Plant Genetics and Genomics: Crops and Models 4

Hank W. Bass
James A. Birchler *Editors*

Plant Cytogenetics

Genome Structure and Chromosome Function

Springer

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Preface

This reference book is intended to provide information for students, instructors, and researchers on a range of topics in plant cytogenetics, including classical cytogenetics of plant genomes and chromosomes from structural or functional perspectives, modern molecular cytology and cytogenetics in the twenty-first century, recent methods, and laboratory exercises suitable for undergraduate or graduate instruction. The book is divided into three sections, each with chapters contributed by leading international scholars in the field. Our hope is that these chapters will supplement the many excellent review articles on plant cytogenetics published in the last 10 years and will provide a lasting contribution as a reference book on this important topic.

The first section, "Structure, Variation, and Mapping in Plant Cytogenetics," covers classical cytology, chromosome aberrations, plant B chromosomes, and cytogenetic mapping by conventional or modern DNA or chromatin-fiber-based techniques. The role of plant chromosomal rearrangements, such as deletions, insertions, and rearrangements, is described, and research tools are explored. The production, detection, and impact of aneuploidy in plants are reviewed in relation to gene dosage and breeding through introgressions. In addition, the supernumerary B chromosomes are reviewed, and their potential research applications examined. This section ends with two chapters on the use of cytogenetics to map plant genomes, from historical cytology with G-banding to fluorescence in situ hybridization (FISH) on chromosome spreads. High-resolution FISH-based mapping using DNA or chromatin fibers highlights the state of the art in plant cytogenetic mapping.

The second section, "Function, Organization, and Dynamics in Plant Cytogenetics," covers the basic elements of chromosomes, their behavior in meiosis, and the epigenetic landscape as surveyed by analysis of DNA methylation and histone modifications. Chapters on plant centromeres and plant telomeres are followed by a chapter on meiotic chromosomes, with emphasis on prophase of meiosis I. The last chapter in this section reviews epigenetic code in plants and a comparison of plants and nonplant eukaryotes.

The third section, "Methods, Informatics, and Instruction in Plant Cytogenetics," provides breadth to the book by covering several major methods used by leading

laboratories as well as including chapters on informatics and laboratory exercises for aspiring or practiced instructors. The techniques for chromosome microdissection and descriptions of their use in several plant genetic applications are covered in the first of four chapters in this section. The next chapter provides detailed methods for the use of antibodies in plant cytogenetics, including immunolocalization and the chromatin immunoprecipitation (ChIP) technique. The next two chapters cover advanced methods in FISH, including extended DNA fiber-FISH and in situ PCR. A chapter on plant cytology in genome databases addresses the growing role of online resources and databases in our access to and comprehension of plant cytogenetics in relation to classic genetic and modern genomic resources. Finally, a chapter for instructors is included to encourage the development or continuation of laboratory courses in plant cytogenetics, an activity deemed important for training future plant cytogeneticists. The chapter includes several modular exercises that can serve as a resource for instructors of new or ongoing courses.

Overall, the book is designed to cover many foundational topics in plant cytogenetics, while reviewing modern research and new techniques that represent the current growth and momentum in the field today. Inclusion of methods and instruction provides a distinct advantage to this reference book. We hope it will stimulate new research and facilitate the hands-on transmission of plant cytogenetic knowledge to students and teachers alike.

Finally, we would like to acknowledge the extraordinary editorial assistance of Dr. Anne B. Thistle. We are deeply appreciative of her dedication and attention to detail.

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Part I
Structure, Variation, and Mapping in Plant Cytogenetics

Chapter 1
Plant Chromosomal Deletions, Insertions, and Rearrangements

Donald L. Auger and William F. Sheridan

Abstract With the exception of a small subset found within mitochondria and chloroplasts, the genes of plants are arranged along an essential set of chromosomes that are found in the nucleus. Within a species, the placement of genes along the chromosomes is expected to be the same in all individuals. This chapter is a primer on several major aberrations of gene order. These aberrations have consequences not only to the individual that harbors them but also to the population at large in terms of genome evolution. Here, we limit our discussion mainly to the effects on the individual. We are particularly interested in the use of these aberrations as experimental tools and include some discussions to that effect.

Keywords Cytogenetics · Deletions · Deficiencies · Insertions · Duplications · Inversions · Reciprocal translocations · Maize B-A chromosomes

Abbreviations

Ctr	Centromeres
Df	Deficiency
Dp	Duplication
EMS	Ethyl methanesulfonate
FISH	Fluorescent in situ hybridization

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In	Inversion
N	Normal
SBE	Starch branching enzymes
TE	Transposable elements

Contents

1.1 Introduction

An analogy useful for explaining genetics to a layperson is to describe the genome as an encyclopedia of instructions necessary to make an organism, in which each gene represents an instruction. Like a traditional encyclopedia, the genome is divided among several volumes or books, which are called chromosomes. Encyclopedias are organized so as to make the data readily available. Chromosomes must be organized as well, so that the cell can access the information correctly and efficiently, when and where needed, but this system of organization is not completely clear. Among members of any given species, the order of genes on a chromosome is generally regarded as canonical – exceptions are considered aberrations. Interestingly, Barbara McClintock, who developed her career and reputation helping to establish this dogma, became one of the earliest dissenters when she described DNA elements capable of being transposed to new sites along the same or even another chromosome. Indeed, extensive sequencing data and other recent techniques are demonstrating that chromosomes are much more labile than was believed even a decade ago. The biological implications of a labile genome affect everything from the individual to the evolution of populations. Here, we offer a primer on some common aberrations from canonical chromosome organization: deficiencies, duplications, and rearrangements.

