CHAPTER 2 DEFICIENCIES AND DUPLICATIONS

Types of structural change

Chromosome structure has been defined by Darlington (1929) as "the potentially linear order of the particles, chromomeres, or genes in the chromosomes". Detailed genetic and cytological analysis of individuals belonging to related species or to isolated populations within a species have shown that they may differ by various kinds of changes in that structure. Exposure to natural and other radiations and to certain chemicals also has resulted in rearrangements of chromosome segments. There are several restrictions on the possible rearrangements that may survive. Only broken ends of chromosomes seem to be capable of fusion, the new arrangements must not carry too great a deficiency or duplication, that is, absence or duplication of genetic material respectively; and as a rule the new arrangement is perpetuated only when one centromere is present per chromosome. Individuals heterozygous for any of the changes have been termed structural hybrids. The change may involve inversion, deletion, or transposition. Inversion is a change in the linear sequence of the genes such that the genes in a segment are in reverse order. Deletion is the loss of a chromosome segment. Transposition is the transfer of a segment to a new position. The segment may be inserted, or it may become attached at the end of a broken chromosome with or without an exchange of segments. When an exchange has occurred, it is termed a chromosomal interchange, a reciprocal translocation, or simply interchange or translocation.

The term interchange will be used here whenever an exchange is known to be present. Based on the definition of structural change given by Darlington and Mather (1949), the changes may be summarized as follows: with respect to arms, the change may be within the same or different arms. With respect to chromosomes, it may be within a single chromosome, between the members of an homologous pair, or between non-homologues. With respect to possession of a centromere, the piece involved may be with or without a centromere. The linear sequence of genes with respect to the centromere may be the same as before or it may be reversed in the transposed segment. The transposition may produce a balanced structural change which can perpetuate itself as in an inversion or in an exchange of terminal or internal segments; or it may produce an unbalanced change, for example a deficient chromosome or one that is acentric (without a centromere) or dicentric (with two centromeres). Acentric chromosomes do not migrate toward either pole as the nucleus divides. They are lost, probably before the next division. The dicentric ones do not survive many cell divisions, except when the centromeres are close together (Steinitz-Sears 1953, Steinitz-Sears 1959). Also, subsequent segregation, or crossing over followed by segregation may produce spores or gametes carrying duplications or deficiencies.

Treatments of diploid tissue with mutagenic agents might be expected to produce all of the types listed above, but treatments of haploid cells or tissue would be expected to produce only those changes that are within a chromosome or between non-homologues unless the chromosome is double or reacts as if it were double at the time of treatment.

With the increased interest in radiations and other mutagenic agents as tools in genetics and in plant and animal breeding, it becomes important to become familiar with the findings as to the kinds of chromosome changes produced in Drosophila, maize, and any other organisms in which detailed cytological and genetical analyses have been made.

Duplication

This term refers to the gain of a segment of a chromosome. The resulting nucleus, cell, tissue, or individual is said to be hyperploid for that segment.

Origin and breeding behavior

A chromosome segment might be transposed and inserted in various positions in its homologue or to a non-homologue, as shown for the 34 segment in Fig. 4 A to D.

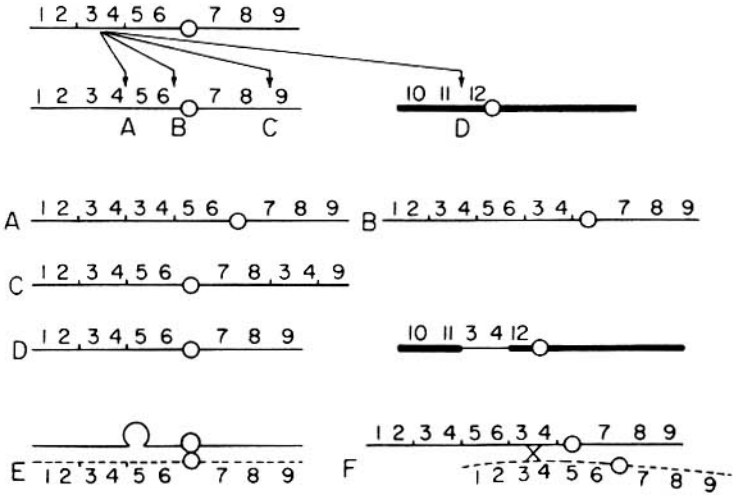


FIG. 4. Types of transpositions (insertions) which may lead to duplication.
A. tandem duplication
B. duplication in the same arm
C. duplication in a different arm
D. duplication in a non-homologue
E. diagram of pachytene configuration in an individual heterozygous for the duplication shown in A, F diagram showing the origin of an additional duplication by crossing over. The shift of a centromere alone has not been shown.

