STUDIES ON GENETIC ORGANIZATION IN HIGHER ORGANISMS: I. ANALYSIS OF A COMPLEX GENE IN *DROSOPHILA MELANOGASTER*[1]

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CURRENT views concerning the nature of elementary genetic units in multicellular organisms focus upon the model of genetic organization which has emerged in recent years from investigations of microbial systems. CHOVNICK (1966) has reviewed the evidence which permits the identification of a genetic unit of function in *Drosophila melanogaster*. This entity, the rosy cistron, was shown to be a solitary unit concerned with the enzyme xanthine dehydrogenase (XDH), located within a short region of the right arm of chromosome 3 at 52.00 (salivary section 87D). Genetic analysis of a large number of mutational alterations within the rosy cistron revealed: (1) there does exist a mechanism for intracistronic genetic recombination in higher organisms; (2) the resolving power of such recombination approaches that seen in microbial systems; (3) utilization of selective procedures for the study of intracistronic recombination permits the demonstration that a cistron in a higher organism consists of a linear order of separable sites capable of independent mutation.

Of the various genetic systems used in studies of genetic organization in Drosophila and other multicellular organisms, the rosy cistron serves as an example of the simplest type. Strong genetic and biochemical evidence argues that it is a structural gene for XDH (GRELL 1962; YEN and GLASSMAN 1965). Mutations restricted to the rosy cistron are homozygous viable, and fall into three classes: (1) a class of "wild-type isoalleles" which produce electrophoretic variants of the enzyme (YEN and GLASSMAN 1965); (2) a "leaky" mutant which has very much reduced enzyme activity (HUBBY 1961); (3) and a large group of mutants which are enzymatically inactive (CHOVNICK *et al.* 1964). Study of this last class of mutants failed to find any evidence of allele complementation (SCHALET, KERNAGHAN and CHOVNICK 1964; CHOVNICK 1966).

A second category of genetic systems which has been studied extensively may be referred to collectively as "complex genes." Most prominent among these are the bithorax (LEWIS 1964), dumpy (CARLSON 1959), lozenge (GREEN and GREEN 1956), Notch (WELSHONS 1965), rudimentary (FAHMY and FAHMY 1959; GREEN 1963), and white (GREEN 1959; JUDD 1959) complexes. Characteristics of these

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systems are: (1) mutants exhibiting morphological anomalies with, in some instances, seemingly unrelated pleiotropic effects; (2) complex patterns of complementation; (3) low recombination frequencies between mutants within any one system. In the absence of a complete understanding of the structural and functional relationships involved, these systems have been interpreted variously. Analysis of the phenotypic effects of mutants of the lozenge complex suggested that the entire complex was most simply interpreted as an integrated physiological unit (CHOVNICK, LEFKOWITZ and FOX 1956). Recently, several other complex gene systems have been interpreted as physiological units. In these cases specific models differing in level of genetic organization have been invoked in explanation of the phenotypic interactions. Thus, the Notch and rudimentary complexes (GREEN 1963; WELSHONS 1965) have been interpreted in terms of a single cistron exhibiting allele complementation (FINCHAM and CODDINGTON 1963), while LEWIS (1964) has shown how the phenotypic effects of bithorax mutants may be fitted to an operon model of genetic organization. Although either or both of the above models might apply in the interpretation of these exceedingly complex situations, real proof has been lacking. This report provides evidence demonstrating that the allele complementation model is the underlying basis for the phenotypic effects seen in the analysis of one complex gene in Drosophila.

The maroon-like locus of *Drosophila melanogaster* (*ma-l*: 1–64.8) was chosen as a model system for the investigation of a complex gene. Maroon-like mutants have a brownish eye color resulting from a reduction in the red (drosopterin) pigments, loss in activity of xanthine dehydrogenase (GLASSMAN and MITCHELL 1959), as well as loss in activity of pyridoxal oxidase (FORREST, HANLY and LAGOWSKI 1961), and aldehyde oxidase (COURTRIGHT, 1967). Chemical and genetic evidence argues that these enzyme activities are associated with distinct molecular species (GLASSMAN 1965; COURTRIGHT 1967). Association of the eye color phenotype with XDH activity is inferred from: (1) mutations in the rosy cistron (discussed above), which lose only the XDH activity, produce the same mutant eye color phenotype as mutations of maroon-like; (2) *In vivo* chemical inhibition of XDH produces a phenocopy of the mutant eye color (KELLER and GLASSMAN 1965). In contrast, the mutation, *lxd* (3–33±), inactivates pyridoxal oxidase, retains a low level of XDH activity and has a wild-type eye color (KELLER and GLASSMAN 1964; GLASSMAN 1965).

MATERIALS AND METHODS

All non-lethal maroon-like mutants used in this study are listed, along with their source, in Table 1. The lethal maroon-like mutants described in Figure 2 arose from X-ray experiments. In addition to maroon-like mutations induced on chromosome *1*, some (as indicated) were induced on a *Y*-bearing duplication of the maroon-like region (SCHALET 1963; SCHALET and FINNERTY 1968).

Derivation of an attached-X *chromosome* with balanced lethal markers proximal to and heterozygous for complementing maroon-like mutants was accomplished by mating X-rayed females of the genotype, $\frac{y\ v\ f\ l5\ ma\text{-}l^{1}\quad +}{y\ v\ +\ +\ ma\text{-}l^{F1}\ l20\cdot y+}$ to $sc^{8}B$ males, and picking up exceptional non-disjunctional daughters associated with the loss of the $y+$ duplication (non-Bar, yellow, vermilion daughters). The derivation of a $y+Yma\text{-}l^{2}$ chromosome is described elsewhere (CHOVNICK 1968).

