A DUPLICATION AND A DEFICIENCY IN *OENOTHERA*

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(With Six Text-figures)

1. Introduction

In the study of the P^s-S position effect in *Oenothera blandina* (Catcheside, 1946, Table 1) a number of exceptional plants with modified expressions of the position effect abnormal in their inheritance were found. The evidence shows that in these plants the P^s-S region of the chromosome was duplicated. In addition corresponding deficiencies were found.

The term 'duplication' has been used for any genetic situation in which a small part of a chromosome has undergone doubling within the haploid set. A considerable variety of such duplications may be expected. The more frequently studied cases are those in which the duplication is *inter-chromosomal*, that is, the two duplicate chromosome portions are attached to separate centromeres. Two cases may be distinguished in this group. In one (Fig. 1 *a*) the duplication is inserted into another normal chromosome different from that from which it has been derived. Cytological evidence of this is provided by pairing and chiasma formation in haploids, e.g. of *Oenothera* (Catcheside, 1932). An example studied in *Drosophila melanogaster* by Dobzhansky (1934) is *Dp* (1; 3) 126, in which a section of the *X*-chromosome from *r* to the right of *B* has been inserted into the third chromosome between *st* and *cu*. In the other type (Fig. 1 *b*) the duplication is present as an extra fragment chromosome, as in certain clones of *Fritillaria imperialis* (Darlington, 1930) and numerous genetic stocks of *Drosophila melanogaster* such as *Dp* (1; *f*) 100 which has a

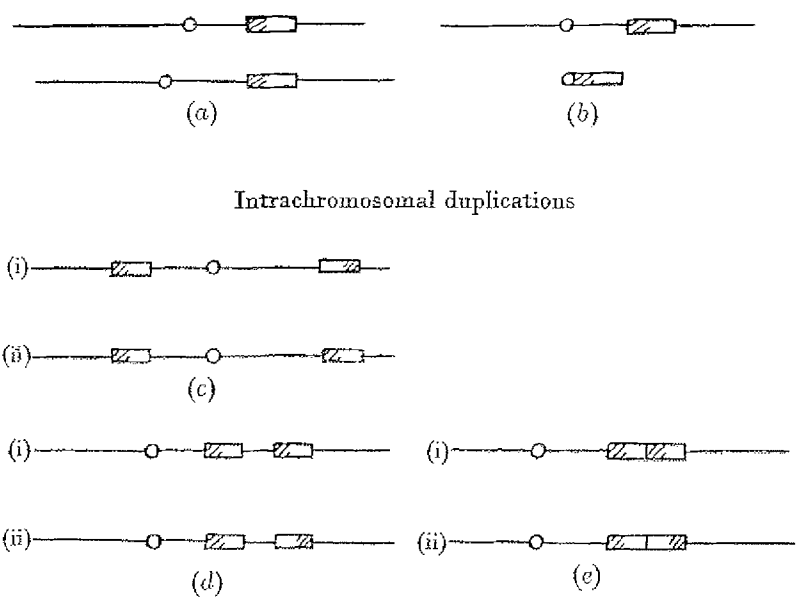

Fig. 1. Principal types of duplication possible in chromosomes. The centromere is shown by means of a circle and the duplicate segments by oblongs, the two ends of which are distinguished by shading.

large duplication two-thirds the length of the *X*-chromosome (Morgan, 1938). Bridges & Brehme (1944) list many others.

Less frequently studied, but no less important, are those cases in which the duplication is *intra-chromosomal*, with the two portions attached to the same centromere. The well-known 'repeats' observed in the salivary chromosomes of *D. melanogaster* (Bridges, 1935) come into this group. In these cases, the duplicated portions may be either *non-contiguous* or *contiguous* (Fig. 1*e*). Non-contiguous duplications may be within the same chromosome arm (Fig. 1*d*) or in separate chromosome arms (Fig. 1*c*). Finally, the two portions may be arranged in the same sense ((i) in Fig. 1) with respect to the centromere and be *direct*, or in opposite, i.e. relatively inverted, senses ((ii) in Fig. 1) and be *reverse*. Thus in the intra-chromosomal group six types of duplication should be distinguishable. In *D. melanogaster*, the two contiguous types are represented by Bar (Bridges, 1936), Hairy wing (Demerec & Hoover, 1939) and Confluens (Schultz, 1941), which are direct, and by eyeless-Dominant (Bridges, 1935) and Star-asteroid (Lewis, 1945), which are reverse.

