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Experiments with the *maroon-like* mutation of *Drosophila melanogaster*

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SUMMARY

Cell lineage analysis of the *maroon-like* mutation of *Drosophila melanogaster* revealed the most extensive degree of non-autonomy reported to date in *Drosophila*: all 1454 gynandromorphs in which *X* chromosome loss uncovered the *ma-l* mutation had *ma-l*$^{+}$ eye colour. In contrast, among 331 gynandromorphs in which *X* chromosome loss simultaneously uncovered the *vermilion* and *maroon-like* mutations, approximately 16% had *v* phenotype but with one possible exception all gynandromorphs again had *ma-l*$^{+}$ eye colour. These results suggest that very small amounts of the *ma-l*$^{+}$ gene product are necessary for wild-type eye colour development and they are therefore compatible with the one cistron–allelic complementation model that has been proposed for the *ma-l* locus. They also provide the best estimate available to date of *In(1)*w^{vc}-induced internal mosaicism: 7%. A preliminary attempt to detect DNA-induced transformants among 6 DNA-injected preblastoderm *ma-l* embryos and at least 80000 of their F_1 to F_4 descendants has yielded completely negative results. An investigation of the maternal effect which *ma-l*$^{+}$ mothers exert on the eye colour of their genetically *ma-l* offspring revealed that, in contrast to earlier observations, this effect is not universal: some phenotypically *ma-l* and intermediate *ma-l* flies were observed in young cultures. The discrepancy between this and earlier observations is probably attributable to as yet uncharacterized nutritional deficiencies in the diet of flies used in this experiment. Cytoplasm drawn from blastoderm *ma-l*$^{+}$ embryos and injected into the posterior region of *ma-l* preblastoderm embryos failed to induce eye-colour alterations in all seven flies which survived the treatment. Injection of the contents of embryos of certain genotypes and developmental stages into *ma-l* pupae 24–48 h old did alter in some instances the eye colour of treated *ma-l* flies. Various tests strongly suggest that these alterations are not due to injection of a substance that has been stored in the egg during oogensis or that has been produced by the embryo itself prior to injection and they therefore preclude the possibility that a simple *in vivo* bioassay for the *ma-l*$^{+}$ substance has been achieved. Rather, they indicate that the observed eye-colour alterations are due to transplantation of blastoderm-stage embryos which remain active long enough within *ma-l* hosts to produce and release a substance into the hosts' haemolymph and that this substance in turn induces phenotypic alterations in the hosts' eye colour. When *v* and *ma-l* eye colour changes are simultaneously

monitored, it appears that injection of embryonic contents into pupae is equally or more effective in modifying the *v* phenotype than in modifying the *ma-l* phenotype. Based on these observations, a tentative hypothesis regarding the time of activation of the *ma-l*$^+$ gene and the relationship between the immediate product of this gene, the maternal substance stored in the egg and the substance released by tissue transplants is proposed.

1. INTRODUCTION

The XDH system constitutes at present one of the most thoroughly studied gene-enzyme systems in *Drosophila*. Its properties have been comprehensively described in three recent reviews (Dickinson & Sullivan, 1975; Courtright, 1976; Finnerty, 1976) and will therefore not be reviewed here. We shall only mention that although a great deal of genetic and biochemical information has been accumulated over the years, more experimental data are required before a theoretical analysis of this promising system, and its potential implications for gene regulation in eukaryotes, can be profitably carried out. The present communication reports some additional characteristics of one gene in this system: *maroon-like* (*ma-l*).

2. MATERIALS AND METHODS

All flies were reared at 22–25 °C on a standard *Drosophila* medium which had not been supplemented with live yeast.

Detailed descriptions of, and references to, most of the genetic variants mentioned in this report appear in Lindsley & Grell (1968). The unstable ring chromosome *In(1)w*^{vc}, *v*, which carries a *v*^{1} allele, was derived for the purpose of these experiments by means of double crossing-over between a rod *X* chromosome carrying the *v*^{1} allele and the unstable ring chromosome *In(1)w*^{vc}. It has the same genetic (Hotta & Benzer, 1973) and cytological (Hinton, 1955) properties as the unstable ring chromosome from which it was derived. The *v*$^+$*Y*$^+$ no. 3 chromosome is a *Y* chromosome carrying the wild-type allele of *vermilion* which has been synthesized by Chovnick (1968) as *B*^{s}*v*$^+$*Y*$^+$ no. 3 but has since lost the *B*^{s} marker. The 2 *ma-l* alleles used in this investigation were *ma-l*^{1} and *ma-l*^{bz} (synonyms: *ma-l*^{F1} and *bz*).