TABLE 1

Non-lethal maroon-like mutants

Mutant	Mutagen	Reference
ma-l^{1}	X ray	BRIDGES and BREHME (1944)
ma-l^{2}	X ray	SCHALET (1961)
ma-l^{F1} *(bz;ma-l*^{bz}*)**	Nitrogen mustard CB 3007	FAHMY and FAHMY (1958)
ma-l^{F2} *(ma-l bz*^{56k}*)**	Methyl Methanesulphonate	FAHMY and FAHMY (unpublished)
ma-l^{F3} *(ma-l*^{65c}*)**	N-ethyl-N-nitrosourethane	FAHMY and FAHMY (unpublished)
ma-l^{F4} *(bz*^{65c}*)**	N-ethyl-N-nitrosourethane	FAHMY and FAHMY (unpublished)
ma-l^{14}	X ray	SCHALET and FINNERTY (1968)
ma-l^{20}	2,4,6-triethyleneimino—1,3,5-triazine (TEM)	*ibid.*
ma-l^{21}	TEM	*ibid.*
ma-l^{23}	TEM	*ibid.*
ma-l^{24}	ethyl methanesulfonate (EMS)	*ibid.*
ma-l^{25}	EMS	*ibid.*
ma-l^{26}	TEM	*ibid.*
ma-l^{27}	TEM	*ibid.*
ma-l^{28}	TEM	*ibid.*
ma-l^{29}	TEM	*ibid.*
ma-l^{30}	EMS	*ibid.*
y+Yma-l^{106}	X ray	*ibid.*
y+Yma-l^{116}	X ray	*ibid.*

* Prior designations.

Enzyme preparations were made following two different procedures: (1) In those experiments which measured XDH activity per fly, enzyme was prepared at 5° C. Flies were homogenized in 5 ml of Tris buffer (pH 7.5) containing 10 mg/ml of crystallized bovine plasma albumin (Armour Pharmaceutical), treated with Norite-A (0.01 g/5 ml) and centrifuged at $>$ 30,000 $\times$ *g*. Norite treatment and centrifugation was repeated and followed by Sephadex G-25 extraction (0.1g/5ml), centrifugation and filtration through sintered glass; (2) In those experiments which measured both XDH and pyridoxal oxidase activity/mg protein, extraction was carried out at 5°C. Flies were homogenized in 0.1M phosphate buffer at pH 7.3, centrifuged at $>$ 40,000 $\times$ *g*. A supernatant sample was taken for protein determination by the method of LOWRY, *et al.* (1951) prior to the addition of albumin (15 mg/ml). Treatment with Norite-A (50 mg/ml) was followed by centrifugation and repeat of the Norite extraction and centrifugation followed by filtration through sintered glass.

Enzyme assays: XDH activity was measured by the method of GLASSMAN (1962). The change in fluorescence associated with the conversion of 2-amino-4-hydroxypteridine (AHP) into isoxanthopterin (ISO) was measured at 30° C using a Photovolt Model 540 Fluorometer equipped with a 335mμ primary filter and a 412 mμ secondary filter. At maximum sensitivity the instrument was standardized at 100 fluorescence units with 3.5 $\times$ 10^{-6}M quinine sulfate, and zeroed with a blank containing the reaction mixture omitting enzyme. Reaction tubes contained 0.02 ml of 10^{-3}M methylene blue, 0.01 ml of 10^{-3}M AHP and 2 ml of enzyme extract supernate.

Pyridoxal oxidase activity was measured by the fluorometric method of KARAM (1965). The change in fluorescence associated with the conversion of pyridoxal to pyridoxic acid was measured at 30° C in the fluorometer equipped with a 335 mμ primary filter, and the secondary filter is a combination of three Turner filters (2-A, 47B, 1-60). The fluorometer was standardized as described above. Reaction tubes contained 0.1ml of 4 $\times$ 10^{-3}M pyridoxal and 2 ml of enzyme extract supernatant.

The maroon-like maternal effect: Genotypically *ma-l* progeny of *ma-l+* heterozygous or homozygous mothers exhibit a maternal effect. Such offspring have very low levels of XDH ac-

tivity and exhibit a wild-type eye color (CHOVNICK and SANG 1968). All experiments were carried out in crosses designed to avoid this complication.

RESULTS

A map of the region of chromosome *1* proximal to the centromere, obtained largely by deletion mapping experiments, is presented in Figure 1. Genetic units whose mutations are lethals are located adjacent to maroon-like. They are readily distinguished from maroon-like mutants, and serve a most useful function in the characterization of the induced genetic lesions involving maroon-like.

A set of 37 maroon-like mutations recovered from X-ray and various chemical induction experiments, both in this laboratory and elsewhere, comprise the raw material of this investigation. Of these, 19 are fully viable (Table 1), while the remaining 18 are homozygous or hemizygous lethal (Figure 1).