Table 1. *Products of exceptional types of pairing and crossing-over in intra-chromosomal duplications*

Type of intra-chromosomal duplication	Products of: Intra-chromosomal pairing with crossing-over between duplicate segments in same chromatid	Products of: Unequal pairing of two homologous chromosomes *or* Intra-chromosomal pairing with crossing-over between duplicate segments in sister chromatids
Inter-arm direct	Inversion of region between duplicate segments	Duplication-deficiency chromosomes for regions distal to duplicate segments
Inter-arm reverse	Centric ring chromosomes of region between duplicate segments + acentric rod	Dicentric + acentric chromosomes
Intra-arm non-contiguous		
Direct	Acentric ring of region between duplicate segments + centric deficient chromosome	Contiguous direct duplication of region including duplicate segments + centric deficient chromosome
Reverse	Inversion of region between duplicate segments	Dicentric + acentric chromosomes
Intra-arm contiguous		
Direct ('Tandem')	Acentric ring + centric chromosomes with duplication singularized	Contiguous direct triplication + singularized chromosome
Reverse	Inversion of region	Dicentric + acentric chromosomes

Some duplications can arise by crossing-over from others. In general this is a special property of the intrachromosomal duplications, but in *D. melanogaster*, inter-arm duplications of the *X*-chromosome may arise from interchromosomal ones. Thus *Dp* (1; 1) 100, described by Morgan (1938), has the duplication attached to the right end of the *X*; it arose by crossing-over in an attached-*X* female carrying the duplication fragment of *Dp* (1; *f*) 100.

The intra-chromosomal duplications have various important properties, dependent upon pairing of the homologous duplicate segments in various ways followed by chiasma formation between the paired duplicate segments. The consequences are summarized in Table 1, the behaviour particular to our present case being described in more detail in § 4.

2. Proof of the duplication in *Oenothera blandina*

The origin of the plants, later interpreted as carrying a *P-S* duplication, was as follows. When *A* $P^r/+P^s$ plants, showing a P^r sepal colour variegation with deep red patches on a green background (Fig. 1*d* of previous paper), were pollinated by $+P^s$ plants, a small

fraction of the progeny proved to be of a novel and unexpected type, which may be called X. Their sepals had deep red patches on a medium red background (Fig. 1*e* of previous paper) and the gametes carrying the abnormality were pollen lethal and nearly inviable as megaspores. These plants had 14 chromosomes and possessed the interchange, and were not distinguishable cytologically from the normal interchange plants.

If we consider the petal colour phenotypes in type X plants coming from interchange heterozygotes with different *S* locus compositions and receiving $+P^s\ s$ in their regular 3.4 chromosome the following facts appear. The X plants from $A\ P^r\ S/+P^s\ S\times +P^s\ s$ have yellow petals (X-1); those from $A\ P^r\ S/+P^s\ s\times +P^s\ s$ have variegated yellow and sulphur petals (X-2); and those from $A\ P^r\ s/+P^s\ S\times +P^s\ s$ have yellow petals (X-3). Now the X-3 plants must have an *S* gene in the 3.11 chromosome and this chromosome must have received it from the 3.4 chromosome of the parent by crossing-over. Equally the X-2 plants have an S gene in their 3.11 chromosome, this time from the 3.11 chromosome of the parent. While X-2 and X-3 each have an *S* gene in their 3.11 chromosomes, there must be a difference between their 3.11 chromosomes since the phenotypic appearance of the petals is different in the two kinds of plants.

The explanation is finally shown when we consider progenies of X-1 plants that have been pollinated by $+P^s\ s$ (Table 4A). In this case, numerous cross-overs of *S* from the 3.11 chromosome to the 3.4 chromosome result in yellow-petalled non-interchange plants. The complementary cross-overs, in which chromosome 3.11 should have received *s* from chromosome 3.4, yield X-type plants with variegated yellow and sulphur petals. Therefore the 3.11 chromosomes of these cross-over plants still possess each an *S* gene, although they have each lost an *S* gene by crossing-over. The inference is that each of these 3.11 chromosomes possessed initially two *S* genes. *Hence, the S locus in the* 3.11 *chromosome of the* X*-type plants is duplicated.* Thus X-1 plants were *S S/s*, X-2 plants *S s/s* and X-3 plants *s S/s*, the locus of the interchange break being to the left of the duplication.

Further, the *S* gene is subject to position effect variegation only when it is situated in the proximal, or left-hand, segment of the duplication. This is shown by different phenotypes of the X-2 and X-3 plants.

That the *P* locus is also duplicated cannot be proved quite so directly. The medium red background to the sepals is circumstantial evidence that the plants carry a P^s gene on each of their 3.11 and 3.4 chromosomes, while the deep red patches are evidence of the presence of P^r in addition. Further evidence is provided by the facts that X plants arising from $A\ P^s\ S/+P^r\ S\times +P^s\ s$ have wholly deep red sepals and yellow petals, while those from $A\ P^s\ S/+P^r\ s\times +P^s\ s$ have wholly deep red sepals and variegated petals. The indication is that these X plants have P^r in the distal segment of the duplication, where it is not subject to variegation. The most satisfactory evidence would be provided by the progenies of the duplication $A\ P^r\ P^r/+P^s$, but these plants have not yet been available.

Recurrence of the same structural abnormality requires a regular origin by exceptional pairing and crossing-over. The origin of a duplication is most easily accounted for by unequal crossing-over, and this in turn requires a pre-existing duplication, i.e. a structural peculiarity that permits asymmetrical pairing. In the present case the duplication must be within the same arm and be direct, otherwise the products of unequal pairing (see Table 1) would be functionally or mechanically inviable. It is most probable that the pre-existing duplication was of the non-contiguous type, otherwise the normal chromosomes would already have a *P-S* duplication and the X types would therefore be triplications.

