THE *P*-LOCUS POSITION EFFECT IN *OENOTHERA*

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(With Two Text-figures)

1. Introduction

The behaviour of a gene, when in the presence of a given set of other genes and under given environmental conditions, may depend upon its position in the chromosomes. Thus, gene *B* normally set between genes *A* and *C*, i.e. as *A B C D* ..., may have a different phenotypic expression when abnormally set through a structural change between genes *A* and *X*, i.e. as *A B X* .... This is the genetic phenomenon known as 'position effect'. Its proof, to the exclusion of mutation or other possible explanations, depends upon showing that the gene itself is identical when in the two positions. This may be demonstrated by transferring the abnormally acting gene to a normal position or by inserting a known normal gene into the abnormal position. Both these removals may be accomplished by crossing-over in suitable heterozygotes.

In *Drosophila melanogaster* numerous cases of position effect have been found, most of them involving changes in the dominance of normal wild-type genes. Two, or possibly three, types of effect may be distinguished, at least superficially. First, an interchange or other structural rearrangement with one break close to a particular locus results in the structural heterozygote showing a change (usually a loss) of dominance of the wild-type allelomorph whether the latter is located on the rearranged chromosome (*R*) or not. This happens, for instance, when *ci* (*cubitus interruptus* in chromosome IV) or its wild-type allelomorph ci^+ is removed to a position adjacent to euchromatin of any other chromosome (Dubinin & Sidorov, 1934*a*, *b*; Stern & Heidenthal, 1944); it is sometimes referred to as the Dubinin-effect. In such cases, the structural heterozygote that is also ci/ci^+ shows the *cubitus interruptus* phenotype, but the structural homozygote does not. *R*(*ci*)/+ genotypes produce *ci* phenotypes in varying degree in contrast to *ci*/+ which is nearly normal. *R*(*ci*)/*ci* genotypes produce more extreme *ci* phenotypes than *ci*/*ci*, the degree of deviation from normality being greatest for those *R*(*ci*) which cause greatest abnormality with +. Also, $R(ci^+)/ci$ give strong *ci* phenotypes, but $R(ci^+)/R'(ci)$ show a shift towards a normal phenotype. Remarkably, those *R*′(*ci*) which cause the most extreme *ci* phenotypes with ci^+ and *ci* are most effective in causing a shift to a normal phenotype with $R(ci^+)$.

In this type it is not clear whether the position effect is restricted in occurrence to those cases in which the affected locus *B*, originally set as ... *A B C D* ..., has a different locus actually adjacent to it, as in ... *A B X* ..., or whether it may also occur when the locus has a new neighbour not actually adjacent to it, as in

... *A B C X* ... or ... *A B C D X* ....

At any rate, such properties are shown by the second type, in which a wild-type gene in the neighbourhood of a breakage point of a structural change may, in a heterozygote carrying a recessive mutant allelomorph on the normal chromosome, be exhibited as a mottled or variegated phenotype made up of apparently normal tissue together with apparently mutant tissue. Where the structural homozygote is viable, it also shows the

variegation. Such behaviour has, for example, been shown (Schultz, 1936; Dubinin, 1936; Saccharov, 1936) to be associated with breaks in the neighbourhood of the w^+ (white) locus of the *X*-chromosome. When the break is just to the left of the w^+ locus, variegation may be exhibited not only for w^+ itself but also for the neighbouring loci rst^+ (roughest), fa^+ (facet), dm^+ (diminutive) and in some cases ec^+ (echinus) with decreasing intensity as the distance of the gene from the breakage locus increases. In variegated white, and likewise in other examples, the effect is commoner, more marked and extends to more genes, i.e. to a greater length of chromosome, if the region affected is translocated to the neighbourhood of heterochromatin, rather than euchromatin (Demerec, 1940). The variegation is partially or completely suppressed by growth at high temperature (Gowen & Gay, 1933*b*) or by the addition of one or more *Y*-chromosomes (Gowen & Gay, 1933*a*).

The third type of position effect in *Drosophila* is characterized by the production of a change that is semi-dominant to the normal wild type as, for example, in the duplications that are responsible for Bar eye (Bridges, 1936) and Hairy wing (Demerec & Hoover, 1939). We are here considering the comparison of *B* and + rather than the position effect shown by the unlike phenotypes of *BB*/+ and *B*/*B*. It is possible that the distinction between this type and the first is more apparent than real. In the case of Bar, Sutton (1943) has shown by comparison with a Bar deficiency that the Bar locus in its normal position has no effect on the phenotype of the fly. The Bar effect is apparently produced by interaction of the Bar locus when in contact with certain other specific loci.

