1980

Linkage studies in Tribolium confusum Duval (Coleoptera, Tenebrionidae)

Halluma Mohamed Edongali

Follow this and additional works at: https://scholarworks.lib.csusb.edu/etd-project

Part of the Entomology Commons

Recommended Citation

https://scholarworks.lib.csusb.edu/etd-project/190

This Thesis is brought to you for free and open access by the John M. Pfau Library at CSUSB ScholarWorks. It has been accepted for inclusion in Theses Digitization Project by an authorized administrator of CSUSB ScholarWorks. For more information, please contact scholarworks@csusb.edu.
LINKAGE STUDIES IN *Tribolium confusum* Duval.
(COLEOPTERA, TENEBRIONIDAE)

A Thesis
Presented to the
Faculty of
California State
College, San Bernardino

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
in
Biology

by
Halluma Mohamed Edongali
August 1980
LINKAGE STUDIES IN *Tribolium confusum* Duval. 
(COLEOPTERA, TENEBRIONIDAE)

A Thesis
Presented to the
Faculty of
California State
College, San Bernardino

by
Halluma Mohamed Edongali
August 1980

Approved by:

Chairman, Biology Department
Graduate Committee

Committee Member

Committee Member

Major Professor

Representative of the Graduate Dean
ABSTRACT

Linkage relationships between an autosomal recessive, fused antennal segments (fas-2), and a new sex-linked dominant, Horned gena (Hg), were studied in Tribolium confusum Duval. Ebony-2 (e2) and pearl (p) were determined to be linked and about two units apart, confirming Dyte and Blackman's (1962) frequency of recombination for e2 and p. However, fas-2 is either not on this linkage group or so far apart that backcrosses give recombination values similar to those obtained when genes are on different linkage groups. Numerous 2-point and 3-point crosses revealed that sex-linked markers red (r) and eye spot (es) and crumpled (cru) are not completely recessive, but that they overlap wild type in expression. The alate prothorax (apt), recessive semilethal, mutation occasionally may overlap wild type. The Horned gena (Hg) mutation is considered incompletely dominant because a high proportion of (Hg) beetles may resemble wild types.

Despite these shortcomings, it has been possible to establish the relative position of Hg in respect to apt and r or apt and es. The order of this gene is r - es - apt - Hg. The cru gene is tentatively placed between es and apt.

A revised map of the sex chromosome is given in Fig. 4.
ACKNOWLEDGEMENTS

I would like to express my special sincere appreciation and gratitude to my major professor and the director of the Tribolium Stock Center, Alexander Sokoloff, for his scientific guidance, assistance, encouragement, friendship and for supplying the stock and equipment to make this study possible.

I am also grateful to Dr. Dalton Harrington and Dr. Ruth C. Wilson of the Biology Department for their understanding and helpful suggestions during the preparation of the manuscript.

My thanks to the faculty and staff members of the Biology Department at the California State College San Bernardino and to Dr. Daryl Faustini for his technical assistance with the scanning electron microscope.

Special thanks and appreciation are expressed to my husband, Ezarug A. Edongali, for his encouragement and understanding; to my children, Sarah and Saife, for their love and inspiration. I would like to thank Mrs. Linda Bobo for her patience, kindness and perfect typing.

My special thanks and appreciation to my parents and my brother, Ramadan M. Kerra, for their support and encouragement throughout my education.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Introduction</td>
<td>1</td>
</tr>
<tr>
<td>II. Materials and Methods</td>
<td>5</td>
</tr>
<tr>
<td>III. Results</td>
<td>14</td>
</tr>
<tr>
<td>IV. Discussion</td>
<td>41</td>
</tr>
<tr>
<td>V. Summary</td>
<td>49</td>
</tr>
<tr>
<td>Literature Cited</td>
<td>50</td>
</tr>
<tr>
<td>Table</td>
<td>Description</td>
</tr>
<tr>
<td>-------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>1.</td>
<td>Phenotypes and the number of progeny from backcrosses involving heterozygous e2 p fas-2 males and females</td>
</tr>
<tr>
<td>2.</td>
<td>Phenotypes expected and actually obtained for T. confusum in P1 crosses involving apt, cru, es, Hg and r males and females</td>
</tr>
<tr>
<td>3.</td>
<td>Phenotypes expected and actually obtained for T. confusum in the backcrosses between heterozygous females and hemizygous males</td>
</tr>
<tr>
<td>4.</td>
<td>Frequency of recombination between the Hg-cru, Hg-r, Hg-es and Hg-apt genes in females heterozygous for the genes as indicated</td>
</tr>
<tr>
<td>5.</td>
<td>Phenotypes expected and actually obtained for T. confusum from Hg cru/+ cru females and apt, es and r males</td>
</tr>
<tr>
<td>6.</td>
<td>Phenotypes expected and actually obtained for T. confusum from triple heterozygous females and Hg cru males</td>
</tr>
<tr>
<td>7.</td>
<td>Male progeny and frequency of recombination between genes in females heterozygous for three genes</td>
</tr>
<tr>
<td>8.</td>
<td>Phenotypes expected and actually obtained for T. confusum from crosses involving Chicago wild type females and triple hemizygous males, and Hg cru (es) females X Chicago wild type males</td>
</tr>
<tr>
<td>Table</td>
<td>Page</td>
</tr>
<tr>
<td>-------</td>
<td>------</td>
</tr>
<tr>
<td>9. Phenotypes expected and actually obtained for <em>T. confusum</em> from crossing Chicago and synthetic wild type females and <em>Hg cru es</em> or <em>Hg cru r</em> males.</td>
<td>32</td>
</tr>
<tr>
<td>10. Phenotypes expected and actually obtained for <em>T. confusum</em> from sib matings in crosses shown in Table 9.</td>
<td>34</td>
</tr>
<tr>
<td>11. Phenotypes expected and actually obtained for <em>T. confusum</em> from crosses between <em>Hg es</em> or <em>Hg r</em> females and <em>apt</em> males.</td>
<td>36</td>
</tr>
<tr>
<td>12. Progeny from heterozygous females when crossed with synthetic wild type males.</td>
<td>39</td>
</tr>
<tr>
<td>13. Linkage data for the X-chromosome of <em>Tribolium confusum</em>.</td>
<td>48</td>
</tr>
<tr>
<td>Fig.</td>
<td>Description</td>
</tr>
<tr>
<td>-------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Fig. 1</td>
<td>Scanning electron micrographs of Horned gena (Hg) in <em>Tribolium confusum</em></td>
</tr>
<tr>
<td>Fig. 2</td>
<td>Map position of <em>apt</em>, <em>es</em>, <em>r</em> and <em>Hg</em> in <em>T. confusum</em></td>
</tr>
<tr>
<td>Fig. 3</td>
<td>Map position of <em>apt</em>, <em>r</em>, <em>es</em> and <em>Hg</em> with the tentative position of <em>(cru)</em> in <em>T. confusum</em></td>
</tr>
<tr>
<td>Fig. 4</td>
<td>Linkage map of the X-chromosome in <em>T. confusum</em></td>
</tr>
</tbody>
</table>

vii
INTRODUCTION

The genetics of Coleoptera, the largest order of insects, is poorly known. Most of the genetic information available for this order comes from studies on flour beetles of the genus Tribolium, carried out in the last 25 years. Within this genus, most of the linkage studies have been carried out in Tribolium castaneum by Sokoloff and his collaborators. These studies have identified ten out of the ten possible linkage groups (review in Sokoloff 1966, 1975 and 1977). Fewer linkage studies have been carried out in Tribolium confusum although numerous mutants are available.

