THE DIFFERENTIATION OF EYE PIGMENTS IN DROSOPHILA AS STUDIED BY TRANSPLANTATION

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Received November 22, 1935

INTRODUCTION

PROMINENT among the problems confronting present day geneticists are those concerning the nature of the action of specific genes—when, where and by what mechanisms are they active in developmental processes? Despite the recognized importance of such questions as these, relatively little has been done toward answering them, a situation not at all surprising considering the difficulty of getting at these problems experimentally. Even so, promising beginnings are being made; from the gene end by the methods of genetics, and from the character end by biochemical methods. Probably the one factor which has played the most significant role in retarding progress in this field is the fact that relatively little is known from a developmental point of view about those organisms that have been studied most thoroughly from the genetic point of view, and, on the other hand, little is known genetically in those organisms that have been most studied from the developmental point of view. One of the two obvious (and alternative) ways of overcoming this difficulty would be to study development in a genetically well known organism. Drosophila, with its numerous mutant types, offers a favorable opportunity for a study of this kind. Several facts have led us to begin such a study on the differentiation of eye color pigments. Many eye color mutants are known, pigments have many advantages for chemical studies, and interactions between tissues of different genetic constitutions with respect to eye pigmentation are already known from studies of mosaics.

In this paper we shall present the detailed results of preliminary investigations (EPHRUSSI and BEADLE 1935a, 1935b, 1935c; BEADLE and EPHRUSSI 1935a, 1935b) which we hope will serve to point out the lines along which further studies will be profitable.

MATERIAL AND METHODS

The technique used in making transplantations in Drosophila has been described elsewhere (EPHRUSSI and BEADLE 1936). In brief, the desired organ or imaginal disc, removed from one larva, the donor, is drawn into

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a micro-pipette and injected into the body cavity of the host. As a rule, operations were made on larvae cultured at 25°C for three days after hatching from the eggs. At this time they are ordinarily about ready to pupate. Some of the stocks developed at slower rates than others, and larvae from these were sometimes used on the fourth day after hatching. In most cases the host larvae pupated within 24 hours after the operation. It is clear, from the above, that the stage of development at the time of operations was not controlled in a very precise way. However, since repetition of experiments at different times and, in some cases, with quite different stocks have given consistent results, we can be reasonably sure that the small differences in stage of development which may have existed between host and implant have not played any significant part.

The reasons for the choice of that stage of development reached shortly before puparium formation as "standard" for the studies reported here are largely those of convenience. At this time the optic discs are of a convenient size for transplantation, injections are readily made, and the host larvae require no more food.

As will be discussed below, implanted optic discs develop in a manner somewhat different from that characteristic of the same disc in its normal position. Because of this, it is not always desirable to compare the pigmentation of an implanted eye with that of a normal one. By dissecting the two eyes, normal and implanted, and observing fragments of the pigmented tissue, one can usually make a good comparison. However, to avoid all difficulty, which becomes important where slight differences are involved, we have practically always made comparisons only between implanted eyes. Thus, a vermilion eye disc implanted in a claret host gives rise to an eye with vermilion pigmentation. This conclusion is reached by comparing the implanted eye with an implanted eye known to be vermilion, obtained by implanting vermilion discs in vermilion larvae. Further comparisons with wild type and with claret control implants enable one to say definitely that the eye in question is vermilion, not wild type and not claret.

List of mutants

A list of the eye color mutants used in the studies reported in this paper is given together with their standard symbols. These mutant types and the genes which differentiate them from wild type will be referred to by symbol only. Other mutant genes were also carried by certain of the stocks used. These are indicated in the tables by symbol only since they presumably have no bearing on the results. These symbols are used generally in Drosophila work; their significance can be found in MORGAN, BRIDGES and STURTEVANT (1925).

bo —bordeaux
bw —brown
ca —claret
car —carnation
cd —cardinal
cl —clot
cm —carmine
cn —cinnabar
g^2 —garnet-2
Hn^r—Henna-recessive
lt —light
ma —maroon
p^p —peach
pd —purpleoid
pn —prune
pr —purple
ras —raspberry
rb —ruby
se —sepia
sed—sepiaoid
sf^2 —safranin-2
st —scarlet
v —vermilion
w —white
w^a —apricot
w^e —eosin

DEVELOPMENT OF IMPLANTED EYES

When an eye transplant is made, the eye disc is injected into the body cavity of the host larva. The implanted disc continues development in the body cavity, and at maturity of the host usually comes to lie in the abdominal cavity. Occasionally, it may lie in the thorax but such cases are exceptional. The location of the implanted eye in the adult fly seems to be determined by purely mechanical factors; it is pushed into that part of the body cavity of the developing individual where the normal organs are least crowded. Usually injections are made toward the posterior end of the larva, but they have also been made near the anterior end, and this seems to have no effect on the final position of the eye. The implanted eye may lie just under the body wall of the adult fly where it is readily visible in the living fly, or it may lie deeply imbedded, in which case it may not be visible without dissection or clearing.

Very often the implanted eye becomes attached to other organs during its development. In females, it is often attached to one of the ovaries. This appears to be brought about mainly by the growth of tracheal tubes. In males the implanted eye may be attached to a testis. Males with an implanted eye sometimes have one testis which retains the ellipsoid shape which is characteristic of a testis at a much earlier stage of development. Such "inhibited" testes may have their sheaths normally pigmented but whether they contain viable spermatozoa is not known.

An implanted eye, which has developed within the body cavity of the host, is inverted as compared with an eye in its normal position. The normal eye has the shape of the head of a mushroom, the outer surface of the eye being represented by the top or convex surface of the mushroom head. An implanted eye disc is detached from its optic ganglion and, after development, its curvature is reversed in such a way that the facets are on the inside and the basement membrane on the outer convex surface. In other respects implanted eyes appear to be perfectly developed and differentiated; particularly, there seems to be no difference in the pigmentation of an implanted and a normal eye.

The optic and antennal imaginal discs in the larval stage are attached to each other. In removing an optic disc for transplantation, the antennal disc is usually left attached and implanted with the optic disc. This is not necessary but is done in routine procedure because it facilitates handling the discs and in most experiments does no harm. In special experiments where it may be desirable to do so, it is easy to remove the antennal disc and implant the optic disc alone. If the antennal disc is not removed and is not injured during dissection, it develops with the implanted eye and gives rise to an antenna, complete with an arista, attached to the eye by the chitinous head parts mentioned below. In most instances antennae developing with implanted eyes are normally everted.

The optic disc gives rise also to certain head parts when it is implanted, and presumably also in its development in the normal position. The exact extent of these head parts which arise from the optic disc has not been determined but they completely surround what would normally be the periphery of the eye and have normally developed bristles. As the developed implanted eye is inverted, these chitinous head parts form a kind of rim around the concave facet-side of the eye with the bristles on the inside.

