DEVELOPMENT OF EYE COLORS IN DROSOPHILA: DIFFUSIBLE SUBSTANCES AND THEIR INTERRELATIONS

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INTRODUCTION

IN EXPERIMENTS reported in a series of papers by the authors an attempt has been made to attack various problems concerned with eye color development in *Drosophila melanogaster* by the use of the technique of transplantation. These experiments have led to the elaboration of a scheme bearing on one phase of the general problem of the relation of genes to eye color development.

Evidence has been presented (BEADLE and EPHRUSSI 1936) indicating that at least three specific diffusible substances are involved in the development of wild type eye color. These are named and defined as follows:

(1) ca^+ *substance*—a substance necessary for the formation of a wild type eye and which cannot be supplied (or is supplied in relatively small quantities) by a claret host.

(2) v^+ *substance*—a substance capable of modifying a genetically vermilion eye in such a way that it develops a color like that of wild type.

(3) cn^+ *substance*—a substance capable of modifying a genetically cinnabar eye in such a way that it develops a color like that of wild type.

It has been assumed that these three substances are related in their formation as steps in a chain of reactions. This can be illustrated in the following way:

$$\rightarrow ca^+ \text{ substance} \rightarrow v^+ \text{ substance} \rightarrow cn^+ \text{ substance}$$

It is the purpose of the present paper to bring together in one place, and to attempt to evaluate, the evidence bearing on:

1. The existence of three different diffusible substances.
2. The relation of these three substances to one another.
3. The relation of various eye color mutants to these substances.

EVIDENCE FOR THREE SUBSTANCES

In planning further experiments it is desirable to know whether we are dealing with three separate diffusible substances, or whether there is only a single substance concerned (or one instead of any two of those postulated) which has different effects when acting under different conditions, at different times, in different concentrations, or in some combination of these.

In dealing with questions concerning amounts of a given diffusible substance, a general qualification with regard to terminology will simplify the presentation of evidence. In previous papers the terms "absence" and "lack" have been used more or less loosely. From the nature of the tests used for such diffusible substances, it is obvious that "absence" can mean only a quantity as small as or smaller than that produced by the test mutants. Thus a claret fly has the smallest quantity of ca^+ substance at present known; such a fly may literally lack this substance or it may merely have a reduced quantity as compared with a wild type fly. The expression "deficiency of substance" is used in this paper in its literal sense to designate reduced quantity or complete absence.

In presenting the reasons for assuming three substances, we shall first consider the arguments indicating that ca^+ substance is different in kind from the other two.

1. A wild type eye disk grown in a claret (*ca*) host gives an eye phenotypically like claret, but the same eye disk grown in a vermilion (*v*) host gives a wild type eye. This shows that a *ca* fly is deficient in something which is present in a *v* host, and, since a *v* fly is deficient in v^+ and cn^+ substances, it is argued that a *ca* host is deficient in a third diffusible substance. It is possible that the difference between *ca* and *v* flies, as hosts to a wild type eye implant, might be explained by assuming differences in time of action of either v^+ or cn^+ substance in *ca* and *v* hosts, but the authors see no simple way of doing this. The difference cannot be explained by assuming that the distinction between a *ca* and a *v* fly is that a *ca* fly has less of the same substance known to be deficient in a *v* fly as compared with a wild type fly, since it is known that a *ca* host is capable of modifying a *v* implant in the direction of wild type (EPHRUSSI and BEADLE, in press); if the *ca* host had less of a substance already deficient in *v*, this modification obviously would not be possible.

2. Since a wild type eye disk grown in a *ca* host does not give rise to a wild type eye, while the same eye disk grown in a *v* host does give rise to a wild type eye, we assume that a wild type eye can itself produce one substance (v^+ substance) but not the other (ca^+ substance).

3. It has been shown that v^+ substance is not present in a wild type fly in detectable quantities until after puparium formation (EPHRUSSI, CLANCY and BEADLE 1936). On the other hand, it has been found that ca^+ substance acts (or at least is taken up by an eye), and therefore must be produced in a wild type fly, before the time of puparium formation (EPHRUSSI and BEADLE, in press).