1.2 Deletions/Deficiencies

Deletion of a chromosomal segment results in a deficiency. When it occurs in a diploid cell, then that cell and its progeny will be hemizygous i.e., it has only one copy of, any gene or locus included in the deficiency. When a whole chromosome is

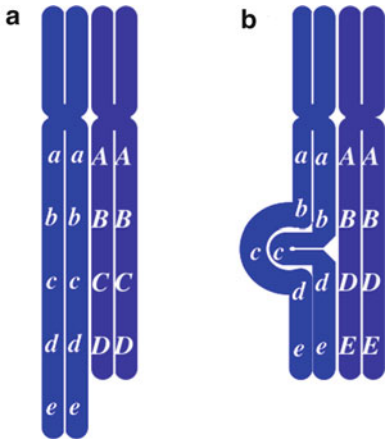


Fig. 1.1 Simple deficiencies. Homologues are lined up as in pachytene with a normal chromosome to the left and deletion chromosome to the right. (**a**) Terminal deficiency. (**b**) Internal deficiency

lost, the resulting cell is said to be monosomic for the remaining homologous chromosome. The word monosomic has also been used to describe larger chromosomal segments that are homologous to large deleted segments. The following discussion focuses on segmental deficiencies rather than losses of whole chromosomes.

A simple case of a chromosomal deficiency is breakage without reunion (Fig. 1.1a). The segment without a centromere is lost quickly in subsequent cell cycles, so the progeny cells are deficient for all loci distal to the breakpoint. In plants with diffuse centromeres, e.g., *Luzula*, a broken piece can be maintained and will not result in a deficiency (Nordenskjold 1961). Internal (interstitial) deficiencies occur when two breaks occur simultaneously in one chromosome, the proximal and distal segments rejoin, and the intervening segment is lost (Fig. 1.1b). McClintock (1931) uses the term "deletion" to describe only this form of deficiency, but the two terms are commonly used interchangeably (see e.g., Burnham 1962, p. 20). Although the deficiency is obvious as shown in Fig. 1.1, small deficiencies are difficult to visualize at pachytene, but larger ones may be visible.

Breaks that occur for unknown reasons are said to occur spontaneously. Breaks can be induced experimentally by means of heat, high-energy radiation, and certain chemicals. Deficiencies seem to be the mode for X-ray-induced mutations. Stadler and Roman (1948), Nuffer (1957), and Mottinger (1970) could not find evidence of base-change mutations when using X-rays; instead these mutations were apparently short deficiencies. Interestingly, the form of induction affects the locations of breaks. Breakages induced by high-energy radiation are more likely to occur in centromeric and heterochromatic regions (Evans and Bigger 1961). X-ray-induced breaks are more likely to be found in heterochromatin both in tomato (Gottschalk 1951; Khush and Rick 1968) and in maize (Longley 1961). In maize exposed to nuclear explosions, the bias toward breaks in heterochromatic regions was not as pronounced (Longley 1961), indicating that fast neutrons are more efficient in producing breaks in euchromatin.

Deficiencies can also be induced by one of the several genetic conditions. For example, in maize, an allele of the *r1* locus, called *r-X1*, induces deficiencies but is better known for inducing monosomies and trisomies in maize (Weber 1973; Lin et al. 1990; Weber and Chao 1994). This allele is itself a small deletion and can only be transmitted maternally. Deficiencies can also result from transposable elements (McClintock 1950) that are oriented in specific ways on a chromosome (Weil and Wessler 1993; English et al. 1995; Martínez-Férez and Dooner 1997). Another source of deficiencies is uneven crossovers or crossovers involving chromosomes with rearrangements. As these conditions are also associated with duplications, they will be explained later.

The rates of spontaneous breaks and other chromosomal abnormalities are high in various interspecific hybrids, where they seem to act as genetic barriers (Ehrendorfer 1959; Endo 1990). For example, certain chromosomes in some wild relatives of wheat possess one or more factors that induce chromosomal breakage of common wheat (*Triticum aestivum* L.) chromosomes (Endo 1990). The chromosomes that bear these factors are called Gc (gametocidal) chromosomes because their effect takes place immediately after meiosis. They somehow condition meiosis so that any of the spores that lack the Gc chromosome undergo chromosomal breaks, which are usually lethal to the gametophyte. The chromosomal aberrations found in surviving gametophytes can be transmitted and stabilized in the subsequent sporophyte generation. As a result, Gc chromosomes have been used as a tool for genetic analysis and manipulation (Endo 2007).

Deficiencies are often lethal in the gametophyte generation and so cannot be transmitted to a subsequent generation, especially in diploid plants. For example, small deficiencies are lethal to the gametophyte of *Vicia faba* L. (Schubert and Reiger 1990). In a study on tomato, the only deficiencies transmitted were smaller deletions in heterochromatin; no euchromatic deficiencies would transmit (Khush and Rick 1967). In maize, McClintock (1944) found that no deficiencies of the short arm of chromosome 9 (9S) were transmitted though the male, but a loss of the distal one-third of 9S was transmitted through the female gametophyte. Later, several very small deletions on 9S involving *shrunken1* (*sh1*) and *bronze1* (*bz1*) were found that were transmissible through both female and male and were also homozygous viable in the sporophyte (Mottinger 1970). Stadler (1933, 1935) described a haploviable deficiency in maize. It was a relatively large terminal deficiency of the long arm of chromosome 10. Although this deficiency could not be transmitted through the male, it could be through the female. It affected the phenotype of both the male and female gametophyte. About half of the pollen grains from plants heterozygous for this deficiency were small but starch-filled. The embryo sacs were also smaller, but seed set was nearly normal. The rule appears to be that female transmission of a deficiency is more likely than male transmission. Deficiencies transmissible in both egg and pollen are rare (McClintock 1944; Mottinger 1970; Patterson 1978). In polyploid plants, the situation is different, ostensibly because the gametophyte carries multiple homologues or homeologues. For example, about 67% of wheat deficiencies are transmitted normally and can be made homozygous (Endo and Gill 1996).

