DEVELOPMENT OF EYE COLORS IN DROSOPHILA: PRODUCTION AND RELEASE OF cn^+ SUBSTANCE BY THE EYES OF DIFFERENT EYE COLOR MUTANTS

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INTRODUCTION

THE evidence given in previous papers by the authors has led to the hypothesis that individuals with the mutant eye color vermilion are deficient in two diffusible substances. These have been designated v^+ and cn^+ substances. It has been shown also that either one or both of these substances are necessary for the development of any one of several other mutant eye colors. The method of establishing the latter fact was to transplant eye anlagen from larvae homozygous for vermilion and for the mutant gene in question (*vvxx*) into wild type larvae. Such implants developed the color characteristic of the mutant *x* (that is, $++xx$) and not that of the combination *vvxx*. A genetically vermilion eye-implant was thus modified by the host into a nonvermilion phenotype. The phenotype *x* thus depends upon v^+ or cn^+ substance or both. These are supplied by the wild type host, but not by the genetically vermilion donor. This is not true of the vermilion-like eye color mutants (cardinal, cinnabar and scarlet); here we have no evidence that the double recessives with vermilion are phenotypically different from vermilion.

Eye disks of the mutants bordeaux (*bo*), clot (*cl*), Henna-recessive (Hn^r), mahogany (*mah*), purpleoid (*pd*), prune-2 (pn^2), purple (*pr*), raspberry-2 (ras^2), sepia (*se*), sepiaoid (*sed*), and safranin-2 (sf^2) grown in vermilion hosts give rise to eyes more or less intermediate in color between their own control implants and control implants of the double recessives with vermilion. It was concluded (EPHRUSSI and BEADLE 1937a) that, in these mutants, the eye itself is incapable of producing sufficient v^+ or cn^+ substance, or both, to give the characteristic pigmentation of the mutant.[1] Since we know that a vermilion host is deficient in both v^+ and cn^+ substances, the above experiments do not distinguish between production of the two different substances by the implant. By growing eye-implants in cinnabar hosts, which are deficient in cn^+ substance but not in v^+ substance, it should be possible to make this distinction; this is the primary purpose of the work reported in this paper.

In studies of the release of diffusible substances by eye-implants, it has

[1] Eyes of the mutants brown (*bw*), claret (*ca*), carnation (*car*), cardinal (*cd*), carmine (*cm*), cinnabar (*cn*), garnet-2 (g^2), light (*lt*), maroon (*ma*), peach (p^p), ruby (*rb*), and scarlet (*st*) grown in vermilion hosts develop colors characteristic of their own genotypes.

been shown that of twenty-five eye color types tested, only nine (claret, carmine, cinnabar, garnet-2, light, maroon, peach, ruby, and scarlet) give evidence of release of v^+ substance when tested in apricot vermilion (w^a v) hosts. Release of v^+ substance is indicated by a modification of the eyes of the hosts toward apricot. On the other hand, all of the eight types of eye-implants tested for the release of cn^+ substance (wild type, bright, cardinal, Henna-recessive, sepia, scarlet, vermilion, and apricot) gave positive results (EPHRUSSI and BEADLE 1937c). In this test, cn^+ substance is detected by a modification of the eyes of apricot cinnabar hosts toward apricot. A second purpose of the present paper is to present the results of additional tests for the release of cn^+ substance by eye-implants of various genotypes.

MATERIAL AND TECHNIQUE

The technique of transplantation used in the experiments reported here has been described by EPHRUSSI and BEADLE (1936). The same series of mutants was tested as was previously used in tests for the release of v^+ substance. In all tests eye disks were taken from larvae approaching puparium formation and implanted in apricot cinnabar larvae of approximately the same age. Shortly after eclosion the eyes of the hosts were examined for implant-host effects. Implants were removed from the hosts and compared with controls made at the same time. Thus, in the case of a sepia implant grown in an apricot cinnabar host, after recording the color of the eyes of the host, the implant was compared with a sepia implant grown in a sepia host.