Complementary to the duplication we should expect to recover a deficient chromosome, provided it is haplo-viable. In the cultures, a deficiency of the P-S region has appeared several times. It provides corroborative evidence for the suggested method of origin of the duplication.

The 3.11+4.12 interchange, from heterozygotes of which the duplication arises, originated through X-radiation of a normal pollen grain having chromosomes 3.4 and 11.12. It is necessary to decide whether the non-contiguous duplication occurs naturally in *Oenothera blandina* or whether it was instead induced at the same time as the interchange. In favour of the former is the fact that the deficiency arises rarely from normal *blandina* itself which has never been in association with the interchange. It is unknown whether the duplication also appears in normal *blandina*, mainly because there has been no ready method available for its recognition. It is to be hoped that some fairly reliable method of recognition through an effect on pollen fertility may become available.

3. Behaviour of the P^s-S deficiency

Plants heterozygous for this deficiency are phenotypically distinct from normal *O. blandina* most conspicuously by their possession of nearly green buds. The whole plant is of a markedly brighter green and has a characteristically different habit. The leaves are relatively broader, and the plant is branched at about half-way up the stem rather than from

Table 2. *Progenies of deficiency plants*

Plant	Genotype	Type of progeny	Progenies
70/40 III 2	$+Df/+P^s$	F_2	27 P^s P^s *blandina*
		Backcross by P^s P^s *blandina*	27 P^s P^s *blandina*
93/34 II 3	$+Df/+P^s$	F_2	31 P^s P^s *blandina*
69/40 V 13	$A\ Df/+P^s$	F_2	27 P^s P^s *blandina*
		Backcross by P^s P^s *blandina*	26 P^s P^s *blandina*, 1 trisomic

the base. The anthers are thin and half the pollen is sterile, being empty and smaller than the normal pollen. Normal *blandina* is homozygous for the gene P^s and therefore shows broad medium red stripes on the sepals; the flower buds are thus predominantly a medium red colour. Plants heterozygous for the deficiency have green sepals with a slight red flush in their upper halves. Such plants, hemizygous for P^s, which have nearly green flower buds at the beginning of the season, become somewhat redder later in the season when the weather is cooler. There is little or no difference in bud pigmentation between plants hemizygous for P^s and those that are P^s P or P^s p. A deficiency for the P^s locus gives an effect similar to the P and p allelomorphs in respect to bud colour.

The deficiency also includes the neighbouring S locus. S S plants and hemizygous S plants have yellow petals, while s s and hemizygous s plants have sulphur coloured (pale yellow) petals.

Plants heterozygous for the deficiency fail to transmit it either through the pollen or through the embryo sac so far as is known. However, rather small numbers of progeny have so far been grown. The available data are collected in Table 2. The 50% of bad pollen evidently represents the expected deficiency pollen which has been killed by the deficiency. Since the deficiency is completely lethal to the pollen, the selfed families may be employed as though they were backcrosses to normal *blandina* as pollen parent. Hence there were no deficient eggs in a sample of 138 tested. It is improbable ($P = 0{\cdot}01$ level) that more than 5·2 such eggs could have been expected and therefore it is probable that

deficient eggs are not more than 3·8% of all functional eggs in plants heterozygous for the deficiency.

The absence of functional deficiency embryo-sacs requires a special explanation. The mere fact that zygotes heterozygous for the deficiency can arise shows that the deficiency is not lethal to the megaspore, embryo-sac or egg *per se*. Probably, instead, the deficiency megaspore is at a total (or nearly total) disadvantage relative to a normal gamete in competition for the production of an embryo-sac. A Renner effect may be expected or else a polarized segregation. Relative to a duplication gamete, however, it may be at a less serious disadvantage. In the origin of the deficiency by unequal crossing-over at megasporogenesis a corresponding duplication should arise, and the two would come into competition in embryo-sac formation in at least a proportion of the megaspore tetrads in which they originate.

Similar plants with a P^s-S deficiency in an interchange, 3.11 chromosome also occur (Table 2). Occasionally chimerical plants that are partly deficient and partly normal have also been observed. None has so far been analysed by growing progenies from it, but in all cases the appearance of the two parts of the plant suggests that the deficient sector has been derived from the normal sector by somatic loss of the P^s-S region. In no case was a whole chromosome missing, so it appears that we may be dealing with a case of intrachromosomal somatic crossing-over of a type analogous to that postulated in Table 1.

4. Behaviour of the P^s-S duplication

So far the duplication has been found only in interchange, 3.11 chromosomes where it may be detected by the reaction of the distal segment with the proximal segment in which P^s (or P^r) and S are subject to position effects. If the interchange is represented by the letter A and the normal chromosomes by +, a plant that is $A\ P^s/+P^s$ or $A\ P^r/+P^s$ shows variegation in its sepal colour with patches of red sharply separated by green tissue. In $A\ P^s/+P^s$ the red is a medium tone, while in $A\ P^r/+P^s$ the red is a deep one. Similarly in $A\ S/+s$ the petals are variegated yellow and pale yellow (sulphur colour), the boundaries again being sharply defined.

