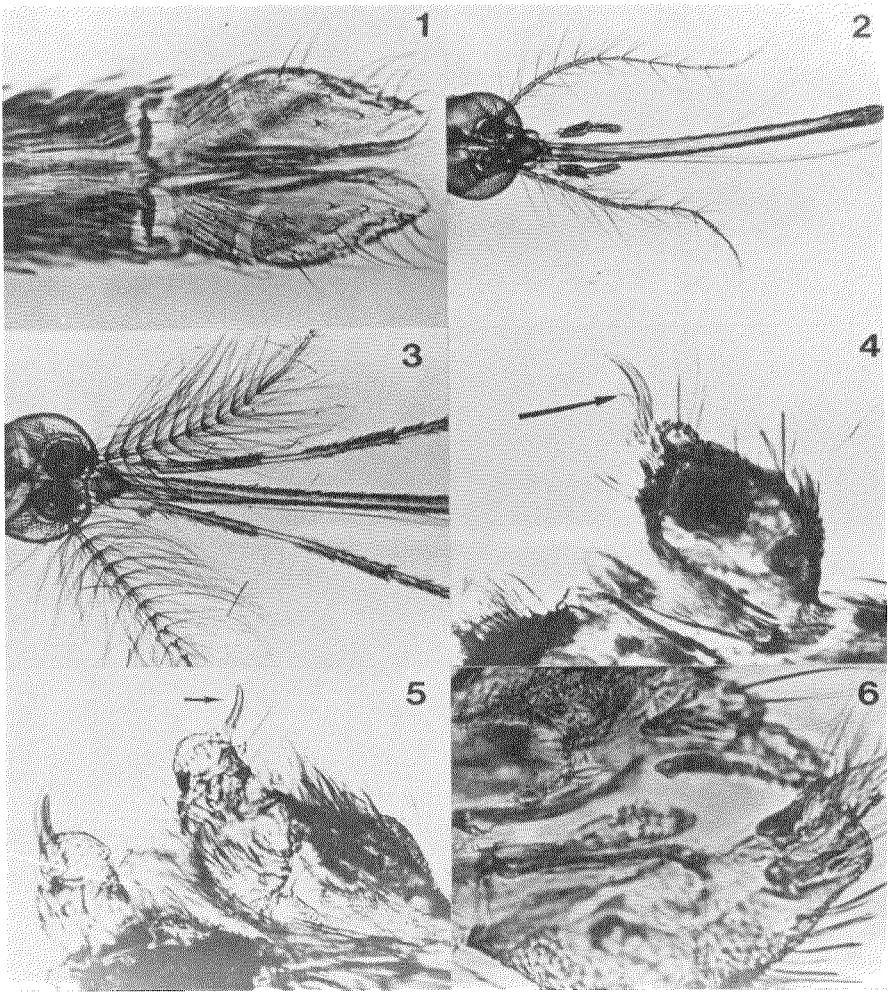

Plate I—Normal and proboscipedia *Aedes aegypti* (L.).

Fig. 1. Distal portion of the proboscis of a wild-type female *Aedes aegypti* (L.).
Fig. 2. Whole head of a wild-type female *Aedes aegypti* (L.).
Fig. 3. Whole head of a wild-type male *Aedes aegypti* (L.).
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. Distal portion of the proboscis of a proboscipedia male showing amorphous mass of tissue.