Deficiencies can also affect the phenotype of the sporophyte plant. The effects depend upon which loci are deleted, what alleles remain in a hemizygous condition, and whether the deficiency is transmissible to future generations. Homozygous deficiencies in maize have been demonstrated to yield phenotypes like those of recessives (Creighton 1937; McClintock 1938a, b, 1941, 1944). Indeed, transmissible deficiencies crossed with known recessive alleles result in expression of the recessive allele. This phenomenon, traditionally called pseudodominance, is the basis for correlating genetic maps, which are based on linkage, with cytological maps, which are based on observations of the chromosomes. Deficiencies have been used as a tool for mapping genes not only in maize but also in other plants such as tomato (Rick and Khush 1961; Khush and Rick 1967, 1968). More recently, deficiencies have been employed for the physical mapping of molecular traits and quantitative traits (Gill et al. 1996; Sutka et al. 1999; Tsujimoto et al. 2001).

1.3 Insertions/Duplications

Insertions involve the transposition of a chromosomal segment to another position on the same chromosome or onto a different chromosome. An insertion without concomitant deletion of that chromosomal region results in duplication and alters the copy number of the duplicated region. Three examples of simple insertions are portrayed in Fig. 1.2, each paired with a progenitor chromosome. Figure 1.2a shows a tandem duplication of the segment *AB*, Fig. 1.2b shows an inverted duplication involving the same segment, and Fig. 1.2c an insertion of a segment that originated from a nonhomologous chromosome. In the heterozygous condition, large insertions are visible at pachytene as unpaired loops or bulges, but small insertions may be undetectable. Of course, chromosomes homozygous for an insertion would be expected to align normally. Segmental duplications seem to be quite common in plants and are often fixed in populations. Different studies have estimated that from 15 to 62% of the rice genome consists of segmental duplications (Vandepoele et al. 2003; Paterson et al. 2004; Wang et al. 2005; Lin et al. 2006).

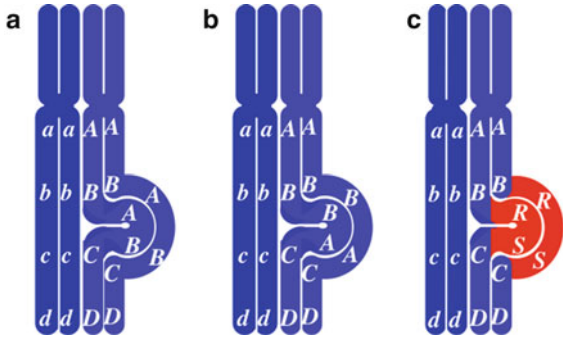


Fig. 1.2 Simple insertions. Homologues are lined up as in pachytene with normal chromosome to the left and insertion chromosome to the right. (**a**) Tandem duplication. (**b**) Inverted duplication. (**c**) Insertion from nonhomologous chromosome

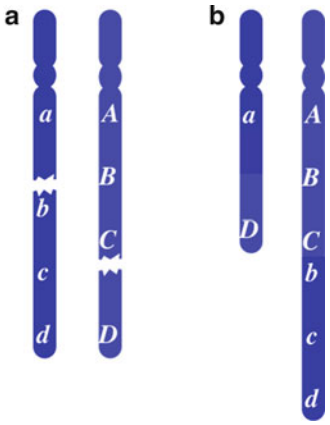


Fig. 1.3 Tandem repeat: simultaneous breaks. (**a**) Breaks in homologues or sister chromatids. (**b**) Ligation with exchange

Several mechanisms for insertions have been proposed. The most direct production of a tandem repeat results from the simultaneous breakage of homologues (Fig. 1.3) or sister chromatids at different locations along the chromosome and exchange and ligation of the broken pieces (Beard 1960). Another results from unequal crossovers (Fig. 1.4), in which nonhomologous loci of homologous chromosomes cross over, a process facilitated by the presence of similar sequences in the two segments. The presence of similar sequences could result from an earlier duplication or from the presence of repetitive sequences such as transposable elements. Note that, if a duplication is produced by either of these two mechanisms, a concomitant deficiency will also result (Figs. 1.3b and 1.4b).

Some models involving aberrant transposition of transposable elements have been developed, and the evidence is strong that these events actually occur (English et al. 1995; Zhang and Peterson 1999). Normally, DNA transposable elements (TE) are flanked by terminal inverted repeats (Fig. 1.5). They are mobilized by a transposase that cleaves the DNA immediately flanking the inverted repeats, causing those flanking ends to be joined to each other. The excised TE is then reinserted at another chromosomal location – the chromosome is cleaved at that location, and the ends of the TE are joined to the ends of the freshly cleaved DNA. In some cases, TE transposition is abnormal, and relatively large chromosomal regions can be rearranged. One example is portrayed in Fig. 1.6, where the terminal repeats are in the same orientation rather than the inverted; this situation can arise when one TE is inserted into another of its own kind. If the transposase uses one terminus from each of the sister chromatids (Fig. 1.6a), the result will be bridging of the sister chromatids at the point of excision (Fig. 1.6b). The excised termini, along with the distal chromosomal regions, are subject to transposition to a new chromosomal location. If the integration site is on the same chromosome arm, proximal to the excision site, it will produce one chromatid with an inverted repeat and another with a deficiency (Fig. 1.6c). Transposition to any other chromosomal region would result in major chromosomal imbalances that are unlikely to be heritable.

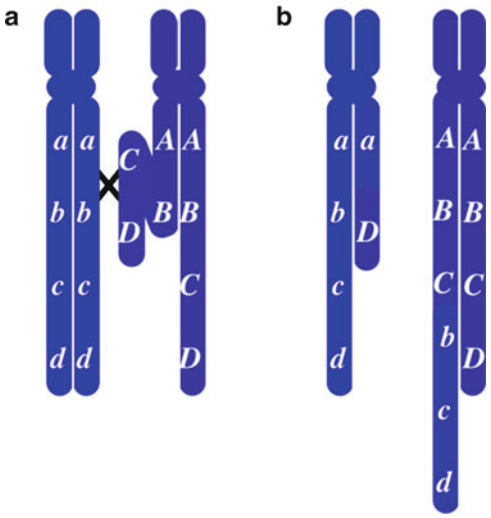


Fig. 1.4 Tandem repeat: uneven crossover. (**a**) Homologues with crossover in nonhomologous region. (**b**) Products of first meiotic division