In very exceptional cases an implanted eye disc may give rise to an external eye. This has happened only four times in about 1200 cases. In one of these, the eye was nearly normal, the facets were on the exterior convex surface, and there was a normally developed antenna attached to the eye by chitinous head parts. In all four cases the supplementary eye was attached to the abdominal wall of the adult fly, presumably at the point of injection. These cases are unusual and probably arise when the optic and antennal discs "plug," in a special way, the hole through which the pipette was inserted.

EXPERIMENTAL RESULTS

Because of the rather complex interrelations of the different types of data to be presented in this paper, they cannot be discussed efficiently until all the data have been presented.

In the following tables the various sex combinations of implant and host are given. In only one case, which will be specifically mentioned, does the sex of either the donor or the host appear to influence the result.

Mutant eye discs in wild type hosts

As a beginning in the study of the differentiation of eye pigment of implanted eyes, it is desirable to know how many eye color mutants are autonomous in their pigment development when implanted in wild type hosts. For the late larval stage, with which we are chiefly concerned in

TABLE 1

Data on the differentiation of mutant eye implants in wild type hosts. Eye color mutant symbols are distinguished from symbols of incidental mutants present in the stocks by being printed in italics. In this and following tables, under the heading "number of individuals," are given the four sex combinations and the total in the following order: female in female, male in female, female in male, male in male, and total.

IMPLANT	HOST	NUMBER OF INDIVIDUALS	PHENOTYPE OF IMPLANT	IMPLANT	HOST	NUMBER OF INDIVIDUALS	PHENOTYPE OF IMPLANT
bo	+	3, 1, 1, 1; 6	?	*pd*	+	2, 0, 2,* 2; 6	*pd*
bw	+	4, 2, 2, 0; 8	*bw*	y *pn*	+	1, 1, 4, 0; 6	*pn*
ca	+	7, 0, 5, 2; 14	*ca*	b *pr*	+	4, 0, 1, 0, 5	*pr*
car	+	5, 0, 6, 0; 11	*car*	sc *ras*	+	2,‡ 0, 0, 0; 2	*ras*
cd	+	4, 0, 0, 0; 4	*cd*	*rb* cv	+	2, 0, 3, 0; 5	*rb*
cl	+	2, 2, 0, 1; 5	*cl*	*se* wo	+	5, 6, 5, 4; 20	*se*
cm	+	1, 0, 2, 1; 4	*cm*	sr *sed*	+	2, 2, 0, 6; 10	*sed*
cn	+	5, 3, 2, 0; 10	+	tk *sf*^{2} abb	+	0, 2, 1, 1; 4	*sf*^{2}
cn	+/*v*	2, 0, 2, 0; 4	+	*st*	+	3, 0, 0, 2; 5	*st*
g^2	+	1, 1, 0, 0; 2	*g*^{2}	*v*	+	11, 6, 8, 5; 30	+
jv *Hn*^{r} h	+	0, 0, 1, 0; 1	*Hn*^{r}	*v*	+/*v*	1, 0, 0, 0; 1	+
lt c	+	0, 1, 2, 1; 4	*lt*	*w*	+	1, 1, 1, 0; 3	*w*
ma	+	2, 2, 2, 2; 8	*ma*	*w*^{a}	+	0, 0, 3, 0; 3	*w*^{a}
p^p	+	2, 0, 1, 1; 4	*p*^{p}				

* One fly in this class had an implanted eye with wild type pigmentation—presumably because of a mistake in the selection of the donor.

‡ One host in this class dissected as mature pupa.

TABLE 2

Data on the differentiation of wild type eye implants in eye color mutant hosts. Arrangement as in table 1.

IMPLANT	HOST	NUMBER OF INDIVIDUALS	PHENOTYPE OF IMPLANT	IMPLANT	HOST	NUMBER OF INDIVIDUALS	PHENOTYPE OF IMPLANT
+	*bo*	0, 0, 0, 1; 1	?	+	*p*^{p}	3,* 2,* 0, 0; 5	+
+	*bw*	2, 1, 1, 0; 4	+	+	*pd*	2, 0, 6, 0; 8	+
+	*ca*	2, 2, 2, 0; 6,* 1*; 13	*ca*	+	y *pn*	2, 4, 0, 1; 7	+
+	*car*	1, 0, 1,‡ 0; 2	+	+	b *pr*	3, 1, 4, 1; 9	+
+	*cd*	4, 1, 0, 0; 5	+	+	sc *ras*	1, 1, 1, 1; 4	+
+	*cl*	2, 2, 5, 1; 10	+	+	*rb* cv	1,‡ 0, 2, 1; 4	+
+	*cm*	2, 1, 0, 0; 3	+	+	*se* wo	5, 1, 8, 1; 15	+
+	*cn*	3,* 1,* 0, 0; 4	+	+	sr *sed*	2, 3, 0, 0; 5	+
+	*g*^{2}	1,* 0, 0, 0; 1	+	+	tk *sf*^{2} abb	1, 0, 1, 2; 4	+
+	jv *Hn*^{r} h	4, 1, 1, 6; 12	+	+	*st*	1, 1, 1, 0; 3	+
+	*lt* c	2, 1, 0, 0; 3	+	+	*v*	3, 2, 5, 2; 12	+
+	*ma*	2, 2, 5, 7; 16	+	+	*w*	6, 0, 0, 0; 6	+

* Sex of donor not determined.

‡ Host dissected as mature pupa.

this paper, the data on this point are presented in table 1. These data show that most of the eye color mutants are autonomous in their pigmentation. The only clearly exceptional cases are those of *v* and *cn*. When implanted in wild type or in heterozygous *v*, the pigmentation of both of these is that characteristic of a wild type eye. In the case of *bo*, the result is not clear because the visible difference between an implant with *bo* pigmentation and one with wild type pigmentation is very slight. This is also true of the two eye color types as seen in normal eyes. Special experiments using other mutants as "intensifiers" of the difference between *bo* and wild type will probably be necessary to determine the behavior of *bo*.