These arguments, considered in relation to one another, offer strong support to the view that ca^+ substance is qualitatively different from the other two.

Turning to the remaining two postulated substances, the evidence that they are qualitatively different is summarized in the following paragraphs. This evidence would be more nearly complete if it were known when cn^+ substance appears in effective concentrations in a wild type fly, but experiments designed to answer this question have not yet been made.

1. The evidence on which the assumption of the two substances, v^+ and cn^+ substances, was originally based, comes from reciprocal transplants involving *v* and *cn*. Using a shorthand method of designating transplants, it has been shown that:

a. *v* in + gives a wild type eye.
b. *cn* in + gives a wild type.
c. *v* in *cn* gives a wild type eye.
d. *cn* in *v* gives a cinnabar eye.

It is difficult to imagine how a wild type eye can be obtained by growing a *v* disk, known to be deficient for something present in a wild type fly (from a), in a *cn* host (c), also known to be deficient for something present in a wild type fly (from b), on the assumption that both *v* and *cn* flies are deficient for one and only one substance. It is perhaps possible to elaborate a formal scheme that would give this result, but such a scheme would involve assumptions at least as elaborate as the simple assumption of two substances.

2. The second argument is based on the impossibility (or at least the difficulty) of accounting for the results of transplantation experiments involving *v* and *cn* on the assumption of a single substance, say substance x, acting in different amounts or at different times. Considering, first, the possibility of a difference in sensitivity of *v* and *cn* eyes to a given amount of substance x, we know (BEADLE and EPHRUSSI 1936) that:

v in *car* gives an eye intermediate between vermilion and wild type,
cn in *car* gives an eye intermediate between cinnabar and wild type.

An essentially similar result was obtained from *v* and *cn* in g^2 transplants. From these results no indication is seen of differences in sensitivity of the two types of implants to a limited amount of substance x. On the other hand, we know (BEADLE and EPHRUSSI, in press) that:

v in *bri* (bright eye color) gives a wild type eye,
cn in *bri* gives an eye intermediate between cinnabar and wild type,

that:

v in *mah* (mahogany eye color) gives a wild type eye,
cn in *mah* gives an eye intermediate between cinnabar and wild type,

and that:

v in *cn* gives a wild type eye,
cn in *cn* gives a cinnabar eye.

These results show that *v* and *cn* eyes are different in sensitivity to given amounts of the assumed substance x, a conclusion contradictory, on any simple assumption, to that indicated above.

Considering biological measurements of the amounts of the assumed substance x produced by two different mutant hosts, it is known (table 2 and above) that:

v in p^p gives an eye close to vermilion,
v in *cn* gives a wild type eye,

from which it must be concluded that the amount of substance supplied to the *v* implant by a *cn* host is *greater* than that supplied by a p^p host. But from results in table 2, we see that:

w^a *cn* in p^p gives an apricot (light) eye,
w^a *cn* in *cn* gives an apricot cinnabar eye,

and a conclusion contradictory to the one just drawn is indicated, namely, that the amount of substance x supplied to an w^a *cn* implant by a *cn* host is *less* than that supplied by a p^p host.

3. The third argument is based on the release of substance by implants of various types (measured by effects on the eyes of the host). Summarizing pertinent results of such tests (EPHRUSSI and BEADLE, in press), it is seen that:

a. + in w^a *cn* modifies the host's eyes toward apricot,
+ in w^a *v* does not modify the host's eyes,

from which we conclude that a wild type implant releases one substance (cn^+ substance) but not the other (v^+ substance);

b. *cn* in w^a *cn* does not modify the host's eyes,
cn in w^a *v* modifies the host's eyes toward apricot,

indicating that a *cn* implant releases v^+ substance but not cn^+ substance;

c. *st* in w^a *cn* modifies the host's eyes toward apricot,
st in w^a *v* modifies the host's eyes toward apricot,

from which it is clear that a *st* implant releases both v^+ and cn^+ substances.