Lastly, it should be mentioned that in *Drosophila* structural changes when homozygous are often less viable or even lethal compared with normals, though whether such lethal effects are properly accounted for as position effects is doubtful (Lea & Catcheside, 1945).

There is no clear evidence for the occurrence of position effects in any other organism except *Oenothera* (Catcheside, 1939). Various cases have been reported where viability or fertility changes are associated with structural changes in the chromosomes. Thus Savchenko (1935) found an interchange in the vetch (*Vicia sativa*) that was less fertile as a homozygote than as a heterozygote. In maize, interchange gametes (or rather gametophytes) seem as viable as normal ones. Thus, when an interchange heterozygote is used as a pollen parent, equal numbers of interchange and of normal pollen tubes effect fertilization. Stadler (1941) showed this to be true of about fifty different interchanges in maize. This is in striking contrast to the behaviour in *Oenothera blandina* where, of ten X-ray induced interchanges, five showed reduced male gametophyte viability (Catcheside, 1935). Similar effects are shown in *Datura* (Blakeslee & Bergner, 1940).

In maize, Jones (1939, 1944) has found various colour and growth changes in the endosperm to be associated with the relocation of chromosome parts. Brink (1932) found plants homozygous for a particular interchange to be slightly earlier than normal ones. Roberts (1942), in an extensive replicated test of a number of maize interchanges, has found similar effects on vigour and earliness in both homozygous and heterozygous interchange plants.

In none of these cases, however, can it be said that the production of gene mutation concurrent with the structural change has been excluded. In one case in *Oenothera blandina* it was possible to give proof of position effect (Catcheside, 1939) though the proof was weak. This weakness lay in the possibility that the single critical plant could itself have been a mutant or even an interloper. These possibilities are removed by the experiments reported below.

2. THE *Ps* POSITION EFFECT IN *OENOTHERA BLANDINA*

The *P* locus lies in arm 3 of chromosome 3.4 of *O. blandina*. A position effect, acting on the *P* locus, was found (Catcheside, 1939) in an X-ray induced interchange between chromosomes 3.4 and 11.12 in which the new chromosomes were 3.11 and 4.12 (Catcheside, 1940). Plants heterozygous for the interchange are characterized by narrower leaves than normal *blandina* and by a peculiar greyish green leaf colour. The interchange set of chromosomes is conveniently referred to as *blandina*-A and by the symbol *A*, the normal set as ^{h}*blandina* and by the symbol +. The position effect was exhibited in relation to P^s, a *P* allelomorph in which the sepals of the flower buds show broad uniformly red stripes separated by narrow green or yellow-green ones (Fig. 1*a*). In heterozygous plants that

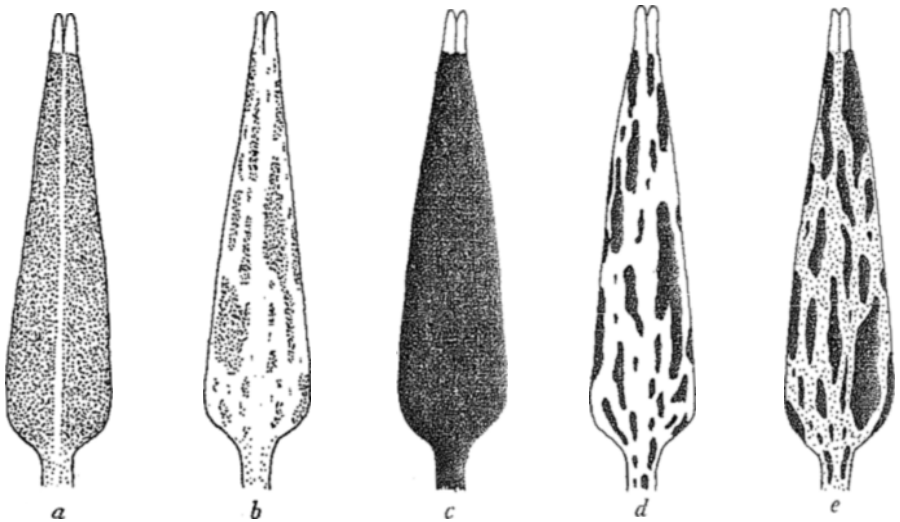

Fig. 1. Diagrams of pigment distribution in buds of various genotypes. The black areas represent deep red, the dotted areas light red and the white areas green tissue. (*a*) P^s P^s *blandina*; (*b*) P^s ^{h}*blandina*/P^s *blandina*-A; (*c*) P^r P^r *blandina* or P^r P^s *blandina* or P^r ^{h}*blandina*/P^s *blandina*-A; (*d*) P^s ^{h}*blandina*/P^r *blandina*-A.

