THE RELATION OF A DOMINANT EYE COLOR IN DROSOPHILA MELANOGASTER TO THE ASSOCIATED CHROMOSOME REARRANGEMENT

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The possibility that the developmental effects of genes are influenced by their neighbors in the chromosomes has only recently become accessible to experiment. The first demonstration of such an effect was given by STURTEVANT (1925). Two Bar genes in *Drosophila melanogaster*, placed in the same chromosome as a result of unequal crossing over, are more effective than two similar genes present in the two chromosomes of a homozygote. The expression of the Bar gene is dependent upon its position in the chromosome.

It was early evident (STURTEVANT 1925, p. 138) that chromosome rearrangements should provide further data for the statement and solution of the problem. Following a chromosome rearrangement, the genes adjoining the break change neighbors. If the behavior of a gene is a function of its relations to its neighbors, at the points of breakage in chromosome rearrangements, changes resembling mutations should be found. In point of fact, the original "Pale-translocation" was detected by virtue of a change inseparable from the locus of the translocation (BRIDGES 1923, BRIDGES and MORGAN 1923).

With the advent of the X-ray technique for producing chromosome rearrangements on a large scale, the problem was really opened to attack. In the X-ray translocations, MULLER and ALTENBURG (1930) and DOBZHANSKY (1930) have shown that most translocations in *Drosophila melanogaster* contain lethal "mutations" at the point of fracture. And, as is usual in the relation between lethal and non-lethal mutants, where many of the former are found, some of the latter also occur.

A few cases involving known mutants at the breaks have been studied in detail. DOBZHANSKY (1932) analyzed a translocation in which the X chromosome was broken at the Bar locus, the second chromosome at vestigial, and an interchange effected. At the point of translocation, in place of the wild-type section present in the treated chromosome, a new allele of Bar was found. The second mutation from wild-type to Bar, in all the

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intensive work done on Drosophila, occurred at the locus of a chromosome rearrangement. STURTEVANT (1928) has already shown that there is no effective wild-type allele of Bar. The "baroid" mutation can therefore not be a simple deficiency, caused by a loss of material due to irregularity of union of the broken pieces. It is, however, possible to understand these data on the assumption of a position effect similar to that which STURTEVANT had shown to be operative in the case of Bar itself.

This assumption is also useful in another series of cases, involving the bobbed locus in the X chromosome. STERN (1931) had noted that in several spontaneous translocations involving X and Y, when a break occurred at the bobbed locus, a mutation to bobbed was found. SIVERTZEV-DOBZHANSKY and DOBZHANSKY (1933) found in a whole series of duplications for the X chromosome, where one break had occurred in the inert region, that a correlated mutation to bobbed was present.

Yet, as was pointed out in the case of baroid and of the duplications for the X chromosome, although the position effect hypothesis provides a sufficient explanation of the data, there are other possibilities, which might be advanced for individual cases. For example, at breakage points, mutations might be more likely to occur (MULLER and ALTENBURG); or there might be actual losses of genic material during the rearrangement, as BRIDGES supposed for the case of the Pale-translocation. The problem is to distinguish between these alternatives.

The dominant eye colors induced by X-ray treatment in *Drosophila melanogaster*, furnish excellent material for such an analysis. A series of these have been reported by WEINSTEIN (1928), MULLER (1930), VAN ATTA (1932), and GLASS (1932). Most of them belong to the class of "eversporting" mutants, showing a distinct variegation in the eye color. The significant point for our present purposes is MULLER'S observation, upheld by later work, that these dominant eye colors are correlated with chromosome rearrangements.

In this paper, we report a new member of this series, together with a comparative analysis of its behavior and that of another member of the series. From the study of the chromosomal structure of our mutant, we have determined the loci of the breakage points. These determinations we have correlated with a study of the allelism of the dominant effect to known recessive eye colors located at these breakage points. The results of this analysis are precisely those to be expected on the position effect hypothesis, and we have been able in this case to exclude the possibility that only a deficiency at the break may account for these effects.

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THE ORIGIN OF PLUM-2, AND ITS ALLELISM TO PLUM-1

Plum-2 occurred as a single female, in the progeny of an X-ray experiment. Freshly hatched Bar males had been treated with X-rays (50 KV, 5 ma, 15 cm, 60 minutes, 0.8 mm Al filter), and mated to females containing attached X chromosomes homozygous for the recessives vermilion (*v*), sable (*s*), garnet-2 (g^2) and forked (*f*). Among about forty-two thousand offspring of this cross ($v\ s\ g^2\ f$ ♀ ♀, B ♂ ♂), one female showed a pale lemon-yellow eye color with numerous ill-defined spots of a darker yellow, instead of the light orange of the vermilion garnet-2 combination (culture No. 27765, July 6, 1932).

This female was mated to an unrelated wild-type male, and in F_1 the mutant eye color appeared in both males and females (table 1).

TABLE 1

$v\ s\ g^2\ f\ \widehat{XX}\ Pm^2/+$ ♀ × +♂

$v\ s\ g^2 f$ ♀	+♂	$v\ s\ g^2 f\ Pm^2$ ♀	Pm^2 ♂
8	12	6	9

This showed that an autosomal dominant was present. Freed of vermilion garnet-2, the new mutant resembled Plum-1, described by MULLER (1930). The ground color of the eye is brownish, with deeper purplish patches, the general hue then appearing as a brownish purple. The interaction with vermilion garnet-2 produced the pale yellow of the original female and her daughters.

By the usual linkage tests it became apparent that no translocation was present, and that Plum-2 was in the second chromosome. The cross of Plum-2 to the standard second chromosome balancer, the dominant mutant Curly wing with its associated inverted sections permitted a stock of the constitution Plum-2/Curly to be established. In this stock only the heterozygotes appear. Curly is known to be lethal when homozygous in stock cultures, and the failure of the Plum-2 homozygotes to survive is due to a recessive lethal effect in the Plum-2 chromosome.