These experiments on release of the two substances by an implant have been made in another way. Two implants, one to supply the substance and one to detect it, were grown in hosts unable to supply either substance (*v* or *v cn*). The results are summarized in table 1. It is seen that a wild type implant does not modify a w^a *v* implant when both are grown, usually in close proximity or in actual contact, in either a *v* or in a *v cn* host. That modification of one implant by another is possible is shown by growing *cn* and w^a *v* implant in *v cn* host; the w^a *v* implant develops an apricot phenotype. On the other hand, there appears to be no modification of a w^a *cn* implant by a wild type implant grown in the same host (*v* or *v cn*). No explanation is offered for the failure of a wild type implant to modify a w^a *cn* eye under these conditions, but this negative result, although it does indicate the operation of an unknown factor, does not invalidate the argument based on release of substances.

Although no one of the above arguments in favor of the assumption of three specific diffusible substances, taken individually, is irrefutable, taken collectively they provide a convincing demonstration of the pragmatic value, if not of the correctness, of the assumption.

RELATION OF THE THREE SUBSTANCES TO ONE ANOTHER

In bringing together the evidence indicating that the three substances are related as successive steps in a chain of reactions, it is convenient first to consider the arguments in favor of assuming ca^+ substance to be in the indicated position in such a chain.

TABLE 1

Influence of one implant on another in double eye disk transplants. In all cases the two implants grown in a given host were from donors of the same sex. In this and table 2, under column heading "number of individuals," sex combinations of donor and recipient are listed in the order female in female, female in male, male in female, male in male, and total.

IMPLANTS		HOST	NUMBER OF INDIVIDUALS	PHENOTYPE OF TEST EYE
SOURCE OF SUBSTANCE	TEST EYE			
0	w^a *v*	*v cn*	2, 1, 0, 0; 3	w^a *v*
+	w^a *v*	*v cn*	1, 1, 1, 1; 4	w^a *v*
+	w^a *v*	*v*	5, 0, 3, 0; 8	w^a *v*
cn	w^a *v*	*v cn*	2, 1, 2, 3; 8	w^a (light?)
+	w^a *cn*	*v cn*	1, 0, 4, 0; 5	w^a *cn*
+	w^a *cn*	*v*	2, 1, 2, 1; 6	w^a *cn*

The primary reason for assuming ca^+ substance to be produced before v^+ and cn^+ substance in the postulated linear series is the fact that a *ca* fly can be shown to have less of both v^+ and cn^+ substances than has a wild type fly. Apparently ca^+ substance is present in full concentration in both *cn* and *v* flies in spite of the fact that such flies are characterized by deficiencies of cn^+ substance or both cn^+ and v^+ substances. Therefore, if the three substances are formed in a linear series of reactions (or its equivalent in terms of the three substances only), ca^+ substance must be formed first; otherwise *v* or *cn* or both *v* and *cn* flies should be deficient in ca^+ substance. The alternative to the assumption that ca^+ substance is formed in the same series with the other two is that it is formed by independent reactions. On this assumption, the reduced quantities of v^+ and cn^+ substances characteristic of a *ca* fly must be ascribed to coincidence, that is, the *ca* gene must be concerned with two independent systems of reactions. This alternative assumption is not excluded by the available evidence, but it appears to be less probable.

As mentioned above, ca^+ substance must be assumed to reach effective concentration in a wild type fly before puparium formation, while v^+ substance reaches an effective concentration only after puparium formation. It should be pointed out that, although this is consistent with the scheme, no particular relation need be assumed between order of formation of these substances and order of reaching effective concentrations.

One may ask how, if ca^+ substance is formed before and in sequence with v^+ and cn^+ substances, a *ca* fly, said to be deficient in ca^+ substance, can form v^+ and cn^+ substances even in limited quantities? As pointed out above, we have, at present, no way of distinguishing between literal absence and presence in reduced amount. We can say only that a *ca* fly has less ca^+ substance than has a wild type fly. Hence the only consequence that must be met following the assumption that ca^+ substance is prerequisite to the formation of v^+ and cn^+ substances is that these two substances be produced at reduced rates in a *ca* as compared with a wild type fly; the evidence is quite in accord with this interpretation.