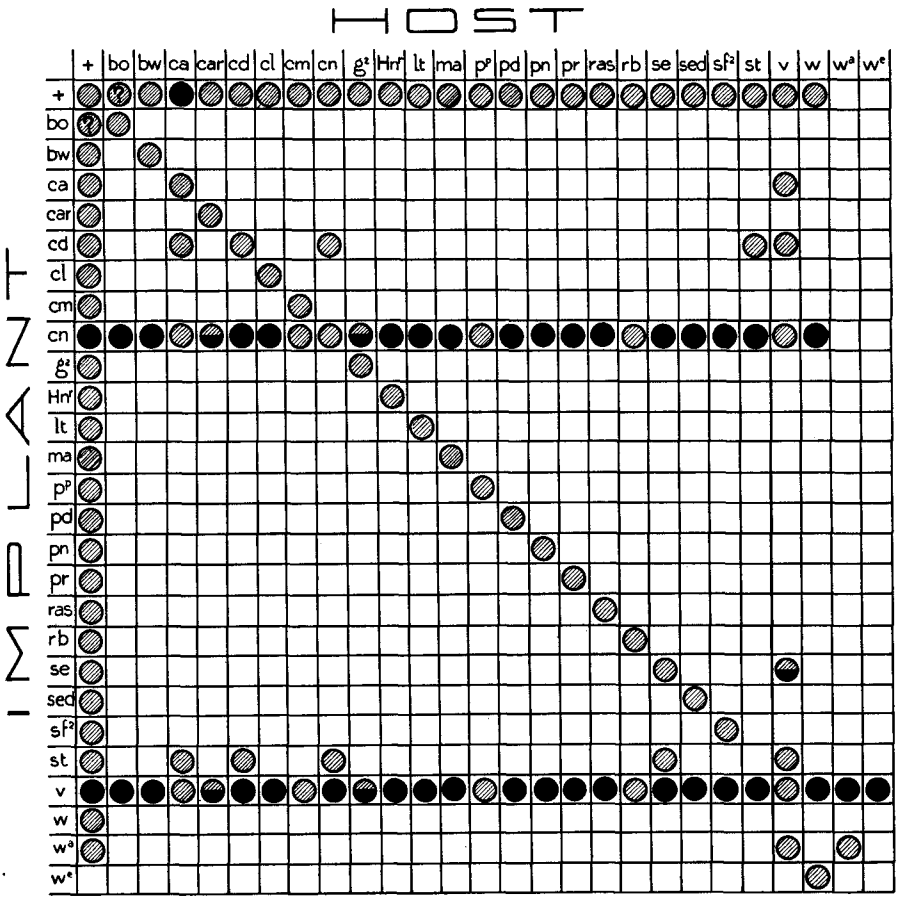

FIGURE 1.—Diagrammatic representation of the results of eye transplants. Shaded circles indicate autonomous development of the pigmentation of the implant. Black circles indicate non-autonomous development of pigmentation. Circles half black and half shaded indicate non-autonomous development of such a nature that the resulting implant is intermediate in color between two controls.

Wild type discs in mutant hosts

Knowing the behavior of the various mutant eye color discs implanted in wild type hosts, the reciprocals of these offer points of interest. The data are summarized in table 2.

It is evident that a wild type disc gives rise to an eye with wild type pigmentation when implanted in any of the mutants except *ca* and possibly *bo*. As in the reciprocal transplant, the result with *bo* is not clear. The significance of this exceptional behavior of + in *ca* transplants will be discussed later.

Vermilion discs in mutant hosts

In the case of a *v* disc implanted into a wild type host, the developing eye is affected by the host in such a way that the final pigmentation is like that of a wild type eye. Before discussing the factor responsible for this change in more detail and its relation to the factor responsible for the fact that a *cn* eye disc implanted into a wild type host develops wild type pigmentation, data should be considered which bear on the question of whether other eye color mutants have anything to do with this "body-to-eye" phase of the *v* reaction. This question can be answered by implanting *v* eye discs into hosts which differ from wild type by various eye color mutants. Such data are given in table 3.

TABLE 3

Data on the differentiation of v eye implants in eye color mutant hosts. Arrangement as in table 1.

IMPLANT	HOST	NUMBER OF INDIVIDUALS	PHENOTYPE OF IMPLANT	IMPLANT	HOST	NUMBER OF INDIVIDUALS	PHENOTYPE OF IMPLANT
v	*bo*	0, 0, 0, 1; 1	+	*v*	*pd*	2, 1, 1, 0; 4	+
v	*bw*	7, 0, 2, 1; 10	+	*v*	y *pn*	2, 0, 1, 1; 4	+
v	*ca*	4, 4, 3, 2, 3,* 2*; 18	*v*	*v*	b *pr*	2, 0, 1, 1; 4	+
v	*car*	5, 0, 2, 1; 8	Interm.	*v*	sc *ras*	0, 1, 0, 1; 2	+
v	*cd*	2, 1, 4, 1; 8	+	*v*	*rb* cv	2,‡ 1, 1, 0; 4	*v*
v	*cl*	2, 3, 1, 0; 6	+	*v*	*se* wo	4, 4, 6, 1; 15	+
v	*cm*	7, 0, 1, 2; 10	*v*	*v*	sr *sed*	0, 1,‡ 0, 0; 1	+
v	*cn*	6, 1, 3, 3; 13	+	*v*	tk sf^2 abb	3, 0, 0, 1; 4	+
v	g^2	2, 0, 3, 2; 7	Interm.	*v*	*st*	3, 0, 3, 2; 8	+
v	jv Hn^r h	1, 0, 2, 1; 4	+	*v*	*w*	8, 2, 2, 1; 13	+
v	*lt* c	2, 0, 0, 0; 2	+	*v*	w^a	0, 0, 0, 1; 1	+
v	*ma*	3, 0, 0, 3; 6	+	*v*	w^e	0, 1, 0, 0; 1	+
v	p^p	3,* 2,* 0, 0; 5	*v*				

* Sex of donor not determined.

‡ One host dissected as mature pupa.

These data show that, when implanted in certain mutant hosts (*bo*, *bw*, *cd*, *cl*, *cn*, Hn^r, *lt*, *ma*, *pd*, *pn*, *pr*, *ras*, *se*, *sed*, sf^2, *st*, and *w*), a *v* optic disc

gives rise to a wild type eye; in others (*ca*, *cm*, p^v, and *rb*), it gives an eye with *v* pigmentation. In *car* and g^2 hosts, a *v* disc gives an eye with pigmentation intermediate between *v* and wild type. Discussion of these relations will be deferred until other evidence is considered.

Cinnabar discs in mutant hosts

Since a *cn* disc implanted in a wild type host gives a result of the same type as the comparable implant of a *v* disc, namely, a wild type eye, the same question arises concerning *cn* as the one stated above for *v*. Data showing the results obtained by implanting *cn* eye discs in eye color mutant hosts are given in table 4. The results, excluding *cn* and *v* hosts, are the same as those for *v*, that is, a *cn* disc gives a wild type eye in the same mutant hosts in which a *v* disc gave a wild type eye, and gives a *cn* eye in the same hosts in which a *v* disc gave a *v* eye. Table 3 shows that a *v* disc in a *cn* host gives a wild type eye. Table 4 shows that the reciprocal transplant does not give this result, that is, a *cn* disc in a *v* host gives a *cn* eye.

TABLE 4

Data on the differentiation of cn eye implants in eye color mutant hosts. Arrangement as in table 1.

