Proboscipedia, A Homeotic Mutant in *Aedes aegypti* (L.) (Diptera: Culicidae)

John Roach Roberts Jr.
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by

John Roach Roberts, Jr.

A thesis submitted to the faculty of Georgia Southern College in partial fulfillment of the requirements for the degree of Master of Science in Biology

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Approved by:

Graduate Dean

Advisor

Member of Thesis Committee

Member of Thesis Committee
ACKNOWLEDGMENT

I am gratefully thankful to my advisor, Dr. W. Keith Hartberg, Assistant Professor of Biology. Without his advice, ideas, and encouragement this work would never have been possible. His assistance and constructive criticism in the preparation of this manuscript is deeply appreciated.

I am also indebted to the faculty of the Department of Biology and especially to my committee members for their suggestions and criticisms. In addition, I would like to thank my colleagues and friends for their help and assistance during the interim of this investigation. Finally, to my wife, Pamela, for her steadfastness and encouragement.
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INTRODUCTION

Great progress has been made in the study of mosquitoes since they were discovered to be vectors of such diseases as malaria, filariasis, yellow fever, and dengue. Until recently little attention has been given to the genetics of mosquitoes. Only during the past two decades have genetic investigations been extensively pursued. Kitzmiller (1963), Davidson and Mason (1963), and Wright and Pal (1967) present comprehensive reviews of the subject.

_Aedes aegypti_ (Linnaeus) is a tropicolitan species, found associated with man throughout the warmer regions of the world. It is euryoecious in respect to habitats and possesses remarkable genetic variability. It has been used extensively in laboratory investigations and a great deal is known about its physiology, bionomics, and genetics. Formal genetics of _A. aegypti_ has been developed with nearly 100 morphological mutants presently isolated. Linkage maps have been constructed and linkage relationships of approximately one-third of the mutants have been determined. Such work is of value to develop a better understanding of mosquitoes and to general genetic concepts.

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
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 *Aedes 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.

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

The present study was undertaken to determine if proboscipedia in *A. aegypti* was an extreme expression of the gene *pe* or a separate but closely linked gene. A palp-extended mutation has not been reported in *A. albopictus*. 
MATERIALS AND METHODS

Strains:

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

Basic Rearing Methods:

Rearing methods were generally similar to those described by Craig and VandeHey (1962). Aedes aegypti was reared at 26 ± 3°C and ambient humidity.

Larvae were reared in white enamel pans (35cm x 25cm x 5cm) covered by clear plexiglass sheets. Each larva was given no less than one cm² of surface area of water. Larvae were fed on a solution of 10cc of Liver Powder N.F. (Nutritional Biochemicals Corporation) in a liter of distilled water. When freshly hatched, larvae were given 60ml of the liver powder solution with an additional 60ml added on or about the third day after hatching. Almost all pupation occurred on day 6 - 7 after hatching except in the cool rearing segments of this investigation. Pupae were segregated according to sex (females being somewhat larger than males).

The segregated pupae were placed in pint paper cups (8.5cm h x 9cm d) containing water soaked cotton covered with brown paper toweling. Each pupation cup was covered
Table 1. Strains of *Aedes aegypti* (L.) used in this investigation.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Strain composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>PALP-EXTENDED</td>
<td>Palp-extended, sex-linked and sex-limited expression. Established from a mutant female collected in Dar es Salaam, Tanzania in 1970 by Dr. W. K. Hartberg. Strain maintained at the Mosquito Genetics Laboratory, Georgia Southern College.</td>
</tr>
<tr>
<td>RED EYE</td>
<td>Multiple marker strain constructed from a synthesis of several strains including GANDA, TRINIDAD, and NEW X. Contains markers - I: sex, red eye; II: spot abdomen, yellow larvae; III: black tarsi. Strain maintained at Vector Biology Laboratory, University of Notre Dame.</td>
</tr>
<tr>
<td>RED, RUST, SMALL ANTENNAE</td>
<td>Mutants red, rust, small antennae. Linkage group I. (sex-linked) Reconstituted after outcrossing. Strain maintained at Vector Biology Laboratory, University of Notre Dame.</td>
</tr>
</tbody>
</table>
with a wax coated cardboard chimney having a lid of fine nylon netting. Forty to eighty pupae were placed in each container. Adults emerged about two days after pupation and were allowed to age for two more days before sex was rechecked to insure adult virginity. Aged adults were better able to survive etherization than newly emerged adults. All matings were conducted with virgin mosquitoes at least four days old. Adults were fed on dry sugar cubes except in the case of the proboscipedia mutants which were maintained on a plug of cotton soaked with a 20% sucrose solution.