If the original transposition had been to a different position in the same chromosome, the duplication shown in Fig. 4 B or C may arise later by crossing over with the normal homologue. If the segment has been inserted in a non-homologue, the donor chromosome has a deficiency. A gamete which includes the recipient and the normal homologue of the donor will carry a duplication. In a stock carrying the duplication such as that shown in C, a cross over between the inserted segment and its homologous segment within the same chromosome may produce a ring and an acentric chromosome.

Different results from crossing over are expected if the inserted segment is in reverse order to those shown in Fig. 4 A to D. For example in Fig. 4 D, a crossover between the two 34 segments gives rise to two interchanged chromosomes, each with one centromere. If the transposed 34 segment is in reverse order, a dicentric and an acentric chromosome are expected. Richardson (1936) has presented diagrams also.

If one member of a pair of homologues has its centromere transposed to a new position in the same chromosome, a crossover that occurs in the region between the two centromeres will produce a dicentric and an acentric chromosome also (Patterson and Stone 1952). No inversion-type pachytene pairing is expected in the heterozygote.

In an individual heterozygous for the duplication shown in Fig. 4 B or C, crossing over between a 3 4 segment in one chromosome and the 3 4 segment in a different position in its homologue produces a chromosome with a duplication also for the 5 6 region between the 3 4 segments as ~~well as an increase in the number of 3 4~~ segments. For the type of chromosome in Fig. 4 C, this would result in a dicentric and an acentric chromosome.

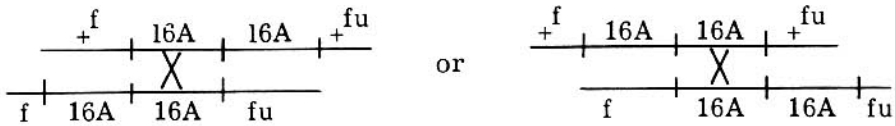
Duplications may arise also if the members of a pair of homologues exchange unequal terminal segments of the same arm, e.g. under X-ray treatment. Tandem repeats may originate in this manner as well as by insertion.

The first duplications were described by Bridges (1919) in *Drosophila melanogaster*. In these a segment of one chromosome was transposed to another location. Bridges (1935) also observed in salivary gland smears of normal stocks fine connecting strands between certain segments (see his drawing of the 2L arm). Since the banding patterns in those segments were similar, he concluded that they were duplications or "repeats", the two homologous segments having been closely associated before smearing.

Phenotypic effects

Several dominant characters in *Drosophila* are associated with duplications in the heterozygous condition. The eyeless-dominant (ey^D) character in *Drosophila* has a segment of unknown source inserted in the middle of chromosome 4 (Sturtevant 1936). The inserted segment is probably a reversed repeat since it bends back on itself in synapsis. The Theta duplication has a segment of the X including the normal alleles of *y*, *sc*, and *bb* attached at or near the centromere of the X to form a short arm (Bridges and Brehme 1944). The Pale character, a specific diluter of eosin eye color, is associated with an insertion of a segment of chromosome 2 into 3. On outcrossing, gametes carrying a duplication are produced.

Another character in *Drosophila* associated with a duplication is Bar eye (B) which is located in the X chromosome and reduces the number of facets in the eye. The original Bar mutation was reported by Tice (1914). Occasional mutations from Bar to normal and to a more extreme type, double-Bar, were observed by Zeleny (1920). By using the adjacent markers, forked bristle (f) and fused veins (fu), Sturtevant (1925, 1928) showed that these mutants were invariably accompanied by crossing over between *f* and *fu*. Also when another allele of Bar, infra-Bar (B^i) became available, he demonstrated that the mutations to normal or the double-type could produce the f B B^i fu or f B^i B fu order in the chromosome. He concluded that unequal crossing over provided the best explanation. It wasn't until 1936 that cytological studies of the salivary chromosomes revealed that these conclusions were correct. The original Bar mutation had a duplication for bands 1 to 6 in section 16A in the X chromosome (Bridges 1936, Muller 1936). The two segments were adjacent to each other and in the same relative order, termed a tandem repeat, as $+\frac{1\ 2\ 3\ 4\ 5\ 6}{16A}+\frac{1\ 2\ 3\ 4\ 5\ 6}{16A}+$. Sutton (1943) found that only the 16 A1-2 bands were essential for the Bar effect. One of the features in the behavior of duplications that are tandem repeats is that when homozygous for the duplication, unequal crossing over occurs occasionally. One of the resulting chromatids has an additional segment, the other has lost one such segment. Such crossovers in the 16 A1-6 segment in a Bar homozygote are indicated in the following diagrams:



Each gives rise to the same two crossovers, but the combinations of outside markers are different. Both types occurred (Sturtevant 1928).

Another feature associated with the Bar duplication is a position effect, a phenomenon first described by Sturtevant (1925). The effect on facet number is different for different distributions of the duplicated segments between the two X chromosomes.

Another such character in Drosophila is Confluens (Co), irregularly thickened veins with some fusion of veins (Schultz 1941). It is a tandem duplication for a section of chromosome 3 including bands 3 C_5 to 3 D_6. The Co phenotype results from duplication of the 3 C_7 band. Deficiency for that band gives a Notch phenotype. Abruptex (Ax), shortened wing veins, is a duplication of this same 3 C_7 band. Hairy wing (Hw) is a tandem duplication of the 1 B 1-2 doublet close to the gene for yellow body (y) (Demerec and Hoover 1939). Certain duplications in Drosophila are fully viable and fertile in both sexes, others are low in viability or in fertility; others are viable in females, but nearly lethal in males.

Nothing comparable to the dominants described above is known in plants at this time. Laughnan (1955) has presented evidence that the A_1 locus in corn has two components, and that certain mutants have lost one of them.

Natural occurrence

Suggestive genetic evidence for the presence of duplications in certain plant species is furnished by the finding of polymeric genes (identical and with cumulative effects). Lamprecht (1953) reported in Pisum five different instances in which a character is determined by two such genes, segregation in F_2 being 15:1 or 9:7. For example F and Fs are two genes which with A give violet flecks on the seed coat; Wa and Wb produce weak growth of leaves and stem. In several of these, the linkage relations of the members of a duplicate set have been determined.

In maize Rhoades (1951) reported linkage data for two duplicate genes (pg 11 and pg 12) and reviewed the previous data. Two of the 14 sets of duplicate loci are located in the same chromosome, w_5, w_6 in 6, and fr_1, fr_2 in 7. His data on pg 11 and pg 12 place pg 12 in the long arm of 9 and pg 11 in the long arm of 6 near w_5 or w_6. The evidence suggests that a segment in the long arm of 9 is a duplication of a segment in the long arm of 6, and that a nearby segment in 6 has been duplicated in the same chromosome.

The interchanges that have occurred naturally in maize, and probably also in Pisum, do not indicate a recurrence of the same or a few interchanges as expected if they arise by crossing over between duplicated segments.

Additional evidence suggesting the presence of duplications in normal diploid plant species has come from cytological observations on haploids. In haploids, occasional rod-shaped or even ring bivalents, are observed at meiosis, whereas only univalents are expected. The presence of duplication could explain such pairing. It would be between partially homologous, i.e. homoeologous, chromosomes. Interchange heterozygotes have been found in the diploid progeny of haploids in maize (Alexander 1956), but the chromosome identification has not been completed.

Other sources

Duplications or duplications plus deficiences may occur among the progeny of other types of structural heterozygotes. The Duplication + Deficiency (Dp-Df) gametes from interchange heterozygotes, in which one or both exchanged segments are short may produce viable offspring. Several of these are listed for Drosophila by Bridges and Brehme (1944) under duplications. These have been referred to as aneuploids in the Drosophila literature, although the chromosome number was normal. They have been reported for maize also (Patterson 1952, Burnham 1932) (see pages 73 and 102).

Also duplications and deficiencies may be found in the progeny of crosses between stocks carrying different interchanges that involve the same chromosomes but with breaks at different loci (see page 107).

Crosses between stocks carrying different inversions involving the same chromosome and in which a segment of the inverted section in one chromosome is common to a segment of the inverted section in the other chromosome (overlapping) are expected to yield duplications and/or deficiencies; some of which may be viable (see page 46).