IMPLANT	HOST	NUMBER OF INDIVIDUALS	PHENOTYPE OF IMPLANT	IMPLANT	HOST	NUMBER OF INDIVIDUALS	PHENOTYPE OF IMPLANT
cn	*bo*	1, 1, 2, 0; 4	+	*cn*	*pd*	1,‡ 0, 5, 1; 7	+
cn	*bw*	3, 0, 0, 3; 6	+	*cn*	y *pn*	3, 0, 0, 0; 3	+
cn	*ca*	2, 0, 1, 0; 3	*cn*	*cn*	b *pr*	0, 1, 2, 0; 3	+
cn	*car*	2, 0, 5, 3; 10	Interm.	*cn*	sc *ras*	0, 0, 0, 1; 1	+
cn	*cd*	3, 3, 2, 2; 10	+	*cn*	*rb* cv	1, 0, 2, 1; 4	*cn*
cn	*cl*	2, 0, 1, 2; 5	+	*cn*	*se* wo	3, 3, 2, 3; 11	+
cn	*cm*	1, 2, 3, 1; 7	*cn*	*cn*	sr *sed*	0, 3, 2, 0; 5	+
cn	g^2	5, 1, 1, 2; 9	Interm.	*cn*	tk sf^2 abb	3, 0, 3, 0; 6	+
cn	jv Hn^r h	1, 0, 3, 0; 4	+	*cn*	*sl*	3, 2, 2, 2; 9	+
cn	*lt* c	0, 0, 2, 1; 3	+	*cn*	*v*	2, 4, 5, 1; 12	*cn*
cn	*ma*	1, 2, 1, 1; 5	+	*cn*	*w*	0, 2, 1, 0; 3	+
cn	p^v	5,* 3,* 0, 0; 8	*cn*				

* Sex of donor not determined.

‡ Host dissected as mature pupa.

Experiments concerning v, cn, and ca

From the data present above, it is seen that, in the cases of *cn* in wild type, *v* in wild type, and wild type in *ca*, the developing eye implant is influenced in its pigmentation by something that either comes or fails to come from some part or parts of the host. Just what this is, whether or not, for example, it is of the nature of a hormone, we cannot yet say. We shall therefore refer to it by the noncommittal term "substance."

Certain obvious questions at once arise concerning the substances con-

cerned in these three cases. For example, is there only one substance? If not, are the different substances related and in what way? What is their relation to the genes concerned in their production? Before attempting to discuss these and related questions, we shall consider additional data which bear on the problem.

Behavior of v in combination with other eye color mutants

By studying the differentiation of pigment in implants which differ from the host tissues by two eye color characters, one autonomous, the other non-autonomous in development, it might be possible to learn something about the interaction of the genes concerned. Data of this nature are summarized in table 5A for the combinations of *v*, $w^a v$ and *v car*. It is seen that the behavior of *v* is here the same as that observed in transplants in which *v* is the only mutant gene concerned. Likewise, *car* and w^a behave in the same way as in simple transplants involving only these mutant genes. This result tells us only that, so far as its behavior in transplants goes, the interaction of the *v* allelomorph with *car* or w^a plus the normal allelomorphs of all the other genes concerned with eye pigmentation is not different from its interaction with car^+ or w^+ under the same conditions. The same kind of result was observed by STURTEVANT (1932) in studies of early cleavage mosaics in *D. simulans* in which the individuals were made up of $v^+ g^+$ and *v g* tissue; here the *v* character is, under certain conditions, not autonomous, but the *g* character is always autonomous.

The relation of Bar and vermilion

In studies of the differentiation of Bar (*B*) eye discs implanted in not-*B* hosts, it was observed that a v^+B disc implanted in a *v* host gives rise to a *B* eye[1] with *v* pigmentation.

This experiment was repeated several times varying both the *v* stocks used as hosts and the *B* stocks which furnished the implants. The result was in all cases the same, indicating that the *B* gene, in addition to influencing the size of the eye in a characteristic way, has an effect closely related to the *v* reaction. The data from the various experiments involving the *v* and *B* mutants, as well as appropriate controls are given in table 5B. It is seen that only in case the host is *v*, does the *B* implant develop *v* pigmentation. An eye disc heterozygous for the *B* gene implanted in a *v* host gives an eye with wild type pigmentation. It follows that, whatever its action may be, the *B* gene effect is recessive in this interaction with *v*. These results suggested that the condition of some process in the *B* eye

[1] It is clear that a *B* disc implanted in a not-*B* host is *B* but whether or not there is any modification of the *B* character such as is observed in mosaics (STURTEVANT, 1932), we have not yet determined.

TABLE 5

Data on various eye implants. Explanations in text.

IMPLANT	HOST	NUMBER OF INDIVIDUALS	PHENOTYPE OF IMPLANT
		PART A	
sc *v* f *car*	+	14, 7, 4, 2; 27	*car*
+	sc *v* f *car*	1, 0, 2, 0; 3	+
sc *v* f *car*	*v*	2, 2, 1, 0; 5	*v car*
v	sc *v* f *car*	2, 0, 4, 1; 7	*v*
sc *v* f *car*	*cn*	1, 3, 3, 2; 9	*car*
$w^a v$	+	0, 0, 1, 1; 2	w^a
+	$w^a v$	6, 4, 2, 0; 12	+
$w^a v$	*v*	0, 0, 1, 1; 2	$w^a v$
v	$w^a v$	0, 2, 0, 4, 6,* 3*; 15	*v*
$w^a v$	*cn*	0, 0, 2, 0; 2	w^a
cn	$w^a v$	4, 5, 1, 2; 12	*cn* (host eyes w^a)
$w^a v$	wa^3	0, 0, 7, 0; 7	w^a
		PART B	
B	y *v* f	0, 0, 3, 0; 3	*v B*
B	*v*	2, 0, 1, 1; 4	*v B*
g^2 f *B*	*v*	0, 0, 3, 2, 5	*v* g^2B
B/+	*v*	3, 2, 0, 0, 5	*B*/+
B	+	0, 0, 3, 0; 3	*B*
B	*B*	0, 0, 0, 1; 1	*B*
se wo	*v*	6, 1, 5, 5; 17	*se* Interm. *v* (sex diff.-text)
		PART C	
cd	*cn*	2, 0, 0, 0; 2	*cd*
cd	*st*	3, 2, 6, 2; 13	*cd*
cd	*v*	2, 0, 3, 0; 5	*cd*
st	*cd*	0, 4, 1, 3; 8	*st*
st	*cn*	4, 3, 2, 3; 12	*st*
st	*v*	3, 0, 2, 0; 5	*st*
st	*se* wo	3, 0, 1, 0; 4	*st*
B	*st*	0, 0, 1, 2; 3	*B*
g^2 f *B*	*st*	0, 0, 1, 0; 1	g^2B
se wo	*cn*	4, 1, 1, 1, 7	*se*
cd	*ca*	3, 2, 5, 1; 11	*cd*
st	*ca*	1, 1, 0, 0; 2	*st*

* Sex of donor not determined.

disc at or after the time of transplantation might be retarded relative to the state of other developmental reactions, and led to experiments in which eye discs from young wild type larvae were implanted in older *v* larvae.

In table 6 data are given from transplants of this kind. In the first experiment, only two transplants were successful in the sense that the implanted discs gave rise to differentiated eyes. Here the age difference

between implant and host, at the time of transplantation, was about 28 hours. One of the two implanted eyes showed *v*-like pigmentation, the other more nearly wild type pigmentation. Unfortunately, in this experiment, there were no satisfactory controls. Later, an experiment was made in which young wild type discs were implanted in older *v* larvae, and at the same time, for a control, wild type discs of the same age and from the same culture dish of larvae were implanted in older wild type larvae. The data (table 6) show that, with an age difference of about 28 hours, the wild type discs implanted in *v* hosts did indeed give eyes with pigmentation approaching in color that of control *v* in *v* implants. The wild type in wild type controls with a similar age difference gave eyes with pigment of the same type as did known wild type control implants. In all cases the young discs implanted in older hosts gave rise to eyes markedly smaller than implanted eyes from transplants where little or no age difference exists between implant and host.