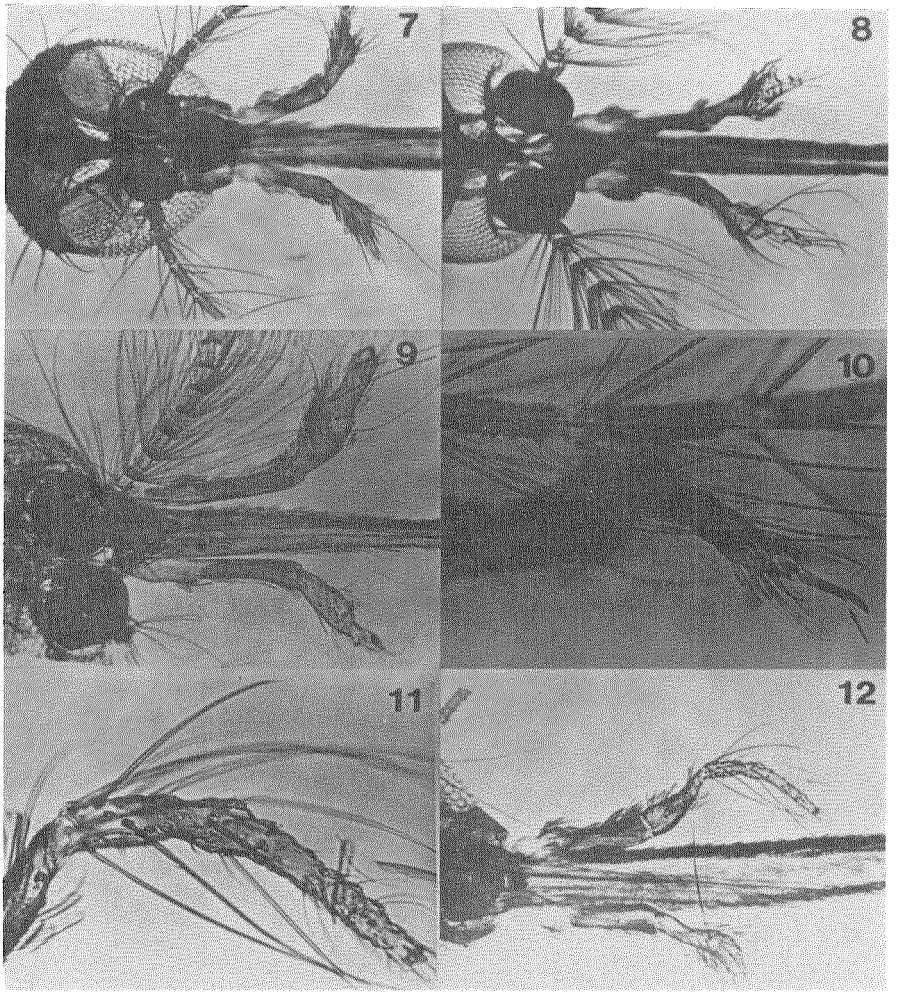

Plate II—Maxillary mutations in the proboscipedia phenotype of *Aedes aegypti* (L.).

Fig. 7. Head and maxillary palps of a proboscipedia female.
Fig. 8. Head and maxillary palps of a proboscipedia male.
Fig. 9. Head and maxillary palps of a proboscipedia male showing clubbed palps.
Fig. 10. Maxillary palp of a proboscipedia female showing tarsal segmentation complete with serrated claw (arrow).
Fig. 11. Maxillary palp of a female proboscipedia showing antenna-like segmentation complete with setae.
Fig. 12. Maxillary palps of a female proboscipedia. Note the nonsymmetrical nature of the palps.

temperature and 30°C; although, there was a highly significant difference between room temperature and 17°C (Table 2).

Proboscipedia males were mated to *re, ru, sma* females to establish linkage intensities with those genes on the first linkage group and to map the location of the

Table 2. The effect of temperature on the penetrance of *prb* in the *pe* population.

Temperature		++ ♀	*pe* ♀	*prb* ♀	++ ♂	*prb* ♂	Total No.	X^{2*}	p
17°C	No.	22	272	19	292	14	619	109.85	p≪.001
	% total	3.55	43.94	3.07	47.17	2.26			
26°C	No.	102	1353	26	1412	9	2902		
	% total	3.51	46.52	0.90	48.66	0.31			
30°C	No.	87	1060	15	1074	6	2242	2.81	.70>p>.50
	% total	3.88	47.28	0.67	47.90	0.27			

* Expected values based on room temperature (26°C).