In Drosophila an individual with an attached-X chromosome and a centromere-bearing fragment of the X may produce by crossing over an exchange in which the fragment is substituted for one arm of the attached-X. In this manner XX flies with duplications for various portions of the X have been obtained for studies of their effects on sex expression, and other characters, for example Dp (1:1) 100 reported by L. V. Morgan (1938) (see Dobzhansky and Schultz 1934).

Individuals with an additional chromosome or a chromosomal fragment with its own centromere are also duplication heterozygotes; but they will be discussed in Chapter 6 which deals with extra chromosomes. There it will be noted that in plants also the addition of a whole chromosome or one arm frequently produces a new dominant phenotype which includes a complex of characters. This is somewhat similar to the dominant "mutations" in Drosophila which have a short duplication.

Deficiency

The loss of a segment of a chromosome or of one or more chromosomes is termed a deficiency. Deficiency-heterozygotes are said to be hemizygous for the missing loci, a term coined by Stadler.

Genetical and cytological tests

This phenomenon was reported first in Drosophila by Bridges (1917). One female from the cross of normal ♀ x w^B/Y ♂ failed to inherit Bar (B) from the male parent, although it did inherit white (w). This female, when crossed, produced a ratio of 2 ♀ : 1 ♂. The missing males were those which had received the X chromosome with the missing Bar gene. Bridges stated that these results could be explained by the loss or inactivation of a section of the X chromosome which carried B and additional alleles necessary for the life of the fly. When heterozygous females were crossed with males carrying rudimentary, or forked, or fused, genes close to Bar as the following map shows:

1.1	55.1	56.5	57.0	59.5
w	r	f	B	fu

, only the crosses with forked produced forked female offspring in F_1 (Bridges 1917). Hence the loss did not include the r or fu loci but

did include f. Females with a normal X carrying Bar and a deficient X produced no crossovers between Bar and forked as shown by the absence of fB or wild type recombinants from the cross with forked males. Females that were $\frac{w \text{ (defic.)}}{+^{w}}$ produced 207 $+^{w}$ and 146 w sons, an inequality of the contrary classes. The white-eyed sons must have resulted from crossing over between w and the deficiency, and represent 41.4% of the sons. The deficiency also had an adverse effect on the viability of heterozygous females.

The extent of the deficiency may be tested not only by crossing with genetic markers known to be in the same general region of the linkage map as shown above, but also by tests for neutralization of the lethal effect by duplications.

Many deficiencies that have been reported since in Drosophila are listed under the Df designation in the list of mutants by Bridges and Brehme (1944). Certain deficiencies are listed also among the lethals, designated as l.

In seed plants, the progeny from pollinations made with X-rayed mature pollen include plants carrying deficiencies which are not transmissible to the next generation. The conclusion is that a pollen grain carrying a deficient sperm is able to accomplish normal fertilization, but the endosperm or embryo will carry the deficiency. If the female parent carries recessive markers, whenever the deficiency carried by the male gamete includes the locus for one of those markers that character is expressed in F_1. This has been termed "pseudodominance".

Progeny from pollinations made in corn with X-rayed pollen as described; and also from X-ray treatment of developing seeds (applied 20 to 50 hours after pollination) from the cross of recessive by dominant markers were grown by Stadler. Plants showing a recessive character were examined cytologically by McClintock (1931). In one such experiment in which the developing seeds were from the cross of yPl by Ypl; two seedlings among a total of 734 were pl, the others all Pl. In one of the pl plants the distal .6 of the long arm of chromosome 6 including Pl had been lost (Fig. 5B). Pollen abortion was about 54%. In another progeny, a glossy seedling appeared.* Cytological examination showed a hump not far from the centromere in the long arm of chromosome 7 (Fig. 5A). The hump may vary somewhat in position with a consequent variation in the amount of non-homologous association, but the general position of the locus can be determined. A short subterminal deficiency may have the same cytological appearance as a short terminal one, unless the chromomeres in the region are distinctive or there is a prominent terminal marker. Examination of several occurrences of deficiency for a particular locus, usually will determine ~~its~~ the position of the gene with greater accuracy.