TABLE 6

Data on the differentiation of wild type eye discs from young larvae implanted in older v larvae. Arrangement under heading "Number of individuals" same as in previous tables.

IMPLANT		HOST			
CONSTITUTION	AGE AFTER HATCHING (HRS.)	CONSTITUTION	AGE AFTER HATCHING (HRS.)	NUMBER OF INDIVIDUALS	PHENOTYPE OF IMPLANT
+	44 to 48	*v*	80±	1, 1, 0, 0; 2	♀ *v* (?) ♂ + (?)
+	43 to 46	*v*	80±	3, 1, 3, 1; 8	Interm. between + and *v*
+	44 to 47	+	80±	4, 0, 1, 0; 5	+

From the data so far discussed, it might be assumed that the difference between *B* in *v* and wild type in *v* transplants is determined merely by the smaller size of the *B* implants. The behavior of young wild type implants in older *v* hosts could then be interpreted in the same way. But there are two arguments against this interpretation. In the first place, we have often obtained, from wild type in *v* transplants where there was no age difference, small fragments of eyes resulting from breakage of the disc during the operation of transplantation. In all cases these "small eyes" had wild type pigmentation. Many of these fragments were smaller than the eyes obtained in the "young in old" transplants. Furthermore, it is known from mosaics that small patches of v^+ tissue in an otherwise *v* eye have wild type pigmentation (STURTEVANT, unpublished). The second argument is one from analogy with the behavior of *se* in *v* transplants discussed below, in which there was little or no age difference between implant and host, but in which the implanted eyes were intermediate between *v* and v^+ (actually intermediate between *se* and *v se*, since *se* is autonomous in its

development). Here the implanted eyes were "normal" in size since the *se* gene does not affect eye size.

Actually, then, it appears probable that the behavior of *B* in *v* implants will find its explanation in terms of the states of certain eye reactions, influenced by the *B* gene, relative to the states of certain developmental reactions in other parts of the organism. Such a situation can, of course, following GOLDSCHMIDT, be expressed in terms of rates of certain eye reactions relative to the rates of other developmental reactions. What the nature of this eye reaction (or reactions) might be, we have, at present, no way of knowing. We shall return later to a consideration of its possible relation to the action of the *v* gene.

The experiments on *se* in *v* transplants mentioned above are summarized in table 5B. Actually these data are the result of three separate experiments, all of which gave the same result. Two *se* stocks were used, the second obtained by outcrossing the first to a *v* stock and recovering *se* flies in the backcross to the *se wo* stock. There was a definite difference between eyes developed from implants of discs from male and female donors; the male discs gave eyes with pigmentation more closely approaching *v se* control implants (*v se* in *v se*) than did female discs. Speculation concerning this effect of *se*, which may be of the same kind as the effect of *B*, will be more profitable when more data are at hand. The nature of the observed sex difference also needs further investigation.

Influence of eye implants on host eye pigmentation

In the above experiments in which $w^a v$ stocks were used, it was observed that $w^a v$ flies in which implanted *cn* eyes had developed, had normal eyes with w^a rather than $w^a v$ pigmentation. Since the $w^a v$ stock used had been recently made up, it was at first thought that this stock might not be pure. However, the same experiment was later repeated with adequate controls and the same result obtained. A *cn* eye implant, then, furnishes something to a $w^a v$ host fly which changes the course of eye pigment formation in such a way that the result is, in effect, v^+ and not *v* pigmentation. Since no such action of wild type or *cn* eye implants on the normal eyes of *v* hosts had been observed previously, a series of transplants was made to check this point carefully. The results were as follows:

Implanted eye disc	Host	Pigmentation of Implant	Pigmentation of Host
+	*v*	+	*v*
cn	*v*	*cn*	*v*
v	*v*	*v*	*v*
+	$w^a v$	+	$w^a v$
cn	$w^a v$	*cn*	w^a
$w^a v$	$w^a v$	$w^a v$	$w^a v$

These results suggest two obvious questions. The first is, why are the eyes of a $w^a v$ host changed by *cn* eye implants to w^a (from v to v^+) while the eyes of a v host are unaffected by such an implant? This change in the $w^a v$ eyes seems to be complete in many cases, that is, the modified eyes show no difference from stock w^a flies. Hence it seems clear that the same proportionate change does not occur in the two cases, detectable in $w^a v$ and not in v hosts. A more probable interpretation assumes that a *cn* eye implant releases into the blood of the host a certain quantity of some substance, presumably the same as that which changes the pigmentation of a v implant in a wild type host, and that this substance is only sufficient in amount to result in the change of a limited amount of pigment from v to v^+. The $w^a v$ eyes have little pigment and this can all be changed, by the available substance, from v to v^+. The normal eyes of a v host, on the other hand, have such a large amount of pigment that the limited supply of substance does not produce a detectable change, even though it may result in a change of the same absolute amount of pigment as in the case of the eyes of a $w^a v$ host. This interpretation obviously can be tested by relatively simple experiments. In fact, we have already observed that, in case the *cn* eye implant is small, the change in $w^a v$ is not complete.

A second question that is apparent from these results is, why is a *cn* eye implant effective whereas a wild type implant has no effect? Both types of eye implants of course have the v^+ gene, and presumably the production of v^+ substance goes on in both. It seems from the data that the *cn* gene produces a change such that the substance in question is released from the implant.

In connection with the influence of an eye implant on the eye color of the host, it is known, from studies of w^+-w gynandromorphs in *D. simulans* (DOBZHANSKY 1931; STURTEVANT 1932), that rate of testis sheath pigmentation is correlated with the amount of w^+ eye tissue present. The substance responsible for the pigmentation of the testis sheath very probably is formed by w^+ eye tissue—if so, it must be able to diffuse from the eye.

Implantation of gonads

In his studies of v-v^+ early cleavage mosaics in *D. simulans*, STURTEVANT (1932) was able to demonstrate clearly a strong correlation between the autonomous or non-autonomous pigmentation of genotypically v eye tissue and the constitution of the gonads with respect to the v gene. Here, if both gonads are v^+ (and female), genetically v eye tissue show v^+ pigmentation in practically all instances. If, on the other hand, both gonads are v (and male), genetically v eye tissue shows v or intermediate pigmentation in all cases. We have pointed out in a preliminary paper (EPHRUSSI and BEADLE 1935a) that it is the constitution of the gonads

with respect to the *v* gene and not with respect to sex that is important. In these experiments of STURTEVANT, there were some exceptions which led him to conclude that, in addition to the gonads, some other organ or part of the fly must be involved in the differentiation of *v* eye tissue in mosaics.

