DEVELOPMENT OF EYE COLOURS IN *DROSOPHILA*: STUDIES OF THE MUTANT CLARET[1]

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Introduction

A previous account of eye transplantation experiments involving the eye colour mutant claret (*ca*, 3–100·7) of *Drosophila melanogaster* (Beadle & Ephrussi, 1936) reported that a wild-type eye disk grown in a claret host develops pigmentation phenotypically like claret. From this it was concluded that a diffusible substance, tentatively called ca^+ substance, is involved in the development of a wild-type eye, and that this substance cannot be supplied by a *ca* host (or is supplied in reduced amounts). In repeating such experiments, it was found that the results were not always uniform; in some cases wild-type eyes grown in *ca* hosts gave eyes phenotypically close to wild type, while in others eyes phenotypically close to *ca* were obtained. Since these experiments were made with larvae of which the ages were only approximately known, it was assumed that the lack of uniformity might mean that we were working at a period of development which included a critical time for the action (or uptake by the eye) of ca^+ substance, and that some experiments had been made before, and others after this time. Additional work has shown that this assumption was correct. It is the primary purpose of this paper to present the evidence bearing on this question.

Transplants made at different ages

Wild type (from crosses of the inbred stocks Florida and Swedish *c*) and *ca* (stock obtained from out-crosses to wild type) larvae of approximately known ages were obtained from eggs laid during 2-hour intervals. Errors from eggs laid in advanced stages of development were minimized by discarding early hatching larvae.

The first experiment was made with larvae taken at 86–89 hours after egg-laying. The results (Table I) show that disks from male donors gave eyes close to *ca*, but that those from female donors gave eyes slightly darker, that is, deviating from *ca* in the direction of wild type. This would suggest that the disks from female donors were more advanced developmentally at the time of transplantation than were the disks from

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male donors. Subsequently, two series of experiments were made using larvae of the same culture dish at different times, at 75–78 hours and at 91½–94 hours. These two experiments therefore should be strictly comparable. The results are summarized in Table I. The phenotypic appearance of the implants in the 75–78-hour experiment was like that of *ca* controls (*ca* eye disks grown in *ca* hosts and examined at the same time

TABLE I

Data on the differentiation of wild-type eye disks implanted in claret hosts. In this and Table II, under the heading "Number of individuals", are given the four sex combinations and the total in the order: female in female, female in male, male in female, male in male, and total.

Age after egg-laying (hours)	Number of individuals	Phenotype of implant
86 –89	2, 2, 1, 2; 7	♂ implants *ca*; ♀ implants slightly darker than *ca*
75 –78	3, 2, 2, 1; 8	*ca*
91½–94	3, 1, 4, 2; 10	Slightly darker than *ca*

as were the implants of the experiment). The implants in the 91½–94-hour experiment were slightly darker than the *ca* controls. In this series there was no indication of a difference dependent on the sex of the wild type donor. Since small differences were being considered, and since the two experiments were made at different times, it is questionable whether there was any real difference in the results of the 86–89-hour and the 91½–94-hour experiments.

The controls for experiments made in connexion with ovary transplants (Table II) confirm the results of the experiments just described. These show that wild-type eye disks transplanted to *ca* hosts at 73¾–

TABLE II

Data on implantation of wild type eye disks and ovaries into ca *hosts. Arrangement under heading "Number of individuals" same as in previous table*

Nature and constitution of implants	Age of donor (hours after egg-laying)	Host: Constitution	Host: Age after egg-laying (hours)	Number of individuals	Phenotype of implanted eye
+ ovary and + eye disk	85 –88	*ca*	85 –88	3, 1, 0, 0; 4	*ca*
+ eye disk	86 –88½	*ca*	86 –88½	0, 3, 0, 0; 3	*ca*
+ ovary and + eye disk	73 –74¾	*ca*	93 –95¾	3, 5, 0, 0; 8	*ca*
+ eye disk	73¾–75¼	*ca*	93¾–96¼	2, 6, 0, 0; 8	*ca*

75½ hours and at 86–88½ hours after egg-laying give eyes phenotypically similar to comparable *ca* control implants.