are P^s ^{h}*blandina*/P^s *blandina*-A, the sepals show more or less numerous streaks of green so that the red pigmentation is broken up into patches of varying size (Fig. 1*b*). The green pigmented areas show a colour which is characteristic of the two lower allelomorphs at the *P* locus, namely, *P* and *p*. P^s is incompletely dominant over *P* and *p*, the buds of P^s *P* and P^s *p* plants being green merely flushed with red. The position-effect sepals show a mosaic of P^s P^s and apparently P^s *P* or P^s *p* tissue with sharp boundaries between the areas. The sepals are variegated in a manner analogous to the eyes in *Drosophila melanogaster* stocks that have a translocation of the w^+ locus to a position close to heterochromatin. In the *Oenothera* case, there is no direct evidence that a translocation of P^s to the neighbourhood of heterochromatin is involved, but the analogous genetic behaviour described below is suggestive of this. The proof that the variegation was a position effect consisted in transferring the affected P^s gene from chromosome 3.11 by crossing-over into a normal 3.4 chromosome. When this was done, the variegation disappeared and the P^s gene was restored to its full activity. Thus there was no evidence that any mutation had been induced in it at the same time that the interchange was produced.

The original proof (Catcheside, 1939) was founded upon a single critical plant. It was, therefore, important to secure confirmatory evidence, particularly by inserting another

P allelomorph, namely, P^r (rubricalyx), which produces uniformly deep red sepals, into the interchange chromosome. The present paper describes the results, which fully confirm the position-effect hypothesis. Briefly, P^s and P^r show variegation when in the 3.11 chromosome, and the variegation extends to the neighbouring *S* locus. It is convenient to record the phenotypes by the symbols P^{se}, P^{re} and S^e in the cases of plants showing the variegation. In addition, various unexpected and complicating phenomena were discovered. These are capable of explanation as duplications (Fig. 1*c*) or deficiencies respectively of the *P*-*S* region; discussion of them is deferred to a later paper, though the identifications of the plants are inserted into the tables of the present paper where desirable.

3. Substitution of P^r for P^s in chromosome 3.11

The technique of substitution was as follows: P^s h*blandina*/P^s *blandina*-A plants were crossed with P^r P^r *blandina* plants and the P^r h*blandina*/P^s *blandina*-A plants picked out amongst the progeny. This could be done readily at the seedling stage using the character of the narrow leaves and their peculiar green colour. These seedlings also show the presence of red pigment in the epidermis of the underside of the mid-veins of the leaves, a character determined by P^r. The flower buds of these plants were deep red (Fig. 1*c*) without any sign of variegation. These doubly heterozygous plants were then back-crossed to P^s P^s *blandina*. In 1940, 717 plants in five families of this backcross were grown; the distribution of these plants amongst the various types is summarized in Table 1.

Table 1. *Progenies of* P^s blandina-*A*/*P* hblandina × P^s P^s blandina

	Family					
Genotype	68/40	69/40	70/40	71/40	72/40	Totals
P^s *blandina*-A/P^s h*blandina*	20	73	47	60	122	322
P^r h*blandina*/P^s h*blandina*	35	97	54	65	119	370
P^s h*blandina*/P^s h*blandina*	.	2	2	1	.	5
P^r *blandina*-A/P^s h*blandina*	.	1	.	1	.	2
P^r P^s *blandina*-A/P^s h*blandina* (duplication)	.	.	.	.	1	1
P^s P^r *blandina*-A/P^s h*blandina* (duplication)	.	1	.	1	.	2
− h*blandina*/P^s h*blandina* (deficiency)	.	2	1	1	2	6
− *blandina*-A/P^s h*blandina* (deficiency)	.	1	2	1	1	5
Trisomics	1	1	.	1	1	4
Totals	56	178	106	131	246	717

The transfer of the affected P^s gene from the 3.11 chromosome to a normal position in a 3.4 chromosome restores its normal behaviour. This was found in five plants which had buds indistinguishable from regular P^s P^s *blandina* plants (Fig. 1*a*).

The P^r gene was transferred into the 3.11 chromosome in five plants, but in the case of three of them the transference involved the establishment of a duplication. Discussion of these three plants is deferred. In the other two plants the sepals showed deep red pigmented areas interrupted by pure green areas forming a green background (Fig. 1*d*). Thus P^r in chromosome 3.11 shows a position effect similar to that of P^s. Restoring this affected P^r gene to a normal 3.4 chromosome, as was done in another similar experiment, restores the normal behaviour of the gene.