Cages were made from gallon-sized cardboard containers (18cm h x 18cm d) covered with fine nylon netting. Plastic shell vials (8cm h x 1.5cm d) with water soaked cotton plugs in the bottom were used for the single pair matings. They were also covered with fine nylon netting.

To obtain eggs, normal females at least 5 days old were provided with a blood meal from an anesthetized mouse. Oviposition occurred about 4 - 5 days after the blood meal.

Eggs were deposited on moist brown paper toweling in a 250 ml beaker 3/4 filled with water. When the oviposition beakers were removed from the cages the water was drained off and the paper with the eggs was allowed to dry slowly at 23±3°C for 48 - 72 hours. The egg papers were dried, stored, and always kept covered with fine nylon netting to prevent accidental oviposition by escaped females.
Completely dried egg papers were stored inside desiccators at 85% R.H. Under these conditions eggs can remain viable for 6 months, although most eggs were hatched within 3 months.

Deoxygenated water (boiled deionized - distilled water at room temperature) was used as a hatching stimulus. Egg papers were placed in one liter of this water for 24 hours and then removed.

Special Rearing Methods:

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.

Technique for Clearing Adult Mosquitoes:

The following materials were used to prepare cleared specimens of adult mosquitoes for microscopic examination:

- 10% solution KOH
  
  (10g KOH in 90ml distilled H₂O)
- Distilled water
- 75% and 95% EtOH
- Clove oil
- Euparal (vert)
Specimens to be cleared were anesthetized with ether and transferred to the KOH solution for 40 minutes at 50°C. Next, the mosquitoes were transferred to distilled water for two hours at 50°C. They were then transferred to 75% and 95% alcohol at room temperature, remaining in the 75% for one hour and in the 95% for 30 minutes. The specimens were finally cleared in clove oil for at least 24 hours at room temperature. The cleared specimens were mounted on slides in euparal (vert), covered with a glass coverslip, and allowed to dry at 50°C for 24 hours. The slides could not be safely placed in a vertical position for at least 5 - 6 days but could be used after the 24 hour period.

Crossing Procedures:

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 x proboscipedia; F_2 progeny used for analysis.

B. Red, rust, small-antenna (re, ru, sma) x proboscipedia; F_2 progeny used for analysis.

C. Palp-extended x proboscipedia; F_1 progeny used for analysis.

D. Wild-type from palp-extended population x proboscipedia; F_1 progeny used for analysis.
E. \( F_1 \) palp-extended from "D" above x proboscipedia; \( F_1 \) progeny used for analysis.

F. \( F_1 \) palp-extended from "D" above x \( F_1 \) wild-type from "D" above; \( F_1 \) progeny used for analysis.

G. \( F_1 \) wild-type from "D" above x \( F_1 \) wild-type from "D" above; \( F_1 \) progeny used for analysis.

Observational Procedures:

Slides of the mutants were observed at 40, 100 and 400 magnifications. Micrographs were made with a 35mm SLR camera back with a microscope adapter on the trinocular head. Individuals used for genetic analysis were etherized and observed with a dissecting microscope.

Calculation of Linkage Intensities:

\( F_2 \) 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 for this data were calculated by a method developed by Serra (1965).

Special Methods:

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

Morphology:

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 prothorasic and mesothorasic 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
12.
Figure 1. Distal portion of the proboscis of a wild-type female *Aedes aegypti* (L.).

