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THE BAR "LOCUS" AND THE v^+ REACTION IN DROSOPHILA MELANOGASTER

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Beadle and Ephrussi,[2] using the transplantation technique (Ephrussi and Beadle[3]), found that the formation of wild type eye pigmentation is dependent upon the presence of at least two substances, the so-called vermilion+ (v^+) and cinnabar+ (cn^+) substances. It was shown that while vermilion eyed flies were completely lacking in both these substances,[1] wild type flies not only possessed them but could supply them to implanted eyes. Thus, the only source of the v^+ and the cn^+ substances for an eye disc implanted into a vermilion host is the eye disc itself, while an eye disc implanted into a wild type host may secure these substances from the host as well. Beadle and Ephrussi reported that imaginal discs of the Bar eye of *Drosophila melanogaster*, when implanted into vermilion hosts, developed vermilion rather than + pigmentation, whereas imaginal discs of heterozygous Bar eyes developed + pigmentation when implanted into vermilion hosts. They offered further evidence to show that the failure of the homozygous Bar eye to form + pigmentation is not due to its smaller size. Beadle and Ephrussi suggested that the failure of the Bar eye to develop + pigmentation may be due to a retardation of certain reactions in the eye relative to the states of certain developmental reactions in other organs, and that this retardation is due to a recessive effect of Bar.

The experiments reported below are the first of a series designed to test further the above hypothesis in order to investigate this phase of the "Bar reaction." It is hoped that as a result some further light may be shed on the Bar reaction as a whole as well as on the method of pigment formation in the eye.

By means of the Ephrussi and Beadle transplantation technique the imaginal discs of the following Bar "alleles" (with wild type eye color) and their heterozygotes with + were implanted into vermilion hosts (with wild type eye size): B, BB, B^i, B^iB^i and B^4 (Bar, double Bar, infra Bar, double infra Bar and Bar^4, respectively). In addition, eyes containing

B in combination with $m(B)$, a semi-dominant inhibitor of *B* located in the second chromosome (Steinberg unpublished), were implanted into vermilion hosts. Both donors and hosts were larvae about to pupate. All experiments were conducted at 25 ± 1°C. In all except two cases the controls were implants of the same Bar genotype into wild type hosts (normal eye size and pigmentation), and the same Bar genotype in combination with vermilion implanted into vermilion hosts. The two cases in which this was not true are B^iB^i/B^iB^i and $B/B;\ m(B)/m(B)$ (modifiedBar). The controls for B^iB^i/B^iB^i were $BB/+$ into + and vBB/v into vermilion. Those for $B/B;\ m(B)/m(B)$ were $B^iB^i/+$ into + and vB^iB^i/v into vermilion. This was done because the facet numbers of B^iB^i/B^iB^i and $BB/+$ and of $B/B;\ m(B)/m(B)$ and $B^iB^i/+$ are alike or very nearly so. In all cases judgments were based on direct comparison of test and control implants; for example, Bar eyes implanted into vermilion hosts were compared at the same time to Bar eyes implanted into wild type hosts and to vermilion Bar eyes implanted into vermilion hosts (cf. table 1). Inasmuch as neither the sex of the donor nor that of the host had any effect on the phenotype of the implant, sex is not considered in the following report.

Before the data are discussed as a whole, it is necessary to call attention to the pigmentation of the homozygous and heterozygous Bar eyes, when implanted into vermilion hosts. As stated above, Beadle and Ephrussi[2] reported that B/B developed vermilion pigmentation, while $B/+$ developed + pigmentation when implanted into vermilion hosts. Reference to table 1 will show that in our experiments *B* implanted into vermilion hosts was slightly but definitely darker than *vB* implanted into vermilion hosts, and that $B/+$ implanted into vermilion hosts was considerably lighter than $B/+$ implanted into + hosts. Since our experiments were conducted at the same temperature and with larvae of the same age as those used by Beadle and Ephrussi, we are at a loss to account for the differences between our results and theirs. The data in table 1 show that *B* anlagen implanted into vermilion hosts develop some + pigmentation and that $B/+$ implanted into vermilion hosts is intermediate in pigmentation; it follows therefore that the effect of the Bar "locus" on the pigment reaction is not a simple recessive effect as Beadle and Ephrussi suggest.

The facet numbers given in table 1 are, in all except two cases (modified Bar and Bar^4), taken from Sturtevant[4] and are used as a standard to indicate the relative sizes of the eyes of the various donors. The Bar "alleles," when implanted into wild eyed hosts, retain the same size sequence as is shown by the non-implanted eyes. In this respect it is of interest to note that the imaginal discs of the various Bar "alleles," as seen in late third instar larvae, also fall into the same size sequence. The data point to some direct correlation between facet number and depth of pigmentation.

The correlation is, however, subject to two exceptions: 1. $B^iB^i/+$ which is approximately one-half as large as B^i/B^i is not only not lighter than B^i/B^i but is considerably darker, and 2. B^4, which is exceeded in facet number only by $B^i/+$ and +, is lighter than $B^iB^i/+$, $B/+$ and $B/B; m(B)/m(B)$, all of which are less than one-half as large as B^4.

These discrepancies indicate that, although both the reduction in facet number and the partial or complete loss (cf. *BB/BB* table 1) of the ability to form the v^+ substance are due to changes at the Bar "locus," they are probably a result of two separate chains of reactions; i.e., the reaction chain resulting in the reduction in facet number is not the one involved in the pigment effect. If this is true, it should be possible to affect one of the reaction systems without affecting the other. It should be possible, for example, to cause a change in facet number resulting from a given genotype without causing any change in the pigment reaction and vice versa. Experiments are under way to test this hypothesis. An alternative but not as probable an explanation of the data is that B^4/B^4 and $B^iB^i/+$ may involve a qualitatively new situation as compared with the other genotypes, thus producing the observed discrepancy.