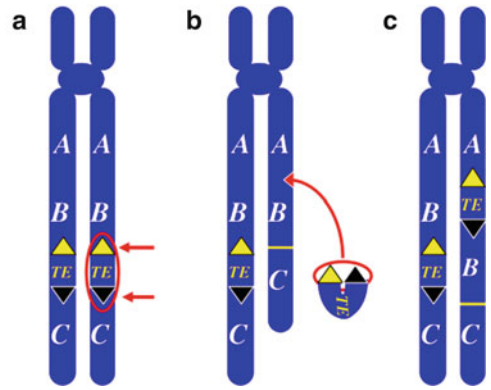


Fig. 1.5 Normal transposition of a transposable element (TE). In this cartoon, the TE is very large relative to the chromosomes. (**a**) Sister chromatids with transposase excising TE at complementary inverted repeats. (**b**) Excised TE with donor locus ligated (*yellow line*). Insertion of TE into new locus. (**c**) Resulting chromosome following transposition of the TE from one location to another within a single sister chromatid

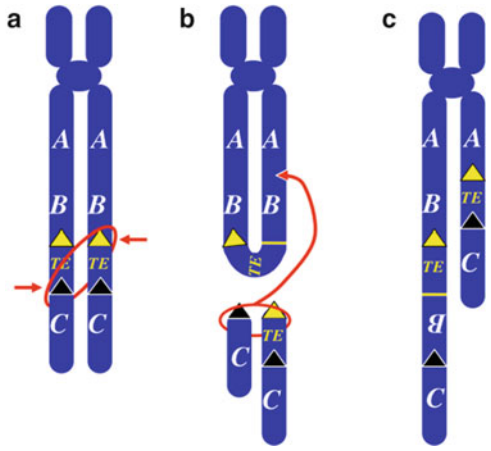


Fig. 1.6 Inverted duplication: transposon mediated. (**a**) Sister chromatids with abnormal DNA transposons having terminal repeats in direct orientation rather than inverted. In this case two ends from sisters interact in transposition. (**b**) Excision site anneals; transposing ends attack proximal site. (**c**) Resulting deletion/duplication with inverted repeat

Insertions such as those portrayed by Fig. 1.2c are more difficult to explain but appear to be common. Pairing between nonhomologous chromosomes in haploid plants of *Antirrhinum majus* L. (Reiger 1957) and *Oenothera blandina* de Vries (Catcheside 1932) indicates the existence of larger interchromosomal duplications. Smaller insertions of one or few loci are also detected. Notable are reports of insertions of organellar DNA into the nuclear chromosomes of *Arabidopsis* (Lin et al. 1999; Stupar et al. 2001), rice (International Rice Genome Sequencing Project 2005), and maize (Lough et al. 2008). The study in maize is especially interesting because it suggests that the insertion of mitochondrial DNA into the nuclear genome is frequent and ongoing (Lough et al. 2008).

Duplications tend to be less deleterious than deficiencies and can regularly be transmitted through the female gametophyte. Male transmission is often inhibited, ostensibly by the inability of aneuploid pollen to compete with euploid pollen (Buchholz and Blakeslee 1932). Whether or not a duplication can be transmitted through the pollen seems to be related to size. Transmission of large duplications and deficiencies typically fails (see e.g., Rhoades and Dempsey 1953; Patterson 1978), but some large duplications are known to be transmitted (Carlson and Curtis 1986; Auger and Birchler 2002). Duplications whose transmission through pollen fails probably include genes that have a dosage-sensitive effect that inhibits the efficient elongation of the pollen tube (Auger and Birchler 2002). Therefore, the longer the duplicated region, the more likely it is to possess such a factor.

A duplication can cause a pollen grain to be noncompetitive because it causes the pollen grain to be essentially aneuploid. Aneuploidy is often associated with abnormal development or function, and this phenomenon is known as an aneuploid syndrome. To understand why aneuploidy might have such effects, consider that, with the exception of organelles, all of the necessary structural and regulatory genes are distributed among an essential complement of chromosomes. For example, all the genes of tomatoes are distributed among 12 chromosomes ($n = 12$). Euploidy is the state of having exact complementary sets. A cell that possesses only one copy of each member of the essential complement is called monoploid (1n) and is considered euploid. Having exactly two copies of each member of the essential complement is diploidy (2n) and is also euploid. The same can be said for any multiple (3n = triploid, 4n = tetraploid, etc.) of the monoploid set as long as it is a perfect multiple; different species have optimal ploidy levels for both the sporophyte and the gametophyte generations. If one (or more) chromosome has a copy number different from those of the other members of the essential complement, the cell is said to be aneuploid. For example, if one chromosome is missing in an otherwise diploid cell ($2n - 1$), the cell is said to be monosomic because one of the essential chromosomes is represented by only one copy. Trisomy ($2n + 1$) describes the condition in which one chromosome exists as three copies in an otherwise diploid cell. Although aneuploidies are aberrant conditions, they are mitotically stable. Therefore, an aneuploid zygote will grow into an organism in which essentially all the cells retain the aneuploidy, and the resulting organism is described as being aneuploid.

Aneuploidy generally has a negative effect on the development and vigor of an individual. A gene that is in a duplicated or deleted segment may yield an amount

of gene product that is altered relative to other gene products in the same cell. Although stoichiometric changes in the products of structural genes, such as enzymes, may contribute to aneuploid syndromes, the copy numbers of regulatory genes appear to be most important (Guo and Birchler 1994). Regulatory proteins typically interact with a collection of target genes, thereby amplifying the potential of regulatory genes to affect the phenotype. To the extent that any one of the downstream products being regulated is rate limiting in some process, alteration of the expression of this downstream product can affect the phenotype. The rate-limiting effects of a deficiency are easier to appreciate, but duplications also affect development and reduce vigor. Duplications may have this effect because many regulatory factors act to down-regulate target genes.