Restricting attention to the viable maroon-like mutants (Table 1), *inter se* crosses leading to the production of heterozygotes reveal that they fall into five groups based upon complementation with respect to eye color (Figure 2). Within any one group, there is no complementation. Crosses between groups led to the indicated pattern of complementation. Thus, Group I mutants are non-complementing, while Group II mutants complement only with Group V. Groups III and IV complement each other and Group V, but do not complement in heterozygotes with Groups I or II. At this time, Groups IV and V are each represented by one mutant. The second major class of maroon-like mutants are those which are lethal in homozygotes or hemizygotes (Figure 1). They are readily classified as maroon-like mutants by the fact that they exhibit a mutant phenotype when heterozygous with all of the viable maroon-like mutants, and thus, might be classified as Group I mutants, with the added feature of a lethal phenotype when homozygous. Crosses between appropriate stocks carrying the various lethal maroon-like mutants reveal that they are lethal with each other in all combinations. Moreover,

Group III — Group IV — Group V

Group II

Group I

Group I	Group II	Group III	Group IV	Group V
ma-l^{F2}	*ma-l*^{2}	*y*+*Yma-l*^{116}	*ma-l*^{1}	*ma-l*^{F3}
ma-l^{14}	*ma-l*^{21}	*ma-l*^{F1}		
ma-l^{20}	*ma-l*^{26}	*ma-l*^{F4}		
ma-l^{23}	*ma-l*^{27}			
ma-l^{24}	*ma-l*^{30}			
ma-l^{25}				
ma-l^{28}				
ma-l^{29}				
y+*Yma-l*^{106}				

FIGURE 2.—Maroon-like complementation map.

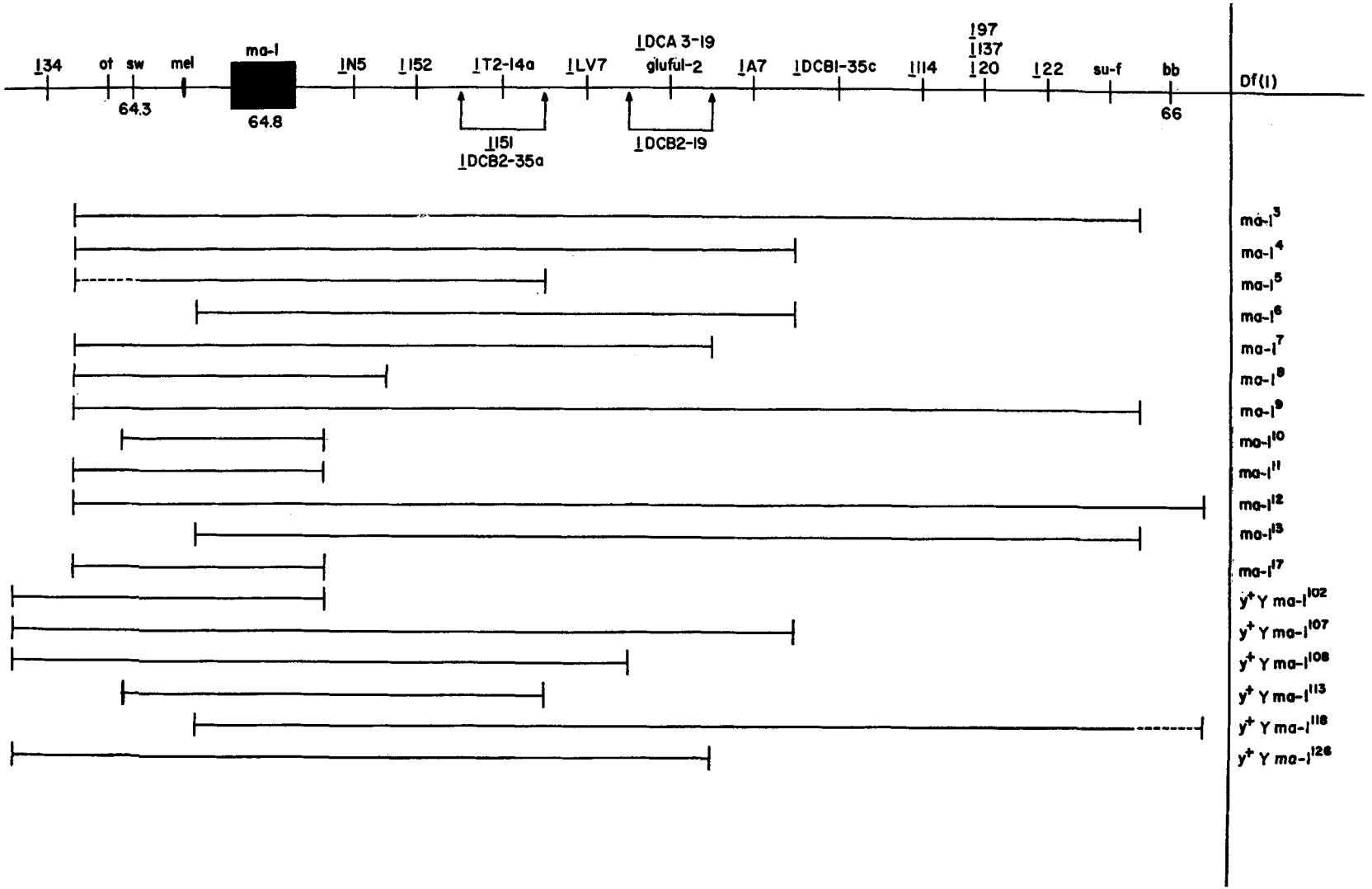

FIGURE 1.—A map of the proximal region of chromosome *1* indicating the extent of the genetic deficiency for each of the lethal maroon-like mutants.