Figure 2. Whole head of a wild-type female *Aedes aegypti* (L.).
Figure 3. Whole head of a wild-type male *Aedes aegypti* (L.).

Figure 4. Distal portion of the proboscis of a proboscipedia female. Note serrated claw (arrow).
Figure 5. Distal portion of the proboscis of a proboscipedia male. Note unserrated claw (arrow).

Figure 6. Distal portion of the proboscis of a proboscipedia male showing amorphous mass of tissue.
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 lacinae 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 A. aegypti produces as severe an expression as the similar mutation in A. albopictus.
Figure 7. Head and maxillary palps of a proboscipedia female.

Figure 8. Head and maxillary palps of a proboscipedia male.
Figure 9. Head and maxillary palps of a proboscipedia male showing clubbed palps.

Figure 10. Maxillary palp of a female proboscipedia showing tarsal segmentation complete with serrated claw (arrow).
Figure 11. Maxillary palp of a female proboscipedia showing antenna-like segmentation complete with setae.
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).

Temperature Effects:

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

Linkage Relationships:

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 this data indicates that there is a normal
Figure 12. Maxillary palps of a female proboscipedia
Note the non-symmetrical nature of the palps.
Table 2. The effect of temperature on the penetrance of prb in the pe population.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>++♀</th>
<th>pe♀</th>
<th>prb♀</th>
<th>++♂</th>
<th>prb♂</th>
<th>Total No.</th>
<th>x²*</th>
<th>p @ .05</th>
</tr>
</thead>
<tbody>
<tr>
<td>17°C</td>
<td>22</td>
<td>272</td>
<td>19</td>
<td>292</td>
<td>14</td>
<td>619</td>
<td>109.85</td>
<td>p &lt;&lt; .001</td>
</tr>
<tr>
<td></td>
<td>% total</td>
<td>3.55</td>
<td>43.94</td>
<td>3.07</td>
<td>47.17</td>
<td>2.26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>26°C</td>
<td>102</td>
<td>1353</td>
<td>26</td>
<td>1412</td>
<td>9</td>
<td>2902</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>% total</td>
<td>3.51</td>
<td>46.62</td>
<td>0.90</td>
<td>48.66</td>
<td>0.31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30°C</td>
<td>87</td>
<td>1060</td>
<td>15</td>
<td>1074</td>
<td>6</td>
<td>2242</td>
<td>2.81</td>
<td>.70 &gt; p &gt; .50</td>
</tr>
<tr>
<td></td>
<td>% total</td>
<td>3.88</td>
<td>47.28</td>
<td>0.67</td>
<td>47.90</td>
<td>0.27</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Expected values based on room temperature (26°C).
Table 3. F$_2$ progeny from the cross female RED-EYE x male proboscipedia

<table>
<thead>
<tr>
<th>PHENOTYPES</th>
<th>FEMALE</th>
<th></th>
<th></th>
<th>MALE</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>++</td>
<td>prb</td>
<td>++</td>
<td>prb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>wild-type</td>
<td>58</td>
<td>11</td>
<td>71</td>
<td>38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>red-eye (re)</td>
<td>48</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>spot abdomen (s)</td>
<td>20</td>
<td>2</td>
<td>28</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>black tarsi (blt)</td>
<td>21</td>
<td>1</td>
<td>27</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>red-eye; spot</td>
<td>11</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>red-eye; black tarsi</td>
<td>14</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>spot; black tarsi</td>
<td>4</td>
<td>0</td>
<td>4</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>re; s; blt</td>
<td>7</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
distribution of the three markers \( x^2 = 5.77; \rho < 0.70 > 0.50 \); 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 \( x^2 = 8.76; \rho > 0.01 \). Additional analysis using the formula from Bhalla and Craig (1967) indicated that proboscipedia is near sex \( (m) \) (cross over value = 3.0 ± 1.21).

