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Linkage studies on Maxillopedia, a homeotic mutant in Tribolium castaneum herbst (Coleoptera Tenebrionidae)

Robert Francis Ferrone

LINKAGE STUDIES ON MAXILLOPEDIA, A HOMEOTIC MUTANT IN *Tribolium castaneum* HERBST (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

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ABSTRACT

Linkage relationships, degree of penetrance and egg viability of the homeotic recessive mutation maxillopedia were studied in *Tribolium castaneum* Herbst. The *mxp* gene has variable expression, complete penetrance and reduced egg viability. Viability reductions are not confined to the egg stage but are evident in pupae that fail to become adults. Further reductions in viability are produced when *mxp* occurs with certain other mutations. In addition, when *mxp* occurs in the heterozygous condition with the Dachs (*Dch*) mutant, a semidominant phenotypic expression of the *mxp* gene is produced at least some of the time.

Two- and three-point backcrosses between known markers for linkage groups II through X and *mxp* were carried out. The gene maxillopedia was found to be linked with known markers for linkage group II. The *mxp* gene is 7.8-5.7 units from Dachs (*Dch*) and 23.4-25.9 units from pearl (*p*). It is also linked with Reindeer (*Rd*) which was recently associated with linkage group II. The mutant genes *Rd* and *mxp* are 29.1-39.9 units apart. A revised map of linkage group II is presented.

The value of homeotic mutants in evolutionary studies of insects is discussed.

ACKNOWLEDGEMENTS

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INTRODUCTION

An hereditary change resulting in "the alteration of one organ of a segmented or homologous series from its own characteristic form to that of some other member of the series" is referred to as homeosis (Darlington and Mather, 1949). Mutant genes giving rise to such alterations by interference with primary processes in embryonic development are called "homeotic mutants" (Goldschmidt, 1945).

The first homeotic mutant in insects was found in Drosophila melanogaster. It modified the mouth parts to structures resembling walking legs (Bridges and Dobzhansky, 1933). Since then, numerous homeotic mutants have been found in well-investigated orders of insects including Tribolium castaneum Herbst and T. confusum Duval, members of the Coleoptera. Many of these mutations markedly affect the appendages of the head region. In T. castaneum the mutant antennapedia (ap), reported independently by Englert and Bell (1963) and Sokoloff and Dawson (1963), is expressed by the modification of the paired antennae into structures resembling walking legs. This gene does not exhibit any decrease in viability (Tagarro, 1973). A sex-linked mutation in T. confusum called labiopedia (lp) causes the normal labial palpi to be replaced by appendages resembling prothoracic walking legs. Expressivity of this mutant is somewhat variable but penetrance is complete (Daly and Sokoloff, 1965). Dawson (1968) reported an homologous mutation in

T. castaneum. From very limited data, he concluded that the gene had an autosomal mode of inheritance, and that the lp mutant appeared to be linked with prothoraxless (ptl) in linkage group IX. In T. castaneum, maxillopedia (mxp) mutants, caused by an autosomal recessive gene, have normal maxillary palpi replaced by appendages resembling walking legs. This gene has considerable variability in expression. An mxp beetle may have almost normal maxillary palpi except for a claw on the terminal segment, or each palpus may develop into a well-developed leg with distinct tarsal claws, tarsal segments, and a tibia-like segment containing tibial spurs. In addition, a segment representing the femur may be present (Hoy, 1966a). The expression of the mxp gene can be intensified by inbreeding and its effect may extend to the labial palpi. As with the maxillary palpi, the most frequent modification in the labial palpi is the additional of tarsal claws and tibial spurs. Occasionally the distal segments of the labial palpi may be modified into a tarsus and partial tibial segment (Hoy, 1966a). (For illustrations of this, see Fig. 18.8f in Sokoloff, 1977 and Fig. 1, E-L.)

From an evolutionary standpoint, genetic studies of Tribolium are of considerable importance since they provide evidence for the Coleopteran phylogeny proposed by Smith (1950) from his cytological studies.

In constructing the phylogeny of Tribolium castaneum, T. confusum, and T. destructor, Smith (1950) found that T. castaneum conformed to the formula for a 9AA + Xy primitive Coleopteran karyotype. Tribolium castaneum has 9 pairs of autosomes and a pair of sex chromosomes. The y chromosome is considerably smaller than the X in the Xy pair. Tribolium confusum, with only 9 pairs of chromosomes, evolved from a T. castaneum-like form by a translocation of a pair of autosomes to the Xy pair. The formation of the so-called neo-X and neo-Y pair resulted in a reduction in chromosome number from 10 to 9 pairs. Tribolium destructor also has 9 pairs of chromosomes and probably evolved from T. confusum following the elimination of the translocated autosomal portion from the X and Y, since the X and y are comparable to the X and y of T. castaneum in size (Smith, 1950).

When considering the genetics of T. castaneum and T. confusum, Smith (1952) postulated that some genes which exhibit autosomal inheritance in T. castaneum should behave like sex-linked genes in T. confusum. Sokoloff, Ackermann and Overton (1967) note that alate prothorax (apt) and prothoraxless (ptl) exhibit autosomal inheritance in T. castaneum, while the similar mutants alate prothorax (apt) and prothoraxlesslike (ptll) in T. confusum are sex-linked. Dawson (1968) found that labiopedia (lp) in T. castaneum is an autosomal gene linked with prothoraxless. Hence, it

would seem that linkage group IX of T. castaneum, identified by the prothoraxless mutant, is the one which became associated with the X and Y in the evolutionary history of T. confusum.

Quantitative values for penetrance in the homeotic mutant maxillopedia (mxp) are lacking and information on linkage relationships of the mutant are unknown. The purpose of the present investigation is to determine values for penetrance and egg viability of the mxp gene and to determine its linkage relationships.

MATERIALS AND METHODS

The following mutants of *Tribolium castaneum*, obtained from the Tribolium Stock Center on the campus of California State College, San Bernardino, were used in this investigation to identify the various linkage groups.

Linkage Group II

1) Dachs (*Dch*) is an autosomal dominant in which the distal segments of the antennae are fused forming a slightly cup-shaped structure. It is linked with pearl on chromosome II (Sokoloff, personal communication).

2) The missing abdominal sternites (*mas*) mutant is an autosomal recessive which causes a reduction in abdominal sternites from five to four leaving an unsclerotized area anterior to the apparent second segment. The whole abdomen is displaced slightly forward and a minute gap is left between the edges of the elytra and the lateral margins of the abdomen. Penetrance, expressivity and viability are good. It is linked with pearl on linkage group II (Sokoloff, 1965).

3) The pearl (*p*) mutant is an autosomal recessive with good viability, complete penetrance, and pleiotropic effects. The major effect of pearl is to produce an eye in the adult that is devoid of pigment (Park, 1937). Marginal ommatidia still appear black, but this is due to a pigmented endoskeletal structure that forms under them (Sokoloff, 1959).

Pearl serves as the anchor gene for linkage group II.