crosses between the lethal maroon-like mutants and a series of other non-*ma-l* mutants in this region (largely lethals) reveals that all of the lethal maroon-like mutations are deficiencies. A summary of the genetic analysis of the lethal maroon-like mutants which permits their classification as deficiencies is presented in Figure 1. The deficiency associated with each of the lethal maroon-like mutants is described by a line under the map. Thus, *Df(1)mal*^{5} behaves in crosses as though it were missing the region from *sw* through *l*T2–14a inclusive. Although it covers *l*34, it has not been tested against *ot*, and thus the left breakpoint of the deficiency has not been defined. This situation is represented by the dotted portion of the line. Of some interest is the fact that *Df(1)ma-l*^{6}, which behaves as a deficiency for the region to the right of *ma-l*, is lethal in heterozygotes with *Df(1)ma-l*^{17} and *y*$^{+}$*Yma-l*^{102}, which are characterized as deficiencies extending to the left of *ma-l*. Since the only known missing region common to these deficiencies is *ma-l*, one might argue that complete loss of the *ma-l* function is lethal. However, since all of the lethal *ma-l* mutants are clearly deficiencies, we prefer to believe that these observations reflect the existence of one or more essential genetic units adjacent to *ma-l*, as yet unidentified by site mutants.

Analysis of the ma-l *complementation pattern:* Considering only the visible eye color phenotype, the pattern of complementation seen in heterozygotes of different viable maroon-like mutants may be interpreted on one or another of three models:

(1) The complementation may be taken to reflect functional distinction, and the existence of several cistrons concerned with separate steps in the production of this phenotype. Following this model, Groups III, IV, and V mutants (Figure 2) may be taken to represent mutations in each of three adjacent cistrons which are transcribed separately. This model requires that the viable Group I mutants be deletions extending into all three cistrons, while the Group II mutants must be deletions involving two adjacent members of the cluster of three cistrons.

(2) The second model, a variant of the first, considers the entire maroon-like region as an operon consisting of three adjacent cistrons. 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 the direction of translation of a polycistronic message produced by this operon. 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 mutants in the first cistron.

(3) A third model would consider maroon-like as a single cistron exhibiting allele complementation. The model argues that maroon-like is a structural gene whose biologically active product (with respect to eye color) is a dimer or higher multiple aggregate of a single polypeptide. Complementation between different mutants then reflects the production of hybrid aggregates which possess some biological activity (FINCHAM and CODDINGTON 1963; SCHLESINGER and LEVINTHAL 1963).

Elimination of Model 1: A genetic fine structure analysis of the maroon-like region is in progress, and this investigation will be reported separately. However,

two points emerge from this study which are pertinent to the present discussion. (A) Groups III, IV, and V mutants map as site mutants in an order consistent with the complementation map (Figure 2), thus permitting the possibility that they are mutants in adjacent cistrons. (B) Two mutants of Group II and four mutants of the viable Group I noncomplementers have been subject to mapping experiments. All behave as site mutants, and the mapping data are consistent with the operon notion of Model 2. That these mutants map as site mutants serves to eliminate the first model which requires that all Group I and II mutants be deficiencies.

XDH activity in complementing mutant heterozygotes: GLASSMAN and PINKERTON (1960) as well as URSPRUNG (1961) have examined the complementation phenotype including XDH activity in the heterozygote, *ma-l*^{F1}/*ma-l*^{1}, a Group III/Group IV (Figure 2) heterozygote. These authors report that, at the enzyme level, the complementation produces only a small fraction (5–10%) of the activity seen in wild-type flies. In addition to confirming the results reported for this heterozygote (Figure 3), the present report provides a more detailed analysis of the complementation pattern. Table 2 presents the results of four separate enzyme activity experiments. At the time of these experiments only five assays could be carried out simultaneously. Consequently, each experiment has a wild-type (Oregon-R) and null activity (homozygous mutant) control for reference. In

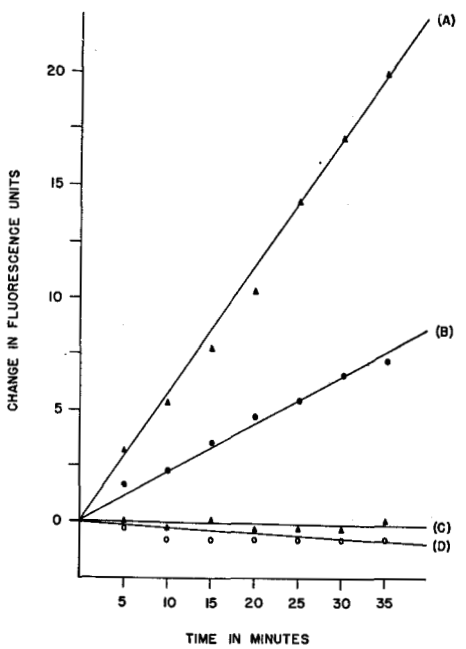

FIGURE 3.—XDH activity measured as change in fluorescence units in time at 30° C in extracts of adult females less than 24 hrs after eclosion. Activity of Oregon-R, wild-type females (A) is presented as fluorescence units/fly while all other genotypes are presented as units/10 flies. (B)— *ma-l*^{1}/*ma-l*^{F1}; (C) — *ma-l*^{1}/*ma-l*^{1}; (D) — *ma-l*^{F1}/*ma-l*^{F1}.