The eye colour of young *v* or *st* flies is bright red. In contrast, eye colour of young *v ma-l* or *ma-l*; *st* flies is light orange. Thus, the use of one of these two combinations increased the resolving power available for *ma-l* eye colour determination. A *v* or *st* background was therefore maintained in many of the experiments reported here. The only exceptions involved attempts to monitor simultaneously changes in eye colour of both *v* and *ma-l*; a *v* phenotype could be accurately distinguished from *v*$^+$ or intermediate phenotype since the ocelli are colourless only in the former case (Nissani, 1975). But there were some uncertainties with respect to the distinction between *ma-l* and intermediate *ma-l*$^+$ eyes of individuals with coloured (non-*v*) ocelli.

Gynandromorphs were generated by means of the *In(1)w*^{vc} unstable ring chromosome or its derivative, *In(1)w*^{vc}, *v* (see above). The crosses are described in

Table 1. *Results of crosses in which* X *chromosome loss uncovered the maroon-like mutation*

Cross no.	Cross	No. of gynandromorphs with both eyes genetically and phenotypically *ma-l*$^+$	No. of gynandromorphs with at least a small genetically *ma-l* eye patch and both eyes phenotypically *ma-l*$^+$	No. of gynandromorphs with *ma-l* eye colour	No. of *XO* flies with *ma-l*$^+$ eye colour	Estimated no. of *XO* flies with *ma-l* eye colour
1	♂♂ *In*(1)w^{vc}, *v*/*y*$^+$*Y* × ♀♀ *y v f ma-l*^{bz}	176	431	0	23	1110
2	♂♂ *In*(1)w^{vc}/*y*$^+$*Y*; *st* × ♀♀ *y ma-l*^{1}; *st*	445	402	0	Not recorded	Not estimated

Table 2. *Results of crosses in which* X *chromosome loss simultaneously uncovered the maroon-like and vermilion mutations*

Cross no.	Cross	No. of gynandromorphs with *v*$^+$ *ma-l*$^+$ phenotype	No. of gynandromorphs with *v ma-l*$^+$ phenotype	No. of gynandromorphs with *v*$^+$ *ma-l* or *v ma-l* phenotypes
1	♂♂ *In*(1)w^{vc}/*y*$^+$*Y* × ♀♀ *y v f ma-l*^{bz}	235	40	1 (*v ma-l*)
2	♂♂ *In*(1)w^{vc}/*y*$^+$*Y* × ♀♀ *y v mel ma-l*^{1}	51	5	0

Tables 1 and 2. The loss of $In(1)w^{vc}$ in the crosses depicted in Table 1 uncovered only the non-autonomous mutation *ma-l*; its loss in the crosses depicted in Table 2, which served as internal controls, simultaneously uncovered both *v* and *ma-l*. The genetic constitution of the eyes in these gynandromorphs was inferred from the phenotype of the surrounding cuticular bristles. This procedure is preferable to others because it circumvents the need to introduce a special eye marker (with a consequent reduction in viability), and because developmental studies (Becker, 1957) and examination of charts of approximately 700 gynandromorphs obtained in unrelated investigations (Nissani, 1975, 1976) and which carried both an eye marker (un^4) and a bristle marker (*y* or sn^3) suggest that this allows a very reliable inference regarding the genetic constitution of the eyes.

The method of injecting cytoplasm into preblastoderm embryos was that of Okada, Kleinman & Schneiderman (1974) except that the microneedles were cut with a razor blade at a 45° angle under a dissecting microscope. The tips of the needles were then examined under a light microscope (× 320) and only needles with an approximate inner diameter of 12 μm and a fine edge were subsequently used for embryonic injection.