Another effect of duplications is that they can alter the rules of genetic segregation. For example, in *Pisum*, five different genes were shown to behave as duplicates with 15:1 ratios or 9:7 ratios (Lamprecht 1953). Ancient duplications can confound both forward and reverse genetic analysis. Consider mutation analysis, which remains a powerful tool for the analysis of gene function. When genes are duplicated, the ability to detect mutant alleles is exponentially diminished. Spontaneous mutations occur at a rate of about 10^{-6} mutations per locus tested (Walbot 1992), whereas ethyl methanesulfonate (EMS)-induced mutation rates can range around 10^{-3} mutations per locus tested (Neuffer et al. 1997). Clearly, having to knock out duplicated genes simultaneously with EMS would lower detection rate below the spontaneous mutation rate of a single gene. An example in which duplicated genes were detected is *orange pericarp* (*orp*) in maize (Wright and Neuffer 1989). The phenotype, in which the pericarp reacts with indole emanating from the mutant endosperm, occurs when two genes, *orp1* and *orp2*, are homozygous for the mutant alleles. Both genes encode the β subunit of tryptophan synthase, but they are found on nonhomologous chromosomes (Wright et al. 1992). The duplication of the *orp* genes appears to have resulted from an ancient polyploidization (Ma et al. 2005). Although the mutations were found in an EMS screen, only the mutation in *orp2* was EMS-induced (Wright and Neuffer 1989). Fortunately, the mutation in *orp1* was previously segregating in the northern flint lines used in the study (Wright 1991).

Gene duplications are important evolutionarily in that they allow for mutations to accumulate that will result in new functions (neofunctionalization) or more specialized functions (subfunctionalization) of one or the other paralogue (Paterson et al. 2004; Wang et al. 2005). Examples of the latter are genes for starch branching enzymes (SBE). Unbranched starch, amylose, becomes branched when enzymes break α-1,4 glycosidic bonds of the linear starch molecules and reattach the starch fragments using an α-1,6 glycosidic bond. Two classes of SBEs are known in plants: one (SBEI) acts preferentially on amylose directly and the other (SBEII) on the partially branched starch (Morell et al. 1997). Cereals have two isoforms of SBEII, which are further subfunctionalized. In maize and rice, SBEIIb is more important for the accumulation of branched starch, amylopectin, in the endosperm (Yamanouchi and Nakamura 1992; Gao et al. 1997), whereas SBEIIa is more active in the leaves. In wheat, the SBEIIa isoform is the one more highly accumulated in the endosperm (Rahman et al. 2001).

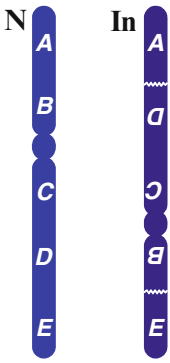


Fig. 1.7 Pericentric inversion. *N* normal (progenitor) chromosome; *In* inversion

1.4 Chromosomal Rearrangements

Chromosomal breakage does not necessarily lead to either a deficiency or a duplication but instead may result in a chromosomal rearrangement in which no chromatin is lost or gained. This process involves two simultaneous breaks followed by reattachment of the segments but not with the original partners. The nature of the chromosomal rearrangement depends on whether the two breaks occur in one chromosome, producing an inversion, or in two different chromosomes, producing a reciprocal translocation. We will first consider inversions.

1.4.1 Inversions

Inversions were first detected in *Drosophila* as "crossover reducers"; certain chromosomes were found to reduce recombination dramatically when heterozygous with their normal homologues. They were given the name inversions after the discovery that the regions between the breaks were inverted relative to the normal progenitor chromosome (Sturtevant 1926).

When two breaks occur on opposite arms of a chromosome, the segments may be reattached so that the centric fragment is reincorporated as an inversion (Fig. 1.7). These cases, in which the centromere is flanked by the breakpoints and is within the inverted region, are known as pericentric inversions (Muller 1940). In Fig. 1.7, the centromere is indicated by a constriction, and loci are designated by letters. Note that no chromatin is lost but that the loci between the breaks have been reattached in inverted positions relative to those of the progenitor chromosome. A pericentric inversion can shift the centromere position and therefore arm ratio. In some cases, the shift is sufficient to allow for the cytological identification of these chromosomes in mitotic cells.

Alternatively, when two breaks occur in one arm of a chromosome, the segments can reattach so that the fragment flanked by the two breaks is incorporated as an

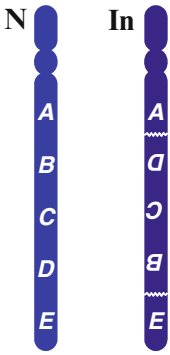


Fig. 1.8 Paracentric inversion. *N* normal (progenitor) chromosome; *In* inversion

inversion (Fig. 1.8). These cases, in which the centromere is outside the inverted region, are known as paracentric inversions (Muller 1940). Again, no chromatin was lost, but the loci between the breaks become inverted relative to those on the progenitor chromosome. In paracentric inversions, the arm ratios remain unchanged.

Because no chromatin is lost, inversions typically have no effect on gene expression. Exceptions are cases in which the breakpoints occur within a gene or in which the rearrangement causes a position effect. Ostensibly, a position effect arises when a gene is placed adjacent to chromatin that will have a *cis*-acting effect on gene expression, most probably heterochromatin. Position effects are apparently rare in plants, although *O. blandina* (Catcheside 1939, 1947) is often cited as an example. Nevertheless, inversion chromosomes can have genetic consequences when they are heterozygous with normal chromosomes. Although paracentric inversions appear to be more common, we will first consider a pericentric inversion because the genetics are more straightforward.