TABLE 2

XDH activity measured as change in fluorescence units in time at 30°C in extracts of adult females, less than 24 hrs from eclosion

Experiment	A					B				
Group	I / I	II / I	III / I	IV / I		II / V	III / V	IV / V	V / V	
Genotype / Time	ma-l^{F2} / ma-l^{F2}	ma-l^{2} / ma-l^{F2}	ma-l^{F1} / ma-l^{F2}	ma-l^{1} / ma-l^{F2}	Ore-R	ma-l^{2} / ma-l^{F3}	ma-l^{F1} / ma-l^{F3}	ma-l^{1} / ma-l^{F3}	ma-l^{F3} / ma-l^{F3}	Ore-R
5	-1.5	0	-0.5	0	4.8	1.5	10.5	13	-0.5	3.8
10	-1	-0.5	-1	-0.5	11.3	8.5	20	23	-1.5	7.8
15	-1.5	-0.5	-2	-0.5	17.0	15.5	30.5	33	-1.5	12
20	-1	-0.5	-1	-0.5	20.5	23	43.5	44	-1.5	17
25	-1	-1.0	-1.5	-1.0	26	30.5		56	-1.5	
30	-2	-1.0	-1.5	-1.0	31.5	36.5			-1.5	

Experiment	C					D				
Group	II / III	III / III	IV / III	IV / IV		V / I	V / III	I / III	III / III	
Genotype / Time	ma-l^{2} / ma-l^{F4}	ma-l^{F1} / ma-l^{F4}	ma-l^{1} / ma-l^{F4}	ma-l^{1}/ma-l^{1}	Ore-R	ma-l^{F3} / ma-l^{F2}	ma-l^{F3} / ma-l^{F4}	ma-l^{F2} / ma-l^{F4}	ma-l^{F4} / ma-l^{F4}	Ore-R
5	0	0	2	-0.5	4.5	1	9	0	-3	4
10	-0.5	0	3	-0.5	11	2	19	0	-2	9.5
15	+0.5	-0.5	5	-1.5	15	1	30	0	-2	14.5
20	0	-1	7	0	15	2	36	1	-2	18
25	-1	-0.5	8	0	20	2	45	1	-1	23.5
30	-1	-1	10	-1	25	1	51	1	-3	26.5

In all experiments the activity for Oregon-R is presented as fluorescence units/fly, while all other genotypes are presented as units/10 flies.

Table 2, XDH activity for Oregon-R is presented as activity/fly, while that for all other genotypes is presented as activity/10 flies. Although carried out at separate times, the controls for the four experiments are quite comparable, and the entire Table may be taken as a single experiment exhibiting the following features:

(1) Those heterozygotes which show no complementation on the basis of eye color phenotype also show no complementation for XDH activity. Thus, Group I mutants, which are classed as non-complementing with all other groups on the

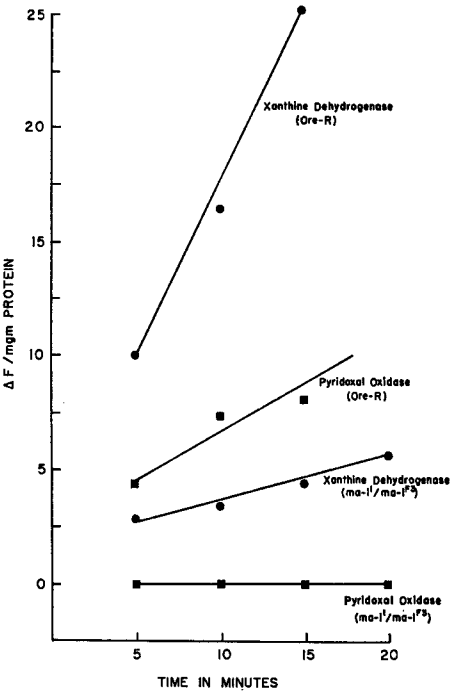

FIGURE 4.—Xanthine dehydrogenase and pyridoxal oxidase activities measured as change in fluorescence units/mg protein in time at 30°C in extracts of adult females of the indicated genotypes.

basis of eye color, show no activity in heterozygotes with mutant members of the other groups (see Experiments A and D, Table 2).

(2) The Group V mutant, *ma-l*^{F3}, which exhibits complementation for eye color with members of all other groups except Group I, also shows enzyme activity in heterozygotes with mutant members of all other groups except Group I (see Experiments B and D, Table 2).

(3) Enzyme activities exhibited by the complementing heterozygotes range from a low of 5% of wild-type seen in the Group III/Group IV heterozygotes (*ma-l*^{F1}/*ma-l*^{1}; *ma-l*^{F4}/*ma-l*^{1}) through 10–12% for the Group II/Group V heterozygote (*ma-l*^{2}/*ma-l*^{F3}) to a high of 25% of wild-type activity seen in the Group III/Group V (*ma-l*^{F1}/*ma-l*^{F3}; *ma-l*^{F4}/*ma-l*^{F3}) and Group IV/Group V (*ma-l*^{1}/*ma-l*^{F3}) heterozygotes (Table 2 and Figure 4).

Pyridoxal oxidase in complementing mutant heterozygotes: We are able to confirm and extend prior reports (FORREST, HANLY and LAGOWSKI 1961; GLASSMAN 1965; COURTRIGHT 1967) concerning pyridoxal oxidase activity in mutants of several loci. Thus mutants of rosy, which show no activity for XDH, appear to be wild-type with respect to pyridoxal oxidase. On the other hand, the mutant *lxd*, which exhibits reduced XDH activity, has no pyridoxal oxidase activity. In addition, all of the viable maroon-like mutants used in this study show no activity for pyridoxal oxidase in homozygotes and hemizygous males.