The procedure of pupal injection was very similar to the above. Young (24–48 h old; about a quarter of the time from the beginning of pupation to eclosion) *y v f* $ma\text{-}l^{bz}$ pupae were collected from the sides of culture bottles and arranged in a row on a double-stick Scotch tape attached to a microscope slide. Subsequently, embryos of the appropriate donor genotype were collected with a fine brush and mechanically dechorionated by rolling them with a dissecting needle on a double-stick Scotch tape attached to a slide. The age of the embryos was then ascertained under the dissecting microscope (Bownes, 1975) and two embryos of the desired developmental stage were placed alongside each pupa. Immediately after, their total cytoplasmic content was drawn with a microneedle of approximately 50 μm inner diameter and injected laterally into the abdominal region of a single *y v f* $ma\text{-}l^{bz}$ pupa. These pupae were then transferred into a fresh food vial and kept at 22 °C in a moisture chamber. The eye colour of young treated flies that eclosed or of treated pupae with fully developed and pigmented eyes was subsequently determined. Most injection experiments were coded, i.e. classification of imagos or pupae with respect to *ma-l* and *v* pigmentation was made by an observer who was unaware of the nature of the treatment administered.

3. RESULTS

(i) *Cell lineage analysis*

Glassman (1957) observed one gynandromorph in which a genetically *ma-l* eye had a $ma\text{-}l^{+}$ phenotype and concluded that *ma-l* is non-autonomous. The purpose of this portion of the experiment was to confirm this conclusion and to apply the technique of genetic fate mapping (Hotta & Benzer, 1973; Nissani, 1975) in an effort to determine which anatomical site(s) are responsible for this non-autonomy. The results of the first part of this analysis are given in Table 1 and, unexpectedly,

they can be summarized by a qualitative statement: all 1454 gynandromorphs observed in these two crosses had *ma-l*$^+$ eye colour. This is true for both *ma-l* alleles tested, for both *In(1)w*^{vc} and *In(1)w*^{vc}, *v* – induced mosaicism and for both *v* and *st* backgrounds. Additional controls are provided by the uniformly *ma-l* colour of the many thousands of males which resulted from the crosses shown in Tables 1 and 2. In addition, with few exceptions which will be taken up in detail later, the same is true for the numerous *XO* males which were also generated by these crosses. The virtual complete non-autonomy of *ma-l* eye colour was also observed in 32 gynandromorphs which had less than 5% of their entire cuticle, and occasionally only a few bristles, *ma-l*$^+$. Although the genotype of internal tissues was not determined in these cases (Janning, 1974), it is likely that in some of these flies this accurately represents the proportion of *ma-l*$^+$ internal tissues as well (Kankel & Hall, 1976).

The crosses in Table 2 served as internal controls. It can be seen from this table that *v*, another non-autonomous eye colour mutation, behaves very differently in mosaic flies: the eyes and ocelli of many *v*//*In(1)w*^{vc} gynandromorphs are genotypically and phenotypically *v*. Again, with one possible exception, all gynandromorphs which resulted from these crosses (Table 2) were phenotypically *ma-l*$^+$.

Taken together, these results suggest that the *ma-l* mutation behaves differently in mosaic individuals from any other mutation that has previously been described in *Drosophila*. That is, unlike most gene mutations, which exhibit autonomous expression, and unlike *vermilion* which is non-autonomous but in which the reason for non-autonomy is the release into the haemolymph of a diffusible substance from either the Malphigian tubes or the fat body but not from any other tissue (Beadle, 1937; Nissani, 1975), the *maroon-like* locus shows a striking degree of non-autonomy: all, or almost all, flies mosaic for *ma-l* and *ma-l*$^+$ tissues have *ma-l*$^+$ eye colour.