TABLE 2

Results of tests of eye color mutants, used as hosts, for effects on v, w^a v, and w^a cn implants.

IMPLANT	HOST	NUMBER OF INDIVIDUALS	PHENOTYPE OF IMPLANT
v	*cm*	2, 3, 5, 5; 15	Close to *v* but darker
v	g^2	2, 1, 2, 0; 5	Intermediate between *v* and +
v	p^p	1, 0, 1, 2; 4	Close to *v* but slightly darker
v	*rb*	3, 1, 3, 2; 9	Close to + but lighter
w^a *v*	*cm*	2, 2, 4, 2; 10	w^a (lighter?)
w^a *v*	g^2	1, 3, 4, 0; 8	w^a
w^a *v*	p^p	1, 2, 3, 0; 6	Close to w^a but lighter
w^a *v*	*rb*	1, 0, 4, 1; 6	w^a (lighter?)
w^a *cn*	cn^3	1, 0, 0, 1; 2	w^a *cn*
w^a *cn*	p^p	1, 2, 1, 2; 6	Close to w^a but lighter
w^a *cn*	*rb*	4, 0, 2, 1; 7	w^a

Certain evidence in favor of the assumption of a sequential relation in formation between v^+ and cn^+ substance has already been considered (BEADLE and EPHRUSSI 1936). A *v* implant (a *v* fly is deficient in cn^+ substance) when grown in a *cn* host (deficient in cn^+ substance) gives a wild type eye, presumably because a *v* eye is capable of making cn^+ substance once it is supplied with the necessary v^+ substance. Other instances of essentially the same kind have been described.

Carrying the analysis of *v* in *cn* transplants one step further, it might be expected that, if the *v* implant in the above produces cn^+ substance in the presence of v^+ substance, a quantity of cn^+ substance would be released. A wild type implant, it will be recalled, releases cn^+ substance. If released in sufficient quantity, we might expect the eyes of the cn host to be modified toward wild type. Since the indications are that a cn eye requires a relatively large amount of cn^{+} substance to influence its appearance appreciably, a test for the release of cn^{+} substance by a v implant was made by growing v implants in $w^{a}\ cn$ hosts (Ephrussi and Beadle, in press). The result was an implant phenotypically like wild type and a relatively strong modification of the eyes of the $w^{a}\ cn$ host toward apricot (w^{a}). The interpretation of this result is as follows. The $w^{a}\ cn$ host supplies the v implant with v^{+} substance. In the presence of this the implant is able to make cn^{+} substance, and consequently develops wild type eye color. An excess of cn^{+} substance is formed which is released from the implant, moves to the eyes of the host, and there results in a modification of the color toward apricot, that is, in the direction of cn^{+}. A similar mutual modification is observed in $w^{a}\ v$ in $w^{a}\ cn$ transplants; the same interpretation is offered.

If the assumption of sequential formation of v^{+} and cn^{+} substances, in the order given, is correct, then there should be a quantitative difference in the magnitude of such mutual influences of implant and host depending on whether the substance first in the sequence (v^{+} substance) is formed by the host or by the implant. Thus in the transplant $w^{a}\ v$ in $w^{a}\ cn$, v^{+} substance should be supplied to the implant in relatively large quantities since it is formed in excess in the two eyes and presumably in other parts of the body, while in the reciprocal transplant, $w^{a}\ cn$ in $w^{a}\ v$, v^{+} substance should be produced in a smaller amount since the single implanted eye is the only source. Hence, the mutual modification in $w^{a}\ v$ in $w^{a}\ cn$ transplants should be strong as compared with that shown by $w^{a}\ cn$ in $w^{a}\ v$ transplants. Actually, this was found to be the case.

An even more striking demonstration of this quantitative difference in mutual modifications, depending on the source of v^{+} substance, is seen in the two transplants v in $w^{a}\ cn$ and cn in $w^{a}\ v$. The effect should be stronger in the first of these since the host supplies v^{+} substance while in the second, v^{+} substance originates in the implant. Actually, in the first, the implant is modified completely to wild type (first effect) and the eyes of the host show a strong modification (second effect). In the second, on the other hand, the eyes of the host show a strong modification (first effect) but the implant is not detectably modified (second effect).

