RESOLUTION OF AN EQUIVOCAAL GENETIC ELEMENT IN
DROSOPHILA MELANOGASTER: ORGANIZATION
OF THE MAROON-LIKE LOCUS

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ABSTRACT

Despite extensive prior study, an understanding of the genetic organization of the maroon-like locus (ma-l:1-64.8) has been elusive. A large-scale, three-point fine structure recombination experiment is described, whose results provide documentation for an inescapable argument that maroon-like is a single element rather than a polycistronic system.

The maroon-like locus (ma-l:1-64.8) of Drosophila melanogaster has been under investigation in this laboratory as one of several interesting model systems for the study of genetic organization in higher eukaryotes. Restricted by cytogenetic analysis to the salivary chromosome region 19D1,2-3 (Lefevre, personal communication), all known mutants of the maroon-like locus exhibit a brownish eye color phenotype reflecting a deficiency in the red (pteridine) pigments. In addition to the eye color defect, mutant individuals are characterized by the absence of xanthine dehydrogenase (XDH) (Glassman and Mitchell 1959), pyridoxal oxidase (PO) (Forrest, Hanly and Lagowski 1961), and aldehyde oxidase (AO) (Courtright 1967), three enzyme activities associated with distinct molecular species (Courtright 1967; Collins, Duke and Glassman 1971).

In prior studies (Chovnick et al. 1969; Duck 1974; Finnerty 1974), a collection of twenty fully viable ma-l mutants were shown to fall into five complementation groups on the basis of eye color, XDH activity and AO activity, which behave as concordant phenotypes in mutant heterozygotes (Figure 1). The significance of our failure to observe any complementation for PO activity is qualified by the relative insensitivity of the assay in our hands (Chovnick et al. 1969).

Three possible models for the organization of this locus are suggested by the complementation pattern (Figure 1).

1. The complementation may be taken to reflect the existence of functionally

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distinct genetic elements, each concerned with separate steps in the production of this phenotypic complex. Following this model, Groups III, IV and V mutants (Figure 1) represent lesions in each of the three genetic elements which are transcribed and translated separately. On this model, the viable Group I and Group II mutants must be deletions, whose extent is described by the complementation map (Figure 1).

2. A variant of the first model would consider the ma-l locus to consist of several contiguous genetic elements, which respond to common transcriptional control to produce a polycistronic message. It differs from the first model in that it predicts that the viable Group I and II mutants would include site mutants as well as deficiencies, the site mutants reflecting direction of transcription and translation. Thus, Group II site mutants would be polar mutants located in the second cistron, while the Group I site mutants might include lesions at sites concerned with regulation or initiation of transcription as well as polar translational mutants in the first genetic element.

3. A third model would consider maroon-like as a single genetic unit exhibiting allele complementation. The model argues that the biologically active ma-l product is a dimer or higher multiple aggregate of a single polypeptide. Complementation between different mutants then would reflect the production of hybrid aggregates which possess some biological activity.

Mutants of ma-l, representative of the five known complementation classes, were subjected to fine structure mapping experiments (Finnerty, Duck and Chovnick 1970; Duck 1974). The results of this study are summarized in Figure 2, which relates the resultant genetic map of mutant sites to the comple-
It should be noted that the genetic map presents an inverted order of \textit{ma-l} mutant sites, which merely reflects the fact that the fine structure experiments were carried out on \textit{In(1)sc}\textsuperscript{a} and \textit{In(1)sc}\textsuperscript{aII-sc}\textsuperscript{ab} chromosomes. Two points from this study are pertinent to the question concerning the genetic organization of the \textit{ma-l} region. (1) Groups III, IV and V mutants map as sites in an order consistent with the complementation map, thus permitting the possibility that they are mutants in adjacent genes. (2) The tested Group I and Group II mutants map in positions consistent with the polar nature of their complementation, and the recombination data serve to eliminate Model 1 which requires that all of the Group I and II mutants be deletions. Of the remaining two models, a strong circumstantial argument in support of the allele complementation model (Model 3) may be developed from: (1) analysis of the levels of XDH, AO and PO activity seen in various mutant heterozygotes (Chovnick et al. 1969; Duck 1974; Finnerty 1974), (2) the fact that all \textit{ma-l} mutants comprise a single recessive lethal complementation group when raised on standard Drosophila medium appropriately supplemented with purine (Bailie and Chovnick, unpublished), and (3) comparative analysis of the recombination results of the maroon-like study with those resulting from the rosy locus (Chovnick, Ballantyne and Holm 1971; Chovnick et al. 1974) suggests that the maroon-like locus may be an order of magnitude smaller than rosy.