This behavior of Plum-2, as well as its appearance, was so similar to MULLER's Plum-1 that a cross was made between the two balanced stocks, Plum-1/Curly and Plum-2/Curly. Only Plum Curly flies appeared in F_1, indicating that Plum-1/Plum-2 is a lethal combination. Since there is no likelihood of the presence of any miscellaneous lethal common to both chromosomes, this demonstrates the allelism of Plum-1 and Plum-2.

PLUM-2 ASSOCIATED WITH AN INVERSION IN THE RIGHT LIMB OF CHROMOSOME II

Three different sets of experiments were carried out, which sufficed to show in some detail what changes from the normal second chromosome are present in Plum-2.

In the first experiment, it became apparent that Plum-2 involves an inversion in the second chromosome. Crossing over was studied in heterozygous females of the composition Plum-2 (*Pm*²)/aristaless (*al*) Bristle (*Bl*) curved (*c*) speck (*sp*) (table 2). In this way, a survey of the crossing

TABLE 2

*Pm*²? *Pm*²?
al (1) *Bl* (2) (3) *c* (4) (5) *sp* ♀ ♀ × *al c sp* ♂ ♂

0	*Pm*²	1258	5	*al Bl c*	1
0	*al Bl c sp*	1240	1, 2	*al c sp*	1
1	*al Pm*²	955	3, 4	*al Bl sp*	10
1	*Bl c sp*	1058		*Pm*² *c*	11
2	*al Bl Pm*²	1	1, 3, 4	*al Pm*² *c*	2
2	*c sp*	1	1, 3, 4	*Bl sp*	1

TOTAL 4539

over throughout the chromosome was obtained. The results of the cross appear in table 2. It is clear at a glance that crossing over in the right limb of the chromosome is markedly suppressed. The percentages for the various intervals follow:

al-Bl	*Bl-Pm*²	*Pm*²*-c*	*c-Pm*²	*Pm*²*-sp*
44.43	0.07	0.53	0.53	0.02

These are to be compared with map values:

al-Bl	*Bl-c*	*c-sp*
54.7	20.8	31.5

The discrepancy with the map value in the left limb is due to undetected double crossing over. In the right limb, however, not only is the total amount of crossing over reduced, but the crossovers occurring in the mid-portion are all doubles. Between Plum-2 and speck, single crossovers are again found. The data may be explained simply on the basis of an inverted section of the right limb of the second chromosome, not including the end. Within an inverted section, only double crossovers are found; outside it single crossovers occur, but with reduced frequency (see STURTEVANT 1931). From this interpretation, it follows that Plum-2 must have its locus close to the ends of the inversion, since none of the rather frequent doubles between the inversion and the normal chromosome involved Plum-2.

The second experiment served to confirm this analysis. A more completely marked chromosome was used opposite the Plum-2 inversion. Crossing over was studied in females heterozygous for Bristle Plum-2, and the multiple stock carrying the recessives aristaless (*al*), dumpy (*dp*), black (*b*), purple (*pr*) curved (*c*), plexus (*px*), and speck (*sp*). These data are shown in table 3, and the computed percentages in table 4. Plum-2 and

purple, eye colors which on occasion are difficult to distinguish, were disregarded in these counts.

TABLE 3

(1) (2) (3) (4) (5) (6)

al dp b pr c px sp ♀ ♀ × *al dp b pr c px sp* ♂♂

*Bl Pm*²? *Pm*²?

(*Pm*² and *pr* disregarded)

0	*Bl*	396	1, 3	*dp b Bl*	6
0	*al dp b pr c px sp*	358	1, 3	*al c px sp*	6
1	*al Bl*	88	2, 3	*b Bl*	8
1	*dp b c px sp*	77	2, 3	*al dp c px sp*	7
2	*al dp Bl*	194	4, 5	*Bl c*	3
2	*b c px sp*	244	4, 5	*al dp b bx sp*	3
3	*al dp b Bl*	49	4, 6	*Bl c px*	2
3	*c px sp*	47	4, 6	*al dp b sp*	2
6	*al dp b c px*	1	1, 4, 5	*dp b px sp*	1
6	*Bl sp*	1	2, 4, 5	*b px sp*	2
1, 2	*dp Bl*	5	2, 4, 5	*al dp Bl c*	2
1, 2	*al b c px sp*	8			

TOTAL 1510

TABLE 4

Crossover values computed from the data of table 3, compared with the map values.

	Data of Table 3	Map Value (BRIDGES)	Difference
al-dp	12.7	13.0	− 0.3
dp-b	31.1	35.5	− 4.4
b-Bl	8.2	6.5	+ 1.7
Bl-c	1.0	20.5	−19.5
c-px	0.7	25.0	−24.3
px-sp	0.4	6.5	− 6.1

As in the first experiment, the values for the left limb are near normal; and in the right limb, crossing over is suppressed. From these data, a further estimate may be made of the region involved in the inverted section, and hence of the loci of the points of breakage in the chromosome. Single crossovers occur between Bristle and Plum-2, giving a point not far to the right of Bristle as the locus of one of the breaks. Only double crossovers are found involving curved and plexus, which implies that these genes lie within the inverted section. Single crossovers between plexus and speck are again found, indicating that the locus of the second break is between plexus and speck. Four double crossovers involving the region between plexus and the end of the inversion were found in 1510 flies; two single crossovers occurred in this experiment between the end of the inversion and speck. It appears that the distal end of the inversion is closer to speck than to plexus. The locus of the right break in the chromosome is two-thirds of the distance from plexus to speck, or at the locus of brown (104.5) in the second chromosome.

A third experiment was carried out to locate the proximal end more exactly. Crossing over was studied in females heterozygous for Bristle Plum-2 and cinnabar (*cn*) whose locus is at 57.5, 2.8 units to the right of Bristle. The results are shown in table 5.