Of particular interest for this investigation would be pyridoxal oxidase activity levels in heterozygotes for maroon-like mutants which show complementation for XDH. Unfortunately, the levels of pyridoxal oxidase activity exhibited by extracts of wild-type flies are low compared to the XDH activity of the same extracts. Either the pyridoxal oxidase assay is not as sensitive as that for XDH or the activity is lost during the extraction procedure, or possibly the actual amount of pyridoxal oxidase/fly is low. Therefore, for this investigation the extraction and assay procedure was changed to permit assay of extracts of large numbers of flies. Following procedures described by KARAM (1965), XDH and pyridoxal oxidase activity were measured in terms of mg protein for all complementing maroon-like mutant heterozygote combinations.

Two features of this study are pertinent: (1) XDH activity/mg protein in all complementing mutant heterozygote extracts when compared to control activity in wild-type extracts exhibits the same relative activities as those seen in comparisons made on a per fly basis. (2) In contrast, the mutant heterozygote extracts show no pyridoxal oxidase activity. Figure 4 summarizes the data obtained for extracts of *ma-l*^{1}/*ma-l*^{Fs} heterozygotes. This genotype exhibits a wild-type complementing eye color, has the highest levels of XDH activity seen in complementing mutant heterozygotes, yet is completely negative with respect to pyridoxal oxidase.

Clearly, if one focuses attention upon pyridoxal oxidase, the observations are consistent with the notion of maroon-like as a single cistron. Moreover, the levels of XDH activity seen in complementing heterozygotes are more reminiscent of allele complementation than those seen between mutants in separate cistrons.

Subunit aggregation as the basis for complementation between ma-l *mutants:* Further evidence in support of the single cistron model comes from experiments which derive from the model of allele complementation that has emerged from the investigation of the *am* mutants in Neurospora (FINCHAM and CODDINGTON 1963) and the alkaline phosphatase mutants in *E. coli* (SCHLESSINGER and LEVINTHAL 1963). These investigations have demonstrated that the biologically active gene product, in each case, is a dimer or higher multiple aggregate of a single polypeptide whose primary structure is determined by the cistron under investigation, and that restoration of some activity may result from union of different mutant monomers to produce hybrid aggregates. Such hybrid enzymes differ from the wild-type enzyme in activity as well as in various physical properties.

Following this model, consider the maroon-like function as that of a structural gene concerned with the production of a polypeptide which is biologically active as a dimer or some higher multiple aggregate. A very sensitive assay of the biological activity of complementing mutants of maroon-like is available by following XDH activity in a fly which is otherwise wild-type. In such a system the genotype has provided the individual with sufficient information for very high levels of XDH activity, and the specific genetic composition of *ma-l* mutants becomes the limiting factor in XDH activity.

Figure 5 presents two simplified forms of the allele complementation model applied to a set of three mutant alleles of maroon-like, *ma-l*^{x}, *ma-l*^{y} and *ma-l*^{z}. In the model, *ma-l*^{x} and *ma-l*^{y} complement each other. The mutant, *ma-l*^{z}, is

Genotype	$ma\text{-}l^x/ma\text{-}l^y$ (A)	$ma\text{-}l^x/ma\text{-}l^y/ma\text{-}l^z$ (B)	Ratio A/B
Monomers produced	$ma\text{-}l^x \longrightarrow x$ $ma\text{-}l^y \longrightarrow y$	$ma\text{-}l^x \longrightarrow x$ $ma\text{-}l^y \longrightarrow y$ $ma\text{-}l^z \longrightarrow z$	
Random aggregation into dimers	$(x+y)^2$ $x \cdot x+2x \cdot y+y \cdot y$	$(x+y+z)^2$ $x \cdot x+y \cdot y+z \cdot z+2xy+2x \cdot z+2y \cdot z$	
If only x·y dimers are active	1/2 of dimers are active	2/9 of dimers are active	
Model I Dosage regulation to produce finite number, 2N, monomers in both genotypes	Total monomers = 2N Total dimers = N Active dimers = 1/2N	Total monomers = 2N Total dimers = N Active dimers = 2/9N	2.25
Model II Each allele produces N monomers	Total monomers = 2N Total dimers = N Active dimers = 1/2N	Total monomers = 3N Total dimers = 3/2N Active dimers = 2/9·3/2N Active dimers = 1/3N	1.5

FIGURE 5.—Two forms of the allele complementation model applied to maroon-like.

known to direct the production of a mutant monomer by virtue of the fact that it does complement certain alleles, but it does not complement either *ma-l*^{x} or *ma-l*^{y}. The general model assumes (1) that biological activity is resident in dimer aggregates which (2) form by random association of monomers, and that (3) only the hybrid dimer, $x \cdot y$, has biological activity. Model I, which is referred to as the dosage regulated model, assumes that there is an adjustment of activity of the various alleles to lead to a common finite number of monomers (and hence dimers) produced by both genotypes. Model II assumes a constant output of monomers per allele. Comparison of the number of active dimers produced by the *ma-l*^{x}/*ma-l*^{y} heterozygote (Genotype A) with that produced by the *ma-l*^{x}/*ma-l*^{y}/

TABLE 3

XDH activity measured as cumulative change in fluorescence units/fly over a 35-min incubation period at 30°C in extracts of adult females