Among the few linkage studies of Tribolium confusum, McDonald and Peer (1961) determined that the genes eyespot (es) and Striped (St) were linked and 38 units apart. Subsequently, Sokoloff added more points to the chromosome map: thickened elytral tips (tet) was located 53 units from es (Sokoloff 1964a); labiopedia (lp) was located 47 units from es away from St; the red gene (r) was located 48 units from lp; and the lethal-1 (l-1) gene 40 units from es. Sokoloff (1965) mapped the pointed abdominal segments (pas) gene between es and lp (about 3 units from lp), and subsequently found that the alate prothorax (apt) gene was also between es and lp (Sokoloff et al., 1967).

Dawson (1970) found that depressed (dep) was located about 3 units from r.
Few linkage studies have been carried out with autosomal mutants. For the autosomes, five of the possible eight linkage groups have been identified: Dyte and Blackman (1962) located the gene ebony-2 (e2) at 2.5 units from pearl eye (p). On the II linkage group, Sokoloff (1964b) found that the pearl eye (p) gene and creased abdominal sternites (cas) are linked about 38 units apart, while the dirty pearl eye (dpe) gene is located between pearl (p) and cas (about 5 units from p and 30 units from cas). Thus, the order of the genes is p-dpe-cas. Weber and Roberts (1966) found a very close linkage between the pearl (p) and pearl riboflavinless (p^r). Sokoloff (1977) found p and fas-2 about 42 to 44 units apart.

On the III linkage group, Sokoloff (1964b) found that the black (b) and melanotic stink glands (msg) are linked. Black is located about 42 units from msg and 41 units from ruby spot (rus), while the genes split (sp) and black (b) are linked but over 43 units apart. Dawson (1966) assigned thumbed (thu), (thu^5) as markers for linkage group IV and disjoined (dj) for linkage group VI. Sokoloff (1962) used ebony (e) as a marker for linkage group V. Subsequently he found that blistered elytra (ble) was located 30-40 units from e.

The purpose of this investigation was to determine whether fas-2 (fused antennal segments-2) was on the same linkage group as e2 and p (as initial results suggested) and to establish the map position of Horned gena (Hg), one of the few dominant mutants identified with the X-chromosome.
These studies are of importance in their own right because they provide additional genetic evidence for mechanisms of inheritance in *Tribolium confusum*.

From a broader perspective, these studies are also important because *T. castaneum* and *T. confusum* are related to each other. Because hybridization between these species is not possible, similarities and differences in their genetic makeup can be established either from comparative genetic or comparative biochemical studies. Evidence for the presumptive existence of homologous genes in *T. castaneum* and *T. confusum* has been provided already by Sokoloff (1964), Sokoloff et al. (1967) and Dawson (1968).

Smith (1951) from his cytological studies of *Tribolium castaneum*, *T. confusum* and *T. destructor* concluded that *T. castaneum*, with 9 pairs of autosomes and a pair of sex chromosomes (an X, as large as one of the larger autosomes, attached to the small y chromosome and forming the Xy pair) conforms with the primitive karyotype of Coleoptera. *Tribolium confusum*, according to Smith (1951), evolved from a *T. castaneum*-like form, by a translocation of a pair of autosomes to the X and y pair (forming a neo-X and a neo-y), with the subsequent reduction of autosomes to 8 pairs. Further, *T. destructor* is proposed to have evolved from *T. confusum* by the subsequent loss of the translocated autosomal portion from the neo-X and neo-y chromosomes, resulting in a species with 8 pairs of autosomes and an Xy pair similar in size to that in the ancestral *T. castaneum*-like
species. Genetic evidence supporting Smith's hypothesis has been provided by Sokoloff et al. (1967) and Dawson (1968).
MATERIALS AND METHODS

Available in the Tribolium Stock Center were the following sex-linkage mutants used in this investigation:

1. **Alate prothorax** (*apt)*, a sex-linked recessive homeotic mutant with semi-lethal effects, produces elytra-like growths on prothorax which are absent in normal present-day beetles (Sokoloff, 1967).

2. **Crumpled** (*cru*) is a recessive mutant in which the dorsal surface of the elytra, normally smooth and not wavy, appears wavy and roughened (Hoy and Sokoloff, 1964).

3. **Eye spot** (*es*) is a sex-linked recessive mutation affecting the eye color, normally dark black. If a shadow is cast over the eye, a reddish spot becomes readily visible in the eye, especially when the beetle's head is rotated. This trait can be identified as soon as the ommatidia are formed in the pupa (McDonald and Peer, 1961).

4. **Red eye** (*r*), a recessive eye color mutation, can be identified readily in the pupa and young imago by the reddish ommatidia. It differs from *es* in that the ommatidia of the whole eye are similarly colored. The ommatidia are light red in young beetles, but they become darker in older beetles (Ho and Sokoloff, 1962).

5. **Horned gena** (*Hg*), a newly discovered mutant in *T. confusum* (Sokoloff, personal communication), appears to be a sex-linked dominant mutation with a variable expression. *Hg* can be recognized
phenotypically by the presence of a horn arising from the gena. The horn may appear on one side of the head but not on the other. Sometimes the horn is pointed and solid, other times the horn may be grooved, or in some beetles, the horn may appear double on each side of the head. In extreme manifestation, the beetle's head is badly deformed and not uniformly sclerotized (Fig. 1).

Preliminary crosses were made between *Horned gena* (Hg) and the other four sex-linked mutants (es), (r), (apt) and (cru) in order to obtain beetles carrying two mutants. Although cru expresses itself strongly in stock, its expression overlaps wild type on outcrossing. For this reason, cru was eliminated from further consideration. Two point crosses gave numerous unusual ratios (see Table 2). In order to explain the unusual ratios and at the same time determine the map position of Hg, it was decided to concentrate on a limited number of three-point crosses. To obtain this material, Hg es female and Hg r female beetles were crossed with apt males. Because exceptional classes were obtained in the F₁, the triple heterozygote females \( \frac{(Hg \ es) +}{+ + (apt)} \) and \( \frac{(Hg \ r) +}{+ + (apt)} \) were crossed with wild type males. If we begin with a female homozygous for sex-linked recessive traits, it is expected, when these females are mated to normal males, that the trait will be passed on to all her sons in typical criss-cross inheritance, but to none of her daughters.

Thus, if we let \( X^m \) be the chromosome marked with a recessive mutant \( m \), and + the normal allele of \( m \), crosses between \( X^m X^m \) (mutant...
females) and $X^+Y$ (normal males) will give $X^+X^m + X^mY$ (phenotypically normal females and mutant males).

In the reciprocal cross, $X^+X^+ X X^mY$, it is expected that both males and females will be phenotypically normal (although the females will be carriers of the trait). Crosses between $F_1$ sibs will produce $F_2$ in which all the females will be normal ($X^+X^+$ and $X^+X^m$) and the males will be normal and mutant in equal proportions ($X^+Y$ and $X^mY$). Thus, this sex-linked trait has skipped a generation, but it has been passed from grandfather to grandson.