Proboscipedia males were mated to RED-EYE females in single pair crosses to establish the linkage group for proboscipedia. Proboscipedia is a sex-linked recessive (designated *prb*) in linkage group 1 (Table 3). Chi-square analysis of these data indicates that there is a normal distribution of the three markers ($x^2 = 5.77$; $p < .70 > .50$); however, the results show that proboscipedia is on linkage group 1. The data further show that proboscipedia is a genetically controlled recessive in which there is nearly a 3:1 distribution of the wild-type to proboscipedia (x^2 = p 8.76; >.01). Additional analysis using the formula from Bhalla and Craig (1967) indicated that proboscipedia is near sex (*m*) (cross over value = 3.0 ± 1.21).

Table 3. F_2 progeny from the cross female RED-EYE x male proboscipedia.

Phenotypes	Female ++	Female *prb*	Male ++	Male *prb*
wild-type	58	11	71	38
red-eye (*re*)	48	0	3	0
spot abdomen (*s*)	20	2	28	7
black tarsi (*blt*)	21	1	27	9
red-eye, spot	11	0	1	0
red-eye, black tarsi	14	0	4	0
spot, black tarsi	4	0	4	5
re; s, blt	7	0	1	0

proboscipedia locus. There was wide variation between the F_2 progeny of the initial P_1 cross. The established linkage map for chromosome 1 (Fig. 13) and that obtained in this investigation using Immer's (1930) tables (Fig. 14) show some variation; however, the gene sequence is similar (Table 4).

Matings between proboscipedia males and members of the palp-extended population were made to further establish the significance of proboscipedia in the

Table 4. F_2 progeny, cross over values, and standard errors from the cross, *re, ru, sma* female x proboscipedia male.

Phenotypes	Female ++	Female *prb*	Male ++	Male *prb*
wild-type	110	4	180	20
red-eye (*re*)	11	0	7	0
rust-eye (*ru*)	7	0	6	0
small-antenna (*sma*)	31	4	47	13
red, rust-eye	33	0	13	0
red-eye, small-antenna	16	1	6	0
rust-eye, small-antenna	4	0	5	0
re, ru, sma	60	0	7	0

Cross over values and S.E.			
re—prb	17.0±3.98	re—ru	9.5±1.38
ru—prb	18.0±3.97	m—prb	6.0±1.46
sma—prb	56.0±2.89	re—m	22.0±2.36
re—sma	30.0±2.36	ru—m	20.0±2.31
ru—sma	32.5±2.46	sma—m	51.0±2.87

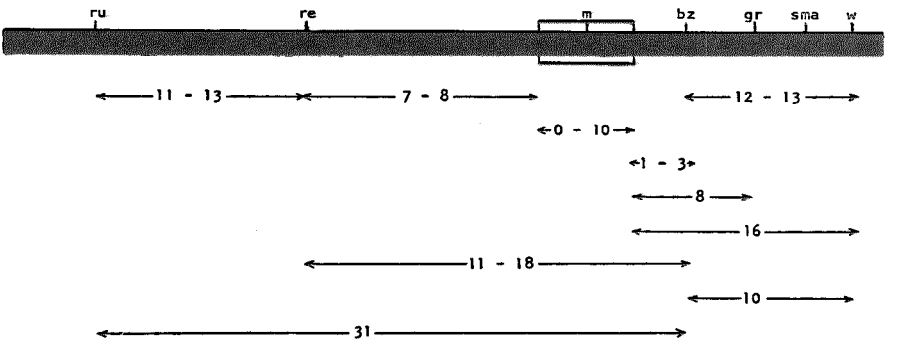

Fig. 13. A tenative linkage map of chromosome 1 of *Aedes aegypti* (Bhalla and Craig, 1970).

palp-extended population. Analysis of crosses between proboscipedia males and females of the palp-extended population indicate a dosage system involving genes *pe* and *prb* in producing the phenotypic results (Table 5). All of the genotypes may not be present in the population but all possible combinations are included. Results of the crosses, as well as the probable genotypes of the parents are given in Tables 6–11.

