GENETICS AND CYTOLOGY OF *DROSOPHILA SUBOBSCURA*

III. TRANSPLANTATION OF EYE-BUDS BETWEEN *DROSOPHILA SUBOBSCURA* AND *DROSOPHILA MELANOGASTER*

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There are in *Drosophila subobscura* four bright red eye-colour mutants, *vermilion* (chromosome I, sex-linked), *scarlet* (chr. II), *poppy* (chr. IV), and *cherry* (chr. V). An attempt is being made to homologize some of these, and certain other genes, with genes in *D. melanogaster*, where the phenotypically similar genes, *vermilion, cinnabar, cardinal* and *scarlet* do not behave identically in transplantation experiments.

As the method depends on the work of Ephrussi & Beadle, the points in their results with *D. melanogaster* relevant to this work will be briefly summarized (see Table 1).

Implants from larvae of most eye-colour mutants are autonomous with respect to their colour developed in wild-type hosts. But implants from vermilion and cinnabar develop the browner colour of the wild-type host's eye. A genetically vermilion eye also develops the wild-type colour in a cinnabar host, though cinnabar in vermilion is autonomous. Ephrussi & Beadle therefore suggested that two diffusible substances essential to the development of the brown pigment were supplied by the wild-type host. The vermilion mutant lacks one, the v^+ substance, which has since been shown to be kynurenine, and cinnabar lacks the cn^+ substance. The behaviour of the reciprocal transplants between the two mutants suggests that the v^+ substance is the precursor of the cn^+ substance. Scarlet and cardinal also lack the brown pigment in the eye, but they are autonomous in wild-type hosts. As hosts, however, they can supply v^+ and cn^+ substances to implants, and are believed to lack the 'brown pigment substrate' in the eye (Beadle & Ephrussi, 1936; Beadle, 1937; Ephrussi, 1942*a*, 1942*b*).

These results provide a basis for a search for homologues of cinnabar and vermilion in other species. If we can find evidence from eye-bud implantations to show that eye colours in another species are due to the absence of those diffusible substances which are lacking in vermilion and cinnabar *D. melanogaster*, we can assume at least that the genes responsible are concerned with the same physiological process; whether they are chemically identical and originated in parallel mutations is less certain, and it is not possible to be sure that they cause the lack of the substances in precisely similar ways.

Eye mutants lacking the cn^+ substance occur in a variety of organisms. In the silkworm, *Bombyx mori*, white eye appears to correspond physiologically to cinnabar, and brown-2*k* to vermilion of *D. melanogaster* (Kikkawa, 1941). The allelomorphs ivory and orange in *Habrobracon* show a similar relationship to cinnabar (Beadle, Anderson & Maxwell, 1938). And the red-eyed *Ephestia* differs from the black in lacking kynurenine (Butenandt, Weidel & Becker, 1940). But whereas genotypically cinnabar brown flies in *Drosophila melanogaster* are changed towards normal by feeding with wild-type extracts, ivory and orange *Habrobracon* can maintain their eye colours as normally cultured, i.e. on wild-type *Ephestia* (Beadle *et al.* 1938). Caspari (1946) has shown recently that there is an increase in the tryptophane content of red-eyed *Ephestia*, as compared with the wild type. He found no such difference between brown and brown vermilion *Drosophila*

melanogaster, though tryptophane is believed to be a near precursor of kynurenine. Becker (1939) considers that the ommatins, which are responsible for the pigmentation of *Drosophila* eyes, are closely related to the eye pigments of *Calliphora* but very different from those of *Ephestia* and other Lepidoptera. Ephrussi, too (1942*b*), emphasizes that the v^+ substance is not produced by the same organs in all insects studied, and that the pigment systems requiring its intervention are not all the same. One should therefore proceed with caution in claiming that genes are homologous on the basis of implantation work only, though one should not assume that differences such as those cited preclude gene homology in the genera discussed. 'Historically' homologous genes, now working against widely different genetic backgrounds, might be expected to differ in some of their effects quite as much as do the examples given.

Table 1

D. melanogaster			*D. subobscura*			
Implant genotype	Host	Implant phenotype	Implant genotype	Host	Implant phenotype	No. of implants
st	+	*st*	*cd* (*s*)	+(*m*)	+	8
cd	+	*cd*	*cd* (*s*)	+(*s*)	+	2
v	+	+	*cd* (*s*)	*cn* (*m*)	+	1
cn	+	+	*cd* (*s*)	*v* (*m*)	*cd*	3
v	*cn*	+	*s* (*s*)	+(*m*)	*s*	3
cn	*v*	*cn*	*s ma* (*s*)	+(*m*)	*s ma*	6
v	*cd*	+	*s ma* (*s*)	*cn* (*m*)	*s ma*	1
v	*st*	+	*pp* (*s*)	+(*m*)	*pp*	5
cn	*cd*	+	*pp* (*s*)	*v* (*m*)	*pp*	1
cn	*st*	+	*pp* (*s*)	*cn* (*m*)	*pp*	2
bri	+	+?	*pp ma* (*s*)	+(*m*)	tomato	5
+	*ca*	*ca*	*ch* (*s*)	+(*m*)	*ch*	3
			+(*s*)	*v* (*m*)	+	2
			v (*m*)	+(*s*)	+	1
			cn (*m*)	+(*s*)	+	1
			+(*s*)	*s ma* (*s*)	+	1
			+(*m*)	*s ma* (*s*)	+	2

Generally accepted scheme for development of brown pigment in *D. melanogaster*:

Tryptophane.....v^+ substance.....cn^+ substance.....brown pigment
(v^+ gene; (kynurenine); cn^+ gene; st^+ and cd^+ genes)

Kynurenine, however, is considered to be widely concerned in eye-pigment formation in the arthropods. It was found by Kotake in the urine of rabbits fed on *l*-tryptophane and is believed to take part in the formation of urochrome; and it has been shown to be produced even by so far removed an organism as a bacterium, fed on a tryptophane diet. It may therefore have been used repeatedly for similar purposes by a variety of organisms.

