A POSITION EFFECT IN *OENOTHERA*

By D. G. CATCHESIDE

Botany School, Cambridge

The position of a gene in the chromosomes has an effect on its expression. That is, its behaviour is determined not only by its own properties, but by those of the whole genotype with which it is associated and especially by those of its neighbours. The phenomenon is particularly well known in *Drosophila melanogaster* (cf. review by Dobzhansky, 1936). So far, little evidence has been forthcoming to show that the position effect also occurs in other organisms.

In maize endosperms structural changes may be detected (Jones, 1937) through the twin spotting shown in respect of certain genes, such as C, whose expression depends partly upon the dose in which they are present. Jones (1939) has found cases of such twin spots in which colourless areas are paired with areas lighter than the surrounding normal cells. In these the relocated C region not only fails to function as usual but prevents normal action of the other C allelomorph. This is probably a position effect. The action of the relocated C gene is shifted to resemble that of the dominant inhibitor of aleurone colour that is thought to be another C allelomorph. A different, though perhaps analogous, property is also shown by maize, for deficiencies, when homozygous, may simulate recessive genes (McClintock, 1938).

The apparent rarity of the phenomenon in plants, and especially the lack of experimental verification, renders its discovery in *Oenothera* very significant. In the latter case we can show that the gene concerned is restored to its normal activity when transferred from the altered chromosome to the original one.

Breeding behaviour of certain *Oenothera blandina* interchanges

A number of interchanges were produced in *Oe. blandina* by X-raying pollen (Catcheside, 1935). Three of them have now been studied in greater detail; the others have been lost owing to their inviability. The three interchange complexes to be reported upon may be designated *blandina*-A, *blandina*-B and *blandina*-C respectively. The first two differ from standard ^{b}*blandina* by one interchange and give with it a ring of four chromosomes (but see below for peculiarity of *blandina*-A); *blandina*-

C differs from the standard by two interchanges involving three chromosomes and gives with it a ring of six chromosomes at meiosis.

Blandina-B, whether homozygous or in combination with standard ^{h}*blandina*, is phenotypically indistinguishable from homozygous standard. The two homozygotes are equally viable and fertile.

Blandina-C, when homozygous, can be distinguished from standard *blandina* by its smaller size, less branching, narrower leaves and less pigmented flower buds. It is also less viable and less fertile. The hybrid with standard is normal in appearance, that is the change in the phenotype associated with the relocation of genes in interchange C is recessive to normal. The change in phenotype is perhaps a position effect, but we cannot prove it because the genes concerned are unknown and attempts to substitute other allelomorphs for them cannot even begin.

Blandina-A in combinations with standard ^{h}*blandina*, or with other complexes (^{h}*Hookeri*, *velans*, *gaudens*, *rubricalyx-α*, ^{h}*purpurata*, *flavens*, etc.), exhibits a definite series of characters by which the combinations can be distinguished from similar ones involving standard ^{h}*blandina* in place of *blandina*-A. These characters are a narrow leaf, a peculiar leaf texture and a considerable reduction in the amount of pigment in the flower buds. It will be shown that the reduction of pigment in the flower buds is the result of a position effect on the gene P^{s}. Homozygous *blandina*-A dies as a seedling. These seedlings can be recognized quite early by their thick-textured, rather reddish cotyledons. Some do not progress beyond this stage, but others produce a few stunted thick leaves before they die. The cause of death is obscure, but appears in part to be a poorly developed root system. So far, it has not been possible to rear any of these crippled plants.

The evidence that the crippled seedlings represent the expected *blandina*-A homozygotes is given in Tables I and II. When *blandina*-A. ^{h}*blandina* is selfed (Table I) a quarter of the offspring are crippled and die. A further quarter look like standard *blandina* and breed true to that type. The remaining half of the plants all have the phenotype of *blandina*-A. ^{h}*blandina* and when selfed repeat the same segregation.

When *blandina*-A. ^{h}*blandina* is pollinated by, or is pollinated on to, standard *blandina* (Table II), one-half of the offspring are standard *blandina* and the other half are *blandina*-A. ^{h}*blandina*. There are no crippled seedlings, and of course none could be expected if they were the *blandina*-A homozygotes.

So far as the offspring have been tested cytologically (about twenty of each kind have been examined) the *blandina* type plants have seven

pairs of chromosomes at meiosis, while the *blandina*-A heterozygotes have a ring of four chromosomes and five pairs. The characteristic *blandina*-A phenotype is always associated with the interchange.