When the P^s-S segment is duplicated, sixteen gametic types of the duplication (Table 5) may be expected, depending upon the allelomorphs present at the four available loci. Several have been recognized and others may have occurred, but the evidence respecting them is inconclusive. Plants heterozygous for the interchange-with-duplication (hereafter referred to as *A-Dp*) are not distinguishable by any conspicuous characters of habit or foliage from corresponding plants lacking the duplication (referred to as *A*). There are slight differences, but it is doubtful whether they allow a reliable diagnosis. Two of the genotypes that have been identified are $A\ P^r\ S\ P^s\ S/+P^s$ and $A\ P^r\ S\ P^s\ s/+P^s\ s$. Both produce a type of sepal variegation in which the dark red areas of epidermal tissue are separated by medium red tissue instead of green tissue. It is as though the P^s genes painted in a medium red background to the dark red patches due to the P^r gene. This is the phenotypic effect that led ultimately to the recognition of the duplication.* The former genotype produces entirely yellow petals, the latter produces variegated yellow-sulphur petals.

* I am greatly indebted to Dr K. Mather (John Innes Horticultural Institution) for his suggestion of what has proved to be the composition of these plants. His idea was based upon knowledge of the patterns produced by two different flower-variegating genes together in the same plants of *Antirrhinum*.

A-Dp appears to be lethal to pollen grains. Heterozygotes, *A-Dp*/+, have considerably in excess of 50% bad pollen, actually about 60–70% bad compared with the 20% found in the normal interchange heterozygotes, *A*/+. In confirmation is the fact that *A-Dp* gametes are not transmitted through the pollen (Table 3B). Further *A-Dp* megaspores are at a marked disadvantage in competition with + megaspores, for *A-Dp* gametes constitute only a small minority of the functional eggs (Table 3A). This is one of the factors that makes work with the duplication rather troublesome.

Table 3A. *Progenies of A-Dp* $P^r\ P^s/+P^s \times +P^s/+P^s$

		♀ Progeny			
Family	Parents	$+P^s/+P^s$	$+P^r/+P^s$	$A\ P^s/+P^s$	$A\text{-}Dp\ P^r\ P^s/+P^s$
12/41	72/40 XI 8 × 1/40 I 1	135	1	1	17
23/42	72/40 XI 8 × 1/40 I 2	106	.	.	8
22/43	23/42 I 1 × 26/42 I 3	36	1	1	1
23/43	23/42 I 1 × 26/42 I 3	11	.	.	.
24/43	23/42 I 1 × 26/42 I 3	53	.	.	3
14/44	22/43 I 3 × 2/43 I 3	66	1	.	5
16/44	24/43 I 1 × 2/43 I 3	89	.	.	4
21/44	21/43 I 9 × 2/43 I 5	44	.	.	3
34/44	11/43 I 19 × 2/43 I 3	11	.	.	.
35/44	11/43 II 1 × 2/43 I 3	26	.	.	.
23/45	14/44 I 6 × 6/44 I 2	112	4	.	4
28/45	19/44 IV 7 × 6/44 I 2	65	1	.	1
29/45	19/44 III 22 × 6/44 I 2	66	2	.	4
30/45	19/44 III 20 × 6/44 I 2	70	2	.	.
31/45	21/44 I 1 × 6/44 I 4	91	1	.	2
32/45	25/44 I 2 × 6/44 I 4	31	.	.	.
33/45	33/44 II 18 × 6/44 I 2	22	1	.	.
34/45	33/44 IV 14 × 6/44 I 2	21	.	.	.
35/45	33/44 VII 12 × 6/44 I 2	65	1	.	6
	Totals	1120	15	2	58

Table 3B. *Progenies of* $+P^s/+P^s \times A\text{-}Dp\ P^r\ P^s/+P^s$

		Progeny			
Family	Parents	$+P^s/+P^s$	$+P^r/+P^s$	$A\ P^s/+P^s$	$A\text{-}Dp\ P^r\ P^s/+P^s$
15/44	2/43 I 3 × 22/43 I 3	50	.	.	.
17/44	2/43 I 3 × 24/43 I 1	72	.	.	.
22/45*	6/44 I 2 × 14/44 I 6	174 (95 *S*) (71 *s*)	1 (1 *S*)	.	.
	Totals	296	1	.	.

* This family from $+P^s/+P^s \times A\text{-}Dp\ P^r\ S\ P^s\ S/+P^s\ s$; 8 plants failed to flower.