DISCUSSION

In the early embryonic development of higher insects, two classes of cells are formed. In the first class, differentiation begins at cleavage. These cells give rise to the larval body and its organs. The second class of cells form imaginal discs (imaginal buds in the Culicidae). These remain in the embryonic state throughout most of the larval period. During

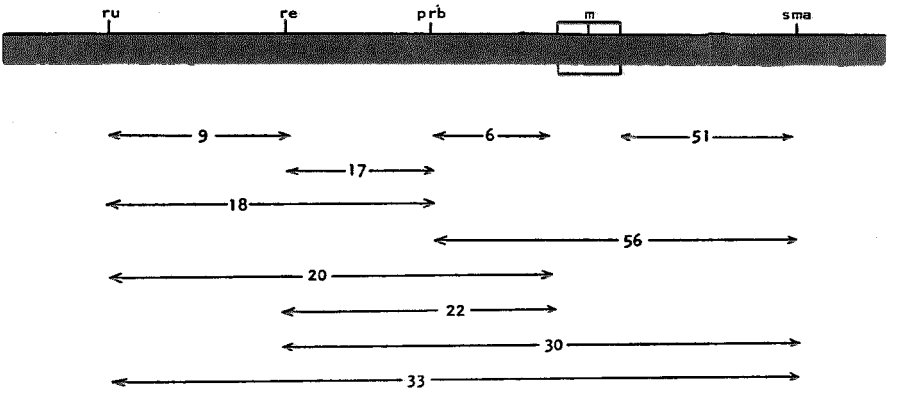

Fig. 14. Linkage map of chromosome 1 of *Aedes aegypti* obtained in this investigation.

metamorphosis from the larva to the pupa, autolysis occurs and the products are used by the mitotically dividing cells of the imaginal discs. These lose their embryonic character and differentiate into adult structures.

Each imaginal disc develops at a different time. In a homeotic mutant, two discs are turned on at once, resulting in two structures with the same basic pattern of differentiation (Villee 1943, 1944, 1945). Hadorn (1968) has been able to produce homeotic effects in disc tissue that has already been determined (a phenocopy). This theory, trans-determination, places primary emphasis for formation of the homeotic structures on the increased rate of proliferation that a previously determined disc area will undergo. Therefore, rate of proliferation is important in homeosis and transdetermination; although, transdetermination occurs in a non-mutated system while homeosis occurs in a system with changes in the genetic material.

Table 5. Proposed dosage system for *Aedes aegypti* in respect to the two mutants—palp-extended (*pe*) and proboscipedia (*prb*).

Phenotype	Genotype	
proboscipedia male	prb pe M / prb pe m	prb pe M / prb + m
	prb + M / prb pe m	prb pe M / + pe m
	+ pe M / prb pe m	prb + M / prb + m
wild-type male	+ pe M / + pe m	prb pe M / + + m
	+ + M / prb pe m	+ pe M / + + m
	+ pe M / prb + m	+ + M / + pe m
	prb + M / + + m	+ + M / prb + m
	prb + M / + pe m	+ + M / + + m
proboscipedia female	prb pe m / prb pe m	prb pe m / prb + m
palp-extended female	prb pe m / + pe m	+ pe m / + pe m
	prb + m / prb + m	prb + m / + pe m
wild-type female	+ + m / + + m	prb pe m / + + m
	+ pe m / + + m	prb + m / + + m

Each of the two mutants, palp-extended (*pe*) and proboscipedia (*prb*), is sex-linked. Recombination between proboscipedia (*prb*), sex (*m*), and the markers *re, ru, sma* on linkage group 1 was determined. Due to the inability to get a female mutant to feed, reciprocal crosses were not possible. The resulting values for linkages between *prb* and *m* were calculated to be 6.0 ± 1.46 chromosomal units using F_2 progeny. In-