(ii) *Injection experiments*

One possible explanation for the cell lineage results is that a diffusible *ma-l*$^+$ substance can be produced by most tissues of the fly and that very small quantities of this substance are sufficient to change genetically *ma-l* eyes into phenotypically *ma-l*$^+$ eyes. If this is the case, then it may be possible to develop a bioassay for this important substance; much in the same way that this was carried out for the *v*$^+$ and *cn*$^+$ substances (reviewed by Ephrussi, 1942). Nevertheless, at least one unsuccessful attempt to develop such an assay has been reported (Hadorn in a personal communication to Glassman, 1965). Hadorn found that extracts of wild-type larvae, pupae or adults injected into *ma-l* larvae had no effect on the eye colour which the injected larvae developed as adults. This failure could indeed mean, as Glassman (1965) suggests, that it is not possible to isolate the active *ma-l*$^+$ substance. However, this failure could be also ascribed to the particular experimental protocol employed by Hadorn. To test this latter possibility it was decided to change this protocol: (1) Inject cytoplasm rather than extracts; (2) Use embryos as source of the *ma-l*$^+$ substance rather than larvae, pupae or adults. This is based on indirect evidence that embryos descended from *ma-l*$^+$ mothers may have this

substance (Glassman, 1965; Sayles, Browder & Williamson, 1973). (3) Treat embryos and pupae, instead of larvae.

We have first injected cytoplasmic content drawn from the central region of *v* embryos which were at the blastoderm stage into the posterior region of *y v f ma-l*^{bz} preblastoderm embryos. Roughly 5% of the egg volume was injected. All seven flies that were obtained had typical *ma-l* phenotype. That is, injection of *ma-l*$^{+}$ cytoplasm, and possibly also of *ma-l*$^{+}$ cells or nuclei, into the posterior region of recipient *ma-l* preblastoderm eggs failed, in a sample of seven flies which survived the treatment, to induce any observable changes in the injected individuals.

The results of injection of embryonic cytoplasm into *y v f ma-l*^{bz} pupae are depicted in Table 3. The first series of injection experiments (group 1, Table 3) indicates that injection of the cytoplasmic content of 2 *v* late blastoderm embryos (after cell membrane formation) into pupae brought about a detectable change in the eye colour of 41% of treated individuals. Group 2 in Table 3 serves as a control group and it shows that the observed eye-colour modifications occur only when the donors' cytoplasm is taken from *ma-l*$^{+}$ individuals. One possible explanation for these phenotypic alterations is that they are caused by injection of a maternal *ma-l*$^{+}$ substance which has been stored in the egg during oogenesis. If this were the case, this could serve as the much needed *in vivo* bioassay for this substance. However, the data summarized in Table 3 serve to rule out this possibility:

(1) Group 6 in this table shows that injection of cytoplasm taken from two unfertilized eggs deposited by *v* (and therefore *ma-l*$^{+}$) females which were mass-mated to *XO* sterile males have no effect on the hosts' eye colour.

(2) Group 7 shows that *ma-l*$^{+}$ blastoderm embryos of *ma-l* mothers can induce eye colour changes; this effect cannot be causally related to the presence of maternal *ma-l*$^{+}$ substance.

(3) Late blastoderm *ma-l*$^{+}$ embryos (group 1, Table 3) appear to bring about a change in a greater proportion of *ma-l* hosts than either early preblastoderm (group 3) or late preblastoderm (group 4) embryos. This difference could be readily explained if it is assumed that the effective injected substance is produced by the embryo itself or, alternatively, if implantation of blastoderm nuclei, and their subsequent effect on the host, is more successful than nuclear transplantation. There is no information regarding the possibility of nuclear or blastoderm cell transplantation into pupae and their subsequent effect on eye colour, but it is known that blastoderm and postblastoderm cells can be successfully cultured and develop larval tissues in adult hosts (Hadorn *et al.* 1968; Chan & Gehring, 1971).

(4) All, or almost all, offspring which result from crossing males with compound third chromosomes to females with a normal set of chromosomes (group 9, Table 3) die during embryogenesis (reviewed by Wright, 1970). This makes it possible to obtain fertilized eggs from *ma-l*$^{+}$ mothers all of which are destined to early death. If the effective substance has been stored in the egg, injection of cytoplasm taken from such embryos should have been as effective as group 1 (Table 3). The fact that this appears not to be the case argues therefore against this possibility.

(5) The three arguments given below against the interpretation that the induced alterations are attributable to injection of substance(s) that have been produced prior to injection by the donor's genome are equally applicable here.