5. The origin of the deficiency and the duplication

The repeated independent occurrence of what is undoubtedly the same deficiency (12 noted, but records probably incomplete) or the same duplication (24 recorded) requires a regular origin by crossing-over. We have seen that the facts can be accounted for if it is supposed that chromosome 3.4 of *O. blandina* has in arm 3 a small non-contiguous direct duplication with the two regions, x_1 and x_2, in one chromosome arm disposed either side of the P^s-S region (Fig. 2*a*). If pairing at zygotene in the 3.4 bivalent is occasionally irregular so that the x_1 segment of one chromosome pairs with the x_2 segment of the other and if crossing-over occurs in these abnormally associated segments, the cross-over products would be respectively a duplication and a deficiency. The duplication would have the structure $4.x_1\ P^s\ S\ x_1\ P^s\ S\ x_2\ 3$ (Fig. 2*b*) and the deficiency the structure $4.x_2\ 3$ (Fig. 2*c*). The bivalent giving such products would have a characteristic unequal chiasma

(Fig. 3). Such bivalents have not been seen in diploid *O. blandina* and might be difficult of detection if the segment $x_1\ P^s S$ were cytologically very short.

The occurrence of the duplication in the normal *blandina* chromosome set has not been observed. The phenotype of a plant heterozygous for such a duplication is a matter for conjecture. Analogy with the duplications recognized suggests it would be little if at all different in appearance from normal *O. blandina*.

Table 4A. *Progenies of A-Dp P^r S P^s S/ + P^s s × + P^s s/ + P^s s*

Family	Phenotypes	+ P^s S	+ P^s s	+ P^r S	+ P^r s	A-Dp P^{rer} S	A-Dp P^{rer} S^e	A-Dp P^{rer} s
23/45		82	30	4	.	1	3	.

TABLE 4B. *Progenies of A-Dp P^r S P^s s/ + P^s s × + P^s s/ + P^s s*

Family	Phenotypes	+ P^s S	+ P^s s	+ P^r S	+ P^r s	A-Dp P^{rer} S	A-Dp P^{rer} S^e	A-Dp P^{rer} s
28/45		(65)		1	.	.	1	.
29/45		(66)		2	.	.	3	1
30/45		(70)		2	.	.	.	.
34/45		(21)		.	.	.	.	.
35/45		(65)		. (1)	.	. (2)	4	.
Totals		(287)		5 (1)	.	. (2)	8	1

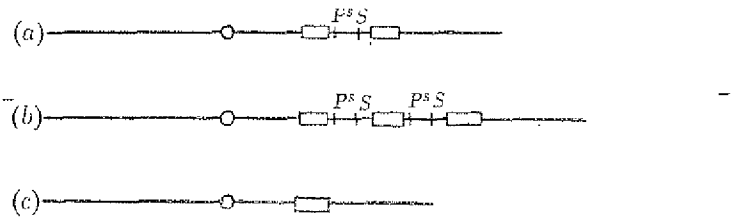

Fig. 2. Inferred structures of chromosome 3.4: (*a*) normal, (*b*) with *P-S* duplication, (*c*) with *P-S* deficiency.

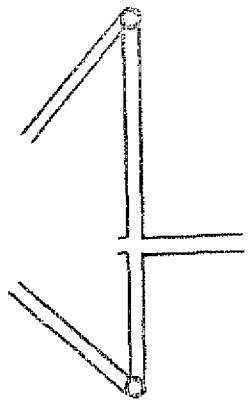

Fig. 3. Type of unequal bivalent expected to follow unequal pairing of duplicate segments.

All the duplications observed have originated in plants heterozygous for the 3.11–4.12 interchange that produces a position effect at the *P* and *S* loci. All the duplications occurred in the 3.11 chromosome of the interchange gamete and the presence of the extra segment was detected by its reaction with the position affected segment contiguous with it.

Actually, when unequal crossing-over occurs in a heterozygous interchange four abnormal products are possible, namely, (1) duplication in interchange 3.11 chromosome, (2) duplication in normal 3.4 chromosome, (3) deficiency in interchange 3.11 chromosome

and (4) deficiency in normal 3.4 chromosome. All except type (2) have been found. Types (2) and (4) should be identical with those arising from unequal crossing over in normal *O. blandina*.

The origin of these four possible types depends upon the fact that two kinds of unequal cross-over figure ought to be possible. These are shown in Fig. 4. Clearly the expected duplications in an interchange chromosome should have the *P-S* segment ($P_1 S_1$ of Fig. 4) of the original interchange chromosome proximal to the *P-S* segment ($P_0 S_0$ of Fig. 4) from the original normal chromosome.

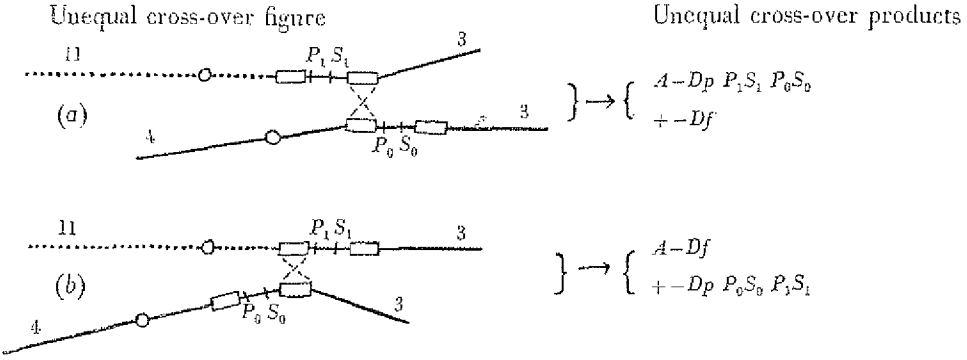

Fig. 4. Types of unequal pairing capable of yielding by crossing-over: (*a*) interchange-duplication and (*b*) interchange-deficiency chromosomes.

