ANALYSIS OF A TRANSLOCATION IN *ASPERGILLUS NIDULANS*

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Influences of the translocation \( T_2(\text{I};\text{VIII}) \) on mitotic and meiotic recombination in \textit{Aspergillus nidulans}

A new \( \text{I;VIII} \)-translocation of \textit{Aspergillus nidulans} was analyzed by meiotic and mitotic recombination. All strains carrying the nitrosoguanidine-induced \( \text{sD205} \)-mutation on \( \text{VIII} \) contained \( T_2(\text{I};\text{VIII}) \), showing the typical complete marker-linkage between linkage groups \( \text{I} \) and \( \text{VIII} \) in mitotic haploids from heterozygous test-diploids. Meiotic linkage data mapped the other breakage point on \( \text{IL} \) proximal to \( \text{suAadE20} \), \( \text{galD} \); but distal to \( \text{anA} \). Mitotic haploids from homozygous \( T_2(\text{I};\text{VIII}) \) diploids established translocation reciprocality, showing two new linkage groups: 1) \( \text{suAadE20} \), \( \text{galD} \) one group, presumably translocated to \( \text{VIII} \); 2) all known group \( \text{VIII} \) markers attached on \( \text{IL} \) distal to \( \text{anA} \). From diploids carrying \( T_2(\text{I};\text{VIII}) \) in coupling or repulsion to \( \text{suAadE20} \), "suppressed" near-diploid mitotic crossover segregants were selected. These were abnormal, as expected, if trisomic for one and monosomic for the other translocation-segments. They spontaneously formed normal diploid sectors by a second mitotic-segregation event in the nonselected translocation-arm, and markers segregated as expected from the postulated \( T_2(\text{I};\text{VIII}) \)-topology. Heterozygous \( T_2(\text{I};\text{VIII}) \) crosses showed a localized reduction of meiotic recombination.

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INFLUENCES OF THE TRANSLOCATION T2(I;VIII) ON MITOTIC AND MEIOTIC RECOMBINATION IN ASPERGILLUS NIDULANS

by

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LITERATURE REVIEW

1. Definition and types of translocations

Translocation refers to the process by which a chromosomal fragment is transferred to a nonhomologous chromosome. The chromosomes involved in the translocation will be viable and normal in cell division provided that they each possess a single centromere. Inviability or deleterious phenotype results if the translocation is associated with lethal conditions or genomic imbalance. Translocations alter linkage relationships and the structural identity of homologous chromosomes. Hence, abnormal marker segregation (identified as unusual linkages with reference to the standard arrangement) and structural heterozygosity (identified as pairing irregularities at meiosis) are genetic and cytological means of identifying heterozygous translocations.

Translocations that involve single transfers of chromosomal fragments are nonreciprocal translocations, either insertional or terminal. Insertional translocation is the transfer of an interstitial segment to an interstitial position in a nonhomologous chromosome. Terminal translocation involving the transfer of a chromosomal segment to the end of another chromosome is believed to be very rare, if it occurs at all. The telomeres of unfragmented chromosomes apparently prevent the addition of extraneous pieces of chromatin distal to them (Swanson, 1966).

Translocations involving more than one segments are reciprocal translocations, if two segments are mutually exchanged; or complex translocations if several segments are transferred. Reciprocal
translocations, also known as segmental interchanges, are the most common viable type of translocations. Complex translocations are very common in some species of plants like Oenothera, Paeonia, Datura. Complex translocations are easily identified by cytological examination of metaphase chromosomes. When there are two interchanges between three nonhomologous chromosomes, a ring or chain of six is seen at metaphase; three interchanges between four nonhomologous chromosomes would result in a ring or chain of eight; and so on. This process can go on until the entire complement is involved when a complete ring may be observed at metaphase. This is known as a translocation complex. Marker segregations in species showing various degrees of complex translocations corresponded to and confirmed their cytological detection. In Oenothera and Paeonia, sub-species with one single linkage group showed the entire complement being translocated (Renner, 1925); while those with two linkage groups showed a ring of translocated complex and one separate tetrad at meiosis (Cleland, 1936). \textit{Rhoeo discolor} represents the complete realization of the translocation complex. These translocation complexes reflect unique and unorthodox evolutionary situations since preservation of genomic stability in these complex translocation heterozygotes require highly complicated chromosomal behavior at meiosis. Further description of this behavior is given in the section on non-random segregation of chromosomes (p. 22).

Fully viable translocations may exist in either heterozygous or homologous states. Homozygous translocated chromosomes behave like normal homozygous chromosomes, if no recessive lethal condition is associated with the translocation. Their identification requires
knowledge of the standard arrangement (the most frequent naturally occurring chromosome complement type) and detailed genetic analysis. Full viability of translocation homozygotes has been reported in plants like maize and barley (Burnham, 1962). In Drosophila, however, less than half of the translocations known are fully viable in homozygous conditions. They either have a lower viability or fertility than the normal individuals, or they are lethal in homozygous conditions (Dobzhansky and Sturtevant, 1931; Bridges and Brehme, 1944). It was postulated that these were associated with small deficiencies or recessive lethal point mutations, since larger deficiencies would be easily seen in the salivary chromosomes. Later, recessive deficiencies were demonstrated in barley (Tuleen, 1960). Tector (1961) in Aspergillus identified amongst irradiated diploid strains, "lethal" translocations which did not affect diploid phenotype; that is, likely small deletions since large deficiencies would lead to abnormal phenotype in A. nidulans.

All the different types of translocations have been identified in natural occurring as well as experimentally induced translocations.

2. Induction of translocations

Most experimental investigations deal with induced translocations. Chromosomal abnormalities can be produced by ionizing radiations (X-rays, neutrons, alpha, beta, and gamma rays) or non-ionizing radiations (ultraviolet light) which produce breaks and gaps in chromosomes or chromatids. They can also be produced by chemical treatments with radiomimetic compounds (alkylating agents, hydroxylamine, antibiotics) which attack the DNA, or with antimetabolites (5-bromodeoxyuridine, fluorodeoxyuridine)
which act during DNA replication. These mutagens have variable yields of induced interchanges depending on a large number of factors: the interaction of the mutagen with the organism used; the dosage delivered; the period of cell cycle affected; etc.

I Radiation induced interchanges

Irradiation with X-rays has been known to produce translocations in various organisms. Among many detailed studies, a selected sample includes Drosophila (Dobzhansky, 1931, 1932; Roberts, 1968, 1970); domestic fowl (Newcomer, 1959); mouse (Reddi, 1965; Lyon, Morris, Glenister and O'Grady, 1970); Zea mays (Anderson, 1935, 1936; Stadler, 1930, 1931); Vicia faba (Scott and Evans, 1967). Indeed, chromosomal rearrangements are so frequent with X-ray treatment that it is preferably avoided as a mutagen in organisms where point mutation is possible.

Fast neutrons have been shown to induce translocations in mouse (Searle, Evans and West, 1969). Thermal neutron treatment has been used on pollen or seeds of maize (Schmidt and Frolick, 1951); such yields of interchanges in maize were reported to be even higher than that of X-irradiation.

In micro-organisms, gamma rays irradiation was reported to have similar effects to that of X-rays in higher organisms. In Aspergillus nidulans, gamma rays irradiation of diploid conidia at dosages resulting in 1% to 10% survival gave frequencies of viable translocations much higher than that of recessive mutations (Tector and Klüfer, 1962). It was established, by tracing the pedigree of various translocations in A. nidulans, that ionizing radiations produced a considerably higher frequency of chromosomal aberrations than non-ionizing
radiations like ultra violet (UV) light (Köhler, 1965), a result consistent with that found in higher organisms (Stadler, 1941).