A sex-linked dominant trait can be identified by the fact that, in crosses between a male carrying the trait and a normal female, the mutant will be passed only to his daughters in the $F_1$ but will be equally distributed among the $F_2$ progeny. Thus:

\[
\begin{array}{c}
P_1 & X^+X^+ & X & X^mY \\
\downarrow \\
F_1 & X^+X^m & ; & X^+Y \\
\downarrow \\
F_2 & X^+X^+ & + & X^+Y \\
& X^+X^m & + & X^mY \\
\end{array}
\]

Among the autosomal mutants were:

1. *pearl* ($p$), a recessive gene which blocks the formation of pigment in the larval ocelli and in the ommatidia of the adult, producing light red or crystalline ommatidia (Graham, 1957).
2. **ebony-2 (e2)**, a recessive mutant phenotypically identical with the semi-dominant \( b \) (Dyte and Blackman, 1962).

3. **fused antennal segments-2 (fas-2)**, a spontaneous recessive mutant, affects the antenna. This appendage appears shorter due to the fusion of segments 3–4 and 5–6 of the funicle (Dawson and Sokoloff, 1964).

For these autosomal genes, **fas-2** males were crossed with **e2** females and the \( F_1 \) were backcrossed to the triple recessive when these became available in the \( F_2 \). The backcross data were examined and \( X^2 \) for viability and for linkage applied according to the methods given by Bailey (1961).

When backcrosses are carried out between individuals heterozygous for three traits (say \(+/a, +/b, +c\)) with the triple homozygotes \((a/a, b/b, c/c)\), the expectation is that eight different classes will be produced. These eight classes will be equally frequent (12.5% or one-eighth if the eight different genotypes are equally viable, and the genes are fully penetrant). If the genes are linked, then the parental combinations will predominate and the other classes will be reduced in frequency depending upon how far the genes are from each other.

The following models illustrate the difference. In Model A, it is assumed that none of the genes is linked. Hence, backcrosses between \(+/a, +/b, +/c\) \( \times \) \( a/a, b/b, c/c \) should give:
<table>
<thead>
<tr>
<th>Frequency (%)</th>
<th>Phenotype</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.5</td>
<td>+</td>
<td>/a, +/b, +/c</td>
</tr>
<tr>
<td>12.5</td>
<td>a</td>
<td>a/a, +/b, +/c</td>
</tr>
<tr>
<td>12.5</td>
<td>b</td>
<td>+/a, b/b, +/c</td>
</tr>
<tr>
<td>12.5</td>
<td>c</td>
<td>+/a, +/b, c/c</td>
</tr>
<tr>
<td>12.5</td>
<td>ab</td>
<td>a/a, b/b, +/c</td>
</tr>
<tr>
<td>12.5</td>
<td>ac</td>
<td>a/a, +/b, c/c</td>
</tr>
<tr>
<td>12.5</td>
<td>bc</td>
<td>+/a, b/b, c/c</td>
</tr>
<tr>
<td>12.5</td>
<td>abc</td>
<td>a/a, b/b, c/c</td>
</tr>
</tbody>
</table>

In Model B, it is assumed that genes a and b are two units apart but gene c is in a separate linkage group. Thus, backcrosses can be represented as +/+ab, +/c X ab/ab, c/c, and the progeny produced by them will be:

<table>
<thead>
<tr>
<th>Frequency (%)</th>
<th>Phenotype</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>.245</td>
<td>+</td>
<td>+/+ab, +/c</td>
</tr>
<tr>
<td>.005</td>
<td>a</td>
<td>a+/ab, +/c</td>
</tr>
<tr>
<td>.005</td>
<td>b</td>
<td>+b/ab, +/c</td>
</tr>
<tr>
<td>.245</td>
<td>c</td>
<td>a+/+b, c/c</td>
</tr>
<tr>
<td>.245</td>
<td>ab</td>
<td>ab/ab, +/c</td>
</tr>
<tr>
<td>.005</td>
<td>ac</td>
<td>a+/ab, c/c</td>
</tr>
<tr>
<td>.005</td>
<td>bc</td>
<td>+b/ab, c/c</td>
</tr>
<tr>
<td>.245</td>
<td>abc</td>
<td>ab/ab, c/c</td>
</tr>
</tbody>
</table>
Model C assumes all three genes are linked and in the order given. The genes \( \text{a} \) and \( \text{b} \) recombine at a frequency of 10\%, \( \text{b} \) and \( \text{c} \) also recombine at a frequency of 10\%, but \( \text{a} \) and \( \text{c} \) recombine at a frequency of 20\%. Theoretically, the backcross between \(+ + +/\text{abc}\) and \(\text{abc}/\text{abc}\) should produce:

<table>
<thead>
<tr>
<th>Frequency (%)</th>
<th>Phenotype</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>.405</td>
<td>+</td>
<td>++ +/abc</td>
</tr>
<tr>
<td>.405</td>
<td>abc</td>
<td>abc/abc</td>
</tr>
<tr>
<td>.045</td>
<td>a</td>
<td>a + +/abc</td>
</tr>
<tr>
<td>.045</td>
<td>bc</td>
<td>+bc/abc</td>
</tr>
<tr>
<td>.045</td>
<td>ab</td>
<td>ab+/abc</td>
</tr>
<tr>
<td>.045</td>
<td>c</td>
<td>++ c/abc</td>
</tr>
<tr>
<td>.005</td>
<td>ac</td>
<td>a + c/abc</td>
</tr>
<tr>
<td>.005</td>
<td>b</td>
<td>+b +/abc</td>
</tr>
</tbody>
</table>

*Triobolium confusum* beetles were reared in "creamers" (3/4 ounce glass containers) in a medium consisting of whole wheat flour supplemented with brewer's yeast in a 19:1 ratio by weight (Sokoloff, 1966). The creamers containing the beetles were maintained in a small incubator, at about 32\(^\circ\) C and about 70\% relative humidity. Under these conditions, the beetles develop in about 28-30 days. Two weeks after starting the crosses, the parents were transferred to another set of creamers with fresh medium. The pupae were isolated from the larvae as they formed by sifting them out of the flour.
Sex can be determined in the pupa by the large genital lobes in the female and the minute ones in the male (see Fig. 3-12 in Sokoloff, 1972). In the adults sex can be determined by examining the genitalia or by the presence of a basal pit on the proximal medial surface of the femur in the males or by its absence in females (Hinton, 1942).
RESULTS

1. **Experiment involving autosomal markers.**

In this experiment the initial crosses were between an $e_2 p \text{fas-2/e}_2 p \text{fas-2}$ female and a normal male. The $F_1$ were classified to sex in the pupa stage and allowed to emerge as adults. All the $F_1$ males and females were normal in respect to these three traits: body color, eye color and antennal segments. A number of $F_1$ males and females were mass-mated to obtain $F_2$. When these were available, the $F_1$ males and females were backcrossed to $e_2 p \text{fas-2}$ homozygotes. When the backcross progeny emerged, they were classified to sex and phenotype.

A summary of the results is given in Table 1.

The data from backcrosses (Table 1) have been examined and $X^2$ for viability and for linkage have been determined according to the methods given by Bailey (1961). The analysis shows that fas-2 is not linked to $e_2$ or $p$, so fas-2 is either not in the same linkage group as $e_2$ and $p$, or so far apart (over 50 units) that these genes produce results similar to those obtained when genes are located on different linkage groups. The genes $e_2$ and $p$ are clearly linked.