On the basis of STURTEVANT'S results we have made transplants of wild type ovaries in *v* hosts to see whether we could influence the pigmentation of the eyes of the host. Such ovary implants develop quite normally and are even capable of forming functional connections with the oviducts of the host (EPHRUSSI and BEADLE 1935b). The results of such experiments with ovary transplants, and which bear on the *v* case, are summarized in table 7.

TABLE 7

Data on transplants of non-v ovaries to v hosts.

CONSTITUTION		NUMBER OF DEVELOPED IMPLANT OVARIES	NUMBER OF INDIVIDUALS		PHENOTYPE OF HOST
IMPLANT	HOST		FEMALE	MALE	
+	*v*	1	17	7	*v*
ca	*v*	1	4		*v*
+	$w^a v$	1	29	5	$w^a v$
cn	$w^a v$	1	1		$w^a v$
+	*v*	2	5	2	*v*
+	*y v f*	3	2		*v*

It is seen that one or two wild type ovaries in a *v* male host or one, two, or even three such ovaries in a *v* female host, have no detectable effect on the *v* color of the eyes of the host. Likewise, neither an implanted wild type nor an implanted *cn* ovary has any influence on the normal eyes of a $w^a v$ host, male or female. These results, then, are entirely negative. Since in all these cases normal *v* ovaries or testes were present in the host, it could be argued that they account for the fact that implanted ovaries are without effect on the host eyes. However, this seems rather improbable as it would involve the assumption that the implanted ovaries produce the necessary substance but that something else produced by either *v* ovaries or *v* testes acts as an inactivating agent on the v^+ substance.

Taken in connection with the results of STURTEVANT which show quite definitely that wild type ovaries do have something to do with the production of the substance which changes the course of pigment formation in *v* eye tissue, our results only corroborate his conclusion that some other organ or part of the body plays an essential role in the production of this substance, i.e., gonads plus an unknown part of the body interact in its formation. Our studies give no clue as to what this unknown might be,

but STURTEVANT has shown that it is not closely related in terms of cell lineage to any surface part of the body, and does not lie in the abdomen (1932).

These results of gonad transplantation in Drosophila show certain obvious differences from those obtained by CASPARI (1933) and KÜHN, CASPARI and PLAGGE (1935) in gonad transplants in *Ephestia kühniella*, likewise made in connection with studies on eye pigmentation. These workers have shown that wild type testes or ovaries implanted in larvae of the red-eyed mutant race *a*, modify the eye pigmentation toward wild type. Here, then, the substance concerned, which they refer to as a hormone, can evidently be formed by the gonads from a wild type race in the absence of other organs or tissues of a^+ constitution. In this case, the substance has an effect on pigmentation in several parts of the organism, in larval skin, larval eyes, eyes of the imago, and in the gonads themselves. The substance can evidently be produced in other parts of the body since a wild type brain implanted in an *a* host modifies, under certain conditions, the pigmentation of the host.

Special experiments with the v-like group of mutants

The four mutants, *v*, *cn*, *st*, and *cd*, are very much alike in their phenotypic appearance. Furthermore, SCHULTZ (1935) has shown that in the development of their pigmentation, they show rather marked similarities and, as a group, are distinct from other mutants. In fact, on the basis of these similarities, he was led to suggest that they might all be found to show the *v*-type of behavior in mosaics. It has already been shown that, although *v* and *cn* are not autonomous in their pigment development in certain kinds of transplants, *st* and *cd* do show autonomous development in eye transplants in wild type hosts. Because of the similarity of *st* and *cd* to each other and to *v* and *cn*, we have used them in certain transplants in which other mutants have not been used (table 5). These data need little discussion. It is evident that both *st* and *cd* show autonomous development of pigment in all the combinations in which they are involved.

It is clear that the *v*-like group of mutants is not homogeneous as regards developmental behavior. In this respect *v* and *cn* are obviously related but not the same, as will be pointed out in more detail below, and *st* and *cd* are different from either *v* or *cn*.

The ca case

As shown by the data already referred to, a wild type eye disc implanted in a *ca* host gives an eye with *ca*-like pigmentation. To account for this result, we must assume that in the development of wild type pigment something must come to the eye from another part or other parts of the

body and that this substance is not formed in a fly homozygous for the *ca* gene. But the data given in tables 3 and 4 show that a *v* eye disc implanted in a *ca* host gives a *v* eye, i.e., not *v ca*, therefore ca^+, and that, similarly, a *cn* disc implanted in a *ca* host gives a *cn* ca^+ eye. Data given in table 5 show that a similar result is obtained if a *st* or a *cd* disc is implanted in a *ca* host, namely, a *st* ca^+ or a *cd* ca^+ eye results. Summary of these results:

+	disc implanted in a *ca* host gives a *ca*	eye
v	disc implanted in a *ca* host gives a *v* ca^+	eye
cn	disc implanted in a *ca* host gives a *cn* ca^+	eye
st	disc implanted in a *ca* host gives a *st* ca^+	eye
cd	disc implanted in a *ca* host gives a *cd* ca^+	eye

In determining that the last four of these results were really *v*, *cn*, *st* and *cd* and not *v ca*, *cn ca*, *st ca*, and *cd ca* respectively, the appropriate double recessive controls were not available, but comparisons were made with *v*, *cn*, *st*, and *cd* control transplants and no differences could be detected. Since *v ca* and *st ca* are both known to be readily separable from *v* and *st* respectively, there is little chance of error in the determinations. The question, of course, is, why is the development of ca^+ pigmentation not autonomous in the first case listed and autonomous in the remaining cases studied? Possibly the four genes *v*, *cn*, *st* and *cd* act, in the implant, in such a way that no ca^+ substance is necessary to give ca^+ pigmentation; that is, a v^+ca^+ implant requires ca^+ substance from the host to develop ca^+ pigmentation, but a *v* ca^+ implant does not require this substance to develop *v* ca^+ pigmentation.

DISCUSSION

From the experimental results considered above, several hypotheses can be suggested concerning the nature of certain of the eye color mutants and the action of the genes which differentiate them from wild type. Alternative hypotheses are obviously possible, and it should be emphasized that those presented are tentative.