TABLE 5

$$\frac{Bl \quad Pm^2}{(1) \quad (2)\ cn} \text{♀ ♀} \times cn \text{♂ ♂}$$

(0)		(1)		(2)		
Bl Pm²	*cn*	*Bl cn*	*Pm*	*Bl Pm cn*	+	TOTAL
2458	2675	4	10	8	8	5163

The crossovers between Plum-2 and cinnabar engage our attention. If cinnabar lies within the inverted section, these are all double crossovers. Then the *Bl Pm² cn* flies carry the inversion, and the contrary crossover class (wild-type) is free of it. Conversely, if cinnabar lies to the left of the inversion, these are single crossovers, and the wild-type flies carry the inversion, the cinnabar flies do not. Tests were made of four crossovers of each type, in which the crossing over was observed in heterozygotes for *al dp b pr c px sp*. It was found that the *Bl Pm² cn* chromosomes carried the inverted section, and the wild-type chromosome did not. The proximal break in the inverted section lies therefore between Bristle and cinnabar, close to the spindle attachment.

Since the proximal break of the $C_{IIR}\ cy$ inversion is close to cinnabar (GRAUBARD 1932) it seemed desirable to make a test of crossing over in the heterozygote for Plum-2 and Curly. If the inverted sections in the two chromosomes correspond, crossing over should increase from the low value observed between either inverted section and the normal chromosome, to an approximately normal amount (STURTEVANT 1931, GRAUBARD 1932). Crossing over was therefore studied in females of the constitution aristaless Curly $C_{IIL}\ cy\ C_{IIR}\ cy$ Lobe^c speck/Plum-2 curved. The data are presented in table 6.

TABLE 6

$$\frac{al \quad Cy\ C_{IIL}\ cy \quad C_{IIR}\ cy\ L^c \quad sp}{(1) \quad (2)\ Pm^2? \ (3) \quad (4)\ c\ (5)\ Pm^2? \ (6)} \text{♀ ♀} \times al\ b\ c\ sp \text{ ♂ ♂}$$

0	*Pm c*	2041	3, 4	*al Cy sp*	4
0	*al Cy L^c sp*	1933	3, 5	*al Cy c sp*	4
2	*al Cy Pm² c*	16	3, 5	*Pm² L^c*	5
2	*L sp*	14	4, 5	*al Cy L^c c sp*	2
6	*Pm² c sp*	1	4, 5	*Pm²*	3
3, 4	*Pm² L c*	1	2, 4, 5	*L c sp*	2

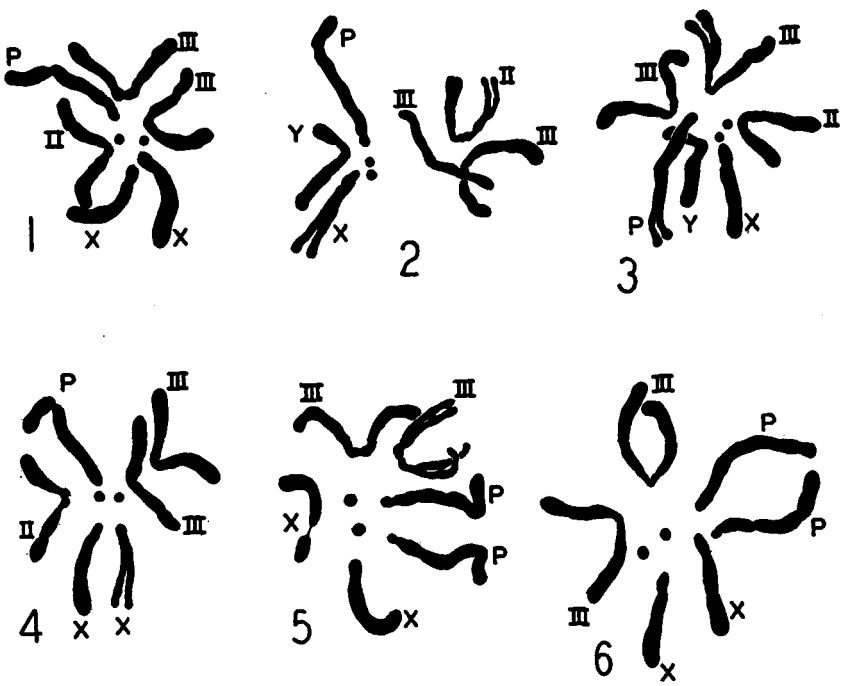
TOTAL 4026

From these data it appears that the two inverted sections do not correspond. The crossover percentages are of the same order of magnitude as

those observed for the right limb in heterozygotes for Plum and a normal chromosome. They are, from the data in table 6:

al-Cy	*Cy-Pm*^{2}	*Pm*^{2}*-L*^{c}	*L*^{c}*-c*	*c-Pm*^{2}	*Pm*^{2}*-sp*
0	0.79	0.34	0.29	0.17	0.02

The total crossing over is 1.61 percent, which is to be compared with a total of 2.1 percent for the right limb in table 4, and 0.50 percent in heterozygotes for the Curly and a normal chromosome (GRAUBARD 1932). The value is intermediate between those observed with the respective heterozygotes for the normal chromosomes.



FIGURES 1–6.—The chromosomes of Plum-2. Metaphase plates of nerve cells. Figures 1–4, heterozygotes; 5–6, homozygotes. The Plum-2 chromosome is designated by the letter "P"; II is the normal second chromosome; III, the third; and X and Y respectively the X and Y chromosomes. Magnification about 6500×.

THE CYTOLOGICAL DETERMINATION OF THE PLUM-2 INVERSION

The genetic data given in the previous section admit of several possible interpretations, equally compatible with the necessary assumption of two breakage points, one between Bristle and cinnabar, the other around the locus of brown. To decide between these, a cytological study was made. The nerve ganglia of full grown larvae from the balanced stock Plum-2 /*Cy* C_{IIL} C_{IIR} were fixed in Navashin's fluid, and paraffin sections prepared which were stained in gentian violet by the usual technique. Only

two types of full grown larvae appear in this stock—Plum-2/*Cy*, and homozygous Plum-2. The homozygotes for Curly usually die in the mid-larval stages, according to the data of SIVERTZEV-DOBZHANSKY (1927).