Table 5. *Genotypes, phenotypes, cross-over duplication products and origins of duplications in* Oenothera blandina

	Genotype of gamete	Expected phenotype with $+^{Ps}\ s$		Expected *A-Dp* cross-overs from heterozygote *A-Dp*/$+^{Ps}\ s$		Most likely *A*/+ genotype yielding *A-Dp* chromosome
				Genotypes	Phenotypes	
1	*A-Dp* $P^s S\ P^s S$	1	*A* $P^s S$	2	II	*A* $P^s S$/$+P^s S$
2	$P^s S\ P^s s$	II	$P^s S^e$	—	—	*A* $P^s S$/$+P^s s$
3	$P^s S\ P^r S$	III	$P^r S$	4 and 2	IV and II	*A* $P^s S$/$+P^r S$
4	$P^s S\ P^r s$	IV	$P^r S^e$	2	II	*A* $P^s S$/$+P^r s$
5	$P^s s\ P^s S$	I	$P^s S$	6	V	*A* $P^s s$/$+P^s S$
6	$P^s s\ P^s s$	V	$P^s s$	—	—	*A* $P^s s$/$+P^s s$
7	$P^s s\ P^r S$	III	$P^r S$	8 and 6	VI and V	*A* $P^s s$/$+P^r S$
8	$P^s s\ P^r s$	VI	$P^r s$	6	V	*A* $P^s s$/$+P^r s$
9	$P^r S\ P^s S$	VII	$P^{rsr} S$	10	VIII	*A* $P^r S$/$+P^s S$
10	$P^r S\ P^s s$	VIII	$P^{rsr} S^e$	—	—	*A* $P^r S$/$+P^s s$
11	$P^r S\ P^r S$	III	$P^r S$	12 and 10	IV and VIII	*A* $P^r S$/$+P^r S$
12	$P^r S\ P^r s$	IV	$P^r S^e$	10	VIII	*A* $P^r S$/$+P^r s$
13	$P^r s\ P^s S$	VII	$P^{rsr} S$	14	IX	*A* $P^r s$/$+P^s S$
14	$P^r s\ P^s s$	IX	$P^{rsr} s$	—	—	*A* $P^r s$/$+P^s s$
15	$P^r s\ P^r S$	III	$P^r S$	16 and 10	VI and VIII	*A* $P^r s$/$+P^r S$
16	$P^r s\ P^r s$	VI	$P^r s$	14	IX	*A* $P^r s$/$+P^r s$

In Table 5 are listed all the sixteen possible interchange chromosome duplications, together with their expected phenotypes (numbers I–IX) when combined with $+^{Ps}\ s$. It will be noticed that four phenotypes (II, V, VIII and IX) should each have a unique genotype, four others (I, IV, VI and VII) should each have two genotypes, while one phenotype (III) should have four genotypes. In the fifth column of this table are given the genotypes of the most probable interchange heterozygote parents (*A*/+) deduced on the principles enunciated above (Fig. 4). When the observed duplications are tabulated on this basis (Table 6) it is found that nineteen are of the expected composition and only five are exceptional, requiring a more complicated origin that will not be discussed at

present. That the majority are of the expected types constitutes an argument strongly in favour of the above framework of principles.

It seems possible that where a given phenotype includes two or four genotypes, the latter might be distinguishable by means of the kinds of cross-overs arising from an *A-Dp*/+ P^s *s* heterozygote and observed in a backcross to + P^s *s blandina*. Two types of pairing may be expected in such a heterozygote. In one case (Fig. 5*a*) the distal duplicate segment of the interchange 3.11 chromosome pairs with the corresponding region of the normal 3.4 chromosome, in the other case (Fig. 5*b*) the proximal segment of 3.11 is so

Table 6. *Relation between genotype of duplications observed and the interchange heterozygotes that have generated them*

	Duplications observed					
	Expected			Not expected		
Interchange heterozygote parent	Composition	Phenotype with + P^s *s*	No. observed	Composition	Phenotype with + P^s *s*	No. observed
A P^s *S*/+ P^r *S*	*A-Dp* P^s *S* P^r *S*	*A* P^r *S*	2	*A-Dp* P^r *S* P^s *S*	*A* P^{rsr} *S*	2
A P^r *S*/+ P^s *s*	*A-Dp* P^r *S* P^s *s*	*A* P^{rsr} S^s	5	*A-Dp* P^r (*S*) P^s S^*	*A* P^{rsr} *S*	1
A P^r *s*/+ P^s *S*	*A-Dp* P^r *s* P^s *S*	*A* P^{rsr} *S*	2	—	—	—
A P^s *S*/+ P^r *s*	*A-Dp* P^s *S* P^r *s*	*A* P^r S^s	9	*A-Dp* P^s *S* P^s *S*	*A* P^s *S*	2
A P^r *S*/+ P^s *S*	*A-Dp* P^r *S* P^s *S*	*A* P^{rsr} *S*	1	—	—	—

* No data are available to show whether the proximal *S*-locus gene was *S* or *s*.