We may conclude then the foregoing discussion as follows. First, injection of the maternal substance alone does not constitute a sufficient condition for a modification of the eye colour of some of the treated pupae and hence a bioassay for this substance was not achieved. Secondly, injection of *ma-l*$^+$ blastoderm stage embryos descended from either *ma-l* or *ma-l*$^+$ mothers does constitute a sufficient condition for eye colour modification of some treated individuals and hence the effect of blastoderm or postblastoderm embryos is fully explained by the presence of the *ma-l*$^+$ gene in the embryo itself. But it is not yet clear whether the alterations are due to transplantation of nuclei and embryonic tissues which remain active long enough within the hosts to produce and release a substance into the host's haemolymph and that this substance in turn changes the host's eye colour or whether the required substance (or its precursor) has been produced already by the embryo prior to injection and that it is the injection of this substance which brings about the change in eye colour. If the latter alternative is correct, then it may be possible to develop a bioassay for this substance and, taken together with group 1 (Table 3), it would indicate that the *ma-l* gene acts very early in development. In contrast, if the former alternative is correct, it would indicate that implanted embryonic cells and nuclei can function long enough in a pupal host to produce and release the necessary substance. Available indirect evidence suggests, but does not prove, that the induced colour changes are attributable to tissue transplantation rather than injection of a substance that has been previously produced by the embryo:

(1) We have injected *y v f ma-l*^{bz} pupae each with the cytoplasmic content of only 1 *v* late preblastoderm embryo. Of the 6 flies that eclosed after this treatment, 5 had *ma-l* phenotype and 1 was intermediate between *ma-l* and *ma-l*$^+$ phenotype. A day or two later these flies were re-examined: 4 flies appeared *ma-l* as before but 1 previously *ma-l* fly and the previously intermediate fly appeared indistinguishable from *ma-l*$^+$ flies. This delayed effect can be best explained by the assumption that the transplanted nuclei continued their development in the host and that they exerted their effect only after they achieved a certain degree of differentiation and proliferation. If, on the other hand, a substance is being transferred, it will be difficult to explain why it has not been utilized prior to eclosion although it was presumably present within the injected individual throughout preimaginal pigment formation in the eye.

(2) Since the biochemical identity of the product of the *ma-l*$^+$ locus is not known it is not possible to determine directly whether or not it is present in the egg at the time the egg is used for injection. An indirect approach is provided by experimental manipulation of the *v* locus. *Vermilion* is probably the structural gene for tryptophan oxygenase (see Dickinson & Sullivan, 1975), an enzyme which converts tryptophan to formylkynurenine which is then converted by another enzyme to kynurenine. This reaction can occur not only in the eyes but also in the larval fat

Table 3. *Results of injection of embryos into pupae: phenotypic alterations of* ma-l

Group no.	Parents from which donor embryos were obtained	Developmental stage of donor	No. of recipients scored for eye colour alteration	No. with *ma-l* eyes	No. with intermediate eyes	No. with *ma-l*$^+$ eyes	Frequency of recipients with altered eye colour (%)
1	*v*	Late blastoderm	56	33	21	2	41
2	*y v f ma-l*^{bs}	Late blastoderm	60	60	0	0	0
3	*v*	Early preblastoderm	21	21	0	0	0
4	*v*	Late preblastoderm	38	36	2	0	5
5	*y v f ma-l*^{bs}	Late preblastoderm	50	50	0	0	0
6	Unfertilized *v* females	—	59	59	0	0	0
7	♂♂ *v/Yma-l*$^+$ no. 2 × ♀♀ *y v f ma-l*^{bs}	Late blastoderm	124	86	17	21	13
8	♂♂ *v/Yma-l*$^+$ no. 2 × ♀♀ *y v f ma-l*^{bs}	Early preblastoderm	58	58	0	0	0
9	♂♂ *C(3L), h*^{1}*/h*^{2}; *C(3R),* + × ♀♀ *v*	Late blastoderm	79	69	10	0	13

Table 4. *Results of injection of embryos into pupae: phenotypic alterations of both* ma-l *and* v