Figures 1–4 show the heterozygotes, Plum-2/*Cy*. These have one second chromosome with a median spindle fiber attachment. The other is a long rod-shaped chromosome, which must be the Plum-2 chromosome. In figures 5 and 6 the homozygous Plum-2 chromosome plates are shown. These have two of the long rods, and no second chromosome with a me-

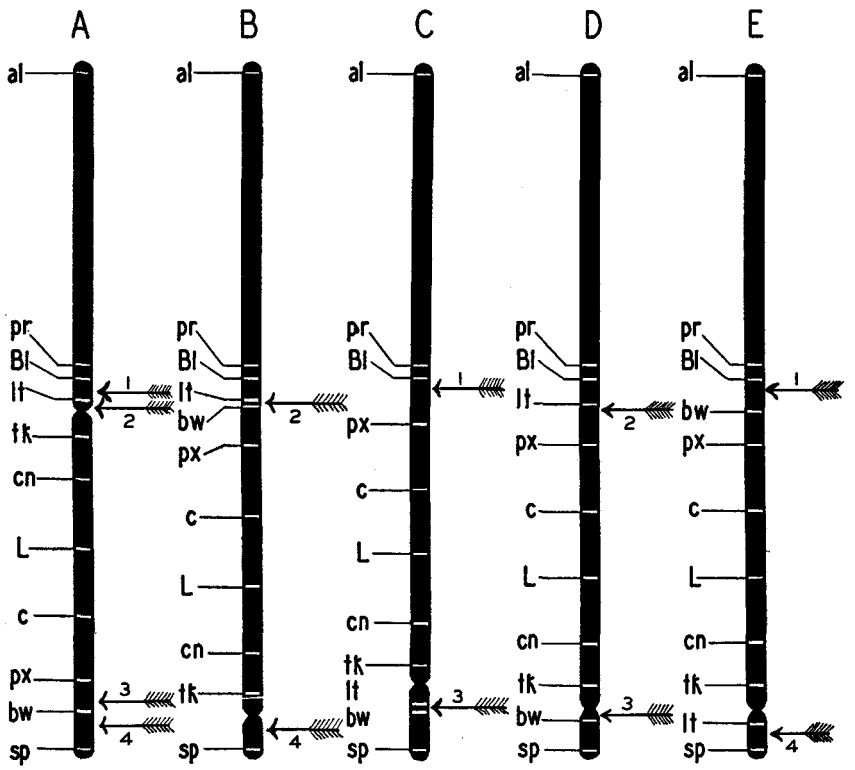


FIGURE 7.—*A*, the structure of the normal second chromosome. *B*, *C*, *D*, and *E*, the various possibilities for the chromosome structure of Plum-2. The spindle attachment is indicated by a constriction, the loci of breakage by arrows.

dian attachment. In the rod-shaped chromosome, the spindle attachment is sub-terminal. The constriction at this place is seen plainly in figure 2, in one of the chromosomes of figure 6 and in both in figure 5. It follows that the break between Bristle and cinnabar occurred to the left of the spindle attachment. Since crossing over in the left limb of the Plum-2 chromosome is normal, this chromosome must have one of the structures represented in figure 7.

A comparison of the topography of the Plum-2 chromosome with that of

the dominant eye colors previously studied presents some points of interest. The "Dilute" series described by VAN ATTA (1932) is very probably homologous with the series of alleles of Plum-1 described by GLASS (1932 and in the press). In these two series, there are four mutants which are associated with inversions. Plum-1 contains two inversions, and Van ATTA's Dilutes 1 and 2 may be interpreted similarly. The remaining member of GLASS's series has not as yet been fully described; the brief account available implies that it contains, as does Plum-2, only one inversion. This gives three out of five cases, all selected on the basis of the eye color alone, in which a double inversion has occurred. It would seem then that a correlation may exist between the formation of an inversion in one limb of chromosome II and in the other. The data are not sufficient at present to be decisive, but it should be remembered that STURTEVANT (1931) has noted a correlation of this kind in the inversions of spontaneous origin which he has studied. The data on the Plum inversions support his interpretation, that the correlation is concerned with the simultaneous origin of the double inversions.

For our present purposes, the most important point is that in all these cases, the dominant eye color is inseparable from the inversion in the right limb; which means that it has its locus at the breakage points (either or both). The rearrangement of chromosomal material at a specific locus is correlated with the appearance of a "mutation" at that locus. It is possible, by a closer analysis of the properties of the "mutation," to obtain a certain measure of information as to its nature.

CHANGES AT THE RIGHT BREAK OF THE INVERSION

It will be recalled that in the case of Plum-2, the right break was shown to have taken place between plexus and speck, at the locus of brown. The work on Plum-1 and its alleles had sufficiently established the loci involved in the changes in this region, for those cases. BRIDGES discovered the suppression of Plum-1 by a duplicating section for the plexus-speck interval (MORGAN, BRIDGES and SCHULTZ 1930). This was extended to the other members of the series by VAN ATTA (1932) and by GLASS (1932) who obtained similar suppressions in all cases. GLASS has added the significant fact that his mutants are all allelic to the recessive eye color brown, located in this region at 104.5.

Further information comes from unpublished work of Dr. BRIDGES. He has made a systematic test of Plum-1 for deficiency of the other mutants available in the plexus-speck region. The heterozygotes with Plum-1 are normal in all cases except those of brown, minus, and abbreviated. The two latter are recessive bristle characters, located respectively at 104.5, and 105.0. Their heterozygotes with Plum-1 show frequently small patches

—mainly single bristles—manifesting the mutant character. This represents a variegation similar to that observed in the cases of mottled eye reported at the white locus (MULLER 1930, PATTERSON 1932). It would appear that in Plum-1 a section of chromosome is involved in the variegation; however, further study of the problem is necessary. BRIDGES has also shown that the lethal effect of homozygous Plum-1 lies outside the plexus-speck region; the homozygote does not survive in the presence of the duplicating segment for this region. In its essentials, the above might serve as a description of the behavior of Plum-2 in these respects.