Linkage Group III

1) The aureate (au) gene is an autosomal recessive which produces an exoskeleton covered with 3 times as many golden "hairs" than normal beetles (Ackermann, 1967; Sokoloff, Hayes, Pease and Ackermann, 1967; and Sokoloff, 1967). Pits at the base of these "hairs" are readily observable and aureate (au) beetles can easily be distinguished from the wild type by their less shiny appearance. This mutant gene exhibits complete penetrance with no variation in expression (Hoy, 1966b). It can be used to identify linkage group III (Sokoloff, Ackermann and Heinze, 1967).

2) The black (b) mutant is an autosomal semidominant in which the normal red-rust or chestnut body color is replaced by a dark black coloration in the homozygote and a bronze coloration in the heterozygote. Black mutants exhibit high viability and good penetrance (Sokoloff, Slatis and Stanley, 1960). This is an excellent marker for linkage group III.

Linkage Group IV

1) The hazel (h) mutant is an autosomal recessive that causes eye color to become a light reddish-brown to a dark brown in place of the normal black (Dawson, 1969). This mutant gene can be used as an alternative marker to identify linkage group IV.

2) The sooty (s) gene is an autosomal semidominant which produces a black body color in place of the reddish-brown

or chesnut. Wild type homozygotes and heterozygotes are nearly indistinguishable. Mutants have a reduced viability of about 20% (Bartlett, Bell and Shideler, 1962 and Sokoloff, 1966). It identifies linkage group IV.

Linkage Group V

1) The jet (j) mutant is an autosomal recessive that produces a body color somewhat darker than sooty (s) and lighter than black (b). It differs from black (b) in that it has a reddish tinge and the appendages are less opaque. Penetrance is complete and viability is low (Park, 1954 and Tagarro, 1973). Nevertheless, jet is an excellent marker for linkage group V.

2) The microcephalic (mc) mutant is an autosomal recessive with considerable variation in expression, complete penetrance and a reduced viability of 10%. In mc beetles the cranial portion of the head is smaller, the compound eyes are reduced in size, there are fewer ommatidia present and, in some cases, the ommatidia are totally absent (Sokoloff and Lasley, 1961). The mc gene is linked with jet and can serve as a marker for linkage group V.

Linkage Group VI

1) Microphthalmic (Mo), a dominant mutant gene with recessive lethal effects, alters the shape of the compound eye and reduces the number of ommatidia present. The dorsal facets are most often eliminated. Less frequently, dorsal and lateral portions are reduced. Ommatidia are often

disarranged from the normal orderly progression of rows. These rows may be incomplete or completely disrupted (Sokoloff, 1960). It is the only marker identifying linkage group VI at this time.

Linkage Group VII

1) The chestnut (c) mutant is an autosomal recessive gene which modifies the color of the compound eye from black to reddish-brown or red. There is no apparent loss of viability from the wild type (Eddleman, 1961). It serves as an alternate marker for linkage group VII.

2) Short antenna (Sa), an autosomal dominant mutation with recessive lethal effects, primarily acts upon the antennae. These may become shorter due to a reduction and/or fusion of the central antenna segments. Viability of heterozygotes is reduced 15% (Bell, 1962). Sa is located on linkage group VII.

Linkage Group VIII

1) The antennapedia (ap) mutant is an autosomal recessive homeotic mutation with pleiotropic effects. The most noticeable effect is a modification of the antennae to leg-like appendages. Lesser effects are the modification of the length and shape of the metathorax and the fusion of the distal segments of the tarsi (Sokoloff, 1977). The ap beetles show complete penetrance and there is no reduction in viability from the wild type (Englert, Shideler and Bell, 1963). This mutation serves to identify linkage group VIII.

2) The mutation short elytra (sh) is an autosomal recessive with variable expression, incomplete penetrance and good viability. The elytra are shortened and the distal abdominal tergite may be visible in sh beetles. In addition, distal portions of the elytra may diverge in varying degrees (Sokoloff, 1962). It serves to identify linkage group VIII.

Linkage Group IX

1) The mutant alate prothorax (apt) is a semidominant homeotic mutation which is often expressed by the production of elytra-like appendages arising from lateral edges of the prothorax in the pupa. Adults and pupae can be recognized by these asymmetrical prothoracic growths. Penetrance is incomplete and viability is poor. Most apt beetles die in the pupa stage (Sokoloff, 1965). This mutant is probably linked with ptl on linkage group IX.

2) The prothoraxless (ptl) mutant is an autosomal semi-dominant with a variable expression. In the heterozygote, the protergum may exhibit a groove at right angles to the midline and may have various indentations at one or the other anterior corners. Homozygotes are severely affected because the prothorax is almost gone and the forelegs are greatly reduced. Homozygote stocks do not live long (Lasley and Sokoloff, 1960). The ptl gene has been assigned to linkage group IX.

Linkage Group X

1) The abbreviated appendages (aa) mutant is an autosomal

recessive that shortens the elytra to three-quarters of the normal length often with elytra divergent exposing the dorsal surface of the abdomen. In some aa beetles the legs and podomeres are shortened. The gene has good penetrance with regard to the effect on the elytra, but variable penetrance in regard to the legs (Sokoloff, 1965). Presently, this gene is the only one available to identify linkage group X.

Linkage Group Unknown

1) The gene maxillopedia (mxp) is an autosomal recessive homeotic mutation of variable expression, which produces leg-like structures in place of normal maxillary palpi (Hoy, 1966a).

2) Reindeer (Rd) is an autosomal dominant mutation. The heterozygote has grossly enlarged proximal antennal segments and may cause a conspicuous branching of the antennae. The proximal bulges are so pronounced that they can be detected with the unaided eye. This mutant was discovered by P. S. Dawson and has not been released for further study.

Studies on penetrance and viability were carried out for the maxillopedia (mxp) mutant of Tribolium castaneum Herbst. Beetles homozygous for the mxp gene were reared in a medium of whole wheat flour and yeast in the proportions of 19:1 and allowed to develop into a productive stock for six months. After that time, pupae were isolated and sexed. Mating pairs of mxp beetles, one month old, were placed in 3/4 ounce glass containers (creamers) for three days and

transferred to new creamers with fresh medium. Another transfer was accomplished three days later. Similar procedures were repeated with some creamers kept in an incubator at 32°C and 70% relative humidity while others were kept at room temperature. Eggs laid were counted and three weeks later the number of emergent larvae was noted. After 5 weeks, maxillary palpi of adults were examined to see if they possessed the mxp trait. A wild type Chicago strain, Chicago +, was used with the same procedure to serve as the control. Similar tests were performed with mxp X + Chicago and with mxp X +/mxp matings. A calculation of penetrance and viability followed.

To determine the linkage group of the mxp gene, crosses were carried out between strains carrying two known markers (when possible) for a particular linkage group and the mxp gene. Beetles homozygous for known markers for each chromosome were crossed with mxp homozygotes. When the F_1 of the cross was obtained, pupae were sexed and isolated in separate containers. Some of the F_1 beetles were mass mated to obtain F_2. The F_1 beetles isolated previously were backcrossed with virgin females and males homozygous for all three traits. Genetic ratios obtained were analyzed taking into account degree of penetrance and genetic recombination due to crossing over according to the methods given by Bailey (1961).

Rearing methods similar to those used for determining penetrance and viability were used for determining linkage relationships.