The vermilion character

Since the pigmentation of a genetically *v* eye can be modified to v^+ by transplanting it to a host which supplies it with what may be called the v^+ substance, it follows that *v* differs from wild type by the absence of this substance. Evidently there is no change in the *v* eye itself which prevents its pigmentation from assuming wild type characteristics. It follows that the mutation $v^+ \rightarrow v$ has resulted in a change such that v^+ substance is no longer formed. Since a *cn* eye disc implanted in a *v* host remains *cn*, the $v^+ \rightarrow v$ mutation has resulted also in preventing the formation of cn^+ sub-

stance. The v^+ gene plays an essential part in the formation of the v^+ and cn^+ substances, but it does not form them directly since any one of several other gene mutations (*ca*, *cm*, p^v, and *rb*) may result in the absence of them. According to this scheme, v^+ substance is necessary for wild type pigmentation. The question then arises, why does a wild type eye disc implanted in a *v* host, which can supply no v^+ substance, develop wild type pigmentation? Two answers are possible: either the v^+ substance has already acted at the time of transplantation or this substance is produced by the eye itself. The fact that, in mosaics, a small patch of v^+ eye tissue in an effectively *v* individual has wild type pigmentation (STURTEVANT, unpublished), shows that the first of these answers cannot be correct, for in this case, the v^+ tissue has been in a *v* tissue environment almost from the beginning of development. We must then conclude that the substance is produced in the eye itself. Actually we have been able to demonstrate that it is produced by a *cn* eye (modification of normal $w^a v$ eyes by an implanted *cn* eye). But, it may be asked, why was it not possible to demonstrate that it is produced by a wild type eye? The answer may be that the substance is produced but cannot get out of the eye, i.e., one of the effects of the *cn* gene is to make eye cells permeable to v^+ substance. The difference in behavior between a wild type and a *B* eye implanted in a *v* host may be accounted for by assuming that one of the effects of the *B* gene is to prevent the formation of v^+ substance in the eye, but not in other parts of the body. This assumption is not necessarily an alternative to the assumption previously suggested that the action of the *B* gene may be explained "in terms of the states of certain eye reactions, influenced by the *B* gene, relative to the states of certain developmental reactions in other parts of the organism." It may well be that it is the formation of v^+ substance that is retarded (in an extreme way) in the eye relative to its formation in other parts of the body. The "young in old" experiments can be formally explained in the same terms. In young wild type discs implanted in older *v* larvae, the time during which v^+ substance can be formed in the implanted eye is much reduced. In a similar way, in a *se* eye, v^+ substance is formed in the eye at a rate so low that, when implanted in a host without v^+ substance, pigmentation intermediate between v^+ and *v* results.

The cinnabar character

The evidence for the existence of a cn^+ substance is the same in kind as that for v^+ substance. It is already evident and will be pointed out in more detail below that the cn^+ substance is different from the v^+ substance. By the same kind of arguments as were presented in the above discussion of the *v* character, it may be concluded that the mutation $cn^+ \rightarrow cn$ produces

a change such that cn^+ substance is no longer formed. According to this interpretation, as in the interpretation of *v*, it is assumed that a wild type eye produces cn^+ substance in its own cells. This would account for the fact that a wild type eye implanted in a *cn* host gives wild type pigmentation.

The claret character

In contrast to the *v* and *cn* cases, two phases of the action of the *ca* gene can be distinguished. First, since a genetically wild type eye cannot develop wild type pigmentation unless some other part of the organism is ca^+, it is concluded that a ca^+ substance is necessary for the formation of wild type pigmentation. This is not formed in the eye itself but comes from some other part of the body. Secondly, since by supplying a *ca* eye with the necessary ca^+ substance by implanting it in a wild type host, we do not produce a change to wild type pigmentation, it is postulated that there is a change in a *ca* eye of such a kind that the addition of ca^+ substance is not sufficient to give wild type pigmentation.

Other eye color mutant characters

By implanting *v* and *cn* eye disc in other eye color mutant hosts, it has been demonstrated that the mutants *cm*, p^p, and *rb* are characterized by lack of both the v^+ and cn^+ substances. In these three mutant types, as in *ca*, there must be two phases of gene action, (1) the failure of the formation of the v^+ and cn^+ substances, and (2) an action in the eye itself, since supplying the two substances by transplantation does not produce a change. The genes *car* and g^2 must be placed in the same class, but in these two cases the formation of v^+ and cn^+ substances is not prevented but only limited.

The other mutants with which we have worked, *bo*, *bw*, *cd*, *cl*, Hn^r, *lt*, *ma*, *pd*, *pn*, *pr*, *ras*, *se*, *sed*, sf^2, *st*, and w^a are characterized by the presence of all the three substances postulated. It cannot be concluded that the normal allelomorphs of the genes differentiating these characters have nothing to do with the production of v^+, cn^+ and ca^+ substances. There is no justification in assuming that, if a given gene concerned with the production of a substance such as we are considering, mutates, the particular mutant allelomorph resulting will be of such a nature as to result in the absence of the substance. KÜHN, CASPARI and PLAGGE (1935) come to such an unjustified conclusion with regard to the t^+ gene in Ephestia.

Relation of the v^+, cn^+ and ca^+ substances

It has been shown from the difference in reciprocal transplants between *v* and *cn* that the v^+ and cn^+ substances are different (BEADLE and EPHRUSSI 1935a). At the same time, it was concluded from the fact that a *v* fly lacks

both substances, that the two substances are related. This conclusion is corroborated by the more extensive data presented in this paper. The strongest indication that two substances are concerned is the fact that a *v* eye disc implanted in a *cn* host gives rise to an eye with wild type pigment. Two other facts strengthen the supposition of two substances: (1) A *B* eye disc implanted in a *v* host gives an eye with *v* pigmentation, but, implanted in a *cn* host, gives wild type pigmentation. (2) A *se* eye disc implanted in a *v* host gives a *se*, partially *v*, eye, but, implanted in a *cn* host, gives a straight *se* eye.

The fact that these substances, although not the same, are developmentally—and presumably chemically—related, is shown by the fact that, if a given mutant is characterized by the absence of one of these substances, it will probably be characterized by the absence of the other also.

Considering the relation of the ca^+ substance to the other two, it is clear that it is different from either for it may be present in the absence of both the others. The fact that the *ca* gene prevents the formation of all three substances (*v* or *cn* discs implanted in *ca* hosts are not modified in their pigmentation) indicates that ca^+ substance is related to the other two.

It may be asked whether, from the relations discussed above, anything can be inferred as to (1) how the v^+, cn^+, and ca^+ substances are related in terms of development, and (2) how the mutant forms of the genes known to be concerned with the production of the three substances produce their effects? A simple, and, it seems to us, plausible, hypothesis may be of help in answering these questions. Such an hypothesis assumes that the ca^+, v^+, and cn^+ substances are successive products in a chain reaction. The relations of these substances can be indicated in a simple diagrammatic way as follows:

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

In such a scheme, we assume that:

1. The mutant gene *ca* in some way produces a change such that the chain of reactions is interrupted at some point prior to the formation of ca^+ substance; hence a *ca* fly lacks ca^+, v^+, and cn^+ substances.

2. Any one of the mutant genes *v*, *cm*, p^p, or *rb* results in a change such that the reaction or reactions leading from ca^+ substance to v^+ substance do not go on; hence the mutants *v*, *cm*, p^p and *rb* lack both v^+ and cn^+ substances but have ca^+ substance. The mutant genes *car* and g^2 slow down this step in the chain of reactions, hence *car* and g^2 flies are characterized by a reduced amount of v^+ and cn^+ substances. The mutant gene *B* interrupts this same step in the chain in the eye, but not in other parts of the body. The mutant gene *se* results in a change such that the ca^+ substance changes to v^+ substance at a reduced rate in the eye, but at a normal rate in other parts of the body.