The above evidence offers strong support not only for the assumption that v^{+} and cn^{+} substances are sequentially related, but as well for the assumption that v^{+} substance is necessary for the formation of cn^{+} substance, that is, that the order is as indicated.

Summarizing the evidence for the postulated relation to one another of ca^{+}, v^{+}, and cn^{+} substances, it is clear that the evidence indicating a relation of ca^+ substance to the other two is relatively weak as compared to that showing a relation of v^+ and cn^+ substances to each other.

RELATION OF VARIOUS EYE COLORS TO THE THREE SUBSTANCES

In studying various eye colors by means of transplantation, it has become increasingly evident that a large number of genes must be concerned, in various more or less direct ways, with the production of the ca^+, v^+, and cn^+ substances. It is the purpose of this section to bring together this evidence.

Because of difficulties inherent in the standard terminology of genetics there is danger, in such an attempt, of implying more than is intended. For example, the substitution in a fly of *cn* for cn^+ alleles is accompanied by a reduction in the amount (possibly to the point of complete absence) of cn^+ substance. From this fact it is inferred that the gene cn^+ is in some way concerned with the reactions leading to the formation of cn^+ substance. In this particular case, very little is known as to *how* the cn^+ gene is concerned with the production of this substance. It is conceivable that immediate products elaborated by the cn^+ gene are necessary for the direct formation of cn^+ substance. On the other hand, there may be a great many reactions separating the cn^+ gene from cn^+ substance, some more, others less, direct. Assuming the latter to be nearer to the truth, it seems probable that cn^+ substance is more closely related to the cn^+ character than it is to the cn^+ gene.

In saying that the cn^+ gene is concerned with the production of cn^+ substance, we assume, since a *cn* fly appears to be essentially normal in all respects except eye color, that the relation is more or less direct. We are aware that in another sense the majority of genes, in being essential to the life of a fly, are "concerned with the production of cn^+ substance." Put on this basis, however, any discussion of the relation of specific genes to specific characters loses a large part of its significance.

Another problem arises in connection with the rôle of various alleles of a gene. For example, is the *cn* allele concerned with the production of cn^+ substance in a purely negative sense by failing entirely to do something essential for the formation of cn^+ substance, does it play the same rôle as the cn^+ allele in a less effective way, or is its rôle essentially different from that of the cn^+ gene? There is also the possibility that the cn^+ allele is concerned with the production of cn^+ substance in a negative way while the *cn* allele is concerned in an active way, say in bringing about destruction of cn^+ substance.

For the sake of simplicity, the following discussion is made in a categorical way. It presents one possible interpretation; in considering it, the above reservations should be kept in mind.

The steps in the postulated chain of reactions leading to the formation of the three diffusible substances are as follows:

$$\rightarrow ca^+ \text{ substance} \rightarrow v^+ \text{ substance} \rightarrow cn^+ \text{ substance}$$
$$(1) \qquad\qquad (2) \qquad\qquad (3)$$

Step 1

No evidence is available indicating that any gene other than ca^+ is concerned with this step. It should be pointed out, however, that negative evidence carries little weight in this connection. First, the experiments are not exhaustive, and second, a given mutation, say $x^+ \rightarrow x$, may result in no modification of a given reaction, but this cannot be taken as evidence that another mutation of this gene, say $x^+ \rightarrow x'$, will likewise result in no modification of the given reaction.

Step 2

The gene v^+ presumably is concerned with the production of v^+ and cn^+ substance since the mutation $v^+ \rightarrow v$ results in a deficiency of both these substances.

A Bar-eyed (*B*) fly is characterized by a reduced quantity (or absence) of v^+ substance (and presumably cn^+ substance) in the eye but not in other parts of the body (BEADLE and EPHRUSSI 1936). This is particularly interesting in view of the discovery by MULLER, PROKOFJEVA and KOSSIKOV (1936) and by BRIDGES (1936) that the Bar character is the result of a small duplication.