Experiment	Complementing heterozygote attached-X, C(1)RM,y v f l5 ma-l^1 +/y v + + ma-l^{F1} l20				Controls			Ratio Column A / Column B
	y+Yma-l^{113}	y+Yma-l^{2}	y+Yma-l^{106}	y+Yma-l^{102}	ma-l^{F1}/ma-l^{F1}	ma-l^{1}/ma-l^{1}	Canton-S	
1	1.0	0.56	..	..	..	0	9.5	1.8
2	1.3	0.81	..	..	..	0	7.0	1.5
3	1.7	1.4	1.0	..	..	0	18	1.2
4	1.9	1.1	.	..	..	..	..	..
	1.8	1.0	..	..	..	..	..	1.8
	2.3	1.3	..	..	..	..	..	..
5	1.4	0.83	..	1.8	0	..	6.0	1.7
6	1.3	0.67	..	1.4	0	..	6.5	2
7	1.3	0.90	0.75	..	0	..	13	1.4
Average	1.6	0.95	0.88	1.6	0	0	10	1.7

Complementing heterozygote females carry the attached-*X* chromosome indicated as well as the indicated *Y* chromosome.

ma-l^{z} heterozygote (Genotye B) reveals that the presence of the *ma-l*^{z} mutant leads to a reduction in the number of biologically active hybrid aggregates. However, the two models of allele complementation differ, and this difference is reflected in the ratio, A/B, of active dimers expected in the two genotypes. The expected ratio for Model I is 2.25, while it is 1.5 for Model II. In contrast, the operon model would argue that the mutant, *ma-l*^{z}, must be a polar mutant involving the cistrons represented by *ma-l*^{x}, and *ma-l*^{y}, and consequently the XDH activity expected for the two genotypes would be the same (A/B=1).

The results of experiments designed to distinguish between these possibilities are summarized in Table 3. Essentially, the experiments involve assay of XDH activity in extracts of females of two genotypes. The attached-*X* chromosome synthesized for this purpose (see MATERIALS and METHODS) is a balanced lethal system, heterozygous for two complementing alleles, *ma-l*^{F1}/*ma-l*^{1}, which are Group III and Group IV mutants (Figure 2). In addition, one female genotype included a doubly marked *Y* chromosome with a duplication of the proximal region of the *X* marked with *ma-l*^{2} on one arm, and the distal tip of the *X* carrying y^{+} on the other arm (CHOVNICK 1968). The *ma-l*^{2} mutant belongs to Group II, does not complement either Group III or IV mutants, but does complement Group V. This genotype serves as the *ma-l*^{x}/*ma-l*^{y}/*ma-l*^{z} of the model (Table 3, Column B). The other experimental genotype (Table 3, Column A) includes the same attached-*X* chromosome, but carries the doubly marked *Y* chromosome, y^{+}*Yma-l*^{113} (Figure 1). In addition to carrying the distal tip of the *X* marked with y^{+}, it also carries a duplication of the proximal region of the *X* from which was deleted the *ma-l* region and several surrounding cistrons. This female serves as the *ma-l*^{x}/*ma-l*^{y} of the model. Both females are y^{+} *v ma-l*$^{+}$ in phenotype. Controls in these experiments are wild-type females (Canton-S) which serve to indicate that the assay system is competent to record high levels of enzyme activity, and either the homozygous mutant, *ma-l*^{1}/*ma-l*^{1} or *ma-l*^{F1}/*ma-l*^{F1} which

indicate that the system is capable of recording null activity. In addition to the two experimental genotypes discussed above, several experiments included females carrying the complementing attached-*X* and either $y^+Yma\text{-}l^{106}$ or $y^+Yma\text{-}l^{102}$. The results with these genotypes will be discussed separately.

The experiment was run seven times, and the data are summarized in Table 3. In all experiments, the wild-type control enzyme was an extract supernate of 5 females. Since wild-type females of all ages have high levels of XDH activity, individuals were chosen from stock cultures without control of age. The broad range of high activities seen in these controls is believed to reflect this variation in age and culture conditions. For all other genotypes used in the experiment, fresh cultures were set up for each experiment, and newly emerged adults were collected on the morning of each run. All adults of the experimental genotypes were only a few hours from eclosion at the time of enzyme extraction. Enzyme extractions and assays were conducted as described above (MATERIALS and METHODS), and thus produced eight measurements for each preparation. Plots of each assay are straight lines whose slopes are presented in Table 3 as cumulative change in fluorescence/fly over the 35 min period of measurement.

In the first two experiments, each enzyme preparation involved 20 flies (except wild-type). Experiment 3 was conducted despite the fact that there were not equal numbers of flies available for extraction. Thus the $y^+Yma\text{-}l^{113}$ extract consisted of 16 flies while all others involved 10 flies each. Experiment 3 demonstrated the feasibility of working with smaller numbers of flies/extract, and all remaining experiments were carried out on extracts of 15 flies. At the time of experiment 4, large numbers of newly emerged flies of the experimental genotypes were available, and three extracts of each genotype were made. Controls were not run on this day, since we were not equipped to carry out more than six assays at a time. The absence of controls on this experiment is not considered serious, since in our total experience with this assay system homozygous mutants have never shown activity, and wild-types always have high XDH activity. Moreover, the data of experiment 4 are entirely consistent with the results of the other experiments. Major features of the experiments summarized in Table 3 are as follows:

1. The addition of the *ma-l*^{2} mutant to the genetic constitution of the complementing heterozygote $ma\text{-}l^{F1}/ma\text{-}l^1$ leads to a depression of the complementation as measured in terms of XDH activity. This depression is seen in every experiment.