Perhaps the strongest evidence in support of the single gene-allele complementation model involves a consideration of the complementation pattern exhibited by a double mutant recombinant, \textit{ma-l}\textsuperscript{F4} \textit{ma-l}\textsuperscript{F5}. Consider the mutants, \textit{ma-l}\textsuperscript{F4} (Group III), \textit{ma-l}\textsuperscript{1} (Group IV) and \textit{ma-l}\textsuperscript{F5} (Group V), which complement...
in all single heterozygous combinations. On a polycistronic operon model (Model 2), the heterozygote, $F_4 + F_3$, should exhibit complementation reflecting the recessive nature of each of these mutants. In contrast, the single cistron-allele complementation model admits the possibility that the doubly mutant product may not complement in heterozygotes with $ma-l^1$ (Case and Giles 1960; Fincham 1966). Following this logic, large-scale experiments were carried out which selected for the double mutant exchange product from heterozygous females, $ma-l^{p1}/ma-l^{p2}$, based upon the prediction that the double mutant, $F_4 F_3$, would not complement $ma-l^1$. Such experiments succeeded, and recombinants were recovered which were classified as representing the double mutant, $ma-l^{p1} ma-l^{p2}$ (Finnerty and Chovnick 1970). They did not complement with $ma-l^{p3}$, $ma-l^{p2}$, or for that matter, with any other mutant allele of $ma-l$. However, even this observation in support of the single cistron model is subject to qualification in the following fashion. Consider that, in fact, there are three adjacent structural genes in a polycistronic operon, and that the recombination experiment involved selective recovery of deficient unequal crossovers from the heterozygote $F_4 + F_3$.

One might argue that such crossovers represent a class of unequal exchanges which produced $F_4 F_3$ recombinants that also were deficient for some part of the region between these sites (i.e., they were deficient for part of the central genetic element, and should be labeled $F_4-F_3$). Certainly, precedent for such an argument is well established in recombination studies of the white locus in *Drosophila melanogaster* (Judd 1961; Green 1963).

Thus, despite extensive investigation of the $ma-l$ locus, the existing published data, albeit consistent with the single cistron-allele complementation model, provides something less than a conclusive argument. Moreover, this discussion serves to illustrate some of the difficulties which plague the direct pursuit of questions bearing upon higher organism genetic organization.

Recent studies in this laboratory have provided a somewhat enlightened view of allele recombination and its resultant products in *Drosophila* (Chovnick, Ballantyne and Holm 1971; Chovnick et al. 1974). This development represents the underlying basis for the present experiment which demonstrates that the double mutant recombinant $F_4 F_3$, discussed above, is not deficient for any part of the $ma-l$ region. Thus, this experiment removes the one qualification to the conclusion drawn earlier (Finnerty and Chovnick 1970), that the $ma-l$ locus represents a single genetic element.