Transplants of eye disks from flies homozygous for various eye color mutant genes to *v* hosts, show by the absence of complete autonomy in eye color development (EPHRUSSI and BEADLE, 1937), that a number of eye color genes are concerned with the production of either v^+ or cn^+ substances, or both, in the eye. Since *v* is deficient in both substances, the test does not distinguish between steps 2 and 3. These genes include the normal alleles of *bo*, *cl*, *Hn*, *mah*, *pn*, *pr*, *ras*, *se*, *sed*, and *sf* and possibly certain others. It is interesting that flies homozygous for the mutant alleles of these genes, used as hosts to *v* and to *cn* eye disks, show no indications of having reduced quantities of v^+ or cn^+ substances in other parts of the body.

As indicated by the failure of flies carrying mutant alleles, when used as hosts to *v* and to *cn* eye disks, to effect a complete modification of the implant to wild type, it is concluded that the normal alleles of the genes *car*, *cm*, *g*, *p*, and *rb* are concerned with the production of v^+ and cn^+ substances in the body; there is no evidence that the mutant alleles of these five genes reduce the amount of v^+ or cn^+ substances produced in the eye (EPHRUSSI and BEADLE, in press). In connection with *cm*, p^p, and *rb*, recent experiments (table 2) have shown that, contrary to the data presented earlier

(BEADLE and EPHRUSSI 1936), flies homozygous for these mutant genes, when used as hosts to *v* implants, do effect a partial modification of the implant in the direction of wild type.

Step 3

It is evident from facts already discussed in this paper that the gene cn^+ must be assumed to be concerned with the production of cn^+ substance (step 3). The normal alleles of the genes *bri* and *mah* evidently are concerned in this step since *cn* implants are only partially modified by *bri* and *mah* hosts while *v* implants are completely modified to wild type by such hosts. As stated above, there is evidence that the gene *mah* is concerned with the production of either v^+ or cn^+ substance in the eye. Since it is known to be concerned with step 3 in the body, it is presumably concerned with the same step in the eye.

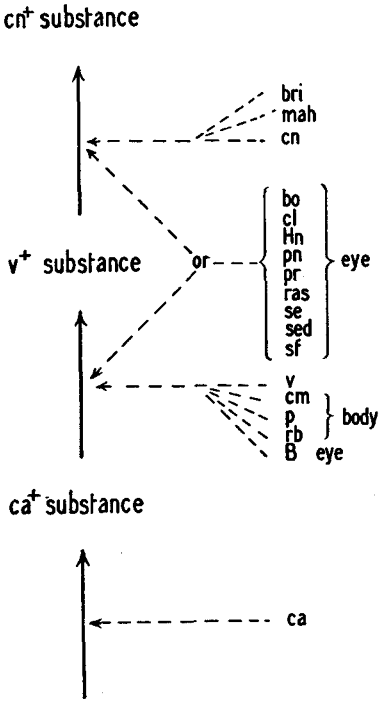

FIGURE 1.—Diagram indicating assumed relations of various genes to the three diffusible substances. Dotted lines indicate the step assumed to be interfered with in the various mutant types, in body, eye, or both. The particular alleles indicated in diagram are not necessarily the ones used in the experiments.

A schematic representation of the relation of the various mutants mentioned above to the three postulated substances is given in figure 1. Although several genes may be indicated as being concerned in the same step in this series, this is not to be taken as indicating a belief that such genes act in an identical manner. Obviously there is no need to assume that one step in the suggested scheme represents one chemical reaction. *A priori*, it is probable that the scheme as presented is little more than a skeleton of a complex series of related reactions; each step may well represent a large number of sub-steps in terms of chemical reactions.

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SUMMARY

An attempt is made to bring together and evaluate evidence indicating that:

1. Three (at least) specific diffusible substances are concerned in the development of the wild type eye color in *D. melanogaster*.

2. These three substances are sequentially related in formation.

3. Formation of the three substances is in the order ca^+, v^+, and cn^+ substance, ca^+ substance being first in the series.

The possible relation of various genes to these three substances is considered.

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