TABLE 1

DATA ON THE VARIOUS EYE IMPLANTS

The hosts in all cases were *v*. *v* pigmentation = 1, + pigmentation = 5 on an arbitrary scale. Values in brackets are those reported by Beadle and Ephrussi[1] in comparable experiments. In all except two cases ($B^iB^i/+$ and $B/B; m(B)/m(B)$, see text) the controls were implants of the same Bar genotype into wild type hosts (wild type controls) and the same Bar genotype in combination with vermilion implanted into vermilion hosts (vermilion controls).

IMPLANT		NUMBER OF INDIVIDUALS	PHENOTYPE OF IMPLANT
GENOTYPE	FACET NUMBER*		
BB/BB	25	10	1.0
B^iB^i/B^iB^i	38	12	1.5 –
$BB/+$	45	15	1.5 –
B/B	68	18	1.5 – (1.0)
$B^iB^i/+$	200	14	3.0
$B/B; m(B)/m(B)$	200	9	3.0
B^i/B^i	348	13	2.0
$B/+$	358	7	3.0 (5.0)
B^4/B^4	?	8	2.5
$B^i/+$	716	19	5.0 –
$+/+$	779	13	5.0 –

* See text.

During the course of these experiments nineteen fragmented eyes were recovered from transplanations involving $+^vB^{\text{``alleles''}}$ eye discs implanted into vermilion hosts. Table 2 lists these cases showing the total number of fragmented eyes recovered and the number of these which did not develop as much pigment as did the unfragmented eyes.[5] In no case was a fragmented eye darker than an unfragmented eye. In the cases of $BB/+$ and B/B it is difficult to be certain that the fragments really were not lighter in pigmentation than the entire eyes, since the latter were already very close to vermilion. Consequently, they are not included in the following discussion.

Five of the fragmented eyes failed to develop the same pigmentation as the intact eyes. The fragmented eye recovered in the $B^i/+$ experiment was broken into four pieces. The two largest developed as much pigment as did the unfragmented eyes, the smallest fragment was lightest (vermilion or very close to vermilion), and the fourth fragment was intermediate in both size and pigmentation. Of the two fragmented eyes recovered in the $+/+$ experiment one consisted of six pieces and the other, of four. Nine of these ranged in size from smaller than B to B, all being close to vermilion in pigmentation. One fragment about the size of $B^iB^i/+$ was only slightly lighter than the unfragmented eyes. In the remaining two cases ($B^iB^i/+$ and $B/B; m(B)/m(B)$) the eyes were each broken into two pieces. In both cases the smaller fragment was vermilion. the larger the same as the intact eyes. In all of the above cases the smaller fragments were as light as or lighter than the larger ones.

TABLE 2

DATA ON FRAGMENTED EYES RECOVERED FROM EXPERIMENTS IN WHICH THE DONORS WERE $+vB^{\text{"alleles"}}$ AND THE HOSTS WERE v^5

IMPLANT GENOTYPE	FACET NO.*	PHENOTYPE OF INTACT EYE	NUMBER OF FRAGMENTED EYES	NUMBER FAILING TO DEVELOP SAME PIGMENT AS INTACT EYES*
$BB/+$	45	1.5−	1	0
B/B	68	1.5−	2	0
$B/B;m(B)/m(B)$	200	3.0	1	1
$B^iB^i/+$	200	3.0	4	1
$B/+$	358	3.0	1	0
$B^i/+$	716	5.0−	6	1
$+/+$	779	5.0−	4	2

* See text.

Of the eleven fragmented eyes which developed the same amount of pigment as did the intact eyes, those arising in the $B^iB^i/+$, $B^i/+$ and $+/+$ experiments are of particular interest. Out of a total of fourteen fragmented eyes recovered in these three experiments, four failed to develop the "normal" pigmentation (one in each of the two former, two in the latter). In the ten remaining eyes many of the fragments, which did develop as much pigment as the unfragmented eyes, were as small as or smaller than those which failed to do so. This makes it seem highly probable that the ability or lack of ability of a fragment to form the v^+ substance involves at least two factors, namely, the size of the fragment itself and the portion of the disc from which the fragment arises. Experiments are planned in which both the size and origin of the fragment will be controlled.

Summary.—The various Bar "alleles" and their heterozygotes with + were implanted into vermilion.

There is an apparent but not entirely consistent correlation between facet number and ability to form the v^+ substance, i.e., smaller eyes show less + pigmentation.

It is suggested that the facet reducing reaction system is not the same as the reaction system affecting pigment formation.

Evidence is offered which indicates that the ability of a fragment to form the v^+ substance is a function of the size of the fragment and of the portion of the disc from which the fragment arises.

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[1] Beadle, G..W., *Genetics*, **22,** 587–611 (1937).

[2] Beadle, G. W., and Ephrussi, Boris, *Ibid.*, **21,** 225–247 (1936).

[3] Ephrussi, Boris, and Beadle, G. W., *Amer. Nat.*, **70,** 218–225 (1936).

[4] Sturtevant, A. H., *Genetics*, **10,** 117–147 (1925).

[5] In experiments involving $+^{v}B^{(\text{alleles})}$ eye discs implanted into + hosts 14 fragmented eyes were recovered. In all 14 cases the fragmented eyes developed the same pigmentation as the intact eyes.