Theoretical Expectations

When individuals heterozygous for three traits are backcrossed to those homozygous for three traits (+/a,+/b,+/c X a/a,b/b,c/c), eight phenotypic classes are expected. These eight classes will occur with equal frequency if the genes are all equally viable and penetrant and are located among distinct linkage groups. If either two or all three genes are linked, then a disproportionately higher phenotypic frequency will be obtained for the parental genotypes and all other classes will be consequently reduced. The magnitude of the skewed frequencies will depend upon the distance between those genes which occur on the same chromosome.

Bailey (1961) gives three illustrative models. In Model A, all three genes belong to distinct linkages. Backcrosses between the hypothetical genotypes noted above should produce the following results:

Phenotype	Genotype	Frequency
+	+/a,+/b,+/c	.125
a	a/a,+/b,+/c	.125
b	+/a,b/b,+/c	.125
c	+/a,+/b,c/c	.125
ab	a/a,b/b,+/c	.125
ac	a/a,+/b,c/c	.125
bc	+/a,b/b,c/c	.125
abc	a/a,b/b,c/c	.125

In Model B, it is presumed that genes a and b are linked and 25 units apart. The backcrosses can be represented as ++/ab,+/c X ab/ab,c/c. The progeny obtained should appear in the following proportions:

Phenotype	Genotype	Frequency
+	++/ab,+/c	.1875
a	a+/ab,+/c	.0625
b	+b/ab,+/c	.0625
c	a+/+b,c/c	.1875
ab	ab/ab,+/c	.1875
ac	a+/ab,c/c	.0625
bc	+b/ab,c/c	.0625
abc	ab/ab,c/c	.1875

In Model C, it is assumed all three genes are linked in the order a, b, c. Genes a and b are ten units apart, b and c are ten units apart and a and c recombine with a

frequency of 20%. Assuming no interference, backcrosses between +++/abc and abc/abc should produce:

Phenotype	Genotype	Frequency
+	+++/abc	.405
abc	abc/abc	.405
a	a++/abc	.045
bc	+bc/abc	.045
ab	ab+/abc	.045
c	++c/abc	.045
ac	a+c/abc	.005
b	+b+/abc	.005

A similar model, Model D, can be devised to illustrate how the triple locus cross is affected by the presence of one dominant allele. Suppose genes A and b are linked and are two units apart but gene c is in a separate linkage group. Backcrosses can be represented as ++/Ab,+/c X +b/+b,c/c. The progeny produced will be in the following proportions:

Phenotype	Genotype	Frequency
+	++/+b,+/c	.245
A	A+/+b,+/c	.005
b	+b/+b,+/c	.005
c	++/+b,c/c	.245
Ab	+b/Ab,+/c	.245
Ac	A+/+b,c/c	.005
bc	+b/+b,c/c	.005
Abc	+b/Ab,c/c	.245

Model E is necessary to show the expected results of a double locus cross when the genes are linked and 25 units apart. The backcross would be A+/+b X +b/+b. Expected frequencies are:

Phenotype	Genotype	Frequency
+	++/+b	.125
A	A+/+b	.375
b	+b/+b	.375
Ab	Ab/+b	.125

Fig. 1. Scanning electron micrographs of normal and maxillopedia (mxp) Tribolium castaneum.

A. Lateral view of the normal head of T. castaneum, wild type (85X). Note shape of maxillary palpus.

B. Ventral view of the normal head of T. castaneum, wild type (80X). Note the appearance of the maxillary and labial palpi.

C. Frontal view of the normal head of T. castaneum, wild type (80X). Note the appearance of the maxillary and labial palpi.

D. Ventral view of Dch+/+mxp in T. castaneum (55X). Note bristle on distal end of one maxillary palpus.

E., F. Ventral view of mxp mutant in T. castaneum (70X and 130X, respectively). Note that both maxillary and labial palpi are modified into leg-like structures.

G.-L. Ventral views of two mxp mutants in T. castaneum (G-35X, H-65X, I-100X, J-300X, K-60X and L-155X). Note tibial spurs are well-defined in all micrographs. In K-L tarsal claws are well-defined on the maxillary palpi and partly visible in the labial palpi.

E

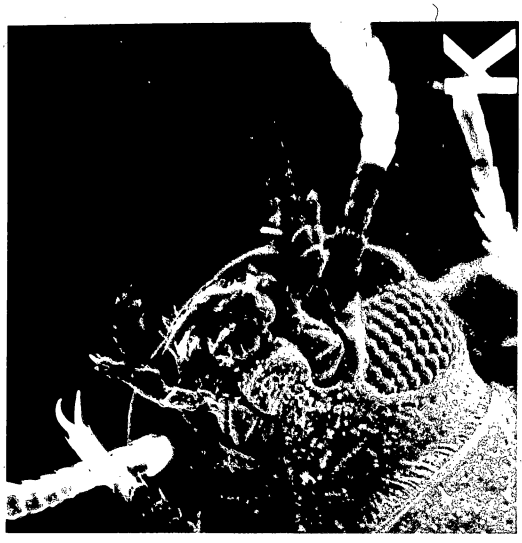
F

C

D

A

B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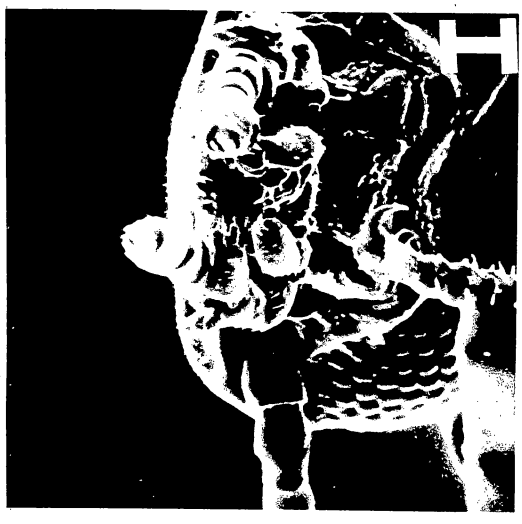
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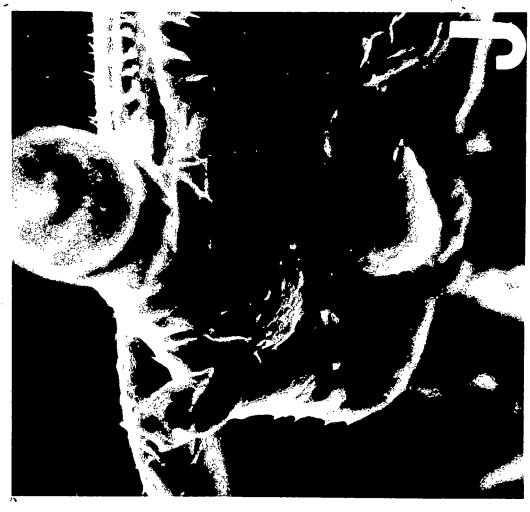
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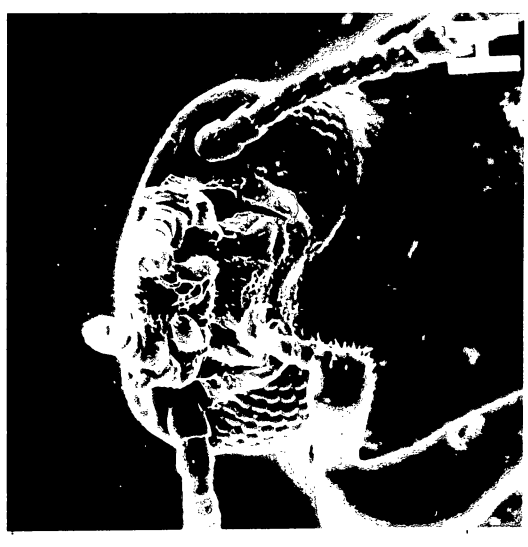
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RESULTS