Inversions behave well in mitosis, but in meiosis homologous chromosomes must pair and align. Inversions cannot properly align linearly with their normal homologues. Compare the normal (N) and pericentric-inversion (In) chromosomes portrayed in Fig. 1.7. The letters in the inverted region are shown upside down to emphasize that loci are not just in a different position but are also in the opposite orientation. For the chromosomes to be paired in a completely linear fashion, either the region between the breakpoints or the ends must be paired to nonhomologous regions. Indeed, nonhomologous pairing is common when the inverted region is relatively small (McClintock 1932). Alternatively, either the distal or inverted regions may remain unpaired, i.e., asynaptic (McClintock 1933). Asynapsis between inversions and their normal homologues also appears to be common (Doyle 1994). Clearly, neither paired nonhomologous regions nor asynaptic regions are subject to genetic recombination, as is reflected by a marked decrease in expected genetic map distances within and immediately adjacent to the inverted region. Interestingly, inverted segments may increase crossover rates elsewhere on the chromosome or even other chromosomes (Stephens 1961). Nonhomologous pairing and asynapsis are not the only causes of map distortion. To see why, we must consider how inversions can align with normal chromosomes with high fidelity.

Fig. 1.9 A pericentric inversion paired with a normal homologue. The centromere is located between *B* and *C*. A crossover is indicated between loci *C* and *D*

When the inverted region is large, the alignment mechanism commonly causes the two chromosomes to pair in a way that is not linear, in what McClintock (1931, 1933) called a reverse loop (Fig. 1.9). In meiosis, each homologue has two chromatids, and in Fig. 1.9, these are separated and distorted so that they can be more easily traced. The centromeres are again shown as constrictions, but the centromeres of sister chromatids are bound to each other. The loci of the normal chromosome are shown with lower-case letters. When a loop configuration is formed in prophase I of meiosis, essentially all loci are correctly aligned and are eligible for genetic crossovers. The exceptions are the inversion breakpoints and the corresponding loci of the normal homologue; the reasons will be explained later in the discussion of reciprocal translocations.

Although the loop formation allows for homologous pairing and recombination along the lengths of the chromosomes, only the crossovers that occur outside the inverted region are readily recovered, because those outside the inverted region result in balanced exchanges just as they do with two normal chromosomes. A crossover between the breakpoints, however, will result in an unbalanced exchange – the two recombinant chromatids will each have a duplication (Dp) and a deficiency (Df). Note that Fig. 1.9 portrays a crossover between loci *C* and *D*. When these four chromatids are separated in meiosis II, they yield one N chromosome, one In chromosome, and two alternative Dp-Df chromosomes (Fig. 1.10). Monoploid spores that possess the Dp-Df chromosomes typically abort. Indeed, pollen and ovule abortion are characteristic of plants that are heterozygous for inversions. Even in the case of reverse loops, therefore, where the degree of homologous pairing is high, the occurrence of a crossover in the inverted region results in recombinant chromatids that are usually lost as a result of gametophyte abortion, and the result is distortion of map distances.

The proportion of gametophytes that abort varies according to the rate of crossovers in the inverted region (Doyle 1994). Some inversion heterozygotes have nearly 50% pollen and ovule abortion, whereas in others abortion rates are hardly detectable. For example, pollen abortion was reported to be nearly 50% in two pericentric inversions in *Vicia faba* (Sjodin 1971) and four pericentric inversions in *Scilla scilloides* (Noda 1974). One determinant appears to be the proportion of linear to

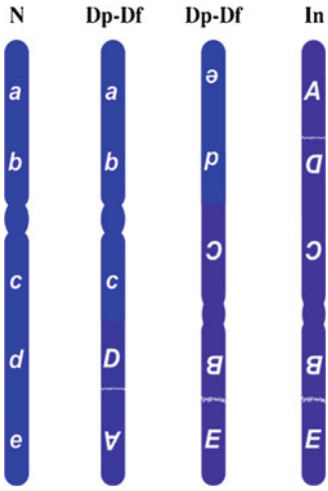


Fig. 1.10 Meiotic products from a single crossover within a pericentric inversion loop

looped meiotic pairings. Recall that linear bivalents are possible only when pairing of the inverted region is nonhomologous, precluding crossovers and Dp-Df products. Such nonhomologous pairing appears to be common for some inversions (see e.g., McClintock 1931, 1933). In some cases, the inverted region may fail to pair at all with the normal homologue (asynapsis; Russell and Burnham 1950), again precluding crossover and production of Dp-Df recombinant chromosomes.

Double crossovers within the inverted region of pericentric inversions can occur but should have little effect on rates of abortion. Two-strand double crossovers produce no Dp-Df chromosomes, whereas four-strand double crossovers cause all four chromosomes to be Dp-Df, and three-strand double crossovers have the same result as single crossovers, i.e., one N, one In, and two Dp-Df (Burnham 1962). Any crossovers that take place outside the inverted region and are concomitant with either single or double crossovers within the inverted region have no effect on the generation of Dp-Df chromosomes.

In maize, pericentric inversion heterozygotes experience abortion rates in the male (pollen) and female (embryo sacs) gametophytes that are generally similar (Anderson 1941; Morgan 1950). The slightly higher rates of male abortion are attributed to the higher crossover rates in male meiosis for the region in question (Rhoades 1941; Morgan 1950). Pollen abortion can be used as a dominant phenotypic trait to identify inversion heterozygotes in gene mapping. Because of the issues of pairing discussed above, map distances will probably be greatly distorted, but information about the placement of the breakpoints relative to other genetic markers can be obtained. A strong reduction in crossovers indicates that a marker is within or near the inversion, whereas considerable recombination indicates that the marker is outside the inversion (Burnham 1962). Although inversions have often been used to map genes (e.g., by Morgan 1950; Russell and Burnham 1950; Rhoades and Dempsey 1953; Ekberg 1974), more recently, extensive mapping projects (e.g., by Bonierbale et al. 1988; Mickelson-Young et al. 1995; Livingstone et al. 1999; Dubcovsky et al. 1996) have commonly revealed previously undetected inversions.