EXPERIMENTAL RESULTS

From the results summarized in table 1 it is seen that in all cases except vermilion and possibly bright, the implant develops pigmentation according to its own genetic constitution; that is, the implant shows autonomous development of color. On the other hand, all implants except cinnabar modify the eye color of the host toward apricot; in such cases the development of color in the host's eyes is not autonomous with respect to the cinnabar component. Implant-host modifications are recorded in a roughly quantitative way. It is seen that this modification varies with the genetic constitution of the implant.

DISCUSSION

It is known from studies of the interaction of different eye color characters that cinnabar in combination with any other eye color except vermilion, cardinal, and scarlet (that is, those belonging to the vermilion group of SCHULTZ, 1932), results in a color different from either component. From these relations we can infer that cn^+ substance is necessary for the

TABLE 1

Development of eye color in implants of various mutants grown in w^a cn hosts, and the effect of the implant on the eye color of the host. Under heading "number of individuals" the arrangement of sex combinations is: ♀ in ♀, ♀ in ♂, ♂ in ♀, ♂ in ♂, and total.

GENOTYPE OF IMPLANT	NUMBER OF IMPLANTS	NUMBER OF INDIVIDUALS	PHENOTYPE OF IMPLANT	EFFECT ON HOST
+		*	+	strong
bo	1	2, 4, 3, 5; 14	*bo*	medium
bri	1	6, 3, 6, 2; 17	*bri***	very weak
bw	1	6, 4, 3, 8; 21	*bw*	weak
ca	1	6, 3, 6, 6; 21	*ca*	medium
car	1	4, 4, 1, 5; 14	*car*	strong
cd	1	7, 0, 4, 3; 14	*cd*	medium
	2	0, 1, 0, 0; 1	*cd*	medium
cl	1	6, 3, 3, 1; 13	*cl*	weak
cm	1	5, 3, 3, 3; 14	*cm*	medium
cn		*	*cn*	none
g^2	1	4, 4, 6, 4; 18	g^2	strong
Hn^r	1	6, 3, 2, 5; 16	Hn^r	weak
lt	1	3, 5, 5, 3; 16	*lt*	strong
	2	1, 0, 0, 0; 1	*lt*	strong
ma	1	2, 5, 6, 2; 15	*ma*	medium
mah	1	7, 5, 7, 8; 27	*mah*	very weak
p^p	1	2, 1, 3, 0; 6	p^p	strong
pd	1	0, 4, 4, 2; 10	*pd*	strong
pn^2	1	4, 2, 2, 2; 10	pn^2	medium
	2	0, 1, 1, 1; 3	pn^2	medium
pr	1	1, 0, 1, 0; 2	*pr*	strong
ras^2	1	9, 5, 8, 5; 27	ras^2	weak
rb	1	3, 4, 7, 2; 16	*rb*	medium
se	1	5, 4, 3, 4; 16	*se*	strong
	2	0, 0, 1, 0; 1	*se*	strong
sed	1	4, 2, 1, 1; 8	*sed*	medium
sf^2	1	3, 0, 2, 1; 6	sf^2	weak
st	1	2, 2, 4, 1; 9	*st*	strong
v		*	+	strong
w	1	0, 4, 1, 0; 5	*w*	strong
	2	0, 1, 0, 0; 1	*w*	strong

* Data previously published (EPHRUSSI and BEADLE 1937c).
** Implants possibly slightly darker than controls (*bo* implants) grown in *bo* hosts).