1) Penetrance of maxillopedia homozygotes

The penetrance of the mxp gene is nearly complete. Out of the 304 adults examined, only two were scored as wild type (see Table 1). One of these, upon close examination, showed a slight modification of one labial palpus. Hoy (1966a) reported that mxp beetles often have affected labial palpi in addition to the alteration of the maxillary palpi. Considering the highly variable expression of mxp, it is possible that the slight modification of the one labial palpus was actually a manifestation of complete mxp penetrance. For the analysis of the data to follow, it was assumed that mxp has complete penetrance.

2) Egg Viability of maxillopedia

The mutant gene mxp appears to have slight effects on viability. Data in Table 2 show that mxp/mxp eggs had a decrease of 11.6% in viability compared to the eggs of the Chicago + strain when the beetles are reared in the incubator. Matings carried out at room temperature and humidity (Table 3) showed a similar reduction of 10.1%. There does not appear to be any decrease in viability when the mxp is in the heterozygous condition (compare Table 2 and Table 3).

Table 1. Number and percentage of phenotypically mxp beetles used to determine percent penetrance in Tribolium castaneum.

Mating pair number	phenotypes		% penetrance
	mxp	+	
1	32	0	100.0
2	30	1	96.7
3	118	0	100.0
4	55	0	100.0
5	69	1	98.6
TOTAL	302	2	99.3

Table 2. Egg Viability in various genotypic crosses of Tribolium castaneum at 32°C and 70% R.H.

Pair mating[α]	Mating Type: +/+ X +/+ eggs	larvae	%	+/+ X mxp/mxp eggs	larvae	%	mxp/mxp X +/+ eggs	larvae	%	mxp/mxp X mxp/mxp eggs	larvae	%
1	138	96	69.5	151	117	77.5	89	74	83.1	152	124	81.5
2	151	123	81.4	-	-	-	-	-	-	110	62	56.4
3	-	-	-	-	-	-	-	-	-	143	74	51.7
TOTAL	289	219	75.8	-	-	-	-	-	-	405	260	64.2

[α]each cross represents a single mating pair successively transferred twice at three day intervals.

Table 3. Egg Viability in various genotypic crosses of *Tribolium castaneum* at room temperature and about 60% R.H.

Pair mating[α]	Mating Type: +/+ X +/+ eggs	larvae	%	+/+ X mxp/mxp eggs	larvae	%	mxp/mxp X mxp/mxp eggs	larvae	%
1	119	103	86.6	168	136	81.0	75	53	70.6
2	101	74	73.3	-	-	-	40	28	70.0
TOTAL	220	177	80.5	-	-	-	115	81	70.4

[α]each cross represents a single mating pair successively transferred twice at three day intervals.

3) Egg Viability and Adult Phenotypic Ratios of maxillopedia homozygote/heterozygous crosses

When mxp heterozygotes and mxp homozygotes were crossed reciprocally, egg viabilities of 77.8% and 79.1% (Table 4) were obtained. These percentages are comparable to the values obtained for crosses between wild type and mxp homozygote beetles in which no decrease in egg viability was noted.

However, a significant deviation from the expected 1:1 ratio of mxp to wild type phenotypes was produced in each case. In both sets of crosses, the mxp class falls short of expected values. In the cross of +/mxp males and mxp/mxp females, a total number of 155 larvae was counted three weeks after the egg number was determined. From these larvae, 119 beetles (76.8%) reached the adult stage. In the cross of mxp/mxp males with +/mxp females, a total number of 235 larvae was counted and, of these, 206 (87.6%) reached the adult stage.

When the resultant adult phenotypic ratios of the two sets of crosses are examined (Table 4), it is apparent that the mxp class is diminished below expected values. In each cross, the wild type class approximates the 50% expected value; 74 out of 155 and 120 out of 235. The mxp class produced values significantly below those expected. Only 37.8% and 41.7% of the total reached the adult stage.

Table 4. Egg Viability and adult phenotypic ratios from (A) mxp/mxp X +/mxp and (B) the reciprocal cross.

A. Mating mxp/mxp X +/mxp	eggs	larvae	%	Adults (N) mxp	+	total	Adults % mxp	+
1	94	65	69.1	17	36	53	32.1	67.9
2	102	90	88.2	28	38	66	42.4	57.6
TOTAL	196	155	79.1	45	74	119[α]	37.8	62.2

B. Mating +/mxp X mxp/mxp	eggs	larvae	%	Adults (N) mxp	+	total	Adults % mxp	+
1	170	138	81.2	57	68	125	45.6	54.4
2	132	97	73.5	29	52	81	35.8	64.2
TOTAL	302	235	77.8	86	120	206[β]	41.7	58.3

[α]76.8% of larvae reached adult stage.

[β]87.6% of larvae reached adult stage.

Apparently mxp significantly reduces the number of larvae that reach adulthood. From observations made during scoring of beetles in these crosses and in others, it seems that a large proportion of deaths of mxp beetles occurs during the process of metamorphosis. In nearly every cross where some mxp homozygotes were expected, dehydrated pupae were observed. That these dead pupae were mostly mxp, was evident by examination of the maxillary palpi.

In attempts to obtain mxp - apt, mxp - ptl and mxp - mas homozygotes for linkage study backcrosses, vast numbers of these dead pupae were observed. Not only does the mxp gene affect pupal emergence by itself, but when it occurs with certain other mutants, the effect is greatly magnified. In fact, it was not possible to obtain any mxp - apt or mxp - mas homozygotes at all, yet both genes in each case could be observed in individual dead pupae.

4) Linkage relationships of maxillopedia mutants

Markers not associated with linkage group II showed no evidence of linkage with maxillopedia (mxp). A brief review of these negative findings follows.

To determine if mxp is a member of linkage group III, the markers black (b) and aureate (au) were used in backcrosses. The backcross b au +/+ + mxp males X b au mxp/b au mxp females and reciprocal crosses, from six and eight successful matings respectively, showed little deviation from the expected 1:1:1:1 ratio when each

marker was considered separately (see Appendix I). However, all classes of phenotypes that expressed mxp had decreased numbers. This is not surprising in the light of the viability reduction of mxp mutants.

The test of linkage for group IV produced similar results. Using hazel (h) and sooty (s) as known markers for group IV, variation from the expected ratios if the genes were not linked to mxp proved of little significance. Backcrosses resulting from fourteen and four successful matings for crosses with female heterozygotes and male heterozygotes, respectively, were performed. In this case, the mxp phenotypic classes were also reduced in number (see Appendix II).