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 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 established by 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 established the significance of proboscipedia in the 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.
Figure 13. A tentative linkage map of chromosome 1 of Aedes aegypti (Bhalla and Craig, 1970).
Figure 14. A tentative map of linkage group 1 \textit{Aedes aegypti}.
Table 4. \( F_2 \) progeny, cross over values, and standard errors from the cross \textit{re}, \textit{ru}, \textit{sma} female x proboscipedia male.

<table>
<thead>
<tr>
<th>PHENOTYPES</th>
<th>FEMALE</th>
<th></th>
<th>MALE</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>++</td>
<td>prb</td>
<td>++</td>
<td>prb</td>
</tr>
<tr>
<td>wild-type</td>
<td>110</td>
<td>4</td>
<td>180</td>
<td>20</td>
</tr>
<tr>
<td>red-eye (\textit{re})</td>
<td>11</td>
<td>0</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>rust-eye (\textit{ru})</td>
<td>7</td>
<td>0</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>small-antenna (\textit{sma})</td>
<td>31</td>
<td>4</td>
<td>47</td>
<td>13</td>
</tr>
<tr>
<td>red, rust-eye</td>
<td>33</td>
<td>0</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>red-eye, small-antenna</td>
<td>16</td>
<td>1</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>rust-eye, small-antenna</td>
<td>4</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>\textit{re}, \textit{ru}, \textit{sma}</td>
<td>60</td>
<td>0</td>
<td>7</td>
<td>0</td>
</tr>
</tbody>
</table>

Cross over values and S.E.

<table>
<thead>
<tr>
<th></th>
<th>FEMALE</th>
<th>MALE</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{re} - prb</td>
<td>17.0 ± 3.98</td>
<td>re - ru</td>
</tr>
<tr>
<td>\textit{ru} - prb</td>
<td>18.0 ± 3.97</td>
<td>m - prb</td>
</tr>
<tr>
<td>\textit{sma} - prb</td>
<td>56.0 ± 2.89</td>
<td>re - m</td>
</tr>
<tr>
<td>\textit{re} - \textit{sma}</td>
<td>30.0 ± 2.36</td>
<td>ru - m</td>
</tr>
<tr>
<td>\textit{ru} - \textit{sma}</td>
<td>32.5 ± 2.46</td>
<td>\textit{sma} - m</td>
</tr>
</tbody>
</table>
Table 5. Proposed dosage system for *Aedes aegypti* in respect to the two mutants - palp-extended (pe) and proboscipedia (prb).