The recombination values between $e_2$ and $p$ from heterozygous females are 1.75 units and from heterozygous males 2.16 units. Dyte and Blackman (1961), who first established linkage between these two genes, give values of 2.5 units. My own data, therefore, are very similar to those obtained by Dyte and Blackman.
Table 1. Phenotypes and the number of progeny for *Tribolium confusum* from backcrosses.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Females</th>
<th>Males</th>
<th>Total</th>
<th>Females</th>
<th>Males</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>528</td>
<td>530</td>
<td>1,058</td>
<td>108</td>
<td>138</td>
<td>246</td>
</tr>
<tr>
<td>e2</td>
<td>4</td>
<td>6</td>
<td>10</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>p</td>
<td>8</td>
<td>8</td>
<td>16</td>
<td>3</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>e2 fas-2</td>
<td>263</td>
<td>297</td>
<td>560</td>
<td>91</td>
<td>91</td>
<td>182</td>
</tr>
<tr>
<td>e2 p</td>
<td>277</td>
<td>342</td>
<td>619</td>
<td>85</td>
<td>133</td>
<td>218</td>
</tr>
<tr>
<td>e2 fas-2</td>
<td>5</td>
<td>10</td>
<td>15</td>
<td>4</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>p fas-2</td>
<td>4</td>
<td>2</td>
<td>6</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>e2 p fas-2</td>
<td>188</td>
<td>211</td>
<td>399</td>
<td>104</td>
<td>110</td>
<td>214</td>
</tr>
</tbody>
</table>

**Total**  
1,277 1,406 2,683 399 480 879

Abbreviations for the traits: fas-2 = fused antennal segments-2, e2 = ebony-2, p = pearl.

*From 29 successful matings.

**From 21 successful matings.
2. Sex-linked traits.

The data obtained from numerous crosses involving beetles carrying single sex-linked traits are summarized in Table 2.

These initial crosses were carried out in order to produce, in stepwise fashion, beetles carrying first two and later at least three genes in heterozygous fashion in order to establish their map position from three-point crosses.

An examination of Table 2 shows that some of the crosses produced progeny according to expectation, i.e. the way one expects sex-linked traits to be passed on from one generation to the next.

Crosses $A_1$ to $A_4$ between crumpled (cru) males and females bearing other sex-linked markers (apt, Hg, es and r) give the expected classes of progeny, but in addition they produce some exceptional beetles. Thus, in cross of $A-1$, cru males X apt females, we expect all $F_1$ males to be apt and all $F_1$ females to be normal. We see in the table that this cross produced in addition some unusual progeny, 4 ++ males and 3 apt and 5 cru females. In cross $A-2$, if females were Hg/Hg, we would expect all $F_1$ males and females to be Hg. There should not be any beetles with + or cru phenotypes. Yet in the $F_1$ there is a very sizable component of cru and + beetles considering the size of the sample (18 cru males and 29 females and 5 ++ females).

$A-3$ crosses should give only normal $F_1$ females and es males. There are 19 unexpected cru females and 29 ++ males which should not appear in $F_1$. The cross $A-4$ should yield only normal females
and red eye males, and we obtained them; but in addition, we obtained 29 exceptional normal males.

In crosses B-1 to B-4, Hg males were mated with females bearing other sex-linked markers (apt, es, cru and r). B-1 gave the expected $F_1$ Hg females and apt males, but in addition, 2 ++ females occurred as an exceptional class. In B-2 we expect the $F_1$ females to be Hg and $F_1$ males to be es, but the Hg did not show in the $F_1$ females and few $F_1$ es males appeared. In addition, ++ males and females appeared.

In B-3 we expect $F_1$ females will be Hg and $F_1$ males will be cru; but just as in B-2, Hg females appeared, and in addition, we obtained an expected class of cru females and ++ males and females.

In the B-4 cross, in addition to the expected Hg females and r males, 53 unusual ++ females and 11 unusual ++ males were obtained.

In the crosses C-1 to C-4, es males were crossed with Hg, apt, cru and r females. The only cross giving the expected results is cross C-2. The cross apt female X es male should give normal females and apt males, and these two classes have been obtained (the shortage of apt males reflects the semilethal nature of this gene). However, in the remaining crosses, exceptional classes have been observed: in C-1 there should not be any beetles with black eyes, but there are 12 exceptional females and 7 males. In C-3 the 41 + males are exceptional, and in C-4 the 3 r females and 2 + males are not expected.
Crosses D-1 and D-4 give the results obtained when *apt* males were crossed with *cru*, *Hg*, *es* and *r* females. D-3 failed, but in the remaining crosses there are exceptional males and females in D-1 (35 + males and 4 *cru* females). In D-2 the + males and females constitute exceptions, and in D-4 the 12 + males are likewise exceptions.

In the cross E-1 to E-4, *r* males were crossed with *Hg*, *apt*, *es* and *cru* females. In E-1 we expect all the *F₁* progeny to be *Hg*, but in addition to the *Hg* males and females, we obtained 10 ++ females and 2 ++ males. In the E-2 cross, in addition to the expected classes, ++ males appeared. In the E-3 cross, we expected the *F₁* females to be normal and the *F₁* males to be *es*. In addition to these two classes (++ and *es*), we obtained some *es* females.

In the E-4 cross we obtained ++ females and *cru* males as expected, and 30 ++ males as an unusual class.

That this phenomenon is not unique can be seen in Table 3, in which the results of backcrossing females heterozygous for *Hg* and another sex-linked recessive gene with males hemizygous for the recessive gene are summarized. In A-1, *Hg*+/+ *cru* females have been crossed back with *cru* males. In A-2, *Hg*+/+ *r* females were crossed back with *r* males. In A-3, *Hg*+/+ *es* females were mated with *es* males. In A-4, *Hg*+/+ *apt* females were crossed with *apt* males. In B-1 to B-4, the same types of heterozygous females were mated with *Hg* males.
Table 2. Phenotype expected and actually obtained for *T. confusum* in *P₁* crosses involving \( ap^{t1}_1 \), \( cru^{2}_2 \), \( es^{3}_3 \), \( Hg^{4}_4 \) and \( r^{5}_5 \) males and females.

<table>
<thead>
<tr>
<th>Cross</th>
<th>Expected</th>
<th>Obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>A. 1.</td>
<td>( apt ) X cru</td>
<td>( \frac{1}{2} ) normal</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Hg X cru</td>
<td>( \frac{1}{2} ) Hg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>es X cru</td>
<td>( \frac{1}{2} ) normal</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>r X cru</td>
<td>( \frac{1}{2} ) normal</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. 1.</td>
<td>apt X Hg</td>
<td>( \frac{1}{2} ) Hg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>es X Hg</td>
<td>( \frac{1}{2} ) Hg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Exceptional class.*
Table 2. Contd.

<table>
<thead>
<tr>
<th>Cross</th>
<th>Expected</th>
<th>Obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td></td>
<td>cru</td>
<td>Hg</td>
</tr>
<tr>
<td>B. 3.</td>
<td>3.</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>4.</td>
<td>r</td>
</tr>
<tr>
<td>C. 1.</td>
<td>Hg</td>
<td>X es</td>
</tr>
<tr>
<td></td>
<td>2.</td>
<td>apt</td>
</tr>
<tr>
<td></td>
<td>3.</td>
<td>cru</td>
</tr>
<tr>
<td></td>
<td>4.</td>
<td>4</td>
</tr>
<tr>
<td>D. 1.</td>
<td>cru</td>
<td>apt</td>
</tr>
</tbody>
</table>

*Exceptional class.
Table 2. Contd.