2. The ratio of XDH activities for the two genotypes, A/B, averages 1.7 over the seven experiments. While significantly greater than 1, and less than 2.25, the observed ratio is not different from that expected on the model of allele complementation which assumes a constant output of gene product per allele.

3. Two additional genotypes were tested. In one case (Column C), in addition to the complementing heterozygote attached-*X*, the females possessed a *Y* chromosome carrying a duplication of the proximal region of *X* which covers the region from *l*34 through *su-f*, but which had a fully viable Group I maroon-like mutation, $ma\text{-}l^{106}$, induced on it. On the operon model such a mutant should be either a deletion involving all three cistrons, a polar mutant in the first cistron, or an operator or promoter mutant. In any case, the operon model would predict that

this mutant would not dilute the complementation activity. However, y^+Y *ma-l*^{106} (Column C, Table 3) behaves like y^+Y *ma-l*^{2}. It depresses the complementation activity in each of three experiments.

4. The fourth genotype tested (Column D) involved the complementing heterozygote attached-*X* carrying y^+Y*ma-l*^{102}. This chromosome is similar in derivation to y^+Y*ma-l*^{106}. However, y^+Y*ma-l*^{102} is an X-ray induced mutation of *ma-l* associated with a deletion of the region immediately to the left of *ma-l*, but still covers the region to the right of *ma-l* (Figure 1). Examination of Column D reveals that it fails to dilute or depress the complementation activity in all experiments, entirely consistent with the genetic evidence that it is a deletion of at least part of the maroon-like cistron. The use of y^+Y*ma-l*^{102} serves as a further control, providing greater confidence in the significance of the complementation depression test.

DISCUSSION

Examination of the pattern of complementation with respect to the visible phenotype exhibited by heterozygotes of different mutants of the maroon-like complex, coupled with site mapping data, provided an enticing argument in favor of considering the maroon-like region as an operon consisting of three adjacent cistrons. However, quantitative analysis of the complementation with respect to XDH and pyridoxal oxidase activities precludes such a model in favor of the notion that maroon-like is a single cistron exhibiting allele complementation. Although the data from the complementation dilution experiment adhere to one version of the simple dimer model of Figure 5, the authors are loath to conclude that the biologically active product of the maroon-like cistron is, in fact, a dimer. It is conceivable that larger aggregates with varying hybrid combinations of differing activities could lead to the same average results as those obtained.

Turning to the question concerning the role of the maroon-like locus in the genetic control of XDH, pyridoxal oxidase, and aldehyde oxidase, the present results are easily adapted to models proposed earlier (GLASSMAN 1965). One model would consider the maroon-like cistron as a structural gene producing a polypeptide which is utilized as a dimer or higher multiple aggregate in control of some step in an unknown biosynthetic path leading to the production of a cofactor or activator common to all three enzymes. On such a model, the XDH and pyridoxal oxidase activity levels serve as indirect assays of the biological activity of the maroon-like product. Differences in the results obtained with the different assays merely reflect differences in sensitivity of the assays. A more specific model directly relates the maroon-like function to the molecular structure of the enzymes whose activities are under study. Thus, two or more monomers of the maroon-like polypeptide, in combination with at least one polypeptide produced by the rosy cistron and possibly a component from the *lxd* cistron comprise XDH, while only one monomer of the maroon-like polypeptide is involved in the structure of pyridoxal oxidase. The relationship of the maroon-like product to aldehyde oxidase cannot be inferred from the present study.

The above models of maroon-like function may be instructive in the interpretation of the patterns of phenotypic effects seen in other cases of complex genes

which seemingly defy interpretation as single cistrons. In these cases the pleiotropic morphological effects associated with mutants may be interpreted as assays of differing sensitivity for the function of some unknown gene product. The differing sensitivities may be either qualitative or quantitative in nature (and possibly both). Thus, a given mutation may have a dramatic effect in one developmental path, a slight effect on another path, and no effect on a third path. If the morphological system under investigation were a simple one (i.e., the common cofactor model of maroon-like function), exclusive use of that morphological variate which was easily quantitized, and exhibited high sensitivity would suffice for the analysis. The pleiotropic effects of the lozenge mutants appear to fit such a model (CHOVNICK *et al.* 1956). However, a second dimension may enter into the situation. The relative sensitivities of the morphological responses to a gene product may vary as a function of the position in the peptide which is altered, or, conceivably, even in the case of a series of different mutant substitutions in the same codon. The phenotypic effects seen in several of the complex gene systems in *Drosophila* might well be instances of such effects.

SUMMARY

The maroon-like mutants of *Drosophila melanogaster* were chosen as a model system for the investigation of a complex gene. Genetic analysis of a series of 37 mutants obtained by X ray and chemical mutagenesis identified one class of 18 mutants as deficiencies extending beyond the maroon-like region. Restricting attention to the remaining 19 mutants, analysis of the pattern of complementation seen in heterozygotes with respect to eye color, xanthine dehydrogenase and pyridoxal oxidase activities provides a compelling argument in favor of one model of genetic organization, that of a single cistron exhibiting allele complementation. On the hypothesis that the observed pattern of complementation reflects the fact that the biologically active product of maroon-like is a dimer or higher multiple aggregate, experiments were carried out which resulted in striking support for the model.

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