3. The mutant gene *cn* stops a reaction essential for the change of v^+ substance to cn^+ substance; hence a *cn* fly lacks cn^+ substance but has the ca^+ and v^+ substances.

On the basis of the above scheme, the results of implanting *v* eye discs in *cn* hosts can be interpreted as follows: The implant produces no v^+ substance, and, because v^+ substance is an essential step in the formation of cn^+ substance, it likewise produces no cn^+ substance. The host can supply v^+ substance to the implant but cannot supply cn^+ substance. With v^+ substance supplied to the implant by the *cn* host, there is no block to the formation of cn^+ substance in the implant itself. The implant therefore develops wild type pigmentation in spite of the fact that normally neither the donor nor the host could have produced the cn^+ substance presumably necessary for the production of wild type pigment.

In a somewhat similar way, the results of transplanting *B* eye discs to *v* and to *cn* hosts can be interpreted. The *B* eye can form no v^+ substance. When transplanted to a *v* host v^+ substance cannot move to it from the host and the pigment developed is therefore *v*. Because of the absence of the prerequisite v^+ substance, the *B* eye normally does not itself produce cn^+ substance. But when a *B* eye disc is implanted in a *cn* host, the *B* implant is supplied with v^+ substance from the host and the reaction or reactions from v^+ to cn^+ substances can then go on in the implant itself and wild type pigment is produced.

The results of implanting *se* eye discs in *v* and *cn* hosts can be interpreted in an essentially similar way.

Eye color mutant groups

The eye color mutants in Drosophila can be grouped according to their phenotypic characteristics, since mutants differentiated by non-allelomorphic genes can look alike (MORGAN, BRIDGES, and STURTEVANT 1925). Recently SCHULTZ (1935) has extended this grouping by studying the time of appearance and the rate of formation of pigment, the distribution of pigment in the eye, and the interaction behavior of the different mutants. It is obvious that we can, on the basis of the results given above, classify the mutants with respect to the presence or absence of the three postulated substances. We may then ask if there is any relation between groups such as made by SCHULTZ and the classification according to these substances. If there is such a relation, it is not evident from the data at hand. As an example, the four mutants, *v*, *cn*, *st*, and *cd*, form one of SCHULTZ'S groups but as we have seen, *v* lacks two substances, *cn* one, while *st* and *cd* have all three.

The above discussion, we hope, has served to indicate some of the possibilities in the application of the method of transplantation to the study

of development in Drosophila. The extension of the studies of certain cases to other stages of development is indicated as a logical next step by which we can hope to get at such questions as concern the time of determination of characters and the time of action of genes associated with these characters.

ACKNOWLEDGMENTS

We are indebted to Professor R. GOLDSCHMIDT and to Doctors M. DEMEREC and N. W. TIMOFEEF-RESSOVSKY for their kindness in supplying various of the stocks used in the work reported in this paper. To Professor CH. PEREZ and Doctor G. TEISSIER we wish to express our thanks for facilities for work at the Biological Station at Roscoff. Members of the staff of the Biological Laboratories of the California Institute of Technology, particularly Professor T. H. MORGAN, have made many helpful suggestions during the preparation of the manuscript. We are grateful to Professor A. H. STURTEVANT for permission to cite unpublished data essential to certain of our arguments.

SUMMARY

Larval optic discs can be successfully transplanted from one larva to another. Such transplanted discs give rise to supplementary eyes, usually lying in the abdominal cavity of the adult fly, which differentiate normally except that they are inverted. The pigmentation of such eyes develops normally.

When optic discs of the mutants *cn* or *v* are implanted in wild type hosts, they give eyes with wild type pigmentation, i.e., under these conditions, the *cn* and *v* characters are not autonomous in their development. Under the same conditions, *bw*, *ca*, *car*, *cd*, *cl*, *cm*, g^2, Hn^r, *lt*, *ma*, p^v, *pd*, *pn*, *pr*, *ras*, *rb*, *se*, *sed*, sf^2, *st* and *w* eye discs implanted in wild type hosts show autonomous development of eye pigment.

In the reciprocals of the above transplants, wild type eye discs implanted in hosts of the mutants mentioned, wild type pigmentation of the implant results in all except one case, a wild type disc implanted in a *ca* host. In this one exception, a genetically wild type eye disc gives an eye with *ca* pigmentation, i.e., ca^+ does not show autonomous pigment development under these conditions.

If *v* eye discs are implanted in eye color mutant hosts, eyes with wild type pigmentation develop in *bo*, *bw*, *cd*, *cl*, *cn*, Hn^r, *lt*, *ma*, *pd*, *pn*, *pr*, *ras*, *se*, *sed*, sf^2, *st*, and *w* hosts, i.e., the *v* character is not autonomous in its development when a *v* eye is transplanted to any one of these hosts. But a *v* eye disc implanted in a *ca*, *cm*, p^v, or *rb* host gives an eye with *v* pigmentation, i.e., the *v* character is autonomous in these cases. It can be concluded that the autonomous or non-autonomous development of the

v character is determined by the genetic constitution with regard to genes other than *v*, of the tissue environment in which the *v* eye develops.

Implanted in eye color mutant hosts other than *v* or *cn*, a *cn* eye disc behaves in the same way as does a *v* eye disc, showing autonomous pigment development in the same mutant hosts as does *v*, and non-autonomous pigment development in the same hosts as does *v*.

Reciprocal transplants involving *cn* and *v* do not give the same result; a *v* eye disc implanted in a *cn* host gives an eye with wild type pigmentation while a *cn* eye disc implanted in a *v* host gives an eye with *cn* pigmentation.

A *B* eye disc implanted to a *v* host gives a *B* eye with *v* pigmentation. This shows that the *B* gene has an effect on the eye somehow related to the effect of the *v* gene but not of such a nature as to modify the pigmentation of the eye in its normal position. This case shows that the autonomous or non-autonomous development of v^+ pigmentation in an implanted v^+ eye may be influenced by the genetic constitution, with respect to genes other than *v*, of the implant itself.

A genetically wild type eye disc from a young larva implanted in an older *v* host shows pigmentation intermediate between *v* and wild type. A *se* eye implanted in a *v* host likewise gives pigmentation of an intermediate nature with respect to the *v* character; here the eye is intermediate between *v se* and *se*. The possible relation of these cases to the *B* in *v* results is considered.

A *cn* eye implanted in a $w^a v$ host gives a *cn* eye, but the eyes of the host are modified from the $w^a v$ to a w^a phenotype.

Ovaries from wild type donors have been implanted in both male and female *v* hosts without any detectable change in the pigmentation of the host eyes.

From the cases of non-autonomous development of the pigmentation of implanted eyes considered in this paper, three substances are postulated, the v^+, cn^+, and ca^+ substances. Their interrelations and the conditions under which they are produced are discussed. A hypothetical scheme accounting for the production and relation of these three substances is suggested, and, in connection with this, questions concerning where and how certain genes might act are considered.

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