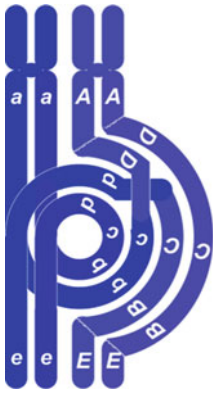


Fig. 1.11 A paracentric inversion paired with a normal homologue. The centromere is located above *A*. A crossover is indicated between loci *C* and *D*

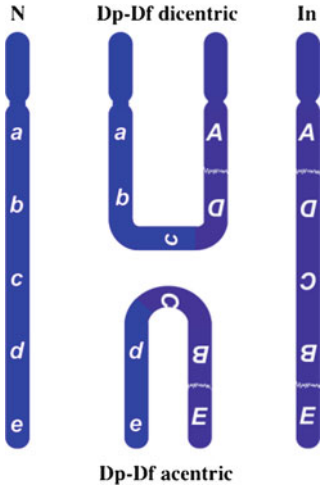


Fig. 1.12 Meiotic products from a single crossover in a paracentric inversion loop

The behavior of paracentric inversions as heterozygotes is essentially the same as that of pericentric inversions, but the genetic consequences are somewhat different. The issues involving nonhomologous pairing, asynapsis, and the formation of a reverse loop are similar. The difference is the consequence of crossovers within a reverse loop. Again, Fig. 1.11 portrays all four chromatids in a separated and distorted fashion to facilitate tracing of the products of a crossover between the *C* and *D* loci. Not only will the two chromatids that are generated by a single crossover be Dp-Df, but also one will have no centromeres (acentric) and the other will have two centromeres (dicentric) (Fig. 1.12). The acentric fragment is typically lost in meiosis I. The dicentric is also Dp-Df, and because the centromeres of the dicentric are from different homologues, they segregate at anaphase I, causing the chromatin between them to bridge and ultimately break. Although the spores that receive the broken remnants of the dicentric typically abort, a deficiency may occasionally be transmissible through the female gametophyte.

When only double crossovers within the inverted region are considered, the results are analogous to those of pericentric inversions. Two-strand double crossovers yield no Dp-Df products, three-strand double crossovers yield products similar to those of a single crossover, and four-strand double crossovers yield two Dp-Df acentrics and two Dp-Df dicentrics. In all of these cases where dicentrics are formed, bridging takes place in anaphase I.

With one exception, crossovers outside of the inverted region have no effect on the generation of acentric and dicentric Dp-Df chromosomes. The exception is the case in which the crossover occurs in the region between the inverted region and the centromere, i.e., the interstitial region. In Fig. 1.11, the *A* locus is located in the interstitial region. A crossover in the interstitial region occurring concomitantly with one or more crossovers within the inverted region creates the opportunity for bridging at anaphase II. Anaphase I bridges result because dicentrics have two centromeres from different homologues, which separate at anaphase I. Depending on which strands are involved in multiple crossovers, a crossover in the interstitial region (not shown) produces a dicentric in which the two centromeres are from sister chromatids; these are separated at anaphase II. More complete discussions of the products of multiple crossovers in paracentric inversions are given by Burnham (1962) and Moore (1976).

The level of pollen abortion experienced by paracentric inversion heterozygotes, like that of pericentric inversions, is expected to be a function of the amount of crossing over that takes place within the inverted segment. Interestingly, in maize the level of ovule abortion is often much less than that of pollen abortion. The explanation appears to be the bridges that occur at anaphase I (Beadle and Sturtevant 1935). In plants, female meiosis tends to produce megaspores in a linear fashion. The embryo sac of maize, like many plants, is monosporic in development; i.e., it develops from just one of the spores (Maheshwari 1950). Monosporic embryo sacs develop from one of the outermost megaspores: in maize, the megaspore most distal to the micropyle. Bridges are believed to cause the Dp-Df chromosomes to be oriented toward the center of the pole at the first division, such that they will be nonrandomly included in the centermost megaspores after the second division. Therefore, the megaspore that develops into the embryo sac will nonrandomly receive either the N or an In chromosome that was not Dp-Df. In male meiosis, the nonrandom distribution of Dp-Df chromosomes is not expected to occur, because divisions are not linear and, more importantly, all four microspores develop into pollen grains.

The use of inversions for genetic analysis and manipulation has been limited, probably by the difficulty of their use. For example, cytological verification of inversions by observation of bridges at anaphase is much easier than observations of reverse loops at pachytene. Even so, bridges and acentric fragments are no guarantee of an intact inversion. Some inversions are capable of producing Dp-Df chromosomes that can be transmitted, though usually through the female. Moore (1976) indicates that bridges and fragment should not be indiscriminately accepted as proof of paracentric inversion heterozygosity. Bridges and acentrics can emerge from breakage and repair in meiosis (Rees and Thompson 1955; Lewis and John 1966). Therefore, care must be taken to observe that bridges and fragments are of uniform sizes.

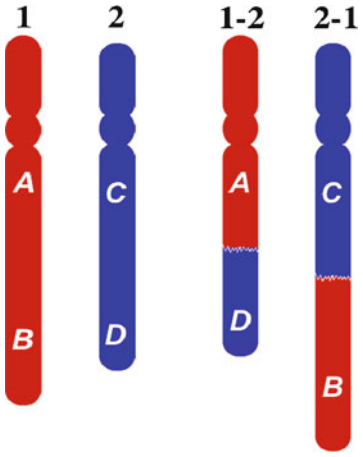


Fig. 1.13 Reciprocal translocations (1-2 and 2-1) with normal progenitors (1 and 2)

1.4.2 Reciprocal Translocations

As explained earlier, reciprocal translocations result from the breakage of two nonhomologous chromosomes and exchange of the broken pieces. Reciprocal translocations are also called translocations, segmental chromosome interchanges, or interchanges (Burnham 1956). Figure 1.13 portrays two nonhomologous chromosomes, numbered 1 and 2. Next to them are two reciprocal translocations, numbered 1-2 and 2-1. The 1-2 chromosome was generated by a break in the long arm of chromosome 1; the lost segment was replaced by a segment produced by a break in the long arm of chromosome 2. The 2-1 chromosome was reciprocally generated. Typically each member of a translocation is designated by the number of the chromosome from which the centromere was derived followed by the number of the chromosome from which the translocated piece was derived. Here we separate the two numbers with a hyphen, but often the second number is presented as a superscript or separated from the first by a comma, e.g., 1^2 and 2^1 or 1,2 and 2,1.