The markers jet (j) and microcephalic (mc) were used in backcrosses to test mxp for linkage in group V. Fourteen successful matings were produced from crosses with female heterozygotes for all those traits and five from crosses with male heterozygotes. When each marker was considered separately, the wild type phenotypic class was the most numerous. This was expected since all three genes reduce viability. The maxillopedia classes were not reduced as much as those of the other markers (see Appendix III).

Microphthalmic (Mo), the only known marker for linkage group VI, was used in backcrosses. Backcrosses of female and male heterozygotes for Mo and mxp to mxp

homozygotes produced six and eight successful matings respectively. Mo-mxp phenotypic classes were considerably below expected values for nonlinkage; however, the wild type class was well-represented in the progeny (see Appendix IV). It appears that mxp occurring in conjunction with the Mo gene drastically reduces viability, although not to the extent observed with alate prothorax (apt) and missing abdominal sternites (mas) mentioned previously.

Backcrosses to test for linkage in group VII were performed with (Sa) Short antenna and (c) chestnut, which are known markers for the group. Phenotypic ratios from nine successful matings with female heterozygotes and from six successful matings with male heterozygotes only vary slightly from the expected 1:1:1:1 ratio when each marker is considered separately (see Appendix V). As in the other crosses, the mxp classes are all reduced due to factors which affect viability and adult survivorship.

For a determination of linkage in group VIII, antennapedia (ap) and short elytra (sh) were used. Three point backcrosses, ap sh +/+ + mxp females X ap sh mxp/ ap sh mxp males and the reciprocal cross, were performed as well as two point crosses between each known marker and mxp. All three groups of crosses failed to show linkage between mxp and the markers for linkage group VIII. The maxillopedia phenotypic classes were below

expected values in most of the crosses (see Appendices VI, VII and VIII).

Due to phylogenetic considerations of great interest, two separate backcrosses were attempted with the two known markers for linkage group IX. Recall that both alate prothorax (apt) and prothoraxless (ptl) are autosomal genes in T. castaneum, but are sex-linked in T. confusum. New genes found to be linked with these would be of great value in supporting the proposed phylogenetic relationship between the two species. Data for ptl backcrosses were difficult to obtain. Only two of the progeny exhibited a ptl phenotype in the two successful crosses when female heterozygotes for ptl and mxp were backcrossed to mxp. Five successful matings were obtained in the reciprocal cross and the ptl and ptl-mxp classes were greatly reduced in number (see Appendix IX). By taking into consideration the more viable mxp and wild type classes, linkage between the two genes was discounted. However, it is possible that ptl and mxp are so far apart that recombination values resemble those of random assortment. As mentioned previously, backcrosses to determine linkage between mxp and apt could not be carried out. Attempts to obtain apt-mxp homozygotes failed. It appears that individuals with both of these genes in homozygous condition are not able to make the transition from pupa to adult, since many partially emerged adults were found to be of the mxp-apt phenotype.

The marker abbreviated appendages (aa) was used to test linkage for mxp in group X. Even considering the difficulty in reading aa homozygotes, linkage between the genes was not demonstrated. Backcrosses with male heterozygous parents had the now familiar decrease in mxp classes (see Appendix X).

Data obtained from backcrosses involving the markers Dachs (Dch) and pearl (p) for linkage group II, which did demonstrate linkage with mxp, are summarized in Tables 5 and 6.

The application of formulas derived by Bailey (1961) shows that linkage occurs between Dch and mxp for backcrosses with female and male heterozygous parents with values of χ^2_L=282.7 (df=1, P<.005) and one of χ^2_L=166.7 (df=1, P<.005), respectively. However, when a different formula accounting for decreased viability of mxp was used, only in female heterozygote backcrosses did a differential viability between the two genes prove significant: χ^2_{mxp}=5.1 (df=1, P<.025). Viability studies for Dachs (Dch) have yet to be performed, but the viability of Dch in the male heterozygous backcrosses appears to be reduced in a similar proportion to the reduction of mxp. The crossover value for backcrosses with female heterozygous parents is 7.8 ± 1.40% and 5.7 ± 1.59% for the reciprocal cross.

Linkage is also demonstrated when pearl (p) and mxp are considered separately. Chi-square values for

Table 5. Backcross progeny from (A) Dch p +/+ + mxp female X + p mxp/+ p mxp male and (B) the reciprocal cross from 12 successful matings each (numbers in parentheses are decimal fractions of the total).

Phenotype	Cross A	Cross B
Dch p (Dch p-bristle)	179 (.45)[α]	81 (.38)[β]
mxp	116 (.29)	72 (.34)
Dch	36 (.10)	18 (.08)
p mxp	35 (.09)	29 (.14)
Dch p mxp	18 (.04)	5 (.02)
+	4 (.01)	3 (.02)
Dch mxp	7 (.02)	3 (.02)
p	2 (.00)	1 (.00)
TOTAL	397	212

[α]38 had one bristle on distal end of one maxillary palpus.

[β]27 had one bristle on distal end of one maxillary palpus.

Table 6. Backcross progeny from (A) Dch p +/+ + mxp females X + p mxp/+ p mxp males and (B) the reciprocal cross when each pair of genes is considered separately.

Phenotype	Cross A	Cross B
Dch	215	99
mxp	151	101
Dch mxp	25	8
+	6	4
TOTAL	397	212
p	181	82
mxp	123	75
p mxp	53	34
+	40	21
TOTAL	397	212
Dch p	197	86
+	120	85
Dch	43	21
p	37	30
TOTAL	397	212

backcrosses, χ^2_L=112.2 for female heterozygote and χ^2_L=49.1 for males, are both highly significant (df=1, P<.005). Again, however, it is only the female heterozygous backcrosses which show mxp having any differential viability: χ^2_{mxp}=5.1 (df=1, P<.025). The crossover value for heterozygous female parents is 23.4 ± 2.12% and 25.9 ± 1.77% for the male. Two point backcrosses attempting to show linkage between p and mxp made without the presence of the Dch allele failed to show linkage. Homozygous pearl (p) beetles for this cross were obtained from a different stock than those used in the three point cross. Further investigation into this may explain these results. Crossover values for Dch and p were 20.2 ± 2.02% for the female and 24.1 ± 2.94% for the male heterozygote backcrosses.

From Table 5, some progeny with the Dch-p phenotype were often observed to possess a bristle at the distal end of one maxillary palpus (see Fig. 1-D). These progeny were not scored as Dch-p-mxp beetles because this bristle was also observed in Dch p +/+ + mxp beetles obtained from Dch p +/+ p + X + + mxp/+ + mxp crosses. It appears that when the Dch and mxp genes are in the heterozygous condition, mxp acts as a semidominant at least some of the time. Whether the alleles Dch and mxp interact to produce this bristle is only conjecture at this point. As

previously mentioned, crosses between + Chicago wild type and mxp homozygotes failed to show any effect of mxp in the heterozygous condition.