**MATERIALS AND METHODS**

The present experiments involve two sets of large-scale matings of $ma-l$ mutant heterozygous females to tester males (Figure 3). Parents were transferred to fresh half-pint culture bottles at two-day intervals through a total of 5 broods of progeny per 20 pairs of parents. Upon removal of parents, 1 ml of 0.2% aqueous purine (Sigma Chemical Co.) was added to each developing culture. At this level of purine supplementation, $ma-l$ mutant progeny lacking XDH activity, or possessing very low levels of XDH activity, die before eclosion. Estimation of total number of zygotes sampled is obtained by omitting purine from a portion of the bottles in each experiment,
and counting total progeny. With the noted exceptions, all chromosomes and markers have been described in several previous reports (CHOVNICK et al. 1969; FINNERTY, DUCK and CHOVNICK 1970). The \( y^{sc} S^{L} m^{a-l^{F}L} m^{a-l^{F}L} \) chromosome (Figure 3) is one of the putative double mutant recombinants recovered by FINNERTY and CHOVNICK (1970) and discussed above. The doubly marked \( Y \) chromosome (Figure 3) \( y^{+} Y \), is one of several \( m^{a-l} \) mutant-bearing \( Y \) chromosomes generated as gross deletions from an \( X Y^{s.} \) chromosome following a protocol described earlier (CHOVNICK 1968). In addition to carrying the mutant, \( m^{a-l} \), the proximal \( X \) duplication covers \( l^{t2-4a} \), a short deficiency distal to (standard chromosome order) the \( m^{a-l} \) locus which is deficient for \( sw \) (1-64,0) (SCHALET, personal communication).

**Tests of selective system survivors:** Phenotypic \( m^{a-l^{+}} \) male survivors carry an exceptional \( X \) chromosome of maternal origin, and a paternal \( y^{+} Y \) chromosome. Diagnosis of the yellow and \( l^{t2-4a} \) markers flanking the \( m^{a-l} \) region of these exceptional chromosomes is made by crossing each male successively to (1) \( C(1)\)RM, \( y v f m a-l/Y m a-l^{108} \) and (2) \( C(1)\)RM, \( y v f m a-l/Y \) (Finnerty and Schulet 1968). The derivation and composition of the \( C(1)\)RM chromosome is described by Schulet (1963). The \( Y \) chromosome carrying \( m a-l^{108} \) covers \( l^{t2-4a} \) (Schulet and Finnerty 1968). Preservation of the exceptional \( X \) for further test is ensured by cross 1. The female survivors carry an exceptional \( X \) chromosome of maternal origin, as well as a \( y^{+} m a-l \) paternal \( X \) chromosome. Flanking marker diagnosis in females is complicated slightly by two factors: (1) Exceptional females that are \( m a-l^{+}/m a-l^{+} \) produce maternally effected progeny (Glassman and Mitchell 1959; Chovnick and Sang 1968) in testcrosses. It should be noted that the complementing mutant heterozygote female survivors do not produce maternally effected progeny. (2) There is a greater than normal frequency of disturbed \( X-Y \) segregation associated with the \( y^{+} Y m a-l^{+} \) chromosome leading to the production of daughters receiving both paternal sex chromosomes. Surviving exceptional \( F_{1} \) females were crossed to \( y^{+} S^{L} m a-l^{F} l^{t2-4a} f S^{R}/y^{-} Y m a-l^{108} \) males. If progeny of this cross do not show a maternal effect, then the exceptional chromosome is readily classified with respect to yellow and \( l^{t2-4a} \) markers, and the chromosome may be preserved for further tests in male progeny carrying \( y^{-} Y m a-l^{108} \). If the progeny do exhibit a maternal effect, then a group of the late eclosing, \( m a-l^{+} \)-like males are collected, and individually tested by the same testcrosses as described above for exceptional males. Finally, all exceptional chromosomes were subjected to complementation testcrosses in order to establish the specific constitution of each recombinant with respect to the \( m a-l \) locus.

**RESULTS AND DISCUSSION**

Figure 3 presents the complete genotypes of the parents used in the large-scale recombination experiments (Experiments 1 and 2, Table 1) designed to question...
TABLE 1

Fine structure recombination experiments with the ma-l^{F4}ma-l^{F3} double mutant