TABLE I*

Progeny of ^{h}blandina . blandina-A *individuals in family* 13/34 *when selfed*

Parent	Offspring: *Blandina*	Offspring: ^{h}*Blandina* . *blandina*-A	Offspring: Crippled seedlings
I 1	50	85	40
I 3	40	75	29
I 4	22	51	19
I 5	16	38	11
I 7	0	5	7
I 8	4	6	5
I 10	3	5	5
I 12	2	4	2
I 13	45	77	37
I 14	4	0	2
II 3	2	2	0
II 4	0	5	1
II 7	4	5	7
II 8	4	6	4
II 14	1	0	0
Totals	197	364	169

In addition, 12 normal *blandina* sibs were tested and gave 122 *blandina* offspring.

* I am indebted to Dr K. Mather for pointing out that the large and small families in this table show some heterogeneity, mainly due to an excess of cripples in the small families. The latter were grown in the winter when conditions of growth were rather poor. It may be that some heterozygotes were misclassified as cripples.

TABLE II

Progeny of blandina-A . ^{h}blandina *backcrossed with* blandina

Parents	Mating type	Offspring: *Blandina*	Offspring: *blandina*-A . ^{h}*blandina*
13/34 I 5 × 251/34 I 1	^{h}*blandina* . *blandina*-A × *blandina*	30	28
13/34 I 5 × 355/34 I 1	^{h}*blandina* . *blandina*-A × P^{r} *blandina*	78	72
13/34 I 13 × 251/34 I 1	^{h}*blandina* . *blandina*-A × *blandina*	33	29
13/34 I 13 × 265/34 I 12	^{h}*blandina* . *blandina*-A × sp *blandina*	63	64
13/34 I 13 × 355/34 I 1	^{h}*blandina* . *blandina*-A × P^{r} *blandina*	52	56
251/34 I 1 × 13/34 I 5	*blandina* . × ^{h}*blandina* . *blandina*-A	82	70
40/35 I 7 × 203/35 II 2	$\frac{\text{sp } {}^{h}\textit{blandina}}{\textit{blandina}\text{-A}}$ × sp *blandina*	14 + 16 sp	16 + 8 sp
41/35 I 11 × 177/35 II 2	$\frac{P^{s}\ \textit{blandina}\text{-A}}{P^{r}\ {}^{h}\textit{blandina}}$ × P^{s} *blandina*	45 P^{r} 1 P^{s}	0 P^{r} 50 P^{s}
	Totals	414	393

CYTOLOGICAL ANALYSIS OF THE INTERCHANGES

The relevant available data for the determination of the chromosomes involved in the three interchanges is summarized in Table III. The

TABLE III

Chromosome configurations at meiosis in combinations of [h]bla ndina *with three induced interchanges and in combinations of these with other complexes*

Complex	[h]*blandina*	*blandina*-A	*blandina*-B	*blandina*-C
[h]*blandina*	2, 2, 2, 2, 2, 2, 2	4, 2, 2, 2, 2, 2	4, 2, 2, 2, 2, 2	6, 2, 2, 2, 2
blandina-A	4, 2, 2, 2, 2, 2	—	6, 2, 2, 2, 2	—
blandina-B	4, 2, 2, 2, 2, 2	6, 2, 2, 2, 2	2, 2, 2, 2, 2, 2, 2	—
[h]*Hookeri*	6, 2, 2, 2, 2	6, 4, 2, 2	6, 4, 2, 2	10, 2, 2
flavens	6, 4, 2, 2	6, 6, 2	6, 2, 2, 2, 2	12, 2
velans	8, 2, 2, 2	8, 4, 2	8, 4, 2	—
gaudens	10, 2, 2	8, 2, 2, 2 (?)	12, 2	—
[h]*purpurata*	4, 2, 2, 2, 2, 2	4, 4, 2, 2, 2	4, 4, 2, 2, 2	—
rubricalyx-α	4, 2, 2, 2, 2, 2	4, 4, 2, 2, 2	4, 4, 2, 2, 2	6, 4, 2, 2

chromosome formulae of the standard tester interchanges that have been used in this investigation are as follows:

[h]*Hookeri*	1.2	3.4	5.6	7.8	9.10	11.12	13.14
flavens	1.4	2.3	5.6	7.8	9.10	11.12	13.14
velans	1.2	3.4	5.8	6.7	9.10	11.12	13.14
gaudens (rubens)	1.2	5.6					
[h]*purpurata*	1.2	3.4	5.6	7.10	8.9	11.12	13.14
[h]*blandina*	1.2	3.4	5.6	7.10	8.13	9.14	11.12
rubricalyx-α	1.2	3.4	5.6	7.14	8.13	9.10	11.12