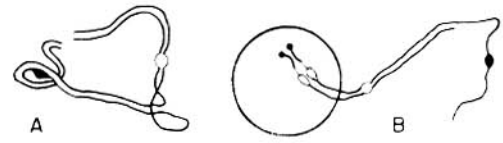


FIG. 5. Camera lucida outline sketches of synapsis of the normal chromosome with its homologue that is deficient.
A. Chromosome 7 deficient for an internal segment including the locus of gl_1 v_5 in the long arm.
B. Chromosome 6 deficient for a terminal segment including the locus of Pl in the long arm. After McClintock, 1931, Mo. Res. Bull. 163: Fig. 19, p. 17 and Fig. 11, p. 12.

If the deficiency is lethal to both pollen and ovules, only normal offspring are produced. Deficiencies that cause less defective gametophyte development are produced also in plants. One of these was described as a haplo-viable deficiency by Stadler (1933, 1935). The plant was heterozygous for a long terminal deficiency (about .78) of the long arm of chromosome 10. It had about 50% of small-sized but starch-filled pollen. The deficiency was not transmitted through the pollen, but was through the ovules. The deficient embryo

* This seedling was glossy and virescent (McClintock, personal comm.)

sacs were smaller but completed the three divisions. Seed-set was good although less than on a normal ear.

Essentially the same technique for locating genes has been used in Drosophila. X-rayed males homozygous for the dominant alleles were crossed on females carrying the recessives. F_1 flies that showed the recessive character were crossed again to the multiple recessive stock and the larvae examined cytologically using the salivary glands. Deficiencies could be recognized by the fact that one member of the synapsed pair was shorter and had a section with certain bands missing (Mackensen, 1935, Alexander, 1959).

Phenotypic effects

In Drosophila many of the dominant characters are associated with small deficiencies in heterozygous condition, e.g. the Minutes (short, fine bristles), Delta (wing veins broadened at junction with margin), Gull (spread wings), Notch (notched wing margins, 131 listed, all in X), Plexate (plexus-like thickening of veins at margin), and Vein (gap in one vein). Pale, a specific diluter of eosin eye color, is associated with a deficiency and a transposed segment. Deficiencies are usually lethal when homozygous, and include a complex of secondary effects. If in the X chromosome, they may be lethal to males. One good example is the class of mutants known as Minutes. Ninety are listed, some in each of the four chromosomes. Heterozygotes have short, fine bristles and in addition secondary effects "such as small body size, larger, somewhat rougher eyes, missing aristae, thin-textured wings with a tendency to plexus venation, missing bristles (usually postverticals), and sterility or low fertility, especially in females" (p. 121, Bridges and Brehme 1944). Certain Minutes may: increase the frequency of somatic segregation, enhance dominance of certain recessive wing venation or bristle characters; or produce a complementary dominant lethal effect with other dominant characters. For most of those given a careful salivary gland chromosome analysis there was a deficiency for one up to several bands. When the deficiency is in the chromocentric portion of any of the chromosomes, the analysis for the extent of the deficiency is not critical. An example of this is Schultz' Minute M (2) S 10 (M = minute; (2) = chromosome 2; S 10 designates this Minute). The heterozygote shows no crossing over in the region from light at 55.0 to cinnabar 57.5, but there is no detectable change in the salivary-gland chromosomes. Metaphase chromosomes show a deficiency of the heterochromatin of the right arm.

Behavior of deficiency homozygotes

In somatic segregation experiments certain X-chromosome loci for lethals were found to act as cell lethals, although surrounded by normal tissue (Demerec 1934, 1936). These were lethal in the hypodermal cells which normally develop the yellow body color used as the linked marker. They were not necessarily lethal in other tissues.

Of 15 X-chromosome loci for visible characters, 13 were cell-lethal while only 10 of 24 X-chromosome loci for lethals were cell lethal. He concluded from this that the visible characters tend to be of greater importance to the individual. The lethals that were studied cytologically were deficient for one or more salivary chromosome bands.

Unequivocal evidence that some recessive mutations are the consequence of homozygous small deficiencies has been obtained in Drosophila and in maize. Individuals with the phenotype of certain known recessives have appeared when they were homozygous for a small deficiency which included the normal alleles of those characters. For example, flies homozygous for a deficiency for the $+^y$ locus have a yellow body, as reported by Ephrussi (1934) and others; and flies homozygous deficient for $+^w$ have white eyes (Panshin 1938).