Group no.	Parents from which donor embryos were obtained	Developmental stage of donor	No. of recipients scored for eye colour alteration	No. with *v ma-l* eyes	No. with *v*$^+$ (and with *ma-l* or intermediate *ma-l*$^+$?)	No. with *v ma-l*$^+$	No. with *v*$^+$ *ma-l*$^+$	Frequency of flies with altered *v* colour (%)	Frequency of flies with altered *ma-l* colour (%)
1	♂♂ + */v*$^+$*Yy*$^+$ no. 3 × ♀♀ *v*	Late blastoderm	114	25	77	0	12	78	⩾ 11
2	+ (Canton strain)	Preblastoderm	8	5	3	0	0	38	⩾ 0
3	Unfertilized + females	—	28	24	4	0	0	14	⩾ 0

body and Malphigian tubes. These organs release kynurenine (the v^+ substance, see Ephrussi, 1942) into the haemolymph and when this chemical reaches the ocelli and eyes, it can be converted into ommochromes. Hence the non-autonomy of *v* (for a review, see Linzen 1974; Nissani, 1975). Now, since *v* females do not produce kynurenine (Beadle, 1937) they certainly do not pass it to their offspring (Graf, 1957). Thus, the only source of a v^+ diffusible substance in a genetically v^+ offspring of *v* mothers is that which is produced by this offspring itself. There is strong evidence that v^+ embryos do not produce kynurenine during embryogenesis and certainly not before they are 6–8 h old (Graf, 1957). Although no direct information is available it is also very unlikely that tryptophan oxygenase or its mRNA is synthesized by 0–4 h old embryos and that, if either one is synthesized, their injection into pupae would result in kynurenine production. It follows that, if injection of the content of blastoderm v^+ embryos of *v* mothers produces a detectable change in genetically *v* eyes towards v^+ eye colour, this change can have been caused only by the activity of the implants in the hosts and not by injection of the v^+ substance which has been produced by the embryos prior to injection. Group 1 in Table 4 indicates that injection of such embryonic contents does alter eye and ocellar colour of *v* hosts towards v^+ phenotype. Indeed, despite the inaccuracies involved in classification of eye colour with respect to the *ma-l* phenotype when both *v* and *ma-l* eye colours are simultaneously monitored (see Materials and Methods), the facts that no flies were observed with *v* *ma-l*$^+$ eye colour and that some flies were almost certainly v^+ *ma-l* suggest that injection is equally or more effective in modifying the *v* phenotype than in modifying the *ma-l* phenotype (Table 4). Because the modification in *v* eye colour is due to tissue transplantation and not to injection of a chemical, it appears very probable that this is also the case for *ma-l*.

(3) Eye colour alterations of treated flies with respect to *ma-l* were very often accompanied by the presence of masses of melanized material within the hosts. This correlation is suggestive of a possible causal relationship between the appearance of these 'residual bodies' (Schubiger & Schneiderman, 1971) and induced *ma-l*$^+$ eye colour. This again is more compatible with an action exerted by the implant within the host rather than with chemical-induced alterations.

(iii) *Maternal effect*

The *ma-l* locus is characterized by a maternal effect that influences the expression of the *ma-l* phenotype: all genetically *ma-l* offspring of heterozygous *ma-l*/*ma-l*$^+$ mothers are reported to have a *ma-l*$^+$ eye colour (Glassman & Mitchell, 1959). This effect persists for about 6–8 days but disappears in old cultures due to yet incompletely understood changes in the food medium. We have studied this phenomenon in 11 different classes, on a *v* or *st* background, with rod, ring and attached *X*'s and with two different *ma-l* alleles. Surprisingly, in all classes some phenotypically *ma-l* and intermediate flies were observed. The proportion of phenotypically *ma-l* offspring out of the total number of expected *ma-l* flies in a given cross ranged from 1% to 80%. All these observations were made not later

than 3 days after the first adults in a given culture eclosed. The reason for the difference between these and earlier observations (Glassman & Mitchell, 1959) is unknown but it is probably related to differences between the food media on which the flies were reared (Chovnick & Sang, 1968).