Table 6. Matings between proboscipedia males and females of the palp-extended population.

palp-extended female x proboscipedia male = P_1

progeny = 0 wild-type females
49 *pe* females
40 *prb* females
30 wild-type males
30 *prb* males

Proposed genotype P_1:

prb + m / + pe m x prb + M / prb pe m

Proposed genotypes of the progeny:

+ pe m / prb pe m	palp-extended female
prb + m / prb pe m	proboscipedia female
prb + M / + pe m	wild-type male
prb + M / prb + m	proboscipedia male

terestingly, Bat-Miriam and Craig (1966) and Quinn and Craig (1971) working with proboscipedia in *Ae. albopictus* placed the proboscipedia locus about 20 units to the left of sex on linkage group 1. This would indicate that there is some degree of difference as well as genetic homology between chromosome 1 of *Ae. aegypti* and *Ae. albopictus*.

Penetrance in the homozygote is near 70%, although there is no penetrance in the heterozygote. Expressivity is variable depending in part on environmental conditions.

The most conspicuous phenotypic effect of proboscipedia is formation of tarsal segments and tarsal claws in place of the labella. The claws are of the same structure as the claws found on the fore- and mid-legs of the adult *Ae. aegypti*. This similarity in structure of either the first or second leg may also influence the formation of the aberrant tarsi on the proboscis. The developmental system of the labella in proboscipedia has been changed to that of tarsi.

The alteration of the maxillary palps by proboscipedia is more difficult to understand. Instead of long palps in the male and short palps in the female, the normal 5-segmented palp is reduced to 3–4 segments of similar structure in both sexes. The last segment is usually clubbed and extends laterally from the proboscis. In extreme cases, the palps are converted into antenna-like structures and even into tarsal-like structures complete with claws.

Table 7. Matings between proboscipedia males and females of the palp-extended population.

wild-type female from *pe* population × proboscipedia male = P_1
progeny = 38 wild-type females
34 palp-extended females
6 proboscipedia females
76 wild-type males
4 proboscipedia males

Proposed genotype P_1:

$$\frac{+\ +\ m}{prb\ pe\ m} \times \frac{prb\ pe\ M}{prb\ pe\ m}$$

Proposed genotypes of the progeny:

N.C.O.*	$\frac{+\ +\ m}{prb\ pe\ m}$	wild-type female
	$\frac{prb\ pe\ m}{prb\ pe\ m}$	proboscipedia female
	$\frac{prb\ pe\ M}{+\ +\ m}$	wild-type male
	$\frac{prb\ pe\ M}{prb\ pe\ m}$	proboscipedia male
C.O.* in the female P_1:	$\frac{prb\ pe\ m}{prb\ +\ m}$	proboscipedia female
	$\frac{+\ pe\ m}{prb\ pe\ m}$	palp-extended female
	$\frac{prb\ pe\ M}{prb\ +\ m}$	proboscipedia male
	$\frac{prb\ pe\ M}{+\ pe\ m}$	proboscipedia male

* N.C.O.=non-cross over, C.O.=cross over.

Table 8. Matings between proboscipedia males and females of the palp-extended population.

wild-type female from *pe* population × proboscipedia male = P_1
progeny = 206 wild-type females
211 palp-extended females
0 proboscipedia females
497 wild-type males
1 proboscipedia male

Proposed genotype P_1:

$$\frac{+\ pe\ m}{+\ +\ m} \times \frac{prb\ +\ M}{prb\ pe\ m}$$

Proposed genotypes of the progeny:

N.C.O.	$\frac{+\ pe\ m}{prb\ pe\ m}$	palp-extended female
	$\frac{+\ +\ m}{prb\ pe\ m}$	wild-type female
	$\frac{prb\ +\ M}{+\ pe\ m}$	wild-type male
	$\frac{prb\ +\ M}{+\ +\ m}$	wild-type male
C.O. in the male P_1:	$\frac{+\ pe\ m}{prb\ +\ m}$	palp-extended female
	$\frac{+\ +\ m}{prb\ +\ m}$	wild-type female
	$\frac{prb\ pe\ M}{+\ +\ m}$	wild-type male
	$\frac{prb\ pe\ M}{+\ pe\ m}$	proboscipedia male

In proboscipedia, the modification of the labella might be explained by proximity to the next developing bud (Christophers 1960) but this explanation is not valid for the maxillary palps.

Homeotic mutations affecting two different segments are not common. Only three examples were found: (1) proboscipedia in *Ae. aegypti*, (2) proboscipedia in *Ae. albopictus*, and (3) proboscipedia in *Drosophila melanogaster*. In *D. melanogaster*, the oral lobes are changed into a labium which resembles a pair of antenna-like or tarsal-like structures (appendages). In addition, the labrum and maxillae are modified to resemble biting mouthparts in the lower insects (Villee 1944). The mutant proboscipedia described in this work for *Ae. aegypti* is essentially phenotypically identical to the mutant proboscipedia in *Ae. albopictus* (Bat-Miriam and Craig 1966, Quinn and Craig 1971).

There are several factors in proboscipedia and other homeotic mutants which differ from certain other classes of mutants. First, the phenotype is variable, from slightly noticeable to extreme expression. Second, the degree of symmetry varies greatly; individuals with complete symmetry are rare (Ville 1942, Quinn and Craig 1971).

Temperature sensitivity is also characteristic of most homeotic mutants. In the present work, lowered rearing temperatures increased penetrance as well as expressivity. Different homeotic mutants of *Drosophila* show different responses to

Table 9. Matings between proboscipedia males and females of the palp-extended population.

F_1 *pe* female (++ (*pe*) female × proboscipedia male) × proboscipedia male

progeny = 3 wild-type females
37 palp-extended females
32 proboscipedia females
41 wild-type males
61 proboscipedia males

Proposed genotype of the F_1 *pe* females × proboscipedia male:

$$\frac{prb\ +\ m}{+\ pe\ m} \times \frac{prb\ +\ M}{prb\ pe\ m}$$

Proposed genotypes of the progeny:

N.C.O	$\frac{prb\ pe\ m}{prb\ +\ m}$	proboscipedia female
	$\frac{prb\ pe\ m}{+\ pe\ m}$	palp-extended female
	$\frac{prb\ +\ M}{prb\ +\ m}$	proboscipedia male
	$\frac{prb\ +\ M}{+\ pe\ m}$	wild-type male
C.O. in the female parent	$\frac{+\ +\ m}{prb\ +\ m}$	wild-type female
	$\frac{prb\ pe\ m}{prb\ pe\ m}$	proboscipedia female
	$\frac{prb\ +\ M}{+\ +\ m}$	wild-type male
	$\frac{prb\ +\ M}{prb\ pe\ m}$	proboscipedia male

Table 10. Matings between proboscipedia males and females of the palp-extended population.

F_1 *pe* female × F_1 ++ male from P_1 = ++ (*pe*) female × proboscipedia male

Progeny = 11 wild-type females
279 palp-extended females
28 proboscipedia females
295 wild-type males
97 proboscipedia males

Proposed genotypes of the F_1:

$$\frac{+\ pe\ m}{prb\ pe\ m} \times \frac{prb\ +\ M}{+\ pe\ m}$$

Proposed genotypes of the progeny:

N.C.O.	$\frac{+\ pe\ m}{+\ pe\ m}$	palp-extended female
	$\frac{prb\ pe\ m}{+\ pe\ m}$	palp-extended female
	$\frac{prb\ +\ M}{+\ pe\ m}$	wild-type male
	$\frac{prb\ +\ M}{prb\ pe\ m}$	proboscipedia male
C.O. in the male F_1:	$\frac{+\ pe\ m}{+\ +\ m}$	wild-type female
	$\frac{prb\ pe\ m}{prb\ pe\ m}$	proboscipedia female

Others are possible due to male determining factor.

temperature shock (Villee, 1943, 1944). Starvation may also increase penetrance and expressivity by changing developmental velocities. All evidence indicates a direct relationship between homeosis and developmental rates.