For the suppression of Plum-2, the duplicating fragment of BRIDGES' "Pale-translocation" was utilized. Crosses of the stock Pale deficiency/Curly Pale duplication/Stubble $C_{IIIR\ Mo}$ with the Plum-2/Curly stock gave individuals of the constitution Plum-2/Curly, Pale duplication. These possess in triplicate the plexus-speck region. They are all, like Plum-1 and its fellows, almost wild-type in appearance, instead of the mottled brownish color of Plum-2. Subsequently, a stock of Plum-2/Curly Stubble $C_{IIIR\ Mo}$/Pale duplication was established by Dr. BRIDGES. As he points out, the suppression of Plum-2 is even more complete than that of Plum-1. The remaining brown facets of the suppressed Plum-1 fly are rarely to be found in the case of Plum-2. The eye is usually completely wild-type. It is clear that the dominant effect is a function of some locus in the plexus-speck region.

The tests with mutants in this region make it highly probable that this locus is brown. The compounds of Plum-2 with brown show an almost homogeneous brown eye color, which darkens in older flies until an ill-defined mottling is discernible. Plum-2, like Plum-1 and the other members of the group, shows in combination with brown, the disproportionate modification characteristic of alleles. There is in addition, another criterion than eye color available in the case of brown. The testis sheath, instead of being bright yellow, as it is in the wild-type, is completely transparent in flies homozygous for this mutant gene. Plum-2 (and Plum-1 as well) has the normal testis color, in the heterozygotes for a wild-type chromosome. The compounds with brown show in all cases a variegated testis, with patches of yellow and white. There is evidently a frequent "mutation" at the brown locus; the mosaic character of the testis sheath confirms the evidence from the eye color relations in a striking fashion.

In the tests with the other mutants in this region (minus, abbreviated, speck) definite results were obtained for Plum-2 only with minus. Heterozygotes for Plum-2 and minus frequently show patches of minus bristles. These are very short stumps, and so are clearly distinguishable from the wild-type. The results in this case are completely parallel to those of Plum-1. In the case of abbreviated, which BRIDGES found to be involved

in the mosaicism in Plum-1, our evidence is not clear. No certain mosaic patches were found in several hundred individuals examined. But abbreviated is occasionally capricious in its manifestation, and in spite of the fact that the tests were made with two different stocks, further study is necessary.

The lethal effect in homozygous Plum-2, like that in Plum-1, was found to lie outside the plexus-speck region. In the case of Plum-1, this is by no means surprising, since two inversions (four breakage points) are concerned, and there may even be more than one lethal. The situation with Plum-2 is somewhat different. The homozygotes survive to a late stage—in certain stocks occasional individuals even hatch—and thus, since only one inversion is involved, it might be supposed that were any recessive locus in the brown region concerned with the lethal effect, a duplicating section for that region should suppress it and permit survival. The experiment performed was as follows: Plum-2/Curly, Pale duplication/Stubble $C_{IIIR\ Mo}$ females were crossed to Plum-2/Curly males. The cross gave:

Pm Cy Sb	*Cy*	Crossovers	Homozygous Curly classes
261	234	30	44

The homozygous Plum individuals should appear in the not Curly classes. These are completely absent, both for the Stubble classes, which do not contain the duplication, and the opposite classes which do. It follows that the lethal effect in Plum-2 lies, as previously stated, outside the plexus-speck region.

PLUM NOT A SIMPLE DEFICIENCY FOR BROWN

The situation may then be epitomized thus: the occurrence of a dominant effect is associated with a breakage at the locus of brown. This breakage is also associated with instability at the brown locus and at neighboring loci. The first statement holds for Plum-2, for all the mutants studied by GLASS, and probably for VAN ATTA'S Dilutes. The second can only be stated definitely for Plum-1 and Plum-2.

The question may be asked, cannot these data be explained on the basis of an unstable chromosome rearrangement of the type described by PATTERSON and PAINTER? The mutant portions would then be interpreted as deficient for the normal alleles of the loci involved. But it does not seem obvious at the moment how the dominant effect can be placed in such a category. Consider the flies of constitution Plum/+ Pale duplication/+. These still show brown patches, although small ones. Now were this effect due simply to a deficiency, such brown patches should not appear, since the normal complement of alleles is present at the brown locus.

Further evidence comes from studies of the behavior of Plum-1 in triploids. Wild-type triploids were crossed to males heterozygous for aristaless dachs Bristle curved speck/dumpy Plum-1. The results were:

2N		3N		Intersexes	
Bl	*Pm*	*Bl*	supp. *Pm*	+	*Bl*
68	59	6	12	43	32

The triploids of constitution Plum-1/+/+, like the diploids with the duplication, have small brown patches in an eye with a red ground color.

In the triploid there is available for comparison evidence as to the eye color of a known brown deficiency. The deficiency corresponding to Pale duplication is lethal in the diploid (BRIDGES and MORGAN 1923, LI 1927). In the triploid, however, it survives. The eye color of such a triploid (Pale deficiency/+/+) is practically normal; there is a slight dullness, but nothing comparable to the brown patches of the Plum-1/+/+ triploid. A simple deficiency of the brown locus does not exhibit the characteristics of Plum. It is possible, by making certain arbitrary assumptions with regard to genic balance, to consider Plum as a deficiency for a definite region having this effect. This is not in accord with our present knowledge of the behavior of deficiencies; but even this possibility can be excluded by procuring short deficiencies for the brown locus.

It is simpler at present to think of the change at the brown locus as a "mutation" of some sort. This is seen to be correlated with a change in chromosome structure. But the rearrangement involved another break in the chromosome. Does a similar situation exist in that case? Are "mutations" to be found in this region?

THE CHANGES AT THE LEFT BREAK OF THE INVERSION

It will be remembered that the left break of the Plum-2 inversion is between the locus of Bristle (54.7) and the spindle attachment. For this region a procedure similar to that adopted for the plexus-speck region was carried out. Not only were tests made with the mutants in the region, but there was also available a viable duplication. This is obtained from the II-Y translocation G, studied by RHOADES (1931), and includes the loci hooked (*hk*, 53.9), purple (*pr* 54.5), and light (*lt*, 55.0). Experiments were made with Plum-1 as well as with Plum-2.