Blandina-A differs from [h]*blandina* by an interchange between two chromosomes. Since in *velans*.*blandina*-A a ring of four chromosomes replaces two of the three pairs of chromosomes in *velans*.[h]*blandina*, the interchange must be between two of 1.2, 3.4 and 11.12. In *flavens*.*blandina*-A a ring of six chromosomes replaces the ring of four and one of the two pairs formed in *flavens*.[h]*blandina*; therefore chromosome 1.2 or 3.4 must have interchanged with 11.12. For, if 1.2 and 3.4 had interchanged, *flavens*.*blandina*-A could form a ring of six chromosomes, a ring of four and two pairs or else a ring of six and four pairs but not two rings of six chromosomes and a pair. In *gaudens*.*blandina*-A there is probably a ring of eight chromosomes and three pairs; there are certainly not less than two pairs of chromosomes at meiosis. If 1.2 had interchanged with 11.12 there could be only one pair of chromosomes in this combination. Therefore the interchange is between 3.4 and 11.12 and *blandina*-A is:

$$1.2 \quad 5.6 \quad 7.10 \quad 8.13 \quad 9.14 \begin{cases} 3.11 & 4.12 \\ 3.12 & 4.11 \end{cases}$$

Both formulae satisfy all the known data. We cannot at present say which of them is correct, but fortunately it is immaterial for a further analysis of the P^s position effect.

The heterozygote *blandina*-A.^{h}*blandina* usually shows a chain of four chromosomes at diakinesis and metaphase I of meiosis instead of a closed ring of four chromosomes. A similar behaviour is also shown in combinations of *blandina*-A with other complexes. Thus ^{h}*Hookeri*.*blandina*-A usually has a ring of six chromosomes, a chain of four and two pairs, while *flavens*.*blandina*-A has a ring of six, a chain of six and a pair. Quantitative data indicate that this is due to a lower chiasma frequency in a particular arm-pair of the interchange association. There is no evidence yet available to show in which arm the failure occurs.

Blandina-B differs from ^{h}*blandina* by an interchange involving two chromosomes. These are the two ^{h}*blandina* chromosomes (1.2 and 3.4) which are in the ring of four chromosomes in *flavens*.^{h}*blandina*, for in *flavens*.*blandina*-B this ring of four chromosomes is replaced by two pairs. Further the interchange has occurred so that the two new chromosomes are substantially like the *flavens* 1.4 and 2.3 chromosomes. The formula of *blandina*-B, which fits all the data, is therefore:

1.4 2.3 5.6 7.10 8.13 9.14 11.12.

Blandina-C differs from standard ^{h}*blandina* by two interchanges involving three chromosomes. One of these is 1.2 or 3.4, because the complex has one more chromosome like ^{h}*Hookeri* than like *flavens*, which differ only in these two chromosomes. A second must be one of the three chromosomes (7.10, 8.13 and 9.14) by which ^{h}*blandina* differs from ^{h}*Hookeri*, because *blandina*-C has only two more chromosomes like ^{h}*blandina* than like ^{h}*Hookeri*. This chromosome cannot be either of the two chromosomes (7.10 and 9.14) by which ^{h}*blandina* differs from *rubricalyx-α*, because *blandina*-C has two more chromosomes like the former than like the latter, and it must therefore be 8.13. The third chromosome is either 11.12 or 13.14; the other of these two gives the one pair with *flavens* and the second pair with ^{h}*Hookeri*. *Blandina*-C therefore has 7.10, 9.14, (1.2 or 3.4) and (11.12 or 13.14); its other three chromosomes are an interchanged arrangement of 8.13, (1.2 or 3.4) and (11.12 or 13.14).

THE $\mathbf{P^s}$ POSITION EFFECT IN *BLANDINA*-A

Standard *blandina* is homozygous for the gene $\mathbf{P^s}$ which is borne in arm 3 of chromosome 3.4. It has derived this gene from the *velans* complex of *O. Lamarckiana*, from which *blandina* has arisen as an alethal interchange complex combining parts of *velans* and *gaudens*. It is not yet possible to specify how it has arisen, except that 3.4 and 11.12 have come from *velans* and 5.6 from *gaudens*, 1.2 being common to these

complexes. It appears that 7.10, 8.13 and 9.14 are new chromosomes compared with those in the races of *Lamarckiana* that have been analysed.