<table>
<thead>
<tr>
<th>Cross</th>
<th>Expected</th>
<th>Obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>D. 2.</td>
<td>Hg X apt</td>
<td>$\frac{1}{2}$ Hg</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>27*</td>
</tr>
<tr>
<td>3.</td>
<td>es X apt</td>
<td>$\frac{1}{2}$ normal</td>
</tr>
<tr>
<td>4.</td>
<td>r X apt</td>
<td>$\frac{1}{2}$ normal</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>64</td>
</tr>
<tr>
<td>E. 1.</td>
<td>Hg X r</td>
<td>$\frac{1}{2}$ Hg</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>10*</td>
</tr>
<tr>
<td>2.</td>
<td>apt X r</td>
<td>$\frac{1}{2}$ normal</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>55</td>
</tr>
<tr>
<td>3.</td>
<td>es X r</td>
<td>$\frac{1}{2}$ normal</td>
</tr>
<tr>
<td></td>
<td>11*</td>
<td>49</td>
</tr>
<tr>
<td>4.</td>
<td>cru X r</td>
<td>$\frac{1}{2}$ normal</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>55</td>
</tr>
</tbody>
</table>

*Exceptional class.

Abbreviations for the traits: 1. apt = alate prothorax, 2. Hg = Horned gena, 3. cru = crumbled, 4. es = eye spot, 5. r = red eye, + = wild type.
Comparison of the theoretical and actual results does not show anything unusual in the backcrosses in the A series when the females were backcrossed to males with recessive sex-linked genes. However, the following exceptional classes can be observed among the females in these backcrosses: In B-1, \textit{Hg}+/+\textit{cru} females X Hg males should give only Hg females, hence the 9 cru, 16 ++ and 98 Hg cru females are exceptional. In B-2, the 7 ++ and 7 Hgr females are exceptional. In B-3, the 3 es and 3 ++ females are exceptional. In B-4, the 29++ females are exceptional. Unfortunately, there is no consistent pattern. In one case, only one exceptional class (+/++) appears. In two cases, two exceptional classes of females appear, but in B-2 the exceptional classes are +/+ and Hgr females, while in B-3 the exceptional classes are +/+ and es/es. Finally in B-1, there are three types of exceptional females (cru, + and Hg cru).

From these two point crosses it is possible to determine the frequency of recombination between these sex-linked genes. This has been done from the male and female progeny in backcrosses between doubly heterozygous females and non-Hg males, and from the male progeny only when the backcrosses involved doubly heterozygous females and Hg males. The recombination values for the five genes involved are shown in Table 4.

Crosses were made between Hg cru/+ cru females and es, apt and r males. These crosses should give one-half Hg and one-half ++ females and one-half Hg cru and one-half cru males. The data are summarized in Table 5. Note that in all these crosses the exceptional
Table 3. Phenotypes expected and actually obtained for *T. confusum* in the backcrosses between heterozygous females and hemizygous males.

<table>
<thead>
<tr>
<th>Cross</th>
<th>Expected</th>
<th>Obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>A. 1.</td>
<td><strong>Hg +</strong></td>
<td><strong>cru</strong></td>
</tr>
<tr>
<td></td>
<td><strong>+ cru</strong></td>
<td>X</td>
</tr>
<tr>
<td>B. 1.</td>
<td><strong>Hg +</strong></td>
<td><strong>cru</strong></td>
</tr>
<tr>
<td></td>
<td><strong>+ cru</strong></td>
<td>X</td>
</tr>
</tbody>
</table>

*Exceptional class.*
Table 3. Contd.

<table>
<thead>
<tr>
<th>Cross</th>
<th>Expected</th>
<th>Obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>B. 3. $Hg^+\frac{+}{es}$ X $Hg$</td>
<td>$Hg$, ++, $es$, $Hg es$</td>
<td>$Hg$</td>
</tr>
<tr>
<td>4. $Hg^+\frac{+}{apt}$ X $Hg$</td>
<td>$Hg$, ++, $apt$</td>
<td>$Hg$</td>
</tr>
</tbody>
</table>

*Exceptional class.

Abbreviations for the traits: $apt$ = alate prothorax, $Hg$ = Horned gena, $cru$ = crumbled, $es$ = eye spot, $r$ = red eye, $+$ = wild type.
Table 4. Frequency of recombination between the \( H_g-cru \), \( H_g-r \), \( H_g-es \) and \( H_g-apt \) genes in females heterozygous for two genes as indicated.

<table>
<thead>
<tr>
<th>Cross</th>
<th>Female</th>
<th>Recombination value* (%)</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. A. ( H_g+_{cru} ) X ( cru ) or ( H_g-cru ) = 53.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. A. ( H_g+_{r} ) X ( r ) or ( H_g-r ) = 41.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. A. ( H_g+_{es} ) X ( es ) or ( H_g-es ) = 45.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. A. ( H_g+_{apt} ) X ( apt ) or ( H_g-apt ) = 30.0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*From females and males in A crosses, but males only in B crosses.
For abbreviations see Table 2.
Table 5. Phenotypes expected and actually obtained for *T. confusum* from **Hg cru/+ cru** females and **es, r, apt** males.

<table>
<thead>
<tr>
<th>Cross</th>
<th>Expected</th>
<th>Obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td></td>
<td>Hg cru</td>
<td>X es</td>
</tr>
<tr>
<td></td>
<td>++ cru</td>
<td></td>
</tr>
<tr>
<td></td>
<td>23</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>5*</td>
<td>12*</td>
</tr>
<tr>
<td>2. Hg cru X apt</td>
<td>++ cru</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>9*</td>
<td>17*</td>
</tr>
<tr>
<td>3. Hg cru X r</td>
<td>++ cru</td>
<td></td>
</tr>
<tr>
<td></td>
<td>64</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>20*</td>
<td>42*</td>
</tr>
</tbody>
</table>

*Exceptional class.

For abbreviations see Table 2.
classes of females were cru and Hg cru, and the exceptional male classes were Hg and +.

Table 6 shows the results of the crosses between F₁ females (obtained in Table 5) which carried 3 mutant genes in heterozygous conditions (i.e. \( \frac{Hg \ cru}{+} + , \frac{Hg \ cru}{+} + , \frac{Hg \ cru}{+} + \)) backcrossed to Hg cru males. In 6-1 we obtained 8 classes of males as expected, while among the females, in addition to the expected classes (Hg, cru and Hg+), we obtained 4 different exceptional classes. In 6-2 also, in addition to the expected males and females, we obtained 5 classes of unexpected females. In the case of 6-3, there are only 5 phenotype classes of males (the other 3 classes did not show up, perhaps because of the semilethal effect of apt gene on those beetles). Among the female progeny, in addition to the expected two classes, we obtained an unexpected group of 25++ and 15 cru females.

Table 7 shows the recombination value for the five sex-linked genes, obtained from crosses involving heterozygous females for three genes and Horned gene crumpled males (Hg cru).

Table 8 summarizes the results of crosses between Chicago wild type females (+/+ and either Hg cru es males or Hg cru r males and a reciprocal cross between Hg cru females (possibly heterozygous for es) with Chicago wild type male. While the sex-ratio among the progeny is approximately 1 female:1 male, again we observe some unusual classes. In cross 8-1 between +/+ female and Hg cru es we expect only Hg females and +/+ males. We see that unexpectedly
Table 6. Phenotypes expected and actually obtained for *T. confusum* from triple heterozygous females and Hg cru males.