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Cross</th>
<th>Total progeny $\times 10^4$</th>
<th>Exceptional chromosomes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Class 1</td>
</tr>
<tr>
<td>1</td>
<td>$ma-l^{F4}ma-l^{F3} / ma-l^{l}$</td>
<td>5.76</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>$ma-l^{F4}ma-l^{F3} / ma-l^{l}$</td>
<td>4.75</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Pooled</td>
<td>10.51</td>
<td>2</td>
</tr>
</tbody>
</table>

whether the recombinant double $ma-l^{F4}ma-l^{F3}$ is, or is not, deficient for any part of the $ma-l$ region normally located between these mutants. The experimental logic, identical for both crosses, is illustrated in Figure 4 for Experiment 1. Figure 4 assumes that the double mutant is not deficient. The only single event that would produce a completely wild-type, $ma-l^{+}$, meiotic product from such a heterozygous female would be a conversion of the $ma-l^{+}$ allele (Recombinant 1, Figure 4). Such an event, involving genetic information transfer of a segment of the region between the $ma-l^{F4}$ and $ma-l^{F3}$ sites on the double mutant strand, will only occur if such a segment exists. However, if one does recover the $ma-l^{+}$ conversion, it might be argued that the deficient region lies immediately to the left or right of the position opposite $ma-l^{l}$ on the double mutant recombinant chromo-

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**Event**

<table>
<thead>
<tr>
<th>Event</th>
<th>Resultant Recombinant</th>
</tr>
</thead>
<tbody>
<tr>
<td>$y \ F_4 + F_3 \ +$</td>
<td>Conversion, $ma-l^{l}$ $y^+ [\ast \ast] l^2-4a^*$</td>
</tr>
<tr>
<td>$y \ F_4 + F_3 \ +$</td>
<td>$l^2-4a^*$</td>
</tr>
<tr>
<td>$y \ F_4 + F_3 \ +$</td>
<td>Conversion, $ma-l^{F4}$ $y [\ast + F_3] l^2-4a^*$</td>
</tr>
<tr>
<td>$y \ F_4 + F_3 \ +$</td>
<td>Crossover $y^+ [\ast + F_3] l^2-4a^*$</td>
</tr>
</tbody>
</table>

**Fig. 4.**—Expected recombinants.
some. However, the experimental protocol also questions this possibility. Consider next a conversion of the \textit{ma-l}^{P4} site on the double mutant strand (Recombinant 2, Figure 4). Such event, involving information transfer from a segment opposite \textit{ma-l}^{P4} on the \textit{ma-l} bearing strand will yield a \textit{ma-l}^{P4} complementing mutant allele as a product only if there is no deficient region remaining on that strand. Similarly, a crossover (Recombinant 3, Figure 4) will yield a \textit{ma-l}^{P4} product only if there is no deficient region remaining on that strand. The experiment is designed to recover the latter two recombinant classes by virtue of the fact that both male and female heterozygotes, \textit{ma-l}^{P4}/\textit{ma-l}, possess approximately 25\% of the wild-type level of XDH activity (CHOVNICK \textit{et al.} 1969) and will survive on the chosen level of purine enrichment. Thus, three classes of recombinants are expected, if the double mutant chromosome is not deficient for any part of the \textit{ma-l} locus. A similar logic obtains for experiment 2 which tests the mutant, \textit{ma-l} against the double mutant.

The reader will recognize that additional recombinant classes are theoretically possible, but none will survive growth on the selective medium. Thus, following the above logic, two classes of recombinants which are \textit{ma-l}^{P4} might be expected. Unfortunately, the \textit{ma-l}^{P4} complementation yields very low levels of XDH activity (CHOVNICK \textit{et al.} 1969)—hence their inability to survive on this medium.

Table 1 summarizes the results of these experiments which produced a total of ten surviving progeny distributed in an apparently random fashion in cultures that assayed more than 10 million zygotes. Nine of the survivors reproduced, and all fell into the expected three classes upon test, as indicated in Table 1. The low frequency of recombination events at the \textit{ma-l} locus is entirely consistent with our past experience with this system (FINNERTY, DUCK and CHOVNICK 1970).

The present data clearly rule out the possibility that the double mutant recombinant, \textit{ma-l}^{P4} \textit{ma-l}^{P4}, fails to complement \textit{ma-l} in heterozygotes because it is deficient for some part of the central region located between \textit{ma-l}^{P4} and \textit{ma-l}^{P4}. In so doing, these data remove the one qualification to the conclusion drawn earlier that the pattern of complementation exhibited by the \textit{ma-l} mutants reflects variation in a single genetic element exhibiting allele complementation.

\textbf{LITERATURE CITED}


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