<table>
<thead>
<tr>
<th>PHENOTYPE</th>
<th>GENOTYPE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>proboscipedia male</strong></td>
<td><strong>prb</strong>  pe  <strong>M</strong> ;  <strong>prb</strong>  pe  <strong>M</strong></td>
</tr>
<tr>
<td></td>
<td><strong>prb</strong>  pe  <strong>m</strong> ;  <strong>prb</strong>  +  <strong>m</strong></td>
</tr>
<tr>
<td></td>
<td><strong>prb</strong>  +  <strong>M</strong> ;  <strong>prb</strong>  pe  <strong>M</strong></td>
</tr>
<tr>
<td></td>
<td><strong>prb</strong>  pe  <strong>m</strong> ;  +  pe  <strong>m</strong></td>
</tr>
<tr>
<td></td>
<td>+  pe  <strong>M</strong> ;  <strong>prb</strong>  +  <strong>M</strong></td>
</tr>
<tr>
<td></td>
<td><strong>prb</strong>  pe  <strong>m</strong> ;  <strong>prb</strong>  +  <strong>m</strong></td>
</tr>
<tr>
<td><strong>wild-type male</strong></td>
<td><strong>+</strong>  pe  <strong>M</strong> ;  <strong>prb</strong>  pe  <strong>M</strong></td>
</tr>
<tr>
<td></td>
<td><strong>+</strong>  pe  <strong>m</strong> ;  +  +  <strong>m</strong></td>
</tr>
<tr>
<td></td>
<td><strong>+</strong>  +  <strong>M</strong> ;  +  pe  <strong>M</strong></td>
</tr>
<tr>
<td></td>
<td><strong>prb</strong>  pe  <strong>m</strong> ;  +  +  <strong>m</strong></td>
</tr>
<tr>
<td></td>
<td><strong>+</strong>  pe  <strong>M</strong> ;  +  +  <strong>M</strong></td>
</tr>
<tr>
<td></td>
<td><strong>prb</strong>  +  <strong>M</strong> ;  +  +  <strong>M</strong></td>
</tr>
<tr>
<td></td>
<td><strong>+</strong>  +  <strong>m</strong> ;  <strong>prb</strong>  +  <strong>m</strong></td>
</tr>
<tr>
<td></td>
<td><strong>+</strong>  +  <strong>M</strong> ;  +  +  <strong>M</strong></td>
</tr>
<tr>
<td><strong>proboscipedia female</strong></td>
<td><strong>prb</strong>  pe  <strong>m</strong> ;  <strong>prb</strong>  pe  <strong>m</strong></td>
</tr>
<tr>
<td><strong>palp-extended female</strong></td>
<td><strong>prb</strong>  pe  <strong>m</strong> ;  <strong>+</strong>  pe  <strong>m</strong></td>
</tr>
<tr>
<td></td>
<td><strong>prb</strong>  +  <strong>M</strong> ;  <strong>prb</strong>  +  <strong>m</strong></td>
</tr>
<tr>
<td></td>
<td><strong>prb</strong>  +  <strong>m</strong> ;  <strong>prb</strong>  +  <strong>pe</strong>  <strong>m</strong></td>
</tr>
<tr>
<td><strong>wild-type female</strong></td>
<td><strong>+</strong>  +  <strong>m</strong> ;  <strong>prb</strong>  pe  <strong>m</strong></td>
</tr>
<tr>
<td></td>
<td><strong>+</strong>  +  <strong>m</strong> ;  +  +  <strong>m</strong></td>
</tr>
<tr>
<td></td>
<td>+  pe  <strong>m</strong> ;  <strong>prb</strong>  +  <strong>m</strong></td>
</tr>
<tr>
<td></td>
<td>+  +  <strong>m</strong> ;  +  +  <strong>m</strong></td>
</tr>
</tbody>
</table>
Table 6. Matings between proboscipedia males and females of the palp-extended population.

palp-extended female x proboscipedia male = P₁

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

Proposed genotype P₁:

\[
\begin{array}{ccc}
\text{pe} & \text{m} \\
\text{prb} & \text{pe} & \text{M} \\
\text{prb} & \text{pe} & \text{m}
\end{array}
\]

Proposed genotypes of the progeny:

\[
\begin{array}{ccc}
\text{pe} & \text{m} \\
\text{prb} & \text{pe} & \text{m}
\end{array}
\text{ palp-extended female}
\]

\[
\begin{array}{ccc}
\text{prb} & \text{pe} & \text{m}
\end{array}
\text{ proboscipedia female}
\]

\[
\begin{array}{ccc}
\text{prb} & \text{pe} & \text{M} \\
\text{pe} & \text{m}
\end{array}
\text{ wild-type male}
\]

\[
\begin{array}{ccc}
\text{prb} & \text{pe} & \text{M} \\
\text{prb} & \text{pe} & \text{m}
\end{array}
\text{ proboscipedia male}
\]
Table 7. Matings between proboscipedia males and females of the palp-extended population.

wild-type female from pe population x 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 $P_1$:

\[
\begin{array}{ccc}
+ & + & m \\
prb & pe & m \\
\end{array} \times \begin{array}{ccc}
prb & pe & M \\
prb & pe & m \\
\end{array}
\]

Proposed genotypes of the progeny:

N.C.O.

\[
\begin{array}{ccc}
+ & + & m \\
prb & pe & m \\
\end{array} \text{ wild-type female}
\]

\[
\begin{array}{ccc}
prb & pe & m \\
prb & pe & m \\
\end{array} \text{ proboscipedia female}
\]

\[
\begin{array}{ccc}
prb & pe & M \\
+ & + & m \\
\end{array} \text{ wild-type male}
\]

\[
\begin{array}{ccc}
prb & pe & M \\
prb & pe & m \\
\end{array} \text{ proboscipedia male}
\]