<table>
<thead>
<tr>
<th>Cross</th>
<th>Expected</th>
<th>Obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>1. Hg cru + ++ r X Hg cru</td>
<td>½ Hg cru, ½ Hg +</td>
<td>Hg cru, + cru +, ++ r, Hg ++, +++, Hg + r, Hg cru r, + cru r</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| 2. Hg cru + ++ es X Hg cru | ½ Hg cru, ½ Hg + | Hg cru +, + cru +, ++ es, Hg + es, ++++, Hg ++, Hg cru es, + cru es | Female | 67 48 3* 9* 2* 6* 3* 0 |
|       |          |          | Male   | 27 16 21 13 6 18 5 14 |

| 3. Hg cru + ++ apt X Hg cru | ½ Hg cru, ½ Hg + | Hg cru +, + cru +, ++ apt, Hg apt, ++++ Hg ++, Hg cru apt, + cru apt | Female | 34 98 0 25* 0 15* 0 0 |
|       |          |          | Male   | 27 27 0 27 0 4 1 0 |

*Exceptional class.
For abbreviations see Table 2.
Table 7. Male progeny and frequency of recombination between $Hg$-cru, $Hg$-r, $Hg$-es, $Hg$-apt, cru-r, cru-es and cru-apt genes in females heterozygous for three genes.

<table>
<thead>
<tr>
<th>Genotype of Mother</th>
<th>Male Progeny</th>
<th>Frequency of Recombination (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$(Hg\ cru)^{++}(r)$</td>
<td>Hg cru 23, r 17, cru 10, Hg r 9, Hg cru r 15, + 17, Hg cru 23, cru r 9</td>
<td>Hg - cru = 42.14, Hg - r = 42.14, cru - r = 52.99</td>
</tr>
<tr>
<td>$(Hg\ cru)^{++}(es)$</td>
<td>Hg cru 27, es 21, cru 18, Hg es 5, Hg cru es 6, + 13, Hg cru 16, cru es 14</td>
<td>Hg - cru = 44.00, Hg - es = 35.00, es - cru = 40.83</td>
</tr>
<tr>
<td>$(Hg\ cru)^{++}apt$</td>
<td>Hg cru 27, apt 0, cru 4, Hg apt 1, Hg cru apt 0, + 27, Hg cru apt 27</td>
<td>Hg - cru = 37.20, Hg - apt = 37.20, cru - apt = 62.79</td>
</tr>
</tbody>
</table>
185 +/- females appear among the progeny. In 8-2 a similar exceptional class of 325 +/- appears. In addition to these exceptional wild type females, there are a few unexpected cru beetles: 3 females and 4 males in cross 8-1, and 7 females and 4 males in cross 8-2. This small number of cru beetles could be due to developmental abnormalities resulting in beetles with cru-like elytra rather than to the effect of the cru gene itself.

In Table 8-3 the results of crossing one of the exceptional females cited in Table 6 (cross 6-2 which unexpectedly had shown es) are given. When crossed with wild type males, this female (which presumably was Hg cru +/- cru es but expressed itself as es) should produce equal frequencies of Hg and + daughters, and Hg cru, cru es, Hg cru es and cru males. Among the progeny, besides the expected Hg++ females, there were 24 exceptional Hg cru, 2 Hg cru es, 2 cru, 2 es and 8 Hg females, and 42 Hg, 43 ++, 25 es, 12 Hg es males.

To rule out the possibility that the abnormal ratios were being produced by an aberrant wild type strain, crosses were made between Hg cru es and Hg cru r males and females derived from the Chicago wild type stock and also between the same males and females derived from the synthetic wild type stock.

The data, summarized in Table 9, showed that the results are not due to an aberrant wild type strain. When progeny of crosses 9-1 and 9-3 are compared, essentially the same exceptional classes are observed, particularly a large component of wild type females.
Table 8. Phenotypes expected and actually obtained from T. confusum from crosses involving Chicago wild type females and triple hemizygous males (1,2) and Hg cru( es) female X Chicago+ males (3).

<table>
<thead>
<tr>
<th>Cross</th>
<th>Expected</th>
<th>Obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>1. Chicago</td>
<td>X Hg cru es</td>
<td>$\frac{1}{2}$ Hg+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Chicago</td>
<td>X Hg cru r</td>
<td>$\frac{1}{2}$ Hg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Hg cru( es) X Chicago</td>
<td>$\frac{1}{2}$ Hg,</td>
<td>$\frac{1}{4}$ Hg cru es,</td>
</tr>
<tr>
<td></td>
<td>$\frac{1}{2}$ ++</td>
<td>$\frac{1}{2}$ Hg cru es,</td>
</tr>
</tbody>
</table>

*Exceptional class.

For abbreviations see Table 2.
Table 9. Phenotypes expected and actually obtained for *T. confusum* from crossing Chicago and synthetic wild type females and Hg cru es or Hg cru r males.

<table>
<thead>
<tr>
<th>Cross</th>
<th>Expected</th>
<th>Obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>1. Chicago ++/+ X Hg cru es</td>
<td>$\frac{1}{2}$ Hg</td>
<td>$\frac{1}{2}$ ++</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>6*</td>
</tr>
<tr>
<td>2. Chicago ++/+ X Hg cru r</td>
<td>$\frac{1}{2}$ Hg</td>
<td>$\frac{1}{2}$ ++</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>6*</td>
</tr>
<tr>
<td>3. Synthetic ++/+ X Hg cru es</td>
<td>$\frac{1}{2}$ Hg +</td>
<td>$\frac{1}{2}$ ++</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>0</td>
</tr>
<tr>
<td>4. Synthetic ++/+ X Hg cru r</td>
<td>$\frac{1}{2}$ Hg</td>
<td>$\frac{1}{2}$ ++</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>240</td>
</tr>
</tbody>
</table>

*Exceptional class. For abbreviations see Table 2.*
constituting one-fourth to one-third of the total. The same can be observed when Chicago and synthetic females are crossed with Hg cru r males (compare crosses 9-2 and 9-4).

Since the number of unexpected wild type females was quite large in experiments 8-1 and 8-2, these exceptional wild type females (not showing Hg), and their Hg sisters were crossed with their normal male sibs. To simplify the classification of the beetles, the cru trait was omitted. The results are shown in Table 10. It is interesting to note that both normal and Hg females, when crossed with their sibs, produced similar results (compare 10A-1 and 10A-2 and 10B-1 and 10B-2).

If the normal females were really normal in respect to Hg, they should not produce any Hg progeny. If the normal males were really normal (non-es), then these crosses should not produce any es females. Both of these exceptions are contradicted by the data. Both apparently + females and Hg females produce Hg male and female progeny, and both produce apparently es females (compare 10A-1 and 10A-2). The same is true for the crosses given under 10B-1 and 10B-2.

The above results suggest that Hg overlaps wild type in its expression. In both males and females (perhaps as many as one-fourth of the beetles) the Hg fails to form horns. This conclusion is further strengthened by the fact that even in one beetle one side of the head may be hornless while the other may bear horns. The
Table 10. Phenotypes expected and actually obtained for *T. confusum* from sib matings in crosses shown in Table 9.