It is clear from the above experiments that if a wild-type eye disk is transplanted to a *ca* host before 80 hours after egg-laying, an eye will develop with *ca*-like pigmentation. From other experiments we know that if the above transplant is made shortly before puparium formation, the implant will develop a colour close to that of wild-type controls. It can be said, therefore, that the critical time with which we are dealing in the case of *ca* lies in that period of development reached some time after 80 hours and before puparium formation (about 106 hours at 25° C. for the wild-type flies used in these experiments). Presumably, before this time, a wild-type eye has received little or no ca^+ substance from other parts of the body. By the time of puparium formation, however, a sufficient amount of ca^+ substance has moved from the body to the eye so that removal of the eye to an environment unable to supply this substance (*ca* host) does not prevent its developing a colour like that of wild type.

These results indicate that certain conclusions arrived at in a previous paper may be erroneous. It was found that vermilion (*v*), cinnabar (*cn*), scarlet (*st*), and cardinal (*cd*) eye disks transplanted to *ca* hosts gave rise to eyes with *v*, *cn*, *st*, and *cd* pigmentation, respectively, that is, not-*ca*. These experiments were done with larvae the ages of which were not accurately controlled. From the experiments reported above on wild type in *ca* transplants, it is evident now that they might all have been made at or after the critical time for the action of ca^+ substance. As a matter of fact, as will be pointed out below, the interpretation of the *v* and *cn* in *ca* experiments is probably even more involved.

From the results obtained by transplanting *v* and *cn* eye disks to *ca* hosts (Beadle & Ephrussi, 1936) it was concluded that the mutant *ca* is characterized by the absence of both v^+ and cn^+ substances. These are specific diffusible substances assumed to be related to ca^+ substance in formation in a manner which can be expressed by the scheme:

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

Additional tests indicate that a *ca* host does not completely lack v^+ substance but has a reduced amount as compared with wild type. Thus there appears to be a slight modification, in the direction of wild type, of a *v* eye disk grown in a *ca* host. The presence of v^+ substance is more clearly shown by growing an w^a *v* (w^a-apricot) eye disk in a *ca* host; here the phenotype of the implanted eye approaches that of straight w^a. A difference in degree of change in *v* and in w^a *v* implants, when acted on by a limited amount of v^+ substance, appears to be a general rule; as such,

it is discussed elsewhere (Ephrussi & Beadle, 1936). Preliminary experiments involving ca^2, differing in origin from *ca*, indicate that a limited amount of cn^+ substance is formed in a ca^2 fly.

There is an obvious bearing of the evidence presented above on the general scheme which attempts to relate the three postulated diffusible substance. Discussion of this will be deferred.

Tests for the production of ca^+ substance by ovaries

In an attempt to determine where ca^+ substance is produced in the body of a wild-type fly ovaries have been tested. Such tests can be carried out by making double transplants into *ca* hosts, a wild-type eye as a detector and, in the same host, the organ to be tested. Experiments in which ovaries were tested are summarized in Table II. These experiments were made with larvae of 73–74¾ hours and 85–88 hours after egg-laying, that is, before the critical time of action of ca^+ substance. The results are entirely negative, showing that an ovary does not produce any appreciable amount of ca^+ substance under the conditions of the test.

Summary

A wild-type eye disk transplanted to a claret host before 80 hours after egg-laying (25° C.) gives rise to an eye with pigmentation like that of claret. If the same transplantation is made shortly before puparium formation (about 106 hours after egg-laying), the resulting implant is phenotypically close to wild type. These results are interpreted by assuming that a specific diffusible substance (ca^+ substance) necessary for wild-type eye colour, moves from the body to the eye in a wild-type fly during some period between 80 and 106 hours after egg-laying, and that transplantation, after this critical time, of an eye from such a fly to a host which cannot supply the substance, does not modify the normal course of pigment development.

The relation of this critical time to experiments previously published is considered.

Tests for the production of ca^+ substance by the ovaries of wild-type flies gave negative results.

References

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