C.O. in the female $P_1$:

\[
\begin{array}{ccc}
prb & pe & m \\
prb & + & m \\
\end{array} \text{ proboscipedia female}
\]

\[
\begin{array}{ccc}
+ & pe & m \\
prb & pe & m \\
\end{array} \text{ palp-extended female}
\]

\[
\begin{array}{ccc}
prb & pe & M \\
prb & + & m \\
\end{array} \text{ proboscipedia male}
\]

\[
\begin{array}{ccc}
prb & pe & M \\
+ & pe & m \\
\end{array} \text{ proboscipedia male}
\]
Table 8. Matings between proboscipedia males and females of the palp-extended population.

wild-type female from pe population x proboscipedia male = P₁

progeny = 206 wild-type females
211 palp-extended females
0 proboscipedia females
497 wild-type males
1 proboscipedia male

Proposed genotype P₁:

\[
\begin{array}{ccc}
+ & pe & m \\
\hline
prb & pe & m \\
\end{array} \times \begin{array}{ccc}
prb & + & M \\
\end{array}
\]

Proposed genotypes of the progeny:

N.C.O. \begin{array}{ccc} + & pe & m \\
\hline
prb & pe & m \\
\end{array} palp-extended female

\begin{array}{ccc} + & + & m \\
\hline
prb & pe & m \\
\end{array} wild-type female

\begin{array}{ccc} prb & + & M \\
\hline
+ & pe & m \\
\end{array} wild-type male

\begin{array}{ccc} prb & + & M \\
\hline
+ & + & m \\
\end{array} wild-type male

C.O. in the male P₁:

\begin{array}{ccc} + & pe & m \\
\hline
prb & + & m \\
\end{array} palp-extended female

\begin{array}{ccc} + & + & m \\
\hline
prb & + & m \\
\end{array} wild-type female

\begin{array}{ccc} prb & pe & M \\
\hline
+ & + & m \\
\end{array} wild-type male

\begin{array}{ccc} prb & pe & M \\
\hline
+ & pe & m \\
\end{array} proboscipedia male
Table 9. Matings between proboscipedia males and females of the palp-extended population.

F₁ pe female from ++(pe) x proboscipedia male x 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 F₁ pe females x proboscipedia male:

\[ \text{prb}^+ \text{m} \times \text{prb}^+ \text{M} \]

Proposed genotypes of the progeny:

N.C.O.  
\[ \begin{array}{c}
\text{prb pe m} \\
\text{prb}^+ \text{m} \\
\text{prb pe m} + \text{pe m}
\end{array} \]

proboscipedia female  
proboscipedia male

\[ \begin{array}{c}
\text{prb}^+ \text{M} \\
\text{prb}^+ \text{M} + \text{pe m}
\end{array} \]

palp-extended female  
wild-type male

C.O. in the female parent.  
\[ \begin{array}{c}
\text{prb pe m} \\
\text{prb}^+ \text{m} \\
\text{prb pe m}
\end{array} \]

wild-type female  
proboscipedia female

\[ \begin{array}{c}
\text{prb} + \text{M} \\
\text{prb} + \text{M}
\end{array} \]

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

$F_1$ pe female x $F_1$ ++ male from $F_1 = ++(pe)$ female x 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 $F_1$:

\[
\begin{align*}
\text{prb} & \quad \text{pe} & \quad m \\
\times & \quad \text{prb} & + & \quad M
\end{align*}
\]

Proposed genotypes of the progeny:

N.C.O.