<table>
<thead>
<tr>
<th>Cross</th>
<th>Expected</th>
<th>Obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>A. 1.</td>
<td>+++</td>
<td>X ++</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. 1.</td>
<td>+++</td>
<td>X ++</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Hg ++</td>
<td>X ++</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Exceptional class.

For abbreviations see Table 2.
presence of $es$ females in the crosses between $es/es \times ++$ may indicate that $es$ also overlaps wild type. Note here that the ratio of $++:es$ or $+:r^r$ is not equal, and neither is the ratio of $+:Hg$.

In an attempt to resolve the problem of exceptional phenotypic classes and at the same time obtain a map of some of the genetic markers used, the more reliable genes were selected for study. To this end, we crossed $Hg\ es$ females with $apt$ males and $Hg\ r^r$ females with $apt$ males. The data, shown in Table 11, show that unusual classes have been obtained. In cross 11-A, one-half $Hg$ and one-half $++$ females were expected, and they were obtained; but in addition, there were $Hg\ es$ and $es$ females. Among the males there should be one-half $Hg\ es$ and one-half $es$ males; but in addition, 58 $Hg$ and 35 $++$ males were recorded. Similar exceptional classes in both sexes were obtained in the crosses 11-B.

The $F_1$ females of all four types given in Table 11A, as well as the $F_1$ females of all four types given in 11B were all crossed with males derived from the synthetic wild type stock. The results are given in Table 12.

Tables 11 and 12 summarize the results from the most critical crosses in these experiments. Table 11 shows that when (A) $Hg\ es$ females and (B) $Hg\ r^r$ females are crossed with $apt$, both of the crosses yield exceptional classes of progeny: The A crosses give exceptional $Hg\ es$ and $es$ females and $Hg\ +$ and $++$ males, while the B crosses produce exceptional $Hg\ r^r$ and $r^r$ females and $Hg\ +$ and $++$ males. When females of all four phenotypes derived from A are
Table 11. Phenotypes expected and actually obtained for *T. confusum* from crosses between *Hg es* or *Hg r* females and *apt* males.

<table>
<thead>
<tr>
<th>Cross</th>
<th>Expected</th>
<th>Obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>A.</td>
<td><em>Hg es + es</em></td>
<td><em>apt</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B.</td>
<td><em>Hg r + r</em></td>
<td><em>apt</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Exceptional class.

For abbreviations see Table 2.
crossed with wild type males, it becomes apparent that both the es and non-es females are actually of the same genetic constitution, i.e. they both are genetically es +/- apt. However, the es gene is not completely recessive, but it overlaps wild type in dominance. Hence, a certain proportion of females appears eye spot even though they are heterozygous for es (i.e. they are +/-es). The crosses between these females and wild type males should give only wild type females and four classes of males (es, apt, + and es apt). The male classes observed are consistent with expectation. The females fall into two classes, + and es, again showing the overlap in dominance of + and es. Similarly, the Hg and non-Hg classes consist of females which are genetically identical (i.e. they are heterozygous for Hg, es and apt), but some Hg females do not show any horns because this trait has incomplete penetrance. If these females are genetically identical, they should produce males that should fall into eight classes. Table 12 crosses A-1 and A-2 show that there are indeed eight classes of males. Furthermore, considering the incomplete penetrance of Hg and the overlap in expression between + and es, when Hg es +/- and apt females are crossed with wild type males, there should be four classes of females among the progeny (Hg, +, es and Hg es) instead of only two types of females (i.e. Hg and non-Hg). Examination of the results of the A-1 and A-2 crosses suggests that this interpretation is correct.
The mutant r also overlaps wild type in its expression. Hence some beetles typically +/r will appear red, while others of the same genotype will appear black. The crosses between Hg r/+ r female X apt male gave Hg and non-Hg black-eyed females as expected, but in addition there were large numbers of exceptional Hg r and r females. When the females of all four phenotypes were mated with wild type males, females that were truly non-Hg were genetically r +/+ apt, but some were red-eyed and other black-eyed. Similarly, among the Hg females, there were some that had red eyes and others had black eyes, and yet they were of the same genotype — namely Hg r +/+ apt. When these females were crossed with wild type males, both types produced male progeny that fell into eight classes. The female progeny produced again belonged to four phenotypic classes (Hg, +, r and Hg r) instead of only two (Hg and +).

Data from males can be used to determine the relative position of the Hg r, apt, Hg, r and es genes.
Table 12A. Progeny from heterozygous females shown in Table 11 when crossed with synthetic wild type males.

<table>
<thead>
<tr>
<th>Cross</th>
<th>Expected</th>
<th>Obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>A. 1. Hg es + ++ apt x syn+/+</td>
<td>(\frac{1}{2}) Hg, (\frac{1}{2}) ++</td>
<td>Hg es, ++ apt, Hg es apt, +++, ++ apt, + es +, Hg + apt, + es apt, Hg ++</td>
</tr>
<tr>
<td>Male</td>
<td>57</td>
<td>97</td>
</tr>
</tbody>
</table>

| B. 2. Hg ++ ++ apt x syn+/+ | \(\frac{1}{2}\) Hg, \(\frac{1}{2}\) ++ | Hg ++, Hg apt, +++, ++ apt | Female 76 92 0 0 32* 0 35* |
| Male | 32 | 22 | 2 | 3 | 36* | 11* | 36* |

| C. 3. es ++ ++ apt x syn+/+ | ++ | es, apt, +, es apt | Female 175* 408 0 0 |
| Male | 289 | 31 | 15 | 35 |

| D. 4. +++ ++ apt x syn+/+ | +++ | ++++, ++ apt | Female 170 0 80* |
| Male | 88 | 11 | 60* |

*Exceptional class. All females are F₁. For abbreviations see Table 2.
Table 12B. Progeny from heterozygous females obtained in Table 11 when crossed with unrelated synthetic wild type males.

<table>
<thead>
<tr>
<th>Cross</th>
<th>Expected</th>
<th>Obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>B. 1.</td>
<td>(r^+) ++</td>
<td>(++) apt</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>(+++)</td>
<td>(++) apt</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>(H_g^++)</td>
<td>(++) apt</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>(H_g r^+)</td>
<td>(++) apt</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Exceptional class. For abbreviations see Table 2.
DISCUSSION

The present linkage studies have shown that:

1. The e2 and p genes are closely linked. Dyte and Blackman (1962) gave a recombination value of 2.5 units. In the present study, recombination values of 2.16 for males and 1.75 for females have been obtained. For these closely linked genes, there is no difference in the frequency of recombination in the two sexes. The fas-2 gene is not located in the same linkage group as e2 and p as it was previously thought. Its linkage relationships remain to be established.

2. Of the five sex-linked genes used, es and r genes are not completely recessive, overlapping wild type in their expression, and the Hg gene is not completely dominant as indicated both by the fact that in Hg beetles one side of the head may bear horns and the other does not and by the appearance of non-Hg beetles where none are expected. The cru gene is not very reliable. Differences in the micro-environment surrounding the pupae from a normal stock may produce adults which resemble cru, and a certain proportion of beetles known to be cru will be misidentified for non-cru or normal beetles. The apt gene, although it has variable expression, is a fairly reliable gene insofar as identification of its phenotype is concerned, but it behaves as a semilethall. The present results indicate that apt is definitely sex-linked, but not always recessive. There were several exceptional apt females (in crosses A-1, Table 2,
and B-2 in Table 12) when there should not be any. The overlap in
dominance of these various genes is responsible for producing
beetles which were exceptions throughout the course of this study.
The exceptional classes were so prevalent in some crosses that
initially it was felt that a new phenomenon of sex-determination
was being observed in \textit{T. confusum}. It was not until the females
of exceptional and expected classes shown in Table 11 were mated
with wild type males (results in Table 12) that the true nature of
the phenomenon could be explained satisfactorily.