Reindeer (Rd) has been included in both linkage group X and group II. According to Dawson (unpublished data), Rd and aa are linked in group X (Sokoloff, personal communication). Using the three point cross Rd-aa-mxp to test for linkage of mxp in group X, no linkage was demonstrated between Rd and aa; however, linkage between mxp and Rd became evident (Table 7). Levels of significance were very high for Chi-square tests for linkage (df=1, $P<.005$) for both sets of backcrosses, $\chi^2_L=244.6$ for female heterozygotes and $\chi^2_L=50.8$ for the males. Differential viability also had a high Chi-square significance value (df=1, $P<.005$) in each backcross, $\chi^2_{mxp}=14.5$ and $\chi^2_{mxp}=25.9$ for female and male heterozygotes, respectively. Crossover values of 29.1 ± 1.22% and 39.9 ± 1.39% were obtained for female and male heterozygous backcrosses.

This disparity between the crossover values in the two sexes can be explained by the existence of different genetic modifiers among males and females. Recently, Dewees (1975) has demonstrated that recombination values can be modified toward a higher or lower value in Tribolium castaneum by selection. Since recombination is under genetic control, these different values for the two sexes are not surprising.

Table 7. Backcross progeny from (A) Rd +/+ mxp female X + mxp/+ mxp male and (B) the reciprocal cross from 16 and 17 successful matings, respectively.

Phenotype	Cross A	Cross B
Rd	517	408
mxp	472	337
Rd mxp	154	193
+	251	301
TOTAL	1394	1239

DISCUSSION

1) Linkage relationships of maxillopedia

Dachs (Dch) and maxillopedia (mxp) have been shown to be closely linked by this present study. These genes are 7.8 units and 5.7 units apart for females and males, respectively. Linkage in the same three point cross was established between pearl (p) and (mxp). These genes are 23.4 units apart for females and 25.9 units apart for males. The map distances between Dch and p, obtained in these studies, were 20.2 for females and 24.1 for males. Sokoloff (unpublished data) established linkage between these two genes with values of 15.44 units apart for females and 19.71 units apart for males. Interference in female backcrosses was moderate, of the order of about .39, while in male backcrosses interference was nil (about 1%).

Linkage was also established between mxp and Rd in this present study. These genes were found to be 29.1 units apart for females and 39.9 units apart for males. Muñoz (unpublished data) found Rd and mas to be linked and 31.90-36.74 units apart. Sokoloff (unpublished data) has found mas and Dch to be closely linked. For females, Dch and mas were found to be 2.28 units apart and 8.74 units apart for males. These figures suggest that Rd is also a gene in linkage group II. The data just described serve to build the linkage map shown in Fig. 2.

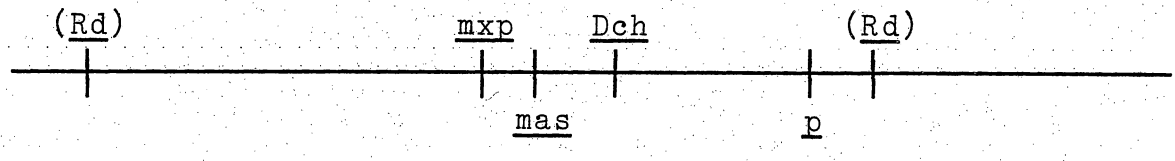


Figure 2. Proposed gene order for data obtained from Table 7 and from previous studies (Muñoz, unpublished data and Sokoloff, unpublished data).

Previous work on linkage group II began with the study of the mutant eye color gene pearl by Park (1937). It subsequently was designated the anchor gene for the group by Sokoloff (1966). Pink ($\underline{p}^{Pk}$) identified by Lasley (1960) was found to be allelic with pearl. It is recessive to wild type, but dominant to pearl. Dewees and Bell (1967) tested $\underline{p}$ and $\underline{p}^{Pk}$ for pseudoallelism from reciprocal crosses of $\underline{p}$ +/$\underline{p}^{Pk}$ $\underline{i}$ X $\underline{p}^{Pk}$ +/$\underline{p}^{Pk}$ + in mass matings. All 36,654 progeny were of the mutant phenotype, which indicated that at this level of resolution no recombination occurs between the two alleles. The pearl and pegleg (pg) mutants were found to be linked and about 30 units apart (Lasley and Sokoloff, 1961).

Ivory ($\underline{i}$) and pearl-like are also allelic autosomal recessive genes in linkage group II. Lemon and Blackman (1967) crossed pearl-like and ivory which produced an F_1 of all mutant beetles. Similar crosses between pearl and ivory by Dewees and Bell (1967) established a recombination frequency of .03%, showing that pearl and ivory are not allelic but closely located genes.

Reduced eye notch (Ren), an autosomal dominant mutation with recessive lethal effects, was also found to be in linkage group II (Bell and Shideler, 1971). Crosses produced a recombination rate of 0.37% with pearl and 31.7% with pegleg (pg). Crosses between pearl and pegleg showed a crossover rate of 30% which suggested a linear gene order

of Ren 0.37 p 30.0 pg, but this was not verified by three point crosses. The 30% crossover rate between pearl and pegleg duplicates the 30% rate obtained by Lasley and Sokoloff (1961) for the two genes.

The information reviewed above and the data accumulated during this study are summarized in Table 8. This also serves as the basis for the linkage map of the second chromosome provided in Fig. 3. Linkage group II is now one of the most thoroughly investigated autosomal linkage groups.

2) The importance of homeotic mutants in evolutionary studies

Homeotic mutants are known to occur in a wide variety of Arthropod groups, particularly in several orders of insects. This is not surprising in view of their common ancestry. These mutants are often used to demonstrate homologies in diverse groups and provide evidence for the structural changes which have occurred in their evolution. A prime example, to demonstrate the usefulness of homeotic mutants in such studies, is the investigation by Bridges and Dobzhansky (1933). They discovered the homeotic mutant proboscipedia in the fruit fly Drosophila melanogaster. This mutant gene produced mouth parts that approximated those of lower orders of insects. The pseudotracheae of the proboscis had disappeared completely in these flies. Changes in shape and

Table 8. Linkage data now available for group II of *Tribolium castaneum*.

MARKER	UNIT DISTANCE
Rd-*mxp*	29.1 - 39.9[α]
Rd-*mas*	31.9 - 36.7[β]
p - *i*	.03
p - *pg*	30
Dch-*mas*	2.3 - 8.7[γ]
Dch-*mxp*	7.8 - 5.7[α]
Dch-*p*	15.4 - 19.7[γ], 20.2 - 24.1[α]
p - *mxp*	23.4 - 25.9[α]
p - *Ren*	close linkage