\[
\begin{align*}
+ & \quad \text{pe} & \quad m \\
+ & \quad \text{pe} & \quad m \\
\text{prb} & \quad \text{pe} & \quad m \\
+ & \quad \text{pe} & \quad m \\
\text{prb} & + & \quad M \\
+ & \quad \text{pe} & \quad m \\
\text{prb} & + & \quad M \\
\text{prb} & \quad \text{pe} & \quad m \\
\end{align*}
\]

palp-extended female
palp-extended female
wild-type male
proboscipedia male

C.O. in the male $F_1$:

\[
\begin{align*}
+ & \quad \text{pe} & \quad m \\
+ & \quad + & \quad m \\
\text{prb} & \quad \text{pe} & \quad m \\
\text{prb} & \quad \text{pe} & \quad m \\
\end{align*}
\]

wild-type female
proboscipedia female

Others are possible due to male determining factor.
Table 11. Matings between proboscipedia males and females of the palp-extended population.

\[ \text{F}_1 \text{ wild-type female} \times \text{F}_1 \text{ wild-type male} \]

\[ \text{F}_1 = ++(\text{pe}) \text{ female} \times \text{prb male} \]

\[ \text{progeny} = 30 \text{ wild-type females} \]
\[ 45 \text{ palp-extended females} \]
\[ 12 \text{ proboscipedia females} \]
\[ 95 \text{ wild-type males} \]
\[ 28 \text{ proboscipedia males} \]

Proposed genotype of the \( \text{F}_1 \):

\[
\begin{array}{c}
+ + m \\
\text{prb pe m}
\end{array}
\quad \times \quad
\begin{array}{c}
\text{prb} + M \\
+ \text{pe m}
\end{array}
\]

Proposed genotypes of the progeny:

N.C.O.\*

\[
\begin{array}{c}
+ + m \\
\text{prb pe m}
\end{array}
\]

wild-type female

\[
\begin{array}{c}
+ \text{pe m}
\end{array}
\]

palp-extended female

\[
\begin{array}{c}
\text{prb} + M \\
\text{prb pe m}
\end{array}
\]

proboscipedia male

\[
\begin{array}{c}
\text{prb} + M \\
+ + m
\end{array}
\]

wild-type male

C.O.\* in the male \( \text{F}_1 \):

\[
\begin{array}{c}
+ + m \\
\text{prb pe m}
\end{array}
\]

wild-type female

\[
\begin{array}{c}
\text{prb pe m}
\end{array}
\]

proboscipedia female

\[
\begin{array}{c}
+ + M \\
+ + m
\end{array}
\]

wild-type male

\[
\begin{array}{c}
+ + M \\
\text{prb pe m}
\end{array}
\]

wild-type male

Others are possible due to male determining factor.

* N.C.O. = non-cross over; C. O. = cross over
DISCUSSION

In the early embryonic development of higher insects, two classes of cells are formed. In the first class, differentiation begins at cleavage. These 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 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 (Ville, 1943, 1944, 1945). Hadorn (1968) has been able to produce homeotic effects in disc tissue that has already been determined (a phenocopy). This theory, transdetermination, 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.
In investigating the mode of inheritance of proboscipedia, it was shown that the mutation is a genetically controlled recessive in a dosage system in the palp-extended population. Moreover, 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 $6.0 \pm 1.46$ chromosomal units using $F_2$ progeny. Interestingly, Bat-Miriam and Craig (1966) and Quinn and Craig (1971) working with proboscipedia in A. 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 A. aegypti and A. 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 A. 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 coverted into antenna-like structures and even into tarsal-like structures complete with claws. 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 A. aegypti, (2) proboscipedia in A. 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 A. aegypti is essentially phenotypically identical to the mutant proboscipedia in A. 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 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, in press). 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 ecdisis 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:

\[ n = \frac{1}{q_n} - \frac{1}{q_0} \]

\[ n = 1/.03 - 1/.10 \]
\[ n = 33 - 10 \]
\[ n = 23 \text{ generations} \]

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

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 (1965b) 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 six systems to follow for mapping. Due to female sterility many of these were impossible to adhere to, especially those dealing with F2 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, 1972) would explain the results projected by the dosage hypothesis. This problem cannot be resolved until a method is found to provide the proboscipedia female with a blood meal sufficient to induce egg production.
LITERATURE CITED


41.