Like the other chromosomal abnormalities, reciprocal translocations can arise spontaneously in a population, or they can be induced by chemical mutagens or irradiation. Other contributory factors that have been noted are age of seed (Gunthardt et al. 1953) and genetic conditions (Beadle 1937; McClintock 1950). The most extensive collection of reciprocal translocations is probably that in maize (Longley 1961). These translocations were induced by various types of radiation, notably that from nuclear-blast testing in the Pacific after the World War II. More than 800 of these translocations still exist and are available through the Maize Genetics Cooperation Stock Center (http://maizecoop.cropsci.uiuc.edu/).

Reciprocal translocations, like inversions, produce no loss of chromatin, so they also have no effect on phenotype. In plants, mutations at the breakpoints, e.g., a chlorophyll mutation in barley (Tuleen 1962), or position effects, e.g., color variegation in *Oenothera* (Catcheside 1939, 1947), are relatively rarely detected. In one experiment, 13 X-ray-induced translocations produced no overt dominant or recessive

mutations, although a number of significant differences for quantitative traits were apparent (Roberts 1942). The low frequency of concomitant mutations in plants may be due to the immediate loss of deleterious mutations at the gametophyte stage (Burnham 1962). In contrast, the majority of translocations in *Drosophila* are lethal or extremely detrimental when homozygous (Bridges and Brehme 1944). Even without mutations, reciprocal translocations have genetic consequences, especially when heterozygous with normal progenitors. The first genetic consequence is new linkage relationships. Note that in the example above (Fig. 1.13), the *A* and *B* loci are linked on the normal chromosome 1 and *C* and *D* are linked on the normal chromosome 2. On the translocations, A is no longer linked with *B* but instead with *D* on the 1-2 translocation chromosome. On the 2-1 chromosome, *C* is linked with *B*. Note also that the dimensions of the chromosomes have changed. Cytologists use the overall length and the short arm/long arm ratio of mitotically and meiotically condensed chromosomes to aid in identification. In Fig. 1.13, the translocation chromosomes clearly differ in both. Unfortunately, the lengths and arm ratios often are not sufficiently reliable for chromosome identification, especially in mitosis. More recently, fluorescent in situ hybridization (FISH) techniques have made chromosome identification more reliable (see e.g., Kato et al. 2004), and these techniques have been employed to identify newly generated reciprocal translocations (Zhang et al. 2009).

In plants that are homozygous for reciprocal translocations, meiosis proceeds normally because each translocation chromosome has a structural homologue with which to pair. The story is different for translocation heterozygotes. At meiosis, reciprocal translocations cannot be correctly paired with normal chromosomes in a linear fashion. Correct pairing requires that one member of the translocation pair with the segments of the normal chromosomes with which it shares homology and that the other pair with the remaining segments of the same two normal chromosomes. Instead of a linear bivalent, the chromosomes form a cross-shaped quadrivalent (Fig. 1.14; again the four chromatids are laid out in a distorted fashion to allow easier tracing). In Fig. 1.14, the chromosomes are identified by the numbers adjacent to the centromeres; letters indicate genetic loci. For the following discussion, the spindle poles are to the left and right.

Plants heterozygous for reciprocal translocations experience pollen and ovule abortion, but the mechanics are different from those of inversions. The two reciprocal members of a translocation are not considered Dp or Df as long as they are together in the same cell. At meiosis, the opportunity arises for the two reciprocals to segregate to different daughter cells and segregate with one of the normal homologues. The daughter cell that receives such a combination will be Dp-Df and will nearly always abort. For a spore produced by meiosis to avoid being Dp-Df, it must receive either both reciprocal members of the translocation or two normal chromosomes. This pattern would result at anaphase I if the 1-2 translocation chromosome at the upper right segregated with its 2-1 reciprocal partner at the lower left and the normal chromosome 1 at the upper left cosegregated with normal chromosome 2 at the lower right (Fig. 1.15). This is called alternate segregation; when chromosomes are drawn out on a flat plane, the alternate nonhomologous centromeres cosegregate.

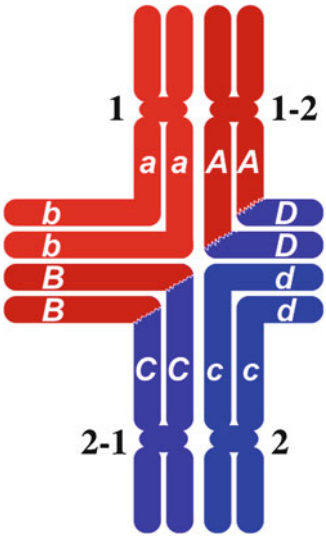
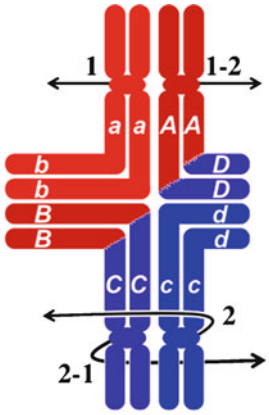


Fig. 1.14 Reciprocal translocation paired with normal homologues



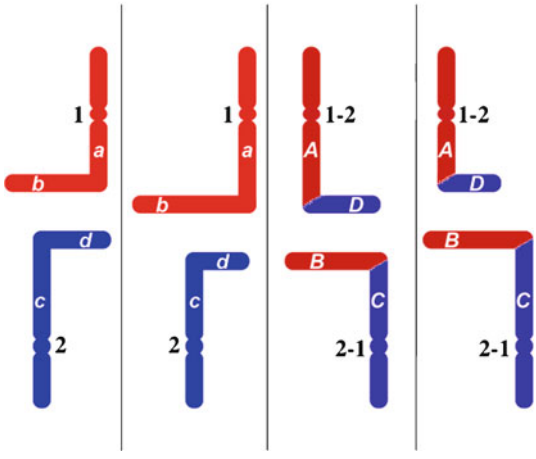


Fig. 1.15 Alternate segregation and products (no crossovers)