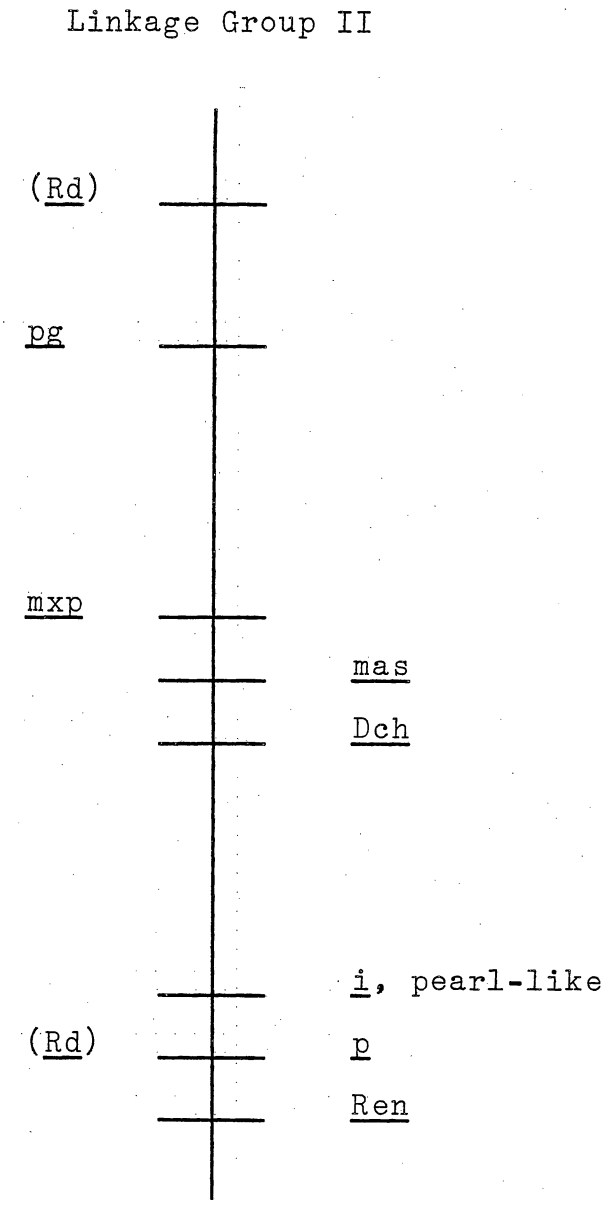
[α]present data.

[β]Muñoz (unpublished data).

[γ]Sokoloff (unpublished data).

Remaining data from Sokoloff, 1977.

Figure 3. Linkage map of group II in Tribolium castaneum.



position were noted in the labium, maxilla and maxillary palpus. In addition, the labrum and labial palpi had developed in place of the oral lobes. This finding supported early claims by MacCloskie (1880) that the fly proboscis contained structures homologous to mouth parts in other insect groups.

MacCloskie (1880), in his detailed study of the proboscis of the house fly and the function of the great tendons in its movement, concluded that these tendons must belong to the mandibles. They were found to closely resemble mandibular tendons of other insect orders and of lobsters in position and function. He concluded that the operculum of the proboscis represents two united mandibles, probably enclosing the labrum. The palpi of the proboscis were said to represent the maxillae.

Insects are presumed to have evolved from a millipede-like ancestor composed of a series of nearly identical leg-bearing segments. This view of insect evolution is supported by the existence of homeotic mutations which transform particular head region appendages into appendages characteristic of thoracic segments. Bridges and Dobzhansky (1933) found that the homeotic mutant proboscipedia in Drosophila melanogaster had the tip of the proboscis modified into a pair of jointed appendages which extended sidewise and were tipped with strong claw-like bristles.

Villee (1942) used this and other information on aristapedia, a homeotic mutant in Drosophila melanogaster in which the arista of the antennae are modified to tarsus-like segments, to demonstrate the homology of insect mouth parts with the antenna-mouthpart-walking leg series of appendages of other arthropods. Stocker (1981) supports the existence of this series in a study of sensory neuron pathways through homeotic organs in Drosophila melanogaster. Displaced leg and antennal neurons by homeotic transformations project exclusively into normal antennal and proboscis centers of the brain. It appears that affinities between the sensory neurons and the specific brain centers are due to homologies between antennal, leg and proboscis neurons and between the three corresponding brain centers.

Many mutants similar to those just described have been reported in the Coleopteran genus of Tribolium. In T. castaneum the mutation antennapedia, an autosomal recessive (Englert and Bell, 1963 and Sokoloff and Dawson, 1963) modifies the antennae into leg-like appendages; and maxillopedia, an autosomal recessive, modifies the maxillary and labial palpi into structures resembling walking legs (Hoy, 1966a). In T. confusum labiopedia, a sex-linked recessive, modifies labial palpi into similar leg-like structures (Daly and Sokoloff, 1965). Dawson (1968) found an homologous mutation, labiopedia (lp), in Tribolium castaneum Herbst.

Garcia-Bellido (1977) developed a model to explain the possible genetic manifestations of homeotic mutations and their mode of operation in development. He found that the transformed organ (allotype) is identical developmentally to the organ it mimics (telotype). Daly and Sokoloff (1965) demonstrated that both allotype and telotype in the same individual can also be equally affected by another mutation. Tribolium confusum flour beetles possessing the mutant gene labiopedia (lp) were crossed with stilted leg (stl) mutants. The mutant stilted legs (stl) produces legs with tarsal segments fused, tibia lengthened, femur reduced and trochanters often absent. The labial legs were affected in the same manner as the walking legs in phenotypically stl-lp beetles.

Garcia-Bellido (1977) concluded further that the function of the wild type allele (autotype) is to repress the developmental characteristic of the telotype in the autotype. He believes that genes have evolved which act to repress a primitive developmental pathway and thus permit the evolution of a new developmental pathway to proceed. In the insects, the evolutionary process has changed the repetition of identical segments into segments with specific characteristics of head, thorax and abdomen. When the genes that produce these specific characteristics become inactive by mutation, the alternative (homeotic) pathway that appears is a thoracic one. It is therefore assumed that the thoracic

pathway is the archetypic one. Sokoloff, Papini and Faustini (1981) in their study of Horned gena (Hg), a homeotic sex-linked dominant with semilethal effects, suggest that the gene is atavistic, returning specialized segments to a more primitive condition. They assert that the gene is of ancient origin and has existed at least since the genera of Tenebrionidae were being evolved. Evidence is drawn from other Coleopteran genera possessing similar horn-like structures in the head region and from cytological studies by Smith (1950), which developed a Coleopteran karyotype phylogeny.

From a review of embryonic studies, Snodgrass (1935) has suggested a five stage evolution of the definitive insect structure from a theoretical wormlike ancestor. In the first wormlike stage, the animal consists of a long segmented part coextensive with the alimentary canal and a short unsegmented preoral part, or prostomium. The mouth is located ventrally between the prostomium and the first segment. Since the prostomium contains the principal sensory ganglion, it is regarded as the archicephalon or primitive head. The second stage is characterized by each body segment acquiring a pair of movable lateroventral appendages with one or two pairs of antennal organs on the prostomium. In the third stage, a protocephalon forms the union of the first postoral somite and the primitive head. The fourth stage is characterized by segregation of the

post-protocephalon segments into three distinct regions. The first region is called the gnathal region since its appendages are destined to become the feeding organs. The thoracic region is located posterior to the gnathal region and its appendages develop into organs of locomotion. In the third region, the appendages are reduced and mostly obliterated. It has been termed the abdominal region. In the fifth stage, the gnathal segments become united with the protocephalon. This definitive head is now composed of the protocephalic appendages or antennae, the prostomium and the four succeeding gnathal segments.