Several allelomorphs of P^s exist and four have been distinguished through the work of Shull (1923), Renner (1925) and Emerson (1931). Their symbols (following Emerson) and characters are as follows:

P^r (*rubricalyx*), flower bud wholly red except for sepal tips, hypanthium red, underside of mid-rib of leaf red, red splotches on underside of leaves and especially the bracts, red papillae on stem.

P^s (striped bud), flower bud with broad red stripes separated by narrow yellow-green ones at the sepal margins, hypanthium striped, red papillae on stem, back of leaf and midrib not red.

P (punctate stem) flower bud and hypanthium green, stem with red papillae.

p (*pervirens*) flower bud green, papillae on stem green or none.

There are various grades of bud striping in hybrids involving P^s from different complexes (e.g. *velans* and ^{h}*Hookeri*) with P or p. It is uncertain how far these are dependent upon modifiers or due to different P^s allelomorphs. Nor can we say whether the manifold effects of these are really due to the same gene or to several closely linked genes. The possible cross-over types, e.g. a pigmented bud combined with unpigmented stem papillae, that might be expected if the latter were true, have not been detected.

The order of dominance, which is often incomplete, is P^r, P^s, P and p. Frequently the heterozygotes are somewhat intermediate between the homozygotes particularly with respect to the bud pigmentation. This is usually the case where P^s and p are concerned and is the reason why the half mutant *rubrinervis* (P^s *velans*.P^s *subvelans*) has a much more pigmented bud than *Lamarckiana* (P^s *velans*.p *gaudens*) from which it arises.

The interchange complex *blandina*-A combined with P^s ^{h}*blandina* has a bud pigmentation much like that of *Lamarckiana*. Instead of wide deeply pigmented stripes separated by narrow yellow-green ones, it has narrower uneven lighter red stripes and much wider yellow-green ones. Moreover, the margins between the stripes are ill-defined and far from straight.

The locus of P^s is on chromosome 3.4, which has interchanged with chromosome 11.12 in the production of *blandina*-A. Two explanations can be suggested for the change in bud pigmentation. The P^s gene in the broken chromosome 3.4 may have mutated at the same time as the

induction of the structural change to another allelomorph, like P or p. Alternatively, the removal of the 3 fragment of 3.4 to a new position in association with 11 or 12 has resulted in a position effect on P^s whereby its activity in bud pigmentation has been reduced. We can show that the latter is correct.

If the position effect hypothesis is correct, the transfer of the gene to its original position in standard *blandina* by crossing-over should restore it to its full activity. This has been accomplished. *Blandina*-A . P^s *blandina* plants were pollinated by P^r *blandina* plants and the F_1 *blandina*-A . P^r *blandina* plants were pollinated by P^s *blandina*. In the backcross there were (cf. Table II) 45 P^rP^s *blandina*, 1 P^sP^s *blandina* and 50 P^s *blandina* . *blandina*-A. These last plants all had the typical reduced pigmentation of the buds. In the production of the single P^sP^s *blandina* plant, which was striped normally, one of the P^s *blandina* gametes must have come from P^r *blandina* by mutation of P^r to P^s, or else through crossing-over of P^s from *blandina*-A to standard *blandina*. The former is not at all likely; it would in fact be the first observation of such a mutation.

We may conclude that *blandina*-A contains a normal P^s gene whose activity is reduced by its relocation adjacent to chromosome material different from that in its usual situation. It would be desirable to have more plants of this critical cross-over type and also to know the behaviour of other P^s allelomorphs in relation to the *blandina*-A interchange. This work is in hand, but its success depends upon the chances of obtaining rare cross-overs.

We may also ascribe the other phenotypic characters exhibited by *blandina*-A, and indeed those of *blandina*-C when homozygous, to position effects. The behaviour of these two interchanges is in striking contrast to that of *blandina*-B, which is indistinguishable from standard *blandina* except by cytological means. It may also be noted that other induced interchanges in *blandina* have shown reduced viability of the gametes, pollen lethality or disadvantageous megaspore competition. All these may be manifestations of position effects. How far the peculiar features of *Oenothera* complexes are to be related to position effects is problematical. Much more information will be needed before we can attempt wider interpretations along these lines.

SUMMARY

In an X-ray induced interchange of *Oenothera blandina*, the capacity of the gene P^s for pigmenting the flower buds is reduced. One of the chromosome breaks is in arm 3 of chromosome 3.4, close to the locus of P^s. When the translocated P^s gene is transferred to normal *blandina* by crossing-over it recovers its normal activity. This is the first experimental demonstration of a position effect in plants.

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