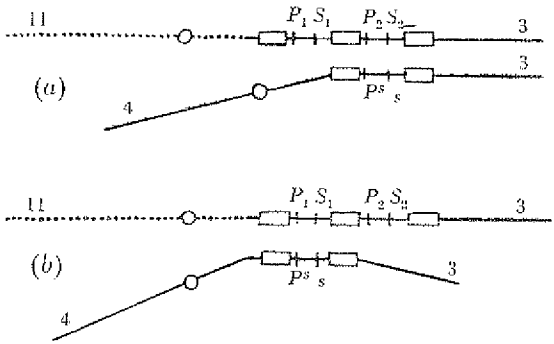

Fig. 5. The two types of pairing possible between an interchange-duplication chromosome and a normal chromosome.

paired. Following crossing-over in the first type the interchange chromosome product would carry the duplication, and would have *s* or P^s and *s* replacing the *P* and *S* locus genes present in the distal part of the duplication. The expectations listed in columns 3 and 4 of Table 5 have been deduced on this basis. Crossing-over in the other type of pairing would yield an interchange chromosome without the duplication and with *s* or P^s and *s* replacing the *P* and *S* locus genes present in the proximal part of the parent duplication. The records suggest that the former type of pairing is by far the commoner one, very few of the cross-overs losing the duplication in the process of their production. The data are, however, not highly critical on the point. The facts are that *A-Dp* P^r P^s/+ P^s gave only 2 *A* P^s chromosomes compared with 58 *A-Dp* P^r P^s chromosomes, while *A-Dp* P^s P^r/+ P^s gave 18 *A-Dp* P^s P^r compared with 10 *A* P^s or *A-Dp* P^s P^s chromosomes (of which probably a majority carried the duplication). It appears that crossing-over between the *P-S* region of a normal chromosome and the distal *P-S* region of an *A-Dp* chromosome is exceptionally frequent. This is also demonstrated by the structurally

homozygous progeny (§ 7). The behaviour supports the view that in *Oenothera* pairing normally commences at the ends of the chromosomes and progresses towards the centromere.

6. Position effect in the duplication

The genes P^s or P^r and S when adjacent to the interchange break are subject to a position effect of the variegation type. These genes show the same behaviour when they are in the interchange-duplication (*A-Dp*) chromosome, but only if they are in the proximal segment of the duplication. When the genes are in the distal segment of the duplication they show no variegation. This is demonstrated by *A-Dp* P^s $P^r/+P^s$ plants which have entirely deep red sepals whereas *A-Dp* P^s $P^s/+P^s$ plants have regular medium red striped buds, and by *A-Dp* s $S/+s$ plants which have wholly yellow petals while *A-Dp* s $s/+s$ plants have sulphur petals. An *A-Dp* P^r $P^s/+P^s$ plant has sepals with patches of deep red disposed like the red patches of *A* $P^r/+P^s$. The intervening areas are uniformly a medium red such as would be expected if the P^s genes were uniformly pigmenting the sepals as in $+P^s/+P^s$ and not showing a variegation as in *A* $P^s/+P^s$, with green tissue intervening between the blocks of medium red. If both P^r and P^s in the *A-Dp* chromosome were showing variegation one would expect to see at least some small well-defined green areas where the deep red of P^r variegation and the medium red of P^s variegation were both absent.

Thus there is a difference in position effect of the same genes in the two sections of the *A-Dp* chromosome. Whatever determines the variegation position effect, presumed to be heterochromatinization sometimes inhibiting the gene, it is evident that it does not spread beyond the end of the proximal segment. Further it is improbable that the x_1 and x_2 segments are heterochromatic, otherwise one would expect that the P and S genes within the distal segment of the *A-Dp* chromosome would also show variegation. The duplication has therefore rather little significance for the interpretation of the position effect. It does show that the effect cannot depend upon the non-contiguous duplicate segments of the *A* chromosome and confirms the belief that the position effect depends solely on the genes being adjacent to heterochromatin.

7. Crossing-over in the duplication

A detailed consideration of the various duplication genotypes and their progenies is hardly profitable at the present time since rather few critical data have been obtained. It is possible that no less than eight of the gametic types (numbers 1, 2, 3, 4, 9, 10, 13 and 14) have occurred in the cultures, but experiments have been confined to just a few of them (especially numbers 3, 9 and 10). The available evidence shows that crossing-over in a duplication heterozygote is very peculiar, partly because it mainly involves the distal segment of the duplication (see § 4) and partly because crossing-over is very free giving recombination fractions apparently in excess of 50%.