The circumstantial evidence presented above suggests that the mouth parts of insects were originally derived from walking appendages. Presumably, during the evolution and specialization of the segments associated with the mouth, a suppressor gene or genes arose that suppressed the formation of legs in the mouth region. The genetic evidence suggests that such a gene could be the wild type allele of maxillopedia. Under normal circumstances, this gene prevents the maxillary palpus-forming cells from coming under the influence of embryonic leg-forming fields and the result is the production of normal maxillary palpi. When this gene mutates, however, it no longer exerts its suppressive effect, and cells which would normally produce maxillary palpi come under the influence of leg-forming genes. These genes then produce leg-like instead of maxillary palpus-like structures.

SUMMARY

The present study shows that maxillopedia (mxp), a homeotic mutant in Tribolium castaneum Herbst, has complete penetrance and reduces egg viability. Viability reductions are not confined to the egg stage but are very evident in pupae that fail to become adults. Further viability reductions are produced when mxp occurs with certain other mutant genes. Beetles which are homozygous for apt and mxp or mas and mxp fail to make the transition from pupae to adult. A similar effect is produced when ptl and mxp occur together.

In addition, when mxp occurs in the heterozygous condition with the Dachs (Dch) mutant, a semidominant effect of mxp is produced at least some of the time. This effect is manifested by one maxillary palpus having a single distal bristle.

The maxillopedia (mxp) gene is linked with known markers for linkage group II. It is 7.8-5.7 units from Dachs (Dch) and 23.4-25.9 units from pearl (p). It is also linked with Reindeer (Rd) which was recently associated with linkage group II. Reindeer (Rd) and maxillopedia (mxp) are 29.9-39.9 units apart.

The value of homeotic mutants in tracing evolutionary history of insects is discussed.

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APPENDICES

Appendix I. Backcross progeny from (A) b au +/++ mxp female X b au mxp/b au mxp male and (B) the reciprocal cross from 8 and 6 successful matings, respectively (numbers in parentheses are decimal fraction of the total).

Phenotype	Cross A	Cross B
b au mxp	78 (.12)	40 (.11)
b au	92 (.15)	49 (.13)
b mxp	73 (.11)	35 (.10)
b	72 (.11)	54 (.15)
au mxp	56 (.09)	36 (.10)
au	74 (.12)	47 (.13)
mxp	88 (.14)	40 (.11)
+	100 (.16)	62 (.17)
TOTAL	633	363

Appendix II. Backcross progeny from (A) h s +/+ + mxp female X h s mxp/h s mxp male and (B) the reciprocal cross from 14 and 4 successful matings, respectively (numbers in parentheses are decimal fractions of the total).

Phenotype	Cross A	B
h s mxp	148 (.16)	1 (.01)
h s	205 (.22)	1 (.01)
h mxp	54 (.06)	9 (.06)
h	50 (.05)	8 (.05)
s mxp	38 (.04)	1 (.01)
s	44 (.05)	1 (.01)
mxp	193 (.20)	50 (.35)
+	203 (.22)	72 (.50)
TOTAL	935	143

Appendix III. Backcross progeny from (A) j mc +/+ + mxp female X j mc mxp/j mc mxp male and (B) the reciprocal cross from 14 and 5 successful matings, respectively (numbers in parentheses are decimal fractions of the total).

Phenotype	Cross A	Cross B
j mc mxp	30 (.11)	0 (.00)
j mc	43 (.16)	2 (.02)
j mxp	16 (.06)	1 (.01)
j	19 (.07)	3 (.04)
mc mxp	12 (.04)	8 (.09)
mc	22 (.08)	2 (.02)
mxp	59 (.21)	26 (.31)
+	76 (.27)	43 (.51)
TOTAL	277	85

Appendix IV. Backcross progeny from (A) Mo +/+ mxp female X + mxp/+ mxp male and (B) the reciprocal cross from 6 and 8 successful matings, respectively (numbers in parentheses are decimal fractions of the total).

Phenotype	Cross A	Cross B
Mo mxp	18 (.04)	3 (.01)
Mo	81 (.19)	48 (.13)
mxp	169 (.39)	169 (.46)
+	165 (.38)	144 (.40)
TOTAL	433	364

Appendix V. Backcross progeny from (A) Sa c +/+ + mxp female X + c mxp/+ c mxp male and (B) the reciprocal cross from 9 and 6 successful matings, respectively (numbers in parentheses are decimal fractions of the total).

Phenotype	Cross A	Cross B
Sa c mxp	44 (.14)	33 (.10)
Sa c	48 (.16)	51 (.15)
Sa mxp	33 (.11)	46 (.14)
Sa	45 (.14)	38 (.11)
c mxp	21 (.07)	27 (.08)
c	39 (.13)	52 (.15)
mxp	35 (.11)	36 (.11)
+	45 (.14)	56 (.16)
TOTAL	310	339

Appendix VI. Backcross progeny from sh +/+ mxp female X sh mxp/sh mxp male from 2 successful matings (numbers in parentheses are decimal fractions of the total).

Phenotype	Cross
sh mxp	34 (.19)
sh	33 (.19)
mxp	52 (.30)
+	57 (.32)
TOTAL	176

Appendix VII. Backcross progeny from (A) ap +/+ mxp female X ap mxp/ap mxp male and (B) the reciprocal cross from 5 and 7 successful matings, respectively (numbers in parentheses are decimal fractions of the total).

Phenotype	Cross A	Cross B
ap mxp	44 (.17)	84 (.20)
ap	70 (.26)	96 (.23)
mxp	68 (.26)	112 (.26)
+	81 (.31)	134 (.31)
TOTAL	263	426

Appendix VIII. Backcross progeny from (A) ap sh +/+ + mxp female X ap sh mxp/ap sh mxp male and (B) the reciprocal cross from 2 and 7 successful matings, respectively (numbers in parentheses are decimal fractions of the total).

Phenotype	Cross A	Cross B
ap sh mxp	6 (.05)	15 (.04)
ap sh	7 (.05)	28 (.08)
ap mxp	22 (.16)	48 (.14)
ap	19 (.14)	61 (.17)
sh mxp	16 (.12)	18 (.05)
sh	19 (.14)	30 (.08)
mxp	25 (.18)	60 (.17)
+	22 (.16)	96 (.27)
TOTAL	136	356

Appendix IX. Backcross progeny from (A) ptl +/+ mxp female X + mxp/+ mxp male and (B) the reciprocal cross from 2 and 5 successful matings, respectively (numbers in parentheses are decimal fractions of the total).

Phenotype	Cross A	Cross B
ptl mxp	0 (.00)	1 (.00)
ptl	2 (.02)	16 (.04)
mxp	73 (.51)	202 (.52)
+	67 (.47)	172 (.44)
TOTAL	142	391

Appendix X. Backcross progeny from (A) aa +/+ mxp female X aa mxp/aa mxp male and (B) the reciprocal cross from 7 and 8 successful matings, respectively (numbers in parentheses are decimal fractions of the total).

Phenotype	Cross A	Cross B
aa mxp	147 (.25)	99 (.23)
aa	154 (.27)	126 (.29)
mxp	156 (.27)	80 (.19)
+	124 (.21)	125 (.29)
TOTAL	581	430