development of any eye color not belonging to the vermilion group. On the basis of this argument, it follows that any eye-implant which shows autonomous development of color when grown in a host deficient in cn^+ substance (apricot cinnabar in this case) must itself be able to make the necessary cn^+ substance. Since there is no evidence that cardinal and scarlet eyes need cn^+ substance to develop their characteristic colors, it does not follow from this behavior alone that the eyes of these mutants are able to produce *cn*⁺ substance. As pointed out in the introduction, eyes of certain mutants do not show autonomous development when grown in vermilion hosts which are deficient in both v^+ and cn^+ substances. It was assumed that such eyes are themselves unable to produce as much of one or the other, or both, of the substances as they would have utilized in their normal environment. The fact that eye-implants of these same mutants grown in apricot cinnabar hosts show autonomous development of color indicates that v^+ substance and not cn^+ substance is the limiting factor when they are grown in vermilion hosts. In other words, eyes of all the mutants studied, when grown in a host able to supply them with a sufficient amount of v^+ substance, produce the cn^+ substance they would normally use.

The above conclusion is confirmed by the fact that eye-implants of all mutants tested with the exception of cinnabar release detectable quantities of cn^+ substance. From the experiments on release of cn^+ substance by eye-implants it is seen that scarlet and cardinal eyes are capable of producing and releasing this substance. Eyes of mutants assumed from other types of evidence to be deficient in v^+ and cn^+ substance (carnation, carmine, claret, garnet-2, peach, ruby, and vermilion, BEADLE and EPHRUSSI 1936), release cn^+ substance when grown in the presence of v^+ substance. This is in agreement with the previous interpretation of the authors which assumed that in these cases the deficiency of the two substances results fron a partial or complete block in the sequence of reactions leading to the formation of v^+ substance. Once this substance is supplied, as it is by a cinnabar host, there should be no further block to the formation of cn^+ substance (EPHRUSSI and BEADLE 1937b, BEADLE and EPHRUSSI 1937a).

The two mutants bright and mahogany show evidence of having reduced amounts of cn^+ substance, but, according to the corresponding type of test, they show no evidence of having a reduced amount of v^+ substance (BEADLE and EPHRUSSI 1937b). It has been assumed that in these mutants the reactions leading to the formation of cn^+ substance are partially blocked. The tests for release of cn^+ substance by eyes of these mutants were positive but very weak. The argument that cn^+ substance is produced at a reduced rate in these mutants as compared with wild type is in general supported.

With regard to the observed differences in magnitude of implant-host effects, several interpretations appear plausible. It seems reasonable to assume that at least two factors influence this effect: 1) the total amount of cn^+ substance produced by the implant, and 2) the amount used by the implant itself. One might assume that the difference between these two quantities represents the amount released. Following these assumptions, the observed implant-host effects measure only this difference. Since at present we have no way of measuring either the total amount produced or the amount used by the eye itself, it is perhaps best to defer further discussion of quantitative aspects of the problem.

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SUMMARY

Larval eye disks of wild type and of the mutants *bo*, *bri*, *bw*, *ca*, *car*, *cd*, *cl*, *cm*, *cn*, g^2, Hn^r, *lt*, *ma*, *mah*, p^p, *pd*, pn^2, *pr*, ras^2, *rb*, *se*, *sed*, sf^2, *st*, *v*, and *w* were transplanted to w^a *cn* larvae. In every case except *v* the implants developed into eyes of a color corresponding to their genotype. Since there is evidence that these mutants, with the exception of *cd*, *cn*, *st*, and *v*, require cn^+ substance for the development of their characteristic colors, it is argued that eye-implants of the mutants tested, when grown in hosts capable of supplying v^+ substance, are themselves able to make the cn^+ substance necessary for the autonomous development of eye color.

All implants tested with the exception of *cn* produced a modification of the eyes of the host in the direction of w^a cn^+. The results of these tests are a direct confirmation of the conclusion stated above.

A comparison is made between these results and the results previously published of the transplantation of eyes of the same mutants to *v* and to w^a *v* hosts. From this comparison it is argued that v^+ substance, not cn^+ substance, is the limiting factor in pigment development in eyes of certain mutants grown in hosts deficient in both v^+ and cn^+ substance, that is, in *v* hosts.

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