Damage caused by UV light are many and varied: delay cell division temporarily; delay synthesis of certain substances; change transport of certain substances across cell membranes; cause chromosomal abnormality; produce mutation; induce dimerization of pyrimidines; break the sugar-phosphate backbone of the DNA molecule when the dosage is exceedingly high (Deering, 1962). In higher organisms, e.g. maize, the main effect of UV light seemed to be the production of deficiencies (Stadler, 1931); breakages and transpositions (Singleton and Clark, 1940). However, it can produce chromosomal interchanges, e.g. in tomatoes (Barton, 1954); in Aspergillus (Köhler, 1962, 1965); and in Neurospora (McClintock, 1945; Perkins, Glassy and Bloom, 1962). UV-irradiation can also induce both chromatid and chromosomal type of aberrations in mammalian cells (Chinese Hamster) grown in vitro (Humphrey, Dewey and Cork, 1963; Chu, 1965). The frequency of induced aberrations is wavelength dependent, the maximum frequency is between 2450Å to 2800Å. Pre-treatment with thymidine analogues significantly increased the UV sensitivity of these mammalian cells as measured by the chromosomal aberrations induced. There was also an indication that chromosomal proteins (structural) played an important role in the induction of aberrations since demolished chromosomes were produced mainly by irradiation at 2800Å, or at 2650Å accompanied by post-irradiation inhibition of protein synthesis with puromycin (Chu, 1965). The effect of UV light on chromosomes in higher organisms is now believed to be the same as ionizing radiations by producing breakage and rejoining. In bacteria, UV has been known for a
long time to have a killing effect. At 2600 Å, its main effect appears to be the production of thymine dimers (Deering, 1962). However, certain resistant strains of bacteria are known to repair such damages (Within, 1969). Major rearrangements induced by UV are few in bacteria, hence it has been used frequently for the induction of mutants. However, small deletions appeared to be rather frequent (Käfer, personal communication).

II Chemically induced interchanges

In Drosophila, interchanges have been induced by exposing adult flies to nitrogen mustard gas (Kauffmann, Gay and Rothberg, 1949). Apart from interchanges, N-methyl-N'-nitro-nitrosoguanidine produced a wide spectrum of mutagenic effects in Drosophila, including deletions, nondisjunctions, point mutations, recessive lethals, etc. (Browning, 1970).

Antibiotics induced both chromatid and chromosomal type of aberrations; the mechanism by which a specific antibiotic acts varies slightly. Streptonigrin induced mainly chromatid aberrations in Vicia faba (Kihlman, 1964; Kihlman and Odmark, 1965), and in human leukocytes (Cohen, Shaw and Craig, 1963). It is believed to produce single stranded DNA breaks (White and White, 1966). Mytomycin C and Daunomycin are believed to produce cross-links in the DNA, since a higher frequency of exchanges were induced. Mytomycin C has been used successfully on Vicia faba root tips (Merz, 1961), and on human leukocytes (Cohen and Shaw, 1966). In human leukocytes, Daunomycin induced mainly interchanges involving two or more chromosomes since multiple chromosomal configurations involving up to ten chromosomes were observed (Vig, Koutras, Paddock and Samuels, 1968). As in all other agents discussed so far, there was no indication of preferential involvement of any particular chromosome in
the translocations. Phleomycin also induced both chromosomal and chromatin aberrations on Vicia root tips (Kihlman, Odmark and Hartley, 1967; Mattingly, 1967).

Antimetabolites that have successfully induced chromosomal aberrations are either nucleotide analogues like 5-bromodeoxyuridine (Somers and Hsu, 1962), or specific inhibitors of DNA synthesis like fluorodeoxyuridine (Taylor, Haut and Tung, 1962).

III Mechanism of induction of translocations

There are two main hypotheses concerning the mechanism of the production of chromosomal aberrations - the "breakage first" hypothesis of Sax (1938) and the "exchange" hypothesis of Revell (1955). The former hypothesis maintained that the initial lesion is a break in the chromosome; the two ends thus produced may either rejoin in the original configuration (restitution) or with other ends produced by another break to form an exchange. If the ends remain unjoined, a terminal deletion results. Revell's "exchange" hypothesis maintained that the primary event is some disturbance in the chromosome (but not a break) which may be repaired with time back to the normal state. This disturbance can "initiate" exchange and is eliminated if exchange follows. All chromosomal aberrations are exchanges produced by the interaction of two such primary events that occur close in space and time. Breaks in the chromosomes are the result of "incomplete exchanges".

Data from the experimentally induced chromosomal interchanges reveal quite a complex situation concerning the mechanism of the repair of chromosomal breaks and the production of an interchange.
a) The storage effect in Drosophila

When Drosophila spermatozoa, treated with a polyfunctional alkylating agent, e.g. triethylene melamine (TEM), was stored in untreated females, the frequency of recovered translocations increased up to ten-fold compared with that obtained without storage. However, the frequency of recessive lethals so recovered remained unchanged (Ratnayake, Strachan and Auerbach, 1967; Schalet, 1955). Monofunctional alkylating agents like ethylenimine (EI) did not have such storage effects on the frequency of viable translocations (Watson, 1964, 1966). It was postulated that storage provided a longer time for repair of breaks leading to a higher yield of translocations. The higher yield of translocations following storage after treatment with polyfunctional alkylating agents, but not monofunctional ones, are consistent with the idea that the higher number of breaks thus produced are repaired more efficiently with storage, leading to an increase in induced interchanges. Further, this indicated that there is an essential difference in the mechanism of intragenic and intergenic mutational events since the frequency of recessive lethals were not increased (Ratnayake, Strachan and Auerbach, 1967).

b) Interaction with DNA or protein synthesis

Experiments with inhibition of DNA synthesis indicated that DNA synthesis was required in the repair of breaks (Taylor, Haut and Tung, 1962; Taylor, 1963; Lozzio, 1969; Yamamoto and Yamaguchi, 1969). The G1 (pre-synthetic) phase of the mitotic cycle was found to be relatively less sensitive to the induction of chromosomal aberrations. Evidence for this was established in various organisms: Chinese Hamster (Bender and
Gooch, 1961; Hsu, Dewey and Humphrey, 1962); Trillium (Grant, 1965); Vicia faba (Scott and Evens, 1967). This is consistent with the idea that repair of breaks is more frequent during DNA replication, hence pre-synthetic cells are repaired more often, thus less sensitive.

Experiments with inhibition of protein synthesis indicated that protein synthesis was also required for the rejoining of chromosomal breaks (Wolff, 1960, 1966; Vogel and Vrba, 1967). Recently, Vig (1970) induced chromosomal aberrations in human leukocytes with daunomycin and observed the ratio of chromosomal exchanges (breaks repaired) to free chromosome fragments (breaks not repaired) in the presence or absence of puromycin (inhibitor of protein synthesis) and fluorodeoxyuridine (inhibitor of DNA synthesis). Inhibition of protein synthesis shifted the ratio in the direction of free chromosome fragments, while inhibition of DNA synthesis did not affect such ratio. He concluded that protein synthesis is required in the rejoining of breaks. Since DNA synthesis was normal in cells inhibited in protein synthesis by puromycin, he further argued that the required proteins are structural proteins rather than the enzymatic proteins of DNA synthesis.