All the observed transfers of either P^s or P^r from 3.4 to 3.11 or vice versa are enumerated in Table 2. In each case, insertion of the gene into 3.11 led to the characteristic position effect, while the removal of the position-affected gene to 3.4 always resulted in the

restoration of its normal behaviour. The observation of fifty-eight critical transfers removes all possible doubt that we are dealing with a genuine position effect. No matter what phenotypic manifestations may follow upon placing P^s or P^r in the interchange chromosome, the P-locus genes are themselves unchanged in their structure, as is shown by their normal behaviour when replaced in a chromosome of normal structure.

Table 2. *Transfers of* P^s, P^r *and* S *between chromosomes* 3.4 *and* 3.11

Transfer		Gene		
From	To	P^s	P^r	S
3·4	3·11	17	7	4
3·11	3·4	17	17	71

4. LOCUS OF THE INTERCHANGE BREAK IN CHROMOSOME 3.11

No detailed cytological localization is possible in *Oenothera*, the pachytene stage being unsuitable for analysis. The experiments designed to demonstrate the position effect (Table 1) showed that the interchange locus must be genetically close to the P locus. There were seven cross-overs amongst 699 plants classified, that is, omitting the duplication and deficiency plants and the four trisomics whose exact constitution was in doubt. Thus there is approximately 1% crossing-over between P and the interchange break locus.

It is important to determine whether the interchange locus is to the left or right of P, i.e. distal or proximal with respect to the centromere. It is known that S (S = yellow petals; s = sulphur-coloured petals) is also carried on chromosome arm 3 (Emerson & Sturtevant, 1932; Catcheside, 1940) and that the order is S-P-centromere, with about 8% crossing-over between S and P. *Blandina* plants homozygous for s and P^s were constructed, being extracted from $S\ P^s/s\ P^r$ by selfing. Several families were then grown (Table 3) from matings of the type $A\ P^r\ s/+P^s\ S\times +P^s\ s$, the type $A\ P^s\ S/+P^r\ s\times +P^s\ s$ and the type $A\ P^r\ S/+P^s\ s\times +P^s\ s$. It was found in making up the heterozygotes that, whereas $A\ s/+S$ had normal yellow petals, $A\ S/+s$ had petals that were a mosaic of yellow- and sulphur-coloured patches. Some of the sulphur patches were large, occupying as much as half a petal or more, but most were quite small. The general effect of the yellow and sulphur mosaic is to give the petals a colour which, seen at a distance, is intermediate between yellow and sulphur. At close range, the patchwork is quite obvious. Moreover, the boundaries between the yellow and sulphur areas are perfectly sharp, there being no appearance of any intergradation between the two colours. Thus S also shows a variegation like that of P^s or P^r and under similar conditions. In both cases the variegation is more extensive earlier in the season, that is, in the buds first produced. Later in the season, in the later produced buds, the variegation becomes much less extensive, and in the case of S variegation is often not at all obvious in the petals of the flowers produced in the cooler weather in September. These are purely physiologically determined variations in expression, dependent upon senescence and environment; there is no difference between progeny grown from early and late flowers of the same plant.

The details of the backcross test progenies are given in Table 3 and summarized in Table 4. The test shows that the P locus is closer to the interchange locus than is the S locus, and that the order of these three points is interchange-P-S. Nearly all cross-overs in the interchange-P interval are also cross-overs between the interchange locus and S; there are only two exceptions (double cross-overs) amongst nineteen cross-overs in the interchange-P interval. There is 1·7% recombination in the interchange-P interval and

8·5% recombination in the *P-S* interval. Thus 0·15% of double cross-overs could have been expected where 0·18% were found.

The assignment of precise linkage values to the region concerned is complicated, especially in the interchange heterozygote by the occurrence of unequal crossing-over leading to duplications and deficiencies. This is rather frequent in comparison with regular crossing-over. In the case of the interchange-*S* region, the value is distorted by the different gametic viabilities of *S*- and of *s*-carrying gametes. However, the data hardly warrant more elaborate treatment.

Table 3. *Three-point test-crosses of S P and interchange break locus in chromosome 3.4*

Family	Genotypes of parents	Phenotypes of offspring: *Blandina* $P^s S$	*Blandina* $P^s s$	*Blandina* $P^r S$	*Blandina* $P^r s$	*Blandina*-A. *blandina* $P^{sc} S^c$	*Blandina*-A. *blandina* $P^{sc} s$	*Blandina*-A. *blandina* $P^{rc} S^c$	*Blandina*-A. *blandina* $P^{rc} s$	Exceptions	Total plants
11/43	$\frac{+P^s S}{A\ P^r s} \times \frac{+P^s s}{+P^s s}$	43	1	.	2	.	.	4 (1)	19	2 *Dp*	72
12/43	$\frac{+P^r s}{A\ P^s S} \times \frac{+P^s s}{+P^s s}$	4	.	19	75	112	6	1	1	2 *Dp*	220
13/43	$\frac{+P^r s}{A\ P^s S} \times \frac{+P^s s}{+P^s s}$	4	.	7	57	87	6	1	.	5 *Dp*	167
14/43	$\frac{+P^r s}{A\ P^s S} \times \frac{+P^s s}{+P^s s}$	.	.	2	46	108	1	.	.	2 *Dp*	159
15/43	$\frac{+P^r s}{A\ P^s S} \times \frac{+P^s s}{+P^s s}$	2	.	6	60	78 (2)	5	.	.	2 *Dp*	155
16/43	$\frac{+P^r s}{A\ P^s S} \times \frac{+P^s s}{+P^s s}$	.	.	11	49	95 (7)	6	.	1		169
19/44	$\frac{+P^s s}{A\ P^r S} \times \frac{+P^s s}{+P^s s}$	2	33	.	.	.	2	25 (1)	5	3 *Dp*; 1 *Df*	72
33/44	$\frac{+P^s s}{A\ P^r S} \times \frac{+P^s s}{+P^s s}$	6 (14)	64	.	.	. (1)	1	50 (19)	5	3 *Dp*; 3 *Df*	166

Notes. Numbers in brackets refer to plants that failed to flower, so petal colour could not be scored. In column listing exceptions, *Dp* are duplications and *Df* are deficiencies.

Table 4. *Summary of linkage data for S-P-interchange break*

Family	Non-cross-overs	Single cross-overs: Region 1	Single cross-overs: Region 2	Double cross-overs: Regions 1 and 2	Total plants
11/43	62	2	5	.	69
12/43	187	5	25	1	218
13/43	144	4	13	1	162
14/43	154	.	3	.	157
15/43	138	2	11	.	151
16/43	144	1	17	.	162
19/44	58	2	7	.	67
33/44	114	1	11	.	126
Total	1001	17	92	2	1112

Note. Duplications, deficiencies, and incompletely scored plants omitted from the reckoning.

The position effect thus extends over a remarkably long genetic distance, about 10 units, much longer than in any of the *Drosophila* cases. It is possible that the cytological distance is small, but there seems to be no likelihood of obtaining direct evidence by observation of the chromosomes.

The data show that the interchange is one of the kinds shown diagrammatically in Fig. 2. There is no direct method available to decide between these alternatives, but on

general grounds (*b*) is the more likely, namely, that the break in 3.4 occurs between P^s and the centromere. If it becomes possible to analyse the pachytene stage of meiosis, the point could be settled. The nature of the duplications obtained shows in an indirect way that this must be the structure, otherwise the duplications would have dicentric chromosomes.

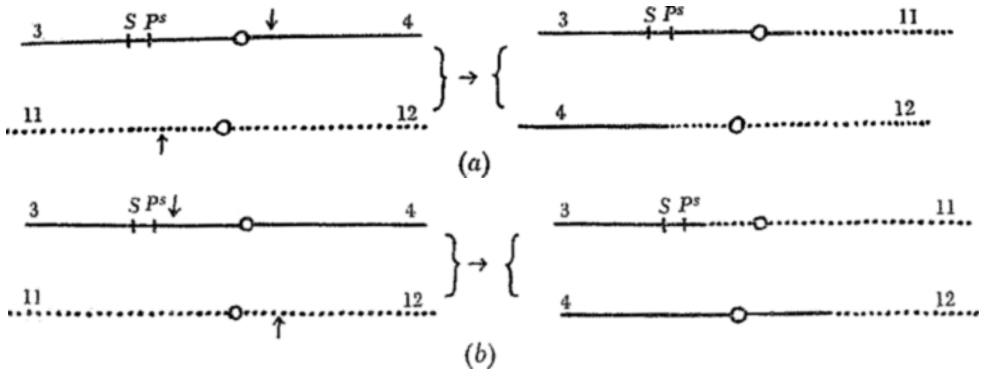

Fig. 2. Diagrams of the possible origins and structures of the 3.11 + 4.12 interchange in *Oenothera blandina*; (*b*) is the more probable on general grounds.

5. EGG AND POLLEN TRANSMISSION OF INTERCHANGE GAMETES

In general, gametophytes carrying the interchange are somewhat less viable in competition with normal gametophytes both as embryo sacs and as pollen tubes that function in fertilization. The available data are given in Table 5. A χ^2 test shows that the forty-eight families available to estimate the relative embryo sac production of normal and interchange megaspores are highly heterogeneous ($\chi^2 = 111{\cdot}6829$ for 47 degrees of freedom). The

Table 5. *Numbers of functional normal and interchange gametophytes yielded by various genotypes*

(A) + P^s S/A P^s S	
+	A
Embryo sacs	
29	33
64	63
55	52
30	28
78	72
8	10
31	33
73	39
94	69
Total 402	399
Pollen grains	
82	70
56	47
10	14
41	28
83	77
48	24
5	3
Total 325	263

(B) + P^s S/A P^r S	
+	A
Embryo sacs	
84	78
113	76
138	125
66	33
57	50
137	132
63	61
42	29
39	33
35	37
68	50
75	80
Total 917	784
Pollen grains	
15	17

(H) + P^s s/A P^s s	
Embryo sacs	
11	15
8	10
Total 19	25

(C) + P^r S/A P^s S	
+	A
Embryo sacs	
46	50
28	21
36	20
100	78
56	50
67	64
120	126
47	66
Total 500	475

(G) + P^r s/A P^s S	
Embryo sacs	
41	40
44	43
99	119
68	94
48	109
68	85
60	109
Total 428	599

(D) + P^s s/A P^s S	
+	A
Embryo sacs	
3	3

(E) + P^s S/A P^r s	
Embryo sacs	
46	23

(F) + P^s s/A P^r S	
Embryo sacs	
36	36
87	79
Total 123	115
Pollen grains	
29	41

(I) + P^s s/A P^r s	
Embryo sacs	
109	86
18	12
Total 127	98

nine genotypes, into which the data may be grouped, show a χ^2 of 55·6487 for 8 degrees of freedom, indicating a high degree of heterogeneity as between genotypes. The χ^2 for the remaining 39 degrees of freedom is 56·0342, P lying between 0·08 and 0·09. Thus the heterogeneity may be ascribed entirely to effects of the different P- and S-locus compositions. The precise way in which P^s and P^r and S and s affect megaspore competition cannot be deduced satisfactorily from the present material. Only nine of the possible sixteen genotypes heterozygous for the interchange are available, and in several of these the data are scanty. It may be said, however, that P^r relative to P^s appears to cause a slightly depressive effect on megaspore viability, while s relative to S causes a more marked depression. No doubt these effects come about through slight Renner effects, but no direct evidence exists.

Amongst fertilizations effected by pollen also there is a small deficiency of those resulting from pollen tubes carrying the interchange.

6. Viability and appearance of interchange homozygotes

During the first generations after the origin of the interchange it was found that interchange homozygotes could not be reared beyond the seedling stage. The early leaves were small, thickened and distorted; and after a shorter or longer time the growing point of the stem went awry and no more growth occurred. The root system was also rather deficient. For most of the period of the experiments described above, the stocks were maintained by crossing to homozygous normals. Occasional selfed progenies were grown and the homozygotes were usually of the expected crippled type.

In one family grown in 1943 the interchange homozygotes seemed more vigorous than usual, and eight of them were successfully overwintered in a box in the cool greenhouse. In 1944 four plants flowered, the others having been lost through planting out at the beginning of a dry spell. This family had arisen from selfing a heterozygote that was P^r *blandina*-A/P^s *blandina*. All the interchange homozygotes were homozygous for P^r. The four plants that flowered all showed P^r variegation though less extensive than in *A* $P^r/+P^s$ plants. The appearance of the sepals is such as would be expected if two P^r variegations were superposed. The plants were not examined for S variegation. The general habit of the plants was like that of the heterozygotes but more extreme, the plants being quite small.

The present case of position effect thus agrees with those cases of w^+ variegation in *Drosophila* which show in homozygotes if the latter are viable, rather than with *ci* position effects which are suppressed in homozygotes.

7. Discussion

The existence of the position-effect phenomenon in *Oenothera* has been clearly established by the above experiments. It remains to consider what bearing the observations may have on theories seeking to explain position effect. In the first place it is quite clear that the P^r, P^s and S genes are themselves unaltered in structure by their presence in the abnormal position. At least the genes recovered from the abnormal position show no abnormality, so that any change in the genes wrought while they are position affected must be temporary and at once reversible when they are removed from the local influence. This behaviour makes it difficult to accept the suggestion of Demerec & Slizynska (1937) that

position-effect variegations are frequent somatic mutations arising as a result of an induced mutability in the affected genes.

The region of the chromosome at the locus of the interchange appears to act as a modifier on various genes more or less closely linked to it, provided in the case of variegations that the genes are actually in the abnormal chromosome. This circumstance limits the possible mechanisms that must be explored. Thus anything closely analogous with mechanisms that would account for the interaction of genes in separate chromosomes in the same nucleus will not suffice.

If we seek for an explanation in terms of diffusion (Sturtevant, 1925) with localized interaction of gene products or competition for precursors, we must suppose that the capacity for diffusion is limited. This limitation is imposed by two circumstances. The diffusion may not spread in certain directions, for genes situated on a separate chromosome are unaffected. In *Drosophila*, particularly, in which there is regularly strong somatic pairing, the homologous parts of the chromosomes must be very close together in a great majority of somatic cells and yet show no interaction comparable to position effect. On the other hand, diffusion along the chromosome for some distance must be possible since genes situated at a considerable distance from the source of the effect are modified in their action.

In contrast to such kinetic hypotheses (Ephrussi & Sutton, 1944) are structural hypotheses (Muller, 1935; Dobzhansky, 1936). The latter postulate reversible modifications of the genes themselves or of their structural interrelations. For instance, it might be supposed that chemical bonds unite neighbouring genes into integrated, larger units, and that within such a unit, as within any large molecule, changes produced in one of its parts would affect the properties of the whole. However, the distances over which position effects may extend are so great in some cases, of the order of 100 gene diameters, that an explanation in terms of such steric effects seems inconceivable (Ephrussi & Sutton, 1944). Moreover, as these authors have also shown, the effects possible by ordinary diffusion at such great distances are so slight as to be completely negligible. A kinetic explanation is possible only if the spread of substances along the chromosome is substantially easier than radially outwards from the chromosome.

The explanation of position effect then must be sought in terms of some factor that will spread along or through a chromosome rather than outwards through the karyolymph. Unless diffusion from or to a gene is preferentially along the chromosome, we must conclude with Ephrussi & Sutton (1944) that the factor responsible is some change in the physical state of the chromosome itself. On analogy with the behaviour of myosin fibres, these authors suggest that the change is one involving the state of extension of the chromosome. In the Diptera, coincident with the occurrence of position effect, the chromosomes show an intimate association in somatic pairing. If, as seems likely, there is a rather precise alignment of homologous parts in somatic pairing, the presence of structural rearrangements in the heterozygous condition could lead through changes in the pairing to alterations in the state of extension of the chromosome in the vicinity of the breakage points. This hypothesis carries with it the corollary that the action should not be confined to the genes in the chromosome carrying the break, but should also appear in its homologue. This expectation is fulfilled in the *cubitus interruptus* position effects (Stern & Heidenthal, 1944) referred to earlier. The data of Dubinin & Sidorov (1934*a*, *b*) add the following corroborative facts, namely that any ci^+ translocations which show

position effects when heterozygous ($R(ci^+)/ci$) give a normal phenotype when homozygous or hemizygous, that is when pairing is complete or absent. Both would lack a local stress.

The cases of Bar and other similar contiguous duplications are also accounted for since the distortion is here intrachromosomal through synapsis of homologous parts of the two duplicated sections. We would therefore expect, as is indeed found, that homozygotes and hemizygotes as well as heterozygotes would have abnormal phenotypes.

The w^+ variegations, and similar ones of bw^+, present greater difficulties, since homozygotes and hemizygotes show the white mottled phenotypes. On analogy with the *ci* cases this is unexpected, for homozygotes show complete pairing and hemizygotes a lack of it. The case can be reconciled with theory on the assumption that the manifestation of the position effect depends upon the affected loci being adjacent to heterochromatin. The heterochromatin of *Drosophila melanogaster* shows non-homologous pairing, or more strictly the heterochromatin of different chromosomes is non-specific in its pairing perhaps because it is not much differentiated. Such non-specificity of pairing should extend to the parts of heterochromatin within one chromosome and such intrachromosomal pairing might well affect genes attached to the heterochromatin. This is the suggestion offered by Ephrussi & Sutton (1944), with the admission that it is tentative.

This explanation would also have to be applied to the *Oenothera* case, although there is no direct evidence that heterochromatin is involved. The evidence is the spreading of the effects along the chromosome coupled with the absence of somatic pairing rendering position effects analogous to the ci^+ and *B* ones improbable. There is no cytological evidence for the occurrence in *Oenothera* of somatic pairing, in fact the appearances are all against its existence in the plant. The possibility of somatic pairing must however be entertained. A number of chimerical plants have been found in *Oenothera*, in which the origin of one component from the other by intrachromosomal somatic crossing-over seems rather probable. The occurrence of somatic crossing-over implies somatic pairing, though it may be only of heterochromatic segments. These exceptional plants are referred to in a subsequent paper on duplication and deficiency in *Oenothera*.

The proposed explanation of the heterochromatin position effects of w^+ does not suggest why these particular position effects should spread further along the chromosome from the seat of action than in the cases of translocations of w^+ to the neighbourhood of euchromatin (Demerec, 1940). The effect could be accounted for if proximity to heterochromatin sometimes caused a persistence of nucleic acid on the genes during the resting stage of the nuclear cycle and if such a coating of nucleic acid inhibited the normal functioning of the gene. Such heterochromatinization of euchromatin bands translocated to the neighbourhood of the heterochromatin has been observed in *Drosophila* by Schultz (1941). This explanation is one of the factors accepted by Prokofyeva-Belgovskaya (1945), who interprets the effect as one of a reduction in the length of the metabolic stage of the affected chromosome region. She also draws attention to two other factors, namely, the effect due to the addition of extra heterochromatin (from the *Y*-chromosome) and an effect dependent upon whether the affected chromosome region was homo- or heterozygous in the parent. I have no sufficiently definite information upon which to judge whether a similar effect of heterozygosity exists in the *Oenothera* case.

A second peculiarity that is not at once accounted for by Ephrussi and Sutton's stress hypothesis, is the fact that the abnormal tissue is often in large patches as though all the cells in the patch were the descendants of one cell in which the abnormality had been

produced. This is very striking in the *Oenothera* case. Rarely, whole branches may appear green budded with the exception of an odd bud here and there which may show red patches on part or the whole of the bud. Even so, the progeny derived from such green buds on wholly green branches are entirely like those from a bud showing the usual variegation. The interchange heterozygotes have red and green variegated sepals, and cross-overs to a normal chromosome of the affected *P* allelomorph, in particular P^r, show it to have an unaltered structure. The observations suggest that the inhibition of the affected gene is by a mechanism that, having occurred by chance in a given cell, persists for a number of cell divisions, rather than by a mechanism that arises by chance in each cell generation and does not persist. No data are available to estimate the persistence time, but it can be stated that the period (in terms of division cycles) is greater at the beginning of the season than at the end; this may be a temperature effect, but one cannot be certain. We need to know whether the suggested intrachromosomal heterochromatin pairing does persist and whether the persistence is governed by external factors such as temperature. Presumably the effect might be secured by a semipermanence of the heterochromatinization earlier referred to. Another possibility that must be explored is the effect upon the differentiation and development of cytoplasmic genes capable of reproduction for a limited period subject to ultimate decay.

A third awkward fact is presented by Lewis (1945) in connexion with the Star-asteroid region of *Drosophila melanogaster* which is a reverse repeat, or reverse contiguous duplication, minute in size and located at the 21 E 1, 2 doublet in the left arm of chromosome II. The Star locus is on the first of these bands and the asteroid locus on the second. The phenotypes of *S ast*/+ + and *S* +/+ *ast* are widely different from one another. Here we have a case where on analogy with intrachromosomal duplications such as Bar, compared with +, we should, on the stress hypothesis, expect no phenotypic difference between the two genotypes. It is difficult to conceive how with the same stresses within each chromosome two genes could be modified differently according to whether they were together in the same chromosome or apart in separate homologues.

While the stress hypothesis will encompass the explanation of a surprising diversity of facts, one cannot avoid the conclusion that other mechanisms are also concerned.

SUMMARY

In *Oenothera blandina*, the genes P^s, P^r and *S* produce a variegated phenotype when they are present in the interchange chromosome 3.11. When transferred by crossing-over to a normal 3.4 chromosome, they produce normal phenotypes. The variegation is therefore a position effect.

The break is 1·7 units from the *P* locus and 8·5 units from the *S* locus, indicating a considerable spread of the position effect along the chromosome. The action is thought to depend on translocation of the *P* and *S* loci to the neighbourhood of heterochromatin.

Theories of the mechanism of position effect are considered, but the *Oenothera* case adds nothing new to the solution of the problem.

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