4. DISCUSSION

A comparative analysis of the differential responses of *ma-l* and *v* eyes to implanted wild-type embryos and to the presence of wild-type tissues in gynandromorphs has produced somewhat puzzling results: *ma-l* showed a much greater degree of non-autonomy in gynandromorphs than *v* but *v* appeared to be equally or more effective than *ma-l* in injection experiments. One way to explain these apparently conflicting results is by speculating that the diffusible substance which is responsible for the change in eye colour of injected flies is not identical to the substance which is responsible for the behaviour of *ma-l*$^+$ in gynandromorphs. Thus, the diffusible substance could be the end result of a set of biochemical reactions of which the immediate *ma-l*$^+$ gene product is a part. In contrast, the maternal substance and the substance responsible for the behaviour of *ma-l* in gynandromorphs could be the immediate product of the *ma-l*$^+$ gene. This product could become available in the limited quantities that are necessary to alter genetically *ma-l* eyes either by its storage in genetically *ma-l* eggs of *ma-l*$^+$ mothers or by transcription of the *ma-l*$^+$ gene prior to or during the first cleavage divisions when the embryo's genome, or a substantial part thereof, contains a *ma-l*$^+$ allele. This explanation is admittedly speculative; its only merits may very well be as a guide for further research and its, in principle at least, refutability.

Regardless of the explanation invoked, the results cast some additional light on certain problems. Firstly, detailed studies of *ma-l* have revealed that mutants at this locus can be classified into a minimum of three distinct complementation groups and that this complex pattern is most likely due to product level intragenic complementation (reviewed by Finnerty, 1976). Our results are compatible with this conclusion since they point to the possibility that very small amounts of the *ma-l*$^+$ gene product may be sufficient to trigger the events which eventually result in *ma-l*$^+$ eye colour. Consequently, a very weak restoration of activity brought about by the association of two singly defective products may be all that is necessary to cause a detectable eye-colour alteration.

Secondly, the virtually complete non-autonomy of *ma-l* affords the best approach available at present to a methodological problem that exists in cell lineage studies. This problem concerns estimating the frequency of internal mosaicism that exists in mosaic generating systems such as $In(1)w^{vc}$. If internal mosaicism is frequent, i.e. many flies that are scored as completely mutant or completely wild-type are internally mosaic, then the distances obtained from genetic fate mapping may not be accurate because a considerable proportion of mosaic flies are not recognized by the standard morphological inspection of the cuticle (Kankel & Hall, 1976). It may be expected from the complete non-autonomy of *ma-l* that most or all

phenotypically *XO* individuals which are internally mosaic for *ma-l* will have *ma-l*$^+$ eye colour. In group 1, Table 1, the number of these males was carefully recorded. Hence, the frequency of such cryptic mosaics can be estimated as $23/431 + 176 + 23$ or 3·65 %. If it is assumed that the reciprocal class (wild-type individuals with some mutant internal tissue) is just as frequent, then the frequency of *In(1)w*^{vc}-mosaic flies which go undetected by morphological inspection of adults is $3{\cdot}65 \times 2$ or 7·3 %. This value is in good agreement with the 7 % estimate of Hotta & Benzer (1973) and the 6–10 % estimate of Kankel & Hall (1976).

Finally, Fox and co-workers have presented evidence that genetic transformation may have been accomplished in *D. melanogaster* (reviewed by Fox, 1976). That is, exogenous DNA can be actually incorporated and expressed in DNA-treated flies and then transmitted to and expressed by their descendants. Because of the non-autonomy of *v* and the presumed mosaic expression of the introduced DNA, *v* is the only locus which has been extensively investigated and for which transformed stocks have been established. These observations on the *v* locus have been recently confirmed in two different laboratories (Germaard, 1976; Limbourg-Bouchon, 1976). Nevertheless, it is important to show that the observed hereditary changes are not due to some special unknown features of the *v* and *su(s)* (which suppresses *v*) loci and that other loci can similarly respond to DNA treatment. All other things being equal, the *ma-l* locus, because of its more extensive eye-colour non-autonomy, should provide a higher yield of transformants than *v*. A preliminary test which included six DNA-injected *y v f ma-l*^{bz} preblastoderm embryos and at least 80000 of their F_1–F_4 descendants yielded completely negative results. However, more experimental evidence is needed before a meaningful evaluation of this failure can be made.

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