Recall that Smith (1951), from his cytological studies of
numerous species of beetles, concluded that the primitive karyotype
includes 9 pairs of autosomes and a pair of \textit{X} and \textit{y} chromosomes
which, at metaphase, are joined in parachute fashion. \textit{Tribolium}
\textit{castaneum} has this primitive appearance. \textit{Tribolium confusum}, on
the other hand, is a derived species: during its evolution from
a \textit{T. castaneum}-like ancestor, a pair of autosomes became translocated
to the \textit{X} and \textit{y} forming a much larger pair of sex-determining chromo-
somes which Smith calls neo-\textit{X} and neo-\textit{y}. The autosomal portion
attached to the \textit{X} remained functional, but the homologue attached
to the \textit{y} became heterochromatinized and nonfunctional. Smith ad-
vanced the hypothesis that some genes acting in autosomal fashion
in \textit{T. castaneum} would behave as sex-linked genes in \textit{T. confusum}.
The discovery of some homeotic mutants (which in \textit{T. castaneum} behave
in autosomal fashion and in \textit{T. confusum} in sex-linked fashion) has
provided evidence to the correctness of Smith's hypothesis. The apt mutant, for example, is an autosomal recessive and linked with prothoraxless (pt1) on linkage group IX of _T. castaneum_. The phenotypically similar prothoraxlesslike (pt11) and alate prothorax (apt) in _T. confusum_ are sex-linked. Thus, it is linkage group IX which in the evolution of _T. confusum_ became translocated to the X and y producing the neo-X and neo-y chromosomes (Sokoloff et al., 1967). The discovery of the autosomal recessive labiopedia which is also linked with _pt1_ in _T. castaneum_ lends further support to Smith's hypothesis (Dawson, 1968).

From the results summarized in Tables 11 and 12, it is possible to produce the linkage map shown in Fig. 2. Fig. 2 shows that the apt, es, Hg and r genes are in the order r-es-apt-Hg. The distance between _r_ and _apt_ is 42.2 units, and between _apt_ and _Hg_ the distance is 38 units. _es_ is located between _r_ and _apt_. The _es_ gene is located to the left of _apt_ and 16.6 units away from it.

Fig. 3 shows the relative position of _cru_ and the other four genes mentioned in the previous paragraph. Tentatively, from the limited results given in Table 6, _cru_ is placed to the right of _es_ but to the left of _apt_. It would be desirable to carry out some crosses between _cru_ and _apt_ and _es_ _cru_ and _apt_ to establish the location of _cru_ more precisely.

Sokoloff (1977) has given maps of the first and second linkage group of _T. confusum_. From the present results, it is possible to
Fig. 2. Map position of r, es, apt and Hg from linkage data obtained in Tables 11 and 12.
Fig. 3. Map position of $r$, $es$, $apt$ and $Hg$ with the tentative position of $cru$ from data obtained in Table 6.
modify his map of the X-chromosomes of \textit{T. confusum} to the extent shown in Fig. 4 and Table 13.

Interestingly, the genetic map shown in Fig. 4 extends well over 115 map units. Comparative genetic evidence has pointed out that the homeotic mutants \textit{apt}, \textit{pas} and \textit{lp} are located in the autosomal portion of the neo-X. Hence, the gene responsible for the Horned gena in \textit{T. confusum} is also located in the autosomal portion of the neo-X. If a comparable gene is present in \textit{T. castaneum}, this gene must be inherited in autosomal fashion in this species of \textit{Tribolium}.

Linkage group II is modified only to the extent that \textit{fas-2} is definitely established as either not being on this linkage group or so far away (50 units or more) that linkage crosses give 50\% recombination. Hence, the \textit{fas-2} gene is tentatively removed from linkage group II.
Fig. 4. Linkage map of the X-chromosome in T. confusum.

The following sex-linked genes are also in this linkage group, but their position is uncertain: tet, ma, ptll, l₁.
Table 13. Linkage data now available for the X-chromosome of *T. confusum*.

<table>
<thead>
<tr>
<th>Linkage</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>es - es</em></td>
<td>1t = 0</td>
</tr>
<tr>
<td><em>es - st</em></td>
<td>= 38</td>
</tr>
<tr>
<td><em>es - lp</em></td>
<td>= 47</td>
</tr>
<tr>
<td><em>apt - lp</em></td>
<td>= 4</td>
</tr>
<tr>
<td><em>apt - es</em></td>
<td>= 53, 16.6*</td>
</tr>
<tr>
<td><em>apt - r</em></td>
<td>= 42.2*</td>
</tr>
<tr>
<td><em>apt - Hg</em></td>
<td>= 30, 38*</td>
</tr>
<tr>
<td><em>r - lp</em></td>
<td>= 48</td>
</tr>
<tr>
<td><em>r - dep</em></td>
<td>= 3**</td>
</tr>
<tr>
<td><em>lp - apt</em></td>
<td>= 4</td>
</tr>
<tr>
<td><em>lp - pas</em></td>
<td>= 3</td>
</tr>
<tr>
<td><em>es - l1</em></td>
<td>= 40</td>
</tr>
<tr>
<td><em>Hg - es</em></td>
<td>= 35 - 46*</td>
</tr>
<tr>
<td><em>Hg - r</em></td>
<td>= 42*</td>
</tr>
<tr>
<td><em>Hg - cru</em></td>
<td>= 37 - 44*</td>
</tr>
<tr>
<td><em>cru - es</em></td>
<td>= 41*</td>
</tr>
<tr>
<td><em>cru - r</em></td>
<td>= 53*</td>
</tr>
</tbody>
</table>

*Present data.*

**Dawson, 1970.

SUMMARY

The present study shows that in Tribolium confusum Duval., the gene fused antennal segments-2 (fas-2), previously thought to be on the linkage group II, is not linked to either ebony-2 (e2) or pearl (p), or it may be very far apart (over 50 units). The data do show that the gene e2 and p are closely linked (about 2 units apart), as Dyte and Blackman (1962) showed.

For the sex-linked genes, Horned gena (Hg) was found to be about 42 units from red (r), 35-46 units from eye spot (es), 30-38 units from alate prothorax (apt), and 37-44 units from crumpled (cru).

Red eye (r) was found to be 53 units from cru, 42.4 units from apt; eye spot (es) is 41 units from cru and 16-53 units from apt.

Neither the r nor the es eyecolor genes nor the cru gene affecting the elytra are completely recessive. They overlap the wild type gene in their expression producing beetles with black eyes or normal elytra. The Horned gena (Hg) is not completely dominant as shown by the fact that, even in single individuals, the gene may fail to express itself, producing only one horn on one side of the head. In addition, many Hg beetles known to be Hg are phenotypically identical to wild type (the horns are absent on both sides of the head).

The most reliable gene in these studies is alate prothorax (apt), a semilethal; but even this gene is not fully recessive, a few +/apt females may appear alate.

A revised map of the X-chromosome is given in Fig. 4.
LITERATURE CITED