Tests for allelism with the following genes in this region were carried out: hooked, purple, Bristle (recessive effects), light, rolled, thick, yellow bristle, and apterous. Of these, only light has its locus between Bristle and the spindle attachment—at the place of breakage in the Plum chromosome. Only light, an orange eye color, shows any signs of an allelic relation; the other tests were all negative, even as regards variegation.

The Plum-light compound is strikingly different from that of Plum with brown. In that case the variegation became almost imperceptible; but with light the mosaic character of the eye becomes more pronounced. On a light tan, almost yellow, ground color, extensive and distinct brown patches are spread. This difference from the brown compound made it necessary to examine the case more critically—a procedure called for in any event by its unusual, although predictable character.

Accordingly, heterozygotes for Plum-2 and a large number of different eye colors were observed. The types used were: carnation, cinnabar, claret, maroon, prune, purple, purploid, scarlet; combinations: purple cinnabar, scarlet claret, and vermilion garnet-2. Of all these, only the heterozygote of Plum-2 with vermilion garnet showed even a slight dilution. The relation of Plum to light remains unique.

There is one other specific relationship found among the eye colors of Drosophila, besides that of allelism. Certain eye colors, normally recessive, show visible effects in the heterozygous condition, when the fly is at the same time homozygous for other specific recessives (MORGAN and BRIDGES 1913, CREW and LAMY 1933, SCHULTZ unpublished). It was then necessary to determine the effect of heterozygous light in the presence of homozygous brown—which might be considered as equivalent to Plum. This was done, and served to exclude the possibility of such a conditioned dominance completely. The combination light brown/brown is practically normal brown; the effect of light upon Plum must therefore be regarded as a case of allelism.

It is then interesting to consider more closely the mosaic nature of the Plum/light eye. The ground color is very similar to the color of the light brown combination. This is a pale yellow, which in old age takes on a pinkish cast. The dilute areas in the Plum/light compound are somewhat darker, but this may be due simply to the effect of the neighboring brown areas. In the double compound Plum/light brown, the whole eye may sometimes look exactly like light brown, except for a few apricot-colored patches. The testis color of light is too close to that of Plum to be of value for the present problem.

In the Plum/light compound, the dilute areas may be interpreted as portions of the eye in which a mutation to light has taken place. This in combination with the dominant effect appears "light brown." The remaining dark areas would then be considered to show only the dominant effect of Plum. If this interpretation be accepted, then the Plum/light brown compound, in which the majority of the ommatidia have the color characteristic of the light brown combination, may indicate that the variegation for light and that for brown are correlated. However, the high frequency of the mutation to brown (Plum/brown is practically homogeneous)

makes it difficult to ascertain this, and special experiments must be devised to distinguish between a spurious association due to a high mutation frequency, and a real correlation. The important point at the present juncture is the indication that the light locus also undergoes variegation.

This can be shown in another way. The chief difficulty in the interpretation of the compounds of light with Plum arises from the dominant effect of Plum. But the dominant effect can be suppressed by the Pale duplication or in triploids, and the behavior of light can then be studied by itself. Therefore flies of the constitution Plum (1 or 2)/lt, Pale duplication were synthesized from crosses of Plum/Curly, Pale duplication/Stubble by light individuals. Instead of the wild-type color of the normal suppressed Plum fly, these showed a distinct purplish ground color, with darker purplish patches, resembling in their distribution those of the Plum/light compound. The variegation at the light locus appears to be a real phenomenon, not dependent on an interaction of a stable light mutation with the unstable brown locus. Results from observations of triploids of the constitution Plum/light/light confirm this. These show also a coarse mottling, and resemble the compounds, with the Pale duplication, just described.

The question again presents itself; can these patches result from a simple deficiency, for the locus of light? This appears unlikely, for several reasons. In the first place, a deficiency involving little more than the locus of light is already known (MORGAN, BRIDGES and SCHULTZ 1931) to have different properties than those observed in the case of Plum. Its compound with light is not as in the Plum/light Pale duplication flies, a purplish color, but closely resembles that of light itself. Moreover, the deficiency is an extreme Minute—which is not an insuperable difficulty, but deserves consideration. Secondly, unless it be postulated that the light locus does not produce its developmental effects autonomously, but is subject to the influence of neighboring tissues—for which there is no evidence at present—the purple, rather than wild-type color of the darker patches remains unaccounted for. Some phenomenon other than simple deficiency must be invoked to explain the results. They are, indeed, easily accounted for either as "mutation" in the orthodox sense or as due to the change brought about by the chromosome rearrangement—a position effect.

There remains the recessive lethal effect of Plum to be placed in its proper setting. It will be remembered that the presence of a duplication for the brown locus was of no avail. The homozygotes for Plum-2 die in very late pupal stages, just before hatching, both in the presence of the duplication and without it. Their eye color and general morphology can be made out on dissection, however, and in certain stocks of Dr. BRIDGES, occasional homozygotes even survive, affording a check of the dissections. Individuals containing Plum-2 in homozygous condition have a pale yellow

eye color, with occasional apricot patches. This is exactly as would be expected from the behavior of Plum-2 with light and brown. The homozygote should resemble, as it does, the compound Plum-2/light brown. In other respects these individuals (even the survivors) are decidedly abnormal. The wings are somewhat broad at the base and flimsy. In a number of cases dissected out of the puparium, patches devoid of ommatidia were found in the eye, and weakly chitinized, blackened places at the joints of the legs.

Does this recessive lethal effect bear any relation to the left break of the inversion? If it does, not one but two "mutations"—possibly on either side of the break—will have been shown to lie here. By the use of the duplication previously mentioned for the loci hooked purple and light, it was in fact possible to obtain viable homozygotes for Plum-2, and establish a stock thereof.

Duplication G is attached to a Y chromosome. Accordingly, males were prepared which carried the Y chromosome with duplication G, and in their second chromosomes Bristle Plum-2 cinnabar/Curly. These were mated to Plum-2/Curly females. The results were:

Plum-2	Curly	*Bl Pm*^{2}	*Cy cn*	*Bl Pm*^{2}
♀ ♀	♂ ♂	♀ ♀	♂ ♂	♂ ♂
32	35	54	25	33

The last named class is homozygous Plum-2 since it is not Curly. The appearance of this class only in the males means that duplication G is the responsible factor in their survival. The locus of the recessive lethal effect in Plum-2 is at the left break of the inversion.

Comparison of these homozygotes with those which occasionally survive without the duplication is interesting. They are practically, normal in viability, except for the slight decrease due to the duplication and the homozygous Plum-2 duplication G stock is easily kept. Their eyes are brown in color, similar to the Plum/brown compound; the effect of light is completely suppressed by the duplication. A similar phenomenon is observed in flies of the composition Plum/light brown, duplication G. These also are clear brown in young flies, with some mottling as age advances. As regards the brown locus, each Plum chromosome behaves to the other as if a mutation to brown were present. This extends to the testis sheath, where the variegation found in the ordinary Plum/brown compound is clearly manifested. The mosaic character is apparently not dependent on heterozygosity for the locus concerned. This was already evident from the presence of patches in the eyes of the homozygote without the duplication. But although the mosaic character persists, none of the abnormalities of the ordinary homozygote appear in the presence of the duplication.

It is clear from the foregoing that at both breaks a complex of changes

has occurred, which cannot readily be interpreted as simple deficiency phenomena. They can be interpreted as resulting from the chromosome rearrangement which is associated with them. An interesting possibility is now raised by the position effect hypothesis. Six alleles of brown have been studied by BRIDGES, one of them possibly a deficiency. None of these shows the dominant effects of the Plum alleles. Are these dominant effects due to a cooperative action of the genes involved in the rearrangement?

THE DOMINANT EFFECT OF PLUM

The obvious method for the analysis of a dominant effect is to consider the mode of its suppression. The data with Pale duplication already have shown that Plum is suppressed by the addition of an extra wild-type allele of brown. It remained possible that a suppression could also be effected by additional allele of light. In that case, the dominant effect would have been due to the interaction of the two loci, neither of which by itself could produce the change.

The data already given for the effect of duplication G on Plum-2 disprove this. All Plum-2 males in that experiment carried the duplication, and were normal Plum-2. A similar experiment was performed with Plum-1. Aristaless dachs Bristle curved speck/dumpy black Plum-1 females were crossed to Curly/translocation G males. In these crosses the translocation appears only in the males, since deficiency G is lethal in heterozygous condition. The Curly males are the class of interest, since these carry the duplication. The results of the cross were:

dp b Cy Pm		*Cy Bl*		*Bl*		*Pm*		Crossovers (mostly minutes)
♀	♂	♀	♂	♀	♂	♀	♂	
89	66	128	49	1	101	0	90	27

There was no suppression of Plum-1 by duplication G, as there was no suppression of Plum-2. It would seem therefore that the dominant effect is concerned solely with the brown locus.

During the course of the work with duplication G, however, it was found that suppression of Plum-1 and Plum-2 could occur even when no additional allele of brown was present. It was found on analysis that the suppression is due to the presence of an extra Y chromosome, an effect similar to that reported by GOWEN and GAY (1933). Not only the dominant eye color, but the instability for minus and light as well are suppressed. And the suppression has been found, by experiments with fragments of the Y, in the presence of only the short arm of this chromosome. These results will be reported separately. They concern us here only in so far as they make it necessary to examine the suppression of Plum by the brown locus more critically.

This is made all the more necessary by a certain parallelism between the effects of Pale duplication and the Y chromosome on the homozygote which contains duplication G. To obtain the former, Plum-2/Curly, Stubble $C_{IIIR\ MO}$/Pale duplication were crossed to homozygous Plum-2 males carrying duplication G. The results were:

Cy Pm Sb	*Cy*	*Cy Pm*	*Pm Sb*	+	*Pm·Sb*
♀ ♀ ♂ ♂	♀ ♀ ♂ ♂	♀ ♂	♀ ♂	♀ ♂	$\overline{Pm}$ ♀
48 48	56 39	1 0	1 56	0 44	2

The not-Curly classes are of interest. The two females did not contain the duplication, and were the extreme homozygous type already described. The "*Pm Sb*" class was brown-eyed, typical homozygous Plum-2 with duplication G. The wild-type males carry in addition the Pale duplication, whose effect on the homozygote is precisely the same as its effect on the heterozygote. It may be remarked, that if Plum could still be considered a simple deficiency for brown, this result would completely exclude the possibility. For the addition of a duplication to the homozygote restores the ratio of alleles characteristic of the heterozygote, and these flies should be Plum. They are, however, as completely suppressed Plum as is the heterozygote. This in itself is not disturbing, as far as the mutation or position effect interpretation is concerned, and can easily be accounted for on these hypotheses.

But homozygous Plum females containing duplication G are also suppressed Plum; this is a constant feature of the stock of homozygous Plum with duplication G. It is, of course, due to the additional Y introduced into the female with the duplication. The suppression is not so complete as that effected by the Pale duplication, but nevertheless makes additional experiments necessary.

The suppression of Plum in single dose in triploids made it possible to pursue the problem further. If the brown locus is really concerned with the dominance, its removal by a deficiency will cause the reappearance of the dominant character, other conditions remaining constant. The material for such an experiment is furnished by the Pale deficiency discussed previously, which is viable in triploids as a fly with somewhat narrowed wings and slight body build. Accordingly, triploids were obtained which contained Plum-1 in single dose. Some already contained Stubble, disregarded in the counts, except for the Pale deficiency flies, which necessarily contain it. These were then crossed to males of the constitution Pale deficiency/Plexate, Pale duplication/Stubble $C_{IIIR\ MO}$. The Plexate character is completely suppressed in triploids, so that in the progeny of this cross, there are only wild-type and Pale deficiency triploids to be distinguished in re-

gard to their behavior with Plum. The disjunction of Plum-1 in the triploid is not random; most of the diploid gametes from a Plum-1 triploid contain the Plum-1 chromosome. Individuals of the desired constitution—Plum-1/Pale deficiency/+ were thus easily obtained. The results of the cross were:

	Intersexes	3*N*		Pale def.	3*N*	
2*N*	+	Supp. *Pm*[1]	+?	*Pm Sb*	*Sb*	Super ♂
210	64	32	25	15	3	5

The distinction between wild-type and Plum in triploids is not easily made. Probably most of the apparent wild-type triploids are really Plum, with the additional suppression of a Pale duplication. But the triploids carrying Pale deficiency and Plum-1 manifest Plum-1 to even a greater extent than does the diploid heterozygote. The ground color is somewhat lighter and the patches more frequent. The Pale deficiency triploids without Plum in this cross as in many others were completely normal in eye color. The loss of the extra brown locus restores the dominant effect of Plum-1. This result is consistent with the assumption that brown is the locus concerned in the dominant effect. It finds corroborative support in certain observations of triploids of the constitution Plum/brown/+. These, instead of the normal suppression of Plum in the triploid, approach the diploid heterozygote in appearance. Further data of this type are desirable, however, although the result already seems clear. The presence of a mutant brown allele reinforces the action of Plum. All in all, the evidence favors the view that the dominant effect in Plum is a specific property of the brown locus.

This is further borne out by the negative results of the experiments with deficiency G for the light locus, which also survives in triploids. Triploid females containing Plum-1 in single dose were crossed to Curly/translocation G males. All not-Curly individuals which receive no Y from the father carry the deficiency. The results were:

2*N*	3*N*				Inter-	
	supp. *Pm*	Supp. *Pm Cy*	*Cy*+?	+?	sexes	Super ♂
135	4	5	7	2	69	3

The not-Curly triploids therefore carry the deficiency, which survives and is almost normal except for bristle length and wing shape. The absence of the light locus produces no effect on the manifestation of Plum. Combined with the failure of the duplication to suppress Plum, these data reinforce the conclusion that the dominant effect of Plum is a function not of the light, but of the brown locus.

DOMINANT EYE COLORS AND THE POSITION EFFECT HYPOTHESIS

The essential requirement of the position effect hypothesis is the regular occurrence of a definite "mutation" with a definite chromosome break. The mutation may involve both, or only one of the genes newly juxtaposed at the place of rearrangement. The important point is the correlated appearance of the mutation and the chromosome fracture at a given locus.

The alternative hypothesis, the frequent occurrence of gene mutations in the ordinary sense at points of breakage, is really one form of the position effect hypothesis. It involves the assumption that the breakage of the chromosome *per se* is responsible for an upset in gene structure. On closer inspection, this means the separation of a gene from its normal neighbors, and implies a relationship of some sort between them.

The other alternative is a loss of material at the point of breakage. This can readily be excluded by the application of the usual tests for deficiency (BRIDGES, MOHR), in those cases where known genes are involved in the mutation.

For the dominant eye colors which are the subject of this paper, the data are consistent with the position effect hypothesis. The work of VAN ATTA, and particularly of GLASS, has shown that in every case, the dominant effect is correlated with a break in a specific region. To their work we have added in several cases the correlated occurrence of a mutation with the other break in the chromosome. And every test for deficiency imposed upon the mutations at both breaks has yielded negative results.

The analysis further indicated no correlative action of the two genes in the production of the dominant effect. This, taken together with VAN ATTA'S and GLASS'S cases in which a similar mutation is found at the brown locus, with a different kind of translocation, would seem to indicate that the separation of brown from its normal neighbors is sufficient to accomplish the mutation.

As regards the light locus, there are, however, data on two translocations in which a breakage has taken place close to light (translocation G, RHOADES 1931 and translocation H, SCHULTZ unpublished) in which there is no mutation at the light locus. These cases, if the break be considered as close enough to light, which makes some caution necessary, provide evidence of an important kind for the position effect hypothesis. If it can be proved that only certain rearrangements give specific effects, the hypothesis becomes established. If a mutation at the light locus occurs only as the result of its juxtaposition to brown or its neighbors, and not when other rearrangements occur, the proof for this case might be regarded as complete. We do not at the present know precisely the relations of brown and light in these inversions; the possibilities are shown in figure 7, and it

should be a simple matter to distinguish between them. If the two eye color genes are really adjacent, a variety of speculations become of interest.

Whether the lethal effect is at the same locus as the eye color mutation touches another question of interest. It is possible that not only the genes adjacent to the breakage, but also those farther removed may be affected. A series of data on changes of dominance of "normal genes" in duplicating sections of chromosome (DOBZHANSKY and STURTEVANT 1932) is suggestive in this respect. The alternative in these cases is an upset of genic balance; but even this must be an ordered change, since the closer the break, the greater the change from the normal dominance effect.

SUMMARY

1. A new dominant eye color in *Drosophila melanogaster*, called Plum-2 is a member of the series of alleles of brown (Plum or Dilute) studied by VAN ATTA and GLASS.

2. The Plum-2 chromosome contains an inversion, cytologically demonstrated, whose ends are respectively near the loci of light and of brown (figure 7). The Plum-2 mutation is inseparable from the ends of the inversion.

3. Plum-2 behaves as an allele to both mutants light and brown. It exhibits instability for these loci, and also for minus (close to brown). Plum-1 exhibits a behavior similar to Plum-2.

4. A series of experiments, designed to test the hypothesis that the mutations at the breaks are due to simple deficiencies, yielded consistently negative results.

5. The dominant effect in Plum-1 and Plum-2 is a function of the brown locus only.

6. The data presented are consistent with the hypothesis that the behavior of genes is a function of their position in the chromosome, that is, depends upon their relation to other genes.

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