But in closely related organisms, where attempts are being made to homologize chromosome arms, tentative attempts at gene homology on the basis of implantation work are permissible and may be useful.

The *Drosophila melanogaster* mutant claret, as well as the mutants *v* and *cn*, has peculiar properties which can be exploited, for though itself autonomous in a wild-type host, implants from wild type into claret develop the claret colour. If the larvae are near to pupation, however, the implant may achieve the wild-type colour (Ephrussi & Beadle, 1936).

Gottschewski & Tan (1937) were able to use these properties to show that the eye colours vermilion, orange and claret in *D. pseudoobscura* depended on the absence of the same diffusible substances as vermilion, cinnabar and claret in *melanogaster*.

In our exper ments eye-buds were dissected out of third-instar larvae and implanted into third-instar hosts. The micro-injector was worked by means of the inside mechanism

of an oil-pressure gauge. When the host fly emerged, the implant was dissected out and compared with eyes of the genotype of implant and host. The implants usually develop among the abdominal organs. With the exception of one implant, which developed with the facets well formed, and on the outside, the implants were inside out, with the pigment on the outside, so that the eyes used for comparison were opened to expose the pigment. When this is done, the wild-type eyes of *subobscura* and *melanogaster* are remarkably alike, though the *subobscura* eye looks much darker normally. But the pigment in the eyes of the red mutants is clearly distinguishable from that in the wild type, either by ordinary light, or using a copper sulphate solution filter. In all experiments Ephrussi & Beadle compared implants only with implanted controls available at the same time. In our experiments each implant was compared with the contents of two eyes, one of the host phenotype and one of donor phenotype. This was considered to be a justifiable saving of labour, as the implants from single recessive donors have so far proved to be clearly distinguishable from one or other of these 'comparison eyes', and indistinguishable from the other. Some use is now being made of the mutant maroon as an 'intensifier' of the colour differences between red and wild-type eyes, just as brown and apricot can be used in *D. melanogaster*. These intensifiers remove the red pigment and thus reveal differences in the brown more clearly. The results with scarlet maroon described below justify the choice of maroon for this purpose. But as the double recessive implants are not in all cases identical with one or other eye used for comparison, it will in future be necessary to make some comparisons with control implants available at the same time.

There is in *D. subobscura* a gene hitherto called cardinal, which, because of its sex-linkage and other properties, was suspected to be homologous with vermilion. (The old name will be retained for descriptive purposes to avoid confusion with *melanogaster* vermilion.) Eye-buds from cardinal larvae were therefore injected into vermilion and cinnabar of *melanogaster*, and into wild type of both species. Implants into the two wild types and into cinnabar were indistinguishable from the host eye. Those in the vermilion host were, however, autonomous. It therefore seems that cardinal *subobscura* is homologous with vermilion *melanogaster*, and, like it, differs from the wild type in lacking kynurenine.

Implantations from scarlet *subobscura* larvae to wild-type *melanogaster* have given one implant indistinguishable from scarlet and two almost indistinguishable but slightly more dilute. The results were checked by injection of eye-buds from larvae homozygous for maroon as well as for scarlet. Three implants showed the typical bright yellow of young scarlet maroon eyes, and one, though darker, was nearer in colour to that of an aged scarlet maroon eye than to maroon. This applied also to two implants from scarlet maroon to cinnabar. It therefore seems certain that scarlet eye-buds develop autonomously in both wild-type and cinnabar hosts, and that scarlet is not homologous with cinnabar, though it might be so with scarlet or cardinal *melanogaster*. Scarlet maroon hosts had no effect on wild-type implants of both species, showing that maroon is not homologous with claret.

Implants from poppy and cherry *subobscura* also appear to be autonomous in wild-type *melanogaster*. Poppy maroon implants, however, do not develop the bright yellow colour typical of poppy maroon eyes, but a dilute 'tomato' colour, which is difficult to assess. As the scarlet maroon implant suggests that maroon is autonomous, one is inclined to pin the responsibility to non-autonomy of poppy. The implant was paler and possibly redder

than maroon, and as poppy alone seemed to be autonomous, it does not seem likely that poppy is homologous with cinnabar. It might behave like the mutant bright in *D. melanogaster*, which produces a limited amount of cn^+ substance (Beadle & Ephrussi, 1937) and is therefore not completely autonomous in wild type. Further work with poppy and maroon will be necessary.

Wild-type *subobscura* implants into vermilion *melanogaster* are, as expected, phenotypically wild type, and vermilion and cinnabar implants to wild type 'colour up' to wild type. This shows incidentally that the *subobscura* wild-type host can supply the same v^+ and cn^+ substances that Ephrussi & Beadle postulate in wild-type *melanogaster*. It would, however, be satisfying to supply kynurenine to the various *subobscura* mutants, to see if the cardinal one, and that only, were affected, and it is desirable that brown vermilion *melanogaster* implants should be made into a variety of *subobscura* mutants.

As a result of this work, the name cardinal in *D. subobscura* has been changed to vermilion.

SUMMARY

1. Cardinal in *Drosophila subobscura*, now called vermilion, is homologous with vermilion in *D. melanogaster*.

2. Scarlet and cherry implants are autonomous in wild-type hosts and are not homologous with cinnabar.

3. Poppy implants are autonomous in wild-type hosts, but poppy maroon implants are pinker in wild-type hosts. Poppy may behave like bright in *D. melanogaster*.

4. Wild type is autonomous in maroon. Maroon is therefore not homologous with claret.

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