Tables 7A and 7B incorporate the data available from the backcrosses

$$A\text{-}Dp\ P^s\ P^r/+P^s \times +P^s/+P^s \quad \text{and} \quad A\text{-}Dp\ P^s\ S\ P^r\ s/+P^s\ s \times +P^s\ s/+P^s\ s.$$

Confining attention to the normal, non-interchange and presumably non-duplication progeny there are seventy-eight cross-overs between P^r and the interchange locus out of a total of 166 plants, i.e. 47% recombination. Further, there are fourteen cross-overs between S and the interchange locus in a total of 127 plants, i.e. 11% recombination. The latter, most surprisingly, is about the same as the 8·5% found in *A* $S/+s$ heterozygotes.

In duplications of the type *A-Dp* P^r $P^s/+P^s$ (Table 3A), 15 cross-overs between P^r and the locus of the interchange were found in a total of 1135 plants, i.e. 1·3 % recombination, again a value close to the 1·75 % found in *A* $P^r/+P^s$ plants. In one family (Table 4A) there were 86 cross-overs between the distal *S* locus and the interchange locus in a total of 116 plants, i.e. 74 % recombination. A reciprocal (Table 3B) showed 96 cross-overs

Table 7A. *Progenies of A-Dp* P^s $P^r/+P^s \times +P^s/+P^s$

Family	Parents	Progeny: $+P^s/+P^s$	$+P^r/+P^s$	*A* $P^s/+P^{s*}$	*A-Dp* P^s $P^r/+P^s$
1/41	69/40 II 12 self	5	19	1	.
2/41	69/40 II 12 × 1/40 I 2	6	12	.	1
36/44	12/43 III 38 × 2/43 I 3	9	2	.	1
37/44	13/43 III 1 × 2/43 I 5	15	11	.	2
38/44	13/43 III 35 × 2/43 I 3	6	6	.	5
39/44	13/43 IV 2 × 2/43 I 5	20	12	4	1
41/44	14/43 IV 13 × 2/43 I 5	4	5	.	1
43/44	15/43 V 7 × 2/43 I 3	28	30	6	7
	Totals	88	78	10	18

* Some or all these plants may be *A-Dp* P^s $P^s/+P^s$.

Table 7B. *Progenies of A-Dp* P^s *S* P^r *s*$/+P^s$ *s* $\times +P^s$ *s*$/+P^s$ *s*

Family	Phenotype $+P^s$ *S*		$+P^s$ *s*	$+P^r$ *S*		$+P^r$ *s*	*A-Dp* (?) P^s S^c	*A-Dp* P^r S^c
36/44	0	(1)*	8	.		2	.	1
37/44	1	(2)	12	.		11	.	2
38/44	2		4	1		5	.	5
39/44	3		17	2		10	4	1
41/44	.		4	3		2	.	1
43/44	2	(9)	17	.	(9)	21	3 (3)	6 (1)
Totals	8	(12)	62	6	(9)	51	7 (3)	16 (1)

* Numbers in brackets are of plants that failed to flower and so could not be scored for *S*.

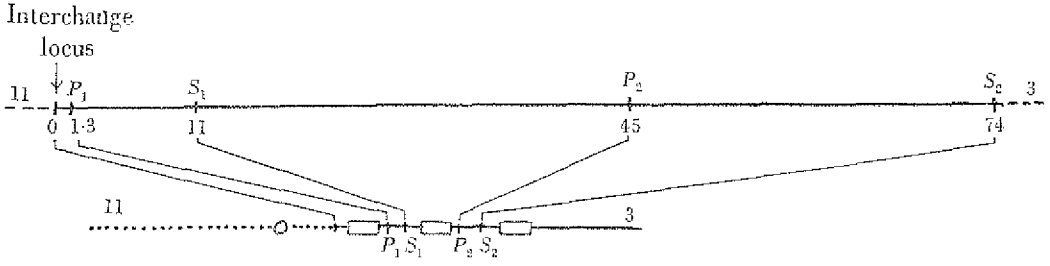

Fig. 6. Preliminary genetical and cytological maps of the interchange-duplication chromosome.

amongst 167 plants, i.e. 57·5 % recombination. These are undoubtedly excessive estimates due to *s* gametes being less viable than *S* gametes. Material in which the viability effect can be estimated is not yet available, so the further consideration of the linkage map of the duplication (Fig. 6) and of the possibility of recombination exceeding 50 % must be deferred. However it does appear that there are local changes in ease of crossing-over along the chromosome.

8. SUMMARY

A direct contiguous duplication of the P^s-*S* region of chromosome arm 3 in *Oenothera blandina* has been found genetically in the interchange chromosome 3.11. Twenty-four separate occurrences of it in the progeny of the interchange heterozygote have been

observed and indicate that the duplication must arise by unequal crossing-over. A corresponding deficiency of the P^s-S region from chromosomes 3.4 and 3.11 has also been found. Crossing-over within the duplication is surprisingly frequent and exhibits various anomalies.

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