Proboscipedia individuals were produced in the second generation of some of the isolated palp-extended individuals; proboscipedia composed about 10% of the palp-extended population in June 1970 (Hartberg 1975). In maintaining the palp-extended population to obtain proboscipedia individuals, the percentage had dropped to about 3% by June 1972. Since proboscipedia is an essentially lethal mutant in respect to ecdysis of adults and also to female sterility, it is selected against. The following formula from Burns (1972) was used to calculate the number of generations required for such a drop:

$$n = 1/qn - 1/qo$$
$$n = 1/.03 - 1/.10$$
$$n = 33 - 10$$
$$n = 23 \text{ generations}$$

Assuming 1 month per generation in *Ae. aegypti* and overlapping generations in the population cage this value appears realistic.

Table 11. Matings between proboscipedia males and females of the palp-extended population.

F_1 wild-type female × F_1 wild-type male P_1 = ++ (*pe*) female × *prb* male
progeny = 30 wild-type females
45 palp-extended females
12 proboscipedia females
95 wild-type males
28 proboscipedia males

Proposed genotype of the F_1:

$$\frac{+\ +\ m}{prb\ pe\ m} \times \frac{prb\ +\ M}{+\ pe\ m}$$

Proposed genotypes of the progeny:

N.C.O.	$\frac{+\ +\ m}{+\ pe\ m}$	wild-type female
	$\frac{prb\ pe\ m}{+\ pe\ m}$	palp-extended female
	$\frac{prb\ +\ M}{prb\ pe\ m}$	proboscipedia male
	$\frac{prb\ +\ M}{+\ +\ m}$	wild-type male
C.O. the male F_1:	$\frac{+\ +\ m}{prb\ pe\ m}$	wild-type female
	$\frac{prb\ pe\ m}{prb\ pe\ m}$	proboscipedia female
	$\frac{+\ +\ M}{+\ +\ m}$	wild-type male
	$\frac{+\ +\ M}{prb\ pe\ m}$	wild-type male

Others are possible due to male determining factor.

Variability in linkage distances have been reported from different laboratories, and in different experiments in the same laboratory (Craig and Hickey 1967, Bhalla and Craig 1970). Crossing over occurs in both sexes at similar rates, although there are small differences between the two sexes (McClelland 1962a). Craig (1965) and O'Meara and Craig (1967) reported that sex and age influence cross over rate in the different linkage groups. In females, initial rates are relatively high but decline rapidly with age to the level characteristics of males. Also high temperatures have a direct effect on cells in meiosis which would have a definite effect on crossing over rates. Specific distances are less important than gene sequence.

Craig and Hickey (1967) suggested 6 systems to follow for mapping. Due to female sterility many of these were impossible to adhere to, especially those dealing with F_2 progeny in which a repulsion phase is involved; also, heterozygous males could not be used. All of the above could lead to and cause the variability in linkage data reported here. In comparing linkage intensities between those established by Bhalla and Craig (1970) and those of this work, it is quite clear that the data gathered from the coupling phases fit more closely with the established map distances than data gathered from the repulsion phases.

The phenotypic characters of the progeny fit well into the projected dosage system in many of the crosses between proboscipedia males and females of the palp-extended population. Others are not exact fits but the presence of a crossover suppressor—enhancer system (Bhalla 1971) would explain the results projected by the dosage hypothesis. This problem cannot be resolved until a method is found to provide the proboscipedia female of *Ae. aegypti* with a blood meal sufficient to induce egg production.

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