Many experiments with other agents seemed to add complexity to the mechanism on induction of translocations. Choice between the two major hypotheses is still uncertain, and the exact mechanism by which mutagenic agents lead to their observable consequences is still obscure. On the whole, it seems that "repair of breaks" is required for the production of an interchange, and this repair involves both DNA synthesis and protein synthesis.
3. Detection and analysis of translocations

I Cytological identification

a) Meiotic pairing structures

Since homologies determine synapsis, pairing of homologous parts in a translocation heterozygote results in the formation of "cross-like" figures at pachytene and "rings" at later stages. This characteristic pairing configuration at pachytene serves as cytological means of identifying translocation heterozygosity. This relationship permits determination of the position of the translocation-breaks provided that the synaptic behavior concerned is exact. If the chromosomes are long enough to allow for crossing over, a ring of four chromosomes is observed at metaphase if chiasmata are formed in each of the paired arms of the "cross" configuration; a chain of four chromosomes results if one arm fails to form a chiasma. This method has been used extensively in plants, e.g. in maize (McClintock, 1930, 1931; Burnham, 1930). Recently, the frequencies of such metaphase configurations were used to estimate map length of the interchange segments in Secale cereale (Sybenga, 1970).

In micro-organisms, cytological identification of chromosomal interchanges is unsuitable due to the small size of their chromosomes. However, few attempts of cytological detection of translocation heterozygosity by pairing structures have been made in fungi (McClintock, 1945; Barry, cited in Fitcham and Day, 1965; Boothroyd, unpublished).

b) Mitotic configurations in organisms with somatic pairing

Pairing of homologous chromosomes in the mitotic cycle has been known as somatic pairing and was first established in Drosophila melanogaster due to the large polytene chromosomes in their salivary glands. Somatic
pairing is also inferred to occur in *Aspergillus nidulans* since mitotic recombination is a common phenomenon in this organism. Polytene chromosomes are very large, banded interphase chromosomes which show intimate pairing of homologous parts. Due to this pairing, structural heterozygosity results in complicated pairing configurations. In *Drosophila*, cytological examination of polytene chromosomes made the identification of structural rearrangements relatively easy. Bridges (1937) showed the correspondence between linkage maps and the banded structures in the polytene chromosomes, and consequently established correspondence between genes and salivary chromosome bands. This further facilitated the mapping of breakage points of structural rearrangements (Painter, 1933).

c) Mitotic and meiotic detection in human cells

In humans, most known translocations have been identified by the clinical syndromes manifested in some aneuploid descendants of normal individuals heterozygous for a translocation (carriers). The affected offsprings may resemble aneuploid 2N+1 types, but occur with high frequencies in certain families. The best known case is the "Translocation Down's Syndrome". Down's Syndrome (also known as Mongolism) is caused by trisomy for chromosome 21 (Lejeune, Gautier and Turpin, 1959) so that the affected individuals have 47 chromosomes instead of the normal complement of 46. However, in a small percentage of Mongoloid patients, the normal number of chromosomes (46) were detected. Detailed analysis showed that they contained an abnormally large chromosome. In these cases, one of the parents was found to be heterozygous for a translocation, with the third 21-chromosome either attached to a chromosome of the D group (13-15/21 translocation) or to a member of the G group (22/21 translocation). Some
of their offspring become effectively trisomic for chromosome 21, if they receive the translocated as well as two normal 21s and are phenotypically indistinguishable from the "standard" trisomic 21 (Thompson and Thompson, 1966; Turpin and Lejeune, 1965; Penrose and Smith, 1966). With the recent development in identifying chromosomes by their heterochromatin banding patterns, detection of structural rearrangements should be greatly facilitated.

Recently, an analogous case of a translocation in man was reported to be associated with a case of Turner's Syndrome (X0) (Sinha and Nova, 1969). Detailed analysis revealed two structurally aberrant chromosomes apparently representing an unidirectional, balanced translocation involving the two X homologues. Other syndromes due to aneuploidy associated with X-autosome translocations in man have also been reported - one case was associated with primary amenorrhea (Mann, Valladmanis, Capp and Puite, 1965) and another was associated with multiple congenital anomalies (Mukherjee and Burdette, 1966).

II Genetic identification of translocations

If a full complement of genes are to be transmitted to the offsprings in a translocation heterozygous mating in absence of crossing over, it requires the two structurally normal chromosomes to be passed on to one gamete and the interchanged chromosomes to another. Other segregation patterns or crossing over between the interstitial regions would result in deficient and duplicated gametes, leading to reduced viability in the offsprings. If markers are available near the breakage point of the translocation, linkage between sterility and genetic markers may be detected. In organisms where the unbalanced gametes are completely
inviab1e, only parental gametes would survive. Thus, a complete genetic
linkage between the two structurally involved chromosomes would be
manifested in the progeny of such a heterozygous translocation mating.
Complete genetic linkage may also be obtained if crossing over is
completely suppressed in the two parents, e.g. in Drosophila, the use
of "crossover suppressed" females which are normally heterozygous for an
inversion. If suitable markers are available, unusual meiotic linkages may
be detected. Hence, three criteria may be used as genetic means of
identifying fully viable heterozygous translocations: 1) reduced viability
of offsprings; 2) complete genetic linkage of structurally affected
chromosomes; 3) unusual linkage of translocated markers.
a) Reduced viability of offsprings

The deleterious effect of translocations known for the longest time
is undoubtedly the reduction in fertility of the affected individuals,
which had led to the first identification of a heterozygous translocation
in plants. It was recognized by Belling in the Florida velvet bean,
Stizolobium, and referred to as "semi-sterility" (1914, 1915, 1925).
Semi-sterility results from the formation of unbalanced gametes which are
usually not functional and can be identified as abortive pollen, reduced
seed set, or empty grains (Burnham, 1948; Stadler, 1935). Reduction in
fertility observed as high mortality in the offsprings or reduced
fecundity in the affected individuals have been reported in a wide
variety of organisms, from Drosophila to boars (Muller and Altenburg, 1930;
Dobzhansky, 1929; Dobzhansky and Sturtevant, 1931; Bridges and Brehme,
1944; Novitski and Sandler, 1957; Henricson and Böckström, 1965). In
maize, linkages between sterility and genetic markers have also been reported (Burnham, 1930; Brink and Cooper, 1931).

In some species of fungi, where examination of ascospore patterns are feasible because of their large linear asci (Neurospora crassa, Sordaria macrospora), detection of translocations or any structural rearrangements is achieved by visual identification of the inviable, presumably unbalanced, meiotic products (McClintock, 1945; Perkins and Ishitani, 1959; Hesolt, 1958; Perkins et al., 1962). In these organisms, inviable meiotic products in crosses heterozygous for rearrangements show up as abortive ascospores recognized by their hyaline appearance and lack of pigmentation. From the numerical distribution of ascoospore patterns, one can differentiate between the presence of translocations and inversions (Esser and Kuenen, 1967). This also permits an estimation of the translocation point from the centromere, if it is no further than 30% recombination away; hence, the extent of chromosome arm involved in the translocation.

In yeast, the ratio of viable to nonviable spores in the asci from a heterozygous translocation mating is of three expected types: 4:0, 2:2, 0:4. The 2:2 asci arise as a consequence of exchange in one of the interstitial regions. Thus, the surviving spores are expected to be non-sister; in other words, a centromere-linked gene, A/a, should segregate in these asci. Also, only one of the two spores in such an asci is expected to carry the translocation. In absence of exchanges, random disjunction of centromeres will lead to either four or no viable spores (Emerson, 1963; Esser and Kuenen, 1967).

In Aspergillus nidulans, the detection of translocation has been
greatly facilitated by means of the parasexual cycle, since tetrad analysis would be extremely tedious to use. Techniques for the detection and analysis of translocations were developed by Käfer (1958, 1962). The principle of detection is the same as the one stated for Drosophila: in absence of crossing over in translocation heterozygotes, all markers of the two linkage groups involved become completely linked. In Aspergillus, heterozygous diploids can easily be made by combining a translocation-strain with a standard tester strain in a heterokaryon (Roper, 1952). Heterozygous diploids arise spontaneously and can be selected for by their wildtype phenotype. Mitotic haploids arise spontaneously as rare mitotic segregants. They seem to result from a process of mitotic nondisjunction by stepwise loss of whole chromosomes via aneuploids (Käfer, 1961). Hence, mitotic haploids carry a random assortment of the homologous chromosomes without crossing over (Pontecorvo and Roper, 1953; Pontecorvo, Tarr-Gloor and Forbes, 1954; Pontecorvo and Käfer, 1958). Such rare mitotic haploid segregants can be selected from a test diploid heterozygous for a translocation. It is desirable to have all linkage groups marked for this kind of analysis. If the translocation is a reciprocal one, unbalanced mitotic haploid segregants are expected to be inviable. Therefore, since only the parental arrangement of the markers carried on the "translocated" linkage groups will provide a balanced genome, haploids of this type would be the only ones recovered from a test diploid heterozygous for a translocation. Thus, markers on the translocated linkage groups become completely linked in mitotic haploids, while those on the normal linkage groups show free recombination. (For detailed techniques on isolation and characterization of mitotic haploids, refer to Material and Methods, p.39).
Therefore, if complete linkage between two previously independent linkage groups is found, this is taken to indicate the presence of a heterozygous translocation between these linkage groups. Even from insertional translocations only the fully balanced haploids of the "parental marker types" are usually isolated, since the selective techniques used select against partially viable duplicated haploid types. When suitable markers are available, complete, detailed mapping of translocations is possible, as outlined by Käfer (1962). Which arm of the linkage group is involved can be determined by mitotic crossing over, if selective markers are available (IR in case of T1(I;VIII), IL in case of T1(I;VII) as well as the translocation analyzed here). Meiotic linkage of the translocation to markers of the affected chromosome arm can then further localize breakage points. So far, only two cases of insertional translocations with linked markers have been mapped fairly well. Both are T(III;VIII), the first one was reported linked to cha on VIII and sc12 on III (Käfer, 1965) and studied in detail by Bainbridge (1970; Bainbridge and Roper, 1966); the other also reported to be an insertional translocation into linkage group III (Clutterbuck, 1970).

4. Effects of translocations on meiotic recombination

Presence of a heterozygous translocation often lead to a reduced frequency of recombinants of those markers carried on the affected chromosome arms. This effect is discussed in detail in the following. Apart from this, it has been reported that translocation heterozygosity could also lead to an increased recombination in the non-affected chromosome arm or even nonhomologous chromosomes not involved in the
translocation - a phenomenon first identified in Drosophila and known as "interchromosomal effects" (Zimmering and Barbour, 1952; Ramel, Goldman and Kjellström, 1965; Hinton, 1964, 1965; Williamson, 1966). Such interchromosomal effects were also reported for translocation heterozygotes of grasshoppers (White, 1963; Hewitt and John, 1965; Hewitt, 1967). However, work in this direction has not been reported in micro-organisms. It would be interesting to confirm these findings in micro-organisms, especially since this type of analysis would be relatively simple in species with a well mapped genome, e.g. fungi.

The effects of translocation heterozygosity on crossing over within structurally affected chromosomes are mainly reductions of crossing over in the various affected regions (discussed in detail below). There was cytological evidence for asynapsis, as well as genetic evidence for reduction of crossing over. Variability in pairing, which is indicative of nonhomologous pairing, has been observed in the salivary gland chromosomes of Drosophila due to the presence of a heterozygous inversion (Hoover, 1937, 1938). It is probable that nonhomologous pairing may also occur in translocation heterozygotes. In maize, variable positions of the center of the "cross-shape" configuration in pachytene in certain translocation heterozygotes have been reported (McClintock, 1932; Burnham, 1932, 1934, 1948). In some figures, there was asynapsis near the center of the cross, but in others there was close pairing in all segments even when the "cross" involved nonhomologous pairing. These observations are expected to have the following genetic implications. First, nonhomologous pairing showing asynaptic regions should be correlated with a reduction of crossing over in the regions affected. Second, close pairing of
nonhomologous regions may lead to nonhomologous crossing over, resulting in the formation of deficiency and duplication progenies, or dicentric and acentric chromatids, and therefore, a reduction in viable progeny. Evidence for this latter type of nonhomologous crossing over had been presented by Green (1959) in Drosophila and by McClintock (1933) and Stadler (1935) in Zea mays.

Reduction in the frequency of crossover progenies in crosses heterozygous for a translocation has been observed in various organisms. In Drosophila, this reduced frequency recovered in translocation heterozygous matings has been known since 1931 (Dobzhansky, 1931, 1934), though relatively little work has been done along these lines compared with those on inversion heterozygous matings. Dobzhansky (1931, 1934) first reported the presence of a heterozygous translocation which reduced the frequency of crossover progenies in the structurally altered chromosomes; the reduction was localized near the breakage points of the translocation. There were attempts to correlate the position of breakage point and the length of interstitial segment with the magnitude of the reduction. The effect of heterozygous translocations on the frequency of recombinant offsprings with respect to their breakage points in Drosophila has been summarized by Burnham (1962) based on information reported by Bridges and Brehme (1944), Dobzhansky and Sturtevant (1931), Brown (1940), and Pipkin (1940). Their results showed that translocations with both proximal breaks, hence short interstitial segments, had no reduction in crossover progenies in the arm with the break, as well as in the non-translocated arm of the same chromosome. When the translocation-breaks were distal, such progenies were greatly reduced near the breaks. When the break was
near the middle of the chromosome arm, frequency of crossover progenies for markers on the entire arm were reduced greatly, the greatest effect being in the region of the translocation-breaks. As the translocation-break moved distally, its effect on markers of the entire arm decreased and a localized reduction at the breakage point was observed. In these experiments, it was difficult to establish with certainty whether the observed reduction in crossover progenies was due to inviability of the crossover offsprings or an actual reduction of crossing over in the chromosome arms involved in the translocation.

Recently, Roberts (1965, 1968, 1970) reported a series of reciprocal translocations in *Drosophila melanogaster* which reduced actual crossing over along the whole chromosome arm. He developed a marker stock with unlinked markers (approximately 50 crossover units) on every major chromosome arm, and exposed males of this stock to irradiation with X-rays to induce crossover suppressors. Individuals with suppressed crossing over were established by screening for unusual linkage of the markers used. These were then subjected to polytene chromosome analysis to identify any structural rearrangements. Results showed that the most predominant type being reciprocal translocations. Since the markers used to detect reduction in crossing over were practically unlinked, a novel type of effect had been selected for, which is different from most of the previously reported effects of translocations which were all localized. Further analysis indicated a correlation between the position of the breakage point of the translocation and the extent of "crossover suppression". Translocations with breaks either both proximal, or both distal, were not recovered as "crossover suppressors". Only types with one distal and one proximal
break were isolated. Apparently, at least one break must be distal to medial for the translocation to be recovered as a "crossover suppressor". By holding one translocation-break constant in the tiny 4th chromosome, he showed that the most effective breaks are located one-third to two-thirds from the chromosomal tips. Reduction of crossing over in the two chromosome arms involved was unequal; the arm with the distal break showed extreme reduction along its whole arm even in the centromere regions which is far from the breakage point. However, crossing over in the arm with proximal break was normal. Further, no multiple crossover progeny was recovered. Since multiple crossover progenies would be genetically balanced and fully viable, they are expected to be selected for if inviability of the unbalanced lethal zygotes was the cause for the reduction of crossover progenies. Therefore, it appeared that it is the actual process of crossing over being impaired in these individuals heterozygous for the translocation. In the homozygous translocation crosses, crossing over was found to be normal, that is, almost at control value. Hence, it seems that it is not so much the presence of a distal break in the rearrangement that interfered with crossing over, but rather, heterozygosity for this type of translocation.

It can be concluded that pairing may be disturbed in the translocation heterozygotes which suppresses the actual process of crossing over. Roberts (1970) further argued that the distal tips of chromosomes are important for "initiating synapsis", since his experiments showed that this structural heterozygosis in the tips reduced crossing over throughout the entire chromosome arm, and presumably caused a disturbance in pairing along that chromosome arm.
Since chromosome pairing in higher organisms has been correlated with the presence of synapticemal complex, the above findings may be related to the impaired function of the synapticemal complex. Comings and Okada (1970) suggested that pairing by the synapticemal complex is nonspecific and merely serves to bring homologous chromosomes into a site by site approximation such that specific molecular base pairing required for crossing over can occur. The mechanism of chromosome alignment is postulated to be the nonspecific pairing of the LC loops (fibres of the lateral element that extend centrally) of the synapticemal complex. That is, as the LC fibres are synthesized they extend out from the opposite lateral elements and join to form the central element, hence complete the formation of the synapticemal complex. It was postulated that LC fibres are synthesized sequentially and those synthesized at the same time associate with one another. On this hypothesis, the sequential order of LC fibre-synthesis in translocation heterozygotes may be out of phase due to the translocation, such that the formation of the synapticemal complex is impaired, or that nonhomologous segments are synapsed due to the nonspecific pairing properties. In both cases, precise molecular pairing is impaired, resulting in no recombination along the chromosome segment affected. Von Wettstein (1971) offered a different interpretation to the pairing process. He suggested that both sister chromatids participate in the biogenesis of one lateral element of the synapticemal complex. The lateral elements are responsible for specific recognition during precise pairing which is initiated at specific initiation points. The biogenesis of the lateral elements may require specific physical continuity of the chromatids and the physical reunion of nonhomologous
chromosomes in a translocation heterozygote may impair its de novo synthesis. Another feasible explanation is that the postulated specific points initiating pairing are disturbed as a result of the translocation such that the chromosomes fail to align and pair. Since pairing is a prerequisite for crossing over, asynaptic chromosomes cannot crossover, hence no recombination is observed along the whole arm of such chromosomes. Roberts' results (1970) seem to favour specific points for the initiation of pairing, at the chromosome tips.

5. Effects of translocations on disjunction of chromosomes

I Non-random segregation of chromosomes

Viable chromosomal aberrations such as inversions and translocations have been shown to cause unusual genetic segregation. In some cases, evidence has been obtained that they likely cause deviations from random assortment of nonhomologous chromosomes. Non-random chromosomal segregations are well established in structural heterozygotes of higher plants and Drosophila (Burnham, 1934; Glass, 1935); the translocation complex of Oenothera and Datura being the extreme example (Cleland and Oehlkers, 1930; Cleland, 1936; Marquardt, 1948). During meiosis, when a translocation ring of two chromosome pairs reaches the metaphase plate, three orientations are possible, each having different genetic consequences. In the "adjacent-1" and "adjacent-2" (open ring) type of arrangement, the ring is oriented so that adjacent chromosomes go to the same anaphase pole. The gametes so formed are both deficient and duplicated (Dp-Df) for certain regions of the involved chromosomes. These heteroploid gametes showed genomic imbalance. The remaining arrangement ("alternate" or "zig-zag" type) differs in that alternate chromosomes go to the same pole. The gametes formed are of two
kinds, those having a set of structurally normal chromosomes and those having a translocated set of chromosomes. They both possess the full complement of genes (orthoploid types), hence are fully viable. In terms of homologous centromeres, "alternate" and "adjacent-1" segregations result in the separation of homologous centromeres to opposite poles; while in the "adjacent-2" segregation they pass to the same pole (nondisjunction). If the three types of orientation occur at random, 2/3 of the gametes produced in a cross heterozygous for a translocation are expected to be inviable. However, this result is not observed. In plants, particularly in maize (Burnham, 1934, 1949, 1950) where the phenomenon has been extensively studied, sterility due to the formation of unbalanced gametes is only 50%, a value lower than expected. This indicated that "alternate" disjunction which gives fully viable gametes is occurring more often than expected by chance, and demonstrated the non-random disjunction of homologous chromosomes involved in a translocation.

In Datura and Oenothera, where complicated structural heterozygosity is a common and natural phenomenon, survival and fertility of these species implied that genomic balance is maintained, hence segregation of homologous chromosomes must be under rigid control. In both Oenothera and Datura species showing translocation complexes, pollen fertility is quite high (Cleland and Oehlkers, 1930; Cleland, 1936). Alternate disjunction occurred in a majority of cases. However, crossing over in the interstitial segment will produce unbalanced gametes (hence abortive spores) even if its segregation is all alternate. Therefore, normal fertility in the complex translocation heterozygotes must imply that crossing over in the interstitial segments is somehow eliminated so that it did not contribute
to the sterility of these individuals. In other words, such individuals must have genetically short interstitial segments. Marquardt (1948) confirmed this hypothesis by showing that induced translocations with long interstitial segments in Oenothera did show partial sterility. He felt that genetically short interstitial segments may result from actual short physical distances; or a reduction in crossing over in the interstitial regions due to disturbances caused by translocation or by proximal heterochromatin.

II Meiotic nondisjunction

Chromosomal aberrations are known to increase meiotic nondisjunction. In the case of heterozygous translocations, earlier work in Drosophila had utilized the occurrence of nondisjunction of the translocated segments as a genetic proof of the presence of breakage and rearrangement involved in the translocations (Dobzhansky, 1930, 1931). The genetic data from several crosses heterozygous for translocations in Drosophila revealed that segments of chromosomes retaining their own spindle fibre attachments always disjoin from their normal homologue at the reduction division; while segments of chromosomes that were translocated were distributed at random with respect to the normal chromosome and markers on these show a nondisjunctional type of segregation (Dobzhansky, 1930, 1931; Muller, 1930). This is perfectly normal and as expected since the behavior of the translocated segments is determined by nonhomologous centromeres.

These studies and many others reported earlier established a reduction of crossing over within the translocated segments. However, no direct correlation was made with respect to this reduction in crossing over and the nondisjunction of the same translocated segments. The first
indication of such a relationship came from a case of high frequency of X-chromosome nondisjunction in Drosophila, which was later shown to involve a X-3 translocation (unpublished data of Anderson, reported in Dobzhansky, 1931). This translocation also reduced crossing over in the X-chromosome and in the right arm of chromosome 3 where the breakage occurred.

Recent work in *Aspergillus nidulans* demonstrated that in fungi, chromosomal aberrations, when heterozygous in crosses, also increase meiotic nondisjunction of the structurally involved chromosomes. In addition, meiotic nondisjunction of other, structurally normal, chromosomes also seemed somewhat increased (Pollard, 1966; Pollard, Käfer and Johnston, 1968).

III Mitotic nondisjunction

Relatively few cases of mitotic nondisjunction in translocation heterozygotes have been reported. A case of preferential mitotic nondisjunction of one translocated element was reported by Roman in maize (1949) and later by Bainchi, Bellini, Contin and Ottaviano (1961). This involved the reciprocal A-B translocations between a member of the regular complement (A) and a genetically inert chromosome (B) of the maize genome. In the mitotic division of the generative nucleus to form the two sperm nuclei, the B-chromosome carrying the translocated A segment (B^A element) of these translocations frequently undergoes nondisjunction resulting in one sperm with two B^A elements (duplicated or hyperploid type) and one sperm with no B^A element (deficient or hypoploid type). The two types of sperm nuclei may be distinguished from one another since subsequent fertilization produces two kinds of seeds: two B^A elements in the endosperm and no
$B^A$ element in the embryo; no $B^A$ in the endosperm and two $B^A$ in the embryo. However, both of the above seeds have the $A^B$ element of the translocation. This demonstrated the preferential mitotic nondisjunction of the $B^A$ element.

In organisms showing mitotic recombination, e.g. *A. nidulans*, it would be interesting to correlate effects of heterozygous translocations on mitotic crossing over and mitotic nondisjunction. However, such experiments have not been studied in detail, and the one case reported on mitotic nondisjunction (Pollard, 1966; Pollard, Kühner and Johnston, 1968) did not establish conclusive results mainly due to the design of their experiments (see discussion).
INTRODUCTION

In diploid strains of Aspergillus nidulans, complete genetic linkage of two previously independent linkage groups in mitotic haploids has been taken to indicate the presence of a heterozygous translocation between these linkage groups (details discussed in Literature Review, p.15). It was postulated that due to heterozygosity for a reciprocal translocation, the two crossover arrangement of translocated chromosomes, in absence of crossing over, result in mitotic haploids duplicated for one translocation-arm and deficient in the other (Fig.1, p.28). These genetically unbalanced haploid progenies are normally inviable, due to their deficiencies. Haploids with the parental arrangements of translocated chromosomes are balanced and fully viable. They would be the only types recovered from diploids heterozygous for a reciprocal translocation, which explains the complete genetic linkage between the two previously independent linkage groups. This hypothesis has two main corollaries. First, in diploids homozygous for the reciprocal translocation, the crossover arrangement of translocated chromosomes would be balanced and viable. Hence, the complete linkage between all markers on the translocated chromosomes should disappear. However, translocated markers would segregate according to their new linkage relationship. Second, in diploids heterozygous for a reciprocal translocation, mitotic recombination in only one of the arms involved in the translocation resulting in its homozygosis should lead to mitotic progenies trisomic for one translocation-arm and monosomic for the other. Such genomic imbalance would manifest observable phenotypic and genetic effects (Käfer, 1962).
FIGURE 1  Expected types of mitotic haploids from a diploid heterozygous for a reciprocal translocation

<table>
<thead>
<tr>
<th>Linkage group A</th>
<th>Linkage group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>b</td>
<td>a</td>
</tr>
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</table>

Possible chromosomal segregations

<table>
<thead>
<tr>
<th>Chromosome arrangement in mitotic haploids</th>
<th>Deficiencies</th>
<th>Duplications</th>
<th>Viability</th>
</tr>
</thead>
<tbody>
<tr>
<td>parental</td>
<td>none</td>
<td>none</td>
<td>balanced fully viable</td>
</tr>
<tr>
<td>crossover</td>
<td>b</td>
<td>a</td>
<td>unbalanced inviable</td>
</tr>
<tr>
<td>crossover</td>
<td>a</td>
<td>b</td>
<td>unbalanced inviable</td>
</tr>
</tbody>
</table>
There are several ways to confirm the above hypothesis.

1) Cytological demonstration of a heterozygous reciprocal translocation by pairing structures. However, this type of analysis in \textit{Aspergillus nidulans} is extremely difficult due to its tiny chromosomes which are unsuitable for light microscopy. Nevertheless, efforts to demonstrate a ring of translocated chromosomes has been successful (Boothroyd, unpublished).

2) Tetrad analysis of inviable meiotic products similar to abortive ascospore pattern in \textit{Neurospora} would be extremely laborious in \textit{Aspergillus} due to the small size of the asci. This leaves genetic evidence confirming the two main corollaries the last and most feasible resource to prove the hypothetical principle on the detection of reciprocal translocations in \textit{Aspergillus nidulans}. Experiments designed in this direction has been long pursued with several likely reciprocal translocations [T₁(I;VII), T(VI;VII)] (Kläfer, unpublished). However, these analyses all failed due to incomplete genetic mapping of the translocations as a result of lack of suitable markers on the translocation-arms and difficulties in obtaining diploids homozygous for the translocation or their analysis (details described in Discussion). In hope to accomplish what previous experiments had failed, a newly identified translocation T₂(I;VIII) was chosen for analysis. It was first identified in descendant strains of a nitrosoguanidine (NTG)-induced mutation ad205 (sd for short) by Nicklewicz (1970). This translocation was chosen because both linkage groups I and VIII are very well mapped with a number of selective markers, including the most useful one, the recessive suppressor of adE20 - suAadE20 (su for short) on the left arm of linkage group I. The present effort intends to perform a complete genetic mapping of the translocation T₂(I;VIII);
to provide direct evidence for the principle on detection of heterozygous reciprocal translocations; and possibly to study some effects of the translocation on meiotic crossing over.
MATERIAL AND METHODS

1. Strains

All strains used in these experiments are descendants of the same wild type haploid strain of *Aspergillus nidulans* used by Pontecorvo and co-workers. Details of all the genetic markers used here are discussed in Pontecorvo, Roper, Hemmons, McDonald and Bufton (1953), Käfer (1958), and Dorn (1967), except for the sulfite mutant sD205 which was induced by Gravel (1969) and described in Gravel, Käfer, Nicklewicz-Borkenhagen, and Zambryski (1970). The map position of all markers used in these experiments are shown in Fig. 2. A description of the markers and their abbreviations in this thesis is given in Table 1.

Strains carrying the translocation T2(I;VIII) were first identified by Nicklewicz (1970). Two first generation descendants from the NTG-induced mutant sD205 were found to carry this translocation as well as the induced marker. It was deduced that the translocation probably arose in the parent strain since it is unlikely that a spontaneous event would occur twice independently in two sister strains. All translocation strains used in these experiments are descendants from one of the first identified T2(I;VIII) strains established by repeated crossing to useful markers on linkage group I and VIII. The pedigree of the translocation is shown in Fig. 3 and 4.

2. Media

Basic media for *Aspergillus nidulans* were used (Pontecorvo et al., 1953) as modified by Käfer (1958) and described in details by Barratt, Johnson and Ogata (1965). Nutritional mutants were characterized on
FIG. 2

Map position of essential markers used in this thesis

su gal riboA an lu pro paba y ad bi

Acr w

phen sC

pyro

lys

sB lac

cho

sD co fw ve cnx fac riboB pal cha
### TABLE 1

Description of all markers used in this thesis

<table>
<thead>
<tr>
<th>New gene symbol*</th>
<th>Linkage group position</th>
<th>Mutant allele used</th>
<th>Abbreviated gene symbol</th>
<th>Description of mutant phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CONIDIAL COLOR MUTANT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>chaA</td>
<td>VIIIR</td>
<td>2</td>
<td>cha</td>
<td>chartreuse conidia</td>
</tr>
<tr>
<td>fwA</td>
<td>VIIIR</td>
<td>1</td>
<td>fw</td>
<td>fawn conidia, epistatic to y, cha</td>
</tr>
<tr>
<td>wA</td>
<td>II</td>
<td>2</td>
<td>w</td>
<td>white conidia, epistatic to y, fw, cha</td>
</tr>
<tr>
<td>yA</td>
<td>IR</td>
<td>1</td>
<td>y</td>
<td>yellow conidia</td>
</tr>
<tr>
<td><strong>CONIDIAL MORPHOLOGY MUTANT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>coA</td>
<td>VIIIR</td>
<td>1</td>
<td>co</td>
<td>compact conidial morphology</td>
</tr>
<tr>
<td>veA</td>
<td>VIIIR</td>
<td>1</td>
<td>ve</td>
<td>velvet appearance of colony present in almost all strains</td>
</tr>
<tr>
<td>ve+</td>
<td></td>
<td></td>
<td></td>
<td>fluffy morphology, like wildtype</td>
</tr>
<tr>
<td><strong>NUTRITIONAL MUTANT</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>adE</td>
<td>IR</td>
<td>20</td>
<td>ad</td>
<td>requiring adenine for growth</td>
</tr>
<tr>
<td>adG</td>
<td>IL</td>
<td>14</td>
<td>-</td>
<td>requiring adenine for growth</td>
</tr>
<tr>
<td>anA</td>
<td>IL</td>
<td>1</td>
<td>an</td>
<td>requiring aneurin for growth</td>
</tr>
<tr>
<td>biA</td>
<td>IR</td>
<td>1</td>
<td>bi</td>
<td>requiring biotin for growth</td>
</tr>
<tr>
<td>choA</td>
<td>VII</td>
<td>1</td>
<td>cho</td>
<td>requiring choline for growth</td>
</tr>
<tr>
<td>cnxB</td>
<td>VIIIR</td>
<td>2</td>
<td>cnx</td>
<td>requiring nitrite for growth</td>
</tr>
<tr>
<td>luA</td>
<td>IL</td>
<td>1</td>
<td>lu</td>
<td>requiring leucine for growth</td>
</tr>
<tr>
<td>lysB</td>
<td>V</td>
<td>5</td>
<td>lys</td>
<td>requiring lysine for growth</td>
</tr>
<tr>
<td>ornB</td>
<td>VIIIR</td>
<td>7</td>
<td>orn</td>
<td>requiring ornithine for growth</td>
</tr>
<tr>
<td>pabaA</td>
<td>IR</td>
<td>1</td>
<td>paba</td>
<td>requiring para-amoeno benzoic acid for growth</td>
</tr>
<tr>
<td>phenA</td>
<td>III</td>
<td>2</td>
<td>phen</td>
<td>requiring phenylalanine for growth</td>
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<td>IR</td>
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<td>pro</td>
<td>requiring proline for growth</td>
</tr>
<tr>
<td>pyroA</td>
<td>IV</td>
<td>4</td>
<td>pyro</td>
<td>requiring pyridoxin for growth</td>
</tr>
<tr>
<td>riboA</td>
<td>IL</td>
<td>1</td>
<td>-</td>
<td>requiring riboflavin for growth</td>
</tr>
</tbody>
</table>
### Cont'd TABLE 1

<table>
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<tr>
<th>New gene symbol*</th>
<th>Linkage group position</th>
<th>Mutant allele used</th>
<th>Abbreviated gene symbol except Fig. 9</th>
<th>Description of mutant phenotype</th>
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<tr>
<td>riboB</td>
<td>VIIIR</td>
<td>2</td>
<td>ribo</td>
<td>requiring riboflavin for growth</td>
</tr>
<tr>
<td>sA</td>
<td>III(R)</td>
<td>2</td>
<td>-</td>
<td>sulfite-requirer, COS-utilizer</td>
</tr>
<tr>
<td>sB</td>
<td>VI</td>
<td>3</td>
<td>-</td>
<td>sulfite-requirer, COS-utilizer; sodium selenate resistant</td>
</tr>
<tr>
<td>sC</td>
<td>III(R)</td>
<td>12</td>
<td>-</td>
<td>sulfite-requirer, COS-utilizer</td>
</tr>
<tr>
<td>sD</td>
<td>VIIIR</td>
<td>205</td>
<td>sD</td>
<td>sulfite-requirer, cannot utilize COS as sulfate source</td>
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<td>SUGAR MUTANT</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>galD</td>
<td>IL</td>
<td>5</td>
<td>gal</td>
<td>cannot utilize galactose</td>
</tr>
<tr>
<td>lacA</td>
<td>VII</td>
<td>1</td>
<td>lac</td>
<td>cannot utilize lactose</td>
</tr>
<tr>
<td>ACETATE MUTANT</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>facB</td>
<td>VIIIR</td>
<td>101</td>
<td>fac</td>
<td>cannot utilize ammonium acetate</td>
</tr>
<tr>
<td>PHOSPHATASE MUTANT</td>
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<td></td>
</tr>
<tr>
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<td>VIIIR</td>
<td>7</td>
<td>pal</td>
<td>cannot utilize beta-glycerophosphate as a phosphate source</td>
</tr>
<tr>
<td>RESISTANT MUTANT</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AcrA</td>
<td>III</td>
<td>1</td>
<td>Acr</td>
<td>resistant to acriflavin</td>
</tr>
<tr>
<td>SUPPRESSOR MUTANT</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>suAadE</td>
<td>IL</td>
<td>1</td>
<td>su</td>
<td>recessive suppressor specific for adE20</td>
</tr>
</tbody>
</table>

* as proposed by Clutterbuck (1968, 1969)
FIGURE 3

Pedigree of T2(I;VIII)

• Barratt et al., 1965
**Käfer, 1965
***unpublished
Fig. 4

$T_2(I;VIII)$ crosses analyzed in this thesis from cross (893) of paba bi; $T_2(I;VIII)$ sD x standard strain (**)

- paba bi; $T_2(I;VIII)$ sD (tested in diploid 1031 *)
  - (1250) su y ad; phen; sB; riboB cha
  - 8 sD+ tested all without $T_2(I;VIII)$

- paba y; $T_2(I;VIII)$ sD (tested in diploid 951 and 1030 *)
  - (1035) wa3; cnx fac riboB cha
  - 3 sD-segregants test, all $T_2(I;VIII)$

- $T_2(I;VIII)$ sD cnx fac riboB cha
  - (1155) orn fw ve+
  - 9 tested
  - 8 sD, all with $T_2(I;VIII)$
  - 1 sD+, without $T_2(I;VIII)$

- $T_2(I;VIII)$ sD fw ve+ fac riboB cha
  - (1156) su gal an paba y ad bi; cha
  - sD strain with $T_2(I;VIII)$ in "coupling diploid" (1238)
    - 14 tested
    - 9 other sD with $T_2(I;VIII)$
    - 5 sD+, no $T_2(I;VIII)$

- su gal an paba y ad; $T_2(I;VIII)$ sD fw ve+ fac riboB cha
  - (1300) ad; Acr; phen; pyro
    - 2 sD strains with $T_2(I;VIII)$ in "repulsion diploids" (1322 and 1348)
    - 3 sD strains with $T_2(I;VIII)$ in "homozygous diploids" (1255 and 1256)
    - 24 other sD strains tested, all with $T_2(I;VIII)$
    - 17 sD+ strains tested, all without $T_2(I;VIII)$

* Nicklewicz, 1970
** refer to Fig. 3.
minimal media (MM) supplemented with the appropriate growth requirements. Sugar mutants were tested on supplemented minimal media with the appropriate sugar substituting for dextrose (Roberts, 1963). Acetate mutants were identified on acetate media (Apirion, 1965). The alkaline phosphatase mutant \textit{palB7} was identified on phosphate-free medium containing beta-glycerophosphate (Dorn, 1965).

The different sulfite requiring mutants (\textit{sB3, sC12, sD205}) were distinguished from one another by their resistance to sodium selenate (Arst, 1968) and response to choline-O-sulfate (COS) (Gravel, 1969).

Acriflavin resistance was identified on complete media (CM) containing 0.025 gm/l. of acriflavin (Roper and Klifer, 1957).

Complete media containing DL-parafluorophenylalanine (pfp) was used to select for mitotic haploids (Lhoas, 1961). Diploid conidia were either inoculated by mass transfers or a conidial suspension was plated onto complete media containing a final pfp-concentration of approximately 1/10,000 w/v.

3. Techniques

Standard methods were used with respect to incubation temperature plating and meiotic analysis (Pontecorvo et al., 1953). Conidial suspensions were made with isotonic saline solution (0.5% NaCl) containing two parts per million of Tween 80 (polysorbate), an emulsifying agent.

Diploids were synthesized using the forced heterokaryon technique of Roper (1952). Mitotic colored diploid recombinants were isolated by picking single colored heads from low density platings of diploids on complete media and purified by streaking on the same medium (Pontecorvo et al., 1953).
Two techniques were used to select for mitotic haploids:

1) "pfp" - details of which may be found in Lhoas (1961),

2) "all - ad" - as follows. Diploids heterozygous for suAladE20 (su) and homozygous for adE20 (ad), i.e. su/+ ad/ad, grow very slowly and produce no conidia on supplemented minimal media lacking adenine ("all - ad"). They frequently produce conidiating sectors which are either mitotic diploid segregants homozygous for su (su/su) or haploid segregants hemizygous for su (su). Selection for the haploid segregants is greatly enhanced if an additional selective marker is present on another chromosome or on the opposite arm of linkage group I; color markers being the most frequently used. Since coincidental occurrence of two mitotic crossing over is rare, suppressed yellow or white segregants are mostly haploid segregants. This simplifies the isolation of mitotic haploids from "all - ad" platings. The "all - ad" selection of mitotic haploids is extremely efficient if a translocation is present on the chromosome arm carrying the selective marker su, previously described for T1(I;VII) (Küfer, 1958, 1962), and applies to the translocation presently analyzed.

Needle platings were made for analysis of "abnormal" segregants (Küfer, 1961). The viability of these "abnormal" segregants is low, hence they are only recovered under noncompetitive situations, i.e. low density platings. The phenotype and extent of sectoring in these "abnormal" segregants depend to a large extent on the amount of space available and the nature of imbalance. Diploids heterozygous for the translocation T2(I;VIII) and the recessive suppressor su, but homozygous for ad, when plated on "all - ad" form "suppressed" diploid sectors. These are unbalanced and abnormal looking. To check for growth type and sectoring,
an uniform patch of abnormal conidia was chosen under the microscope and the conidia were lightly touched three or four times with a fine platinum needle. The needle was then rinsed off in 0.6 ml. of saline, and a five fold dilution of the original suspension was made. Low density, complete media platings of both suspensions were made and incubated for four to six days. Further dilutions and platings were made if the desired low density was not achieved. Suspensions were also plated onto test media to test for the growth requirements of the selected abnormal "suppressed" recombinants.

The haploid state of the selected conidia may be established by several criteria. First, absence of heterozygous markers: the allele for acriflavin resistance in Aspergillus nidulans, Acr, is semi-dominant; diploids heterozygous for this locus show distinctive sectoring phenotypes on complete media supplemented with acriflavin and is used to indicate their diploid nature. Other recessive visual marker, e.g. color markers, or nutritional markers may also be used to identify the ploidy of the conidia. Diploids show the wildtype phenotype (green in the case of color markers) because the recessive markers are present in heterozygous states. Therefore, colored segregants are either haploids or mitotic recombinants. Second, morphology of the conidia: in Aspergillus nidulans, the size and shape of conidial heads observed under the light microscope can be used to differentiate between diploid and haploid conidia. A combination of these criteria firmly establishes the ploidy state of the selected conidia from either "pfp" or "ada" selective techniques.
EXPERIMENTS AND RESULTS

The purpose of this investigation was a three-fold one: 1) to analyze and map in detail a translocation, T2(I;VIII) using mitotic and meiotic recombination; 2) to study, in general, the effects of a heterozygous translocation on patterns of mitotic recombination; and 3) to establish the frequency of meiotic recombination in the chromosome arms involved in this translocation in crosses heterozygous and homozygous for it.

1. Analysis and mapping of T2(I;VIII)

I. Detection of T2(I;VIII)

Techniques for the detection and analysis of translocations were developed by Käfer (1958, 1962). Complete linkage of markers on two previously independent linkage groups in mitotic haploids is taken as an indication that the diploid is heterozygous for a reciprocal translocation involving these two groups (previously discussed in Literature Review and Introduction). Since the linkage between the linkage groups is either complete in the presence of a translocation, or random otherwise; only small samples of mitotic haploids (about ten) is needed to establish conclusive evidence for the presence of a heterozygous translocation.

To identify T2(I;VIII) in diploids heterozygous for the translocation, strains suspected to carry the translocation were forced into diploids with standard tester strains via heterokaryon formation. Both selective techniques were used to recover mitotic haploids. Diploids heterozygous for T2(I;VIII) showed complete linkage of all markers on linkage groups I and VIII in mitotic haploids. This technique was used
repeatedly throughout this thesis in establishing the ideal well marked T2(I;VIII) strain, as well as in mitotic and meiotic mapping of the translocation.

II Identification of chromosome arm by mitotic crossing over

In diploid heterozygous for a selective marker and a translocation, selection for homozygosis of the selective marker carried on one of the arms involved in the translocation leads to genomic imbalance typified by an abnormal phenotype and reduced viability. This may be used to identify the chromosome arm involved in the translocation provided that suitable selective markers are available. For a detailed description of the identification of chromosome arms involved in T2(I;VIII) by mitotic crossing over, refer to section 2.I of Experiments and Results.

III Meiotic mapping of T2(I;VIII)

Meiotic mapping of breakage points of the translocation are done as follows. Meiotic linkage of the translocation to markers on the affected linkage groups can be determined in crosses of a T2(I;VIII) strain to a standard translocation-free strain; recombinants of various marker arrangements may then be isolated and tested for presence of T2(I;VIII) in diploids (as described in section 1.I, Experiments and Results).

Presence of T2(I;VIII) was first identified in a recombinant carrying the NTG-induced new sulfite mutant \textit{sD205} (Nicklewicz, 1970). It was deduced that the translocation must have arisen with the induction of the mutant (refer to section 1, Material and Methods). This strain and a sister strain were crossed with strains carrying the desired markers on VIII in several generations to establish a T2(I;VIII)-recombinant well marked on linkage group VIII (crosses 1250, 1035, 1155, Fig.4). This was then crossed with a strain carrying the desired markers on I such that
the ideal T₂(I;VIII) strain carrying the desired markers on I and VIII could be established for meiotic and mitotic analyses of the translocation (cross 1156, Fig. 4). The number and the sD-genotype of recombinants from the T₂(I;VIII)-heterozygous crosses analyzed for T₂(I;VIII) in diploids with standard strains are shown in Fig. 4.

Results showed that all recombinants, from five generations of outcrossing of the sD strain to standard strains gave complete linkage of the translocation and the marker sD, i.e. all sD strains carried T₂(I;VIII), while the corresponding wildtype strains (sD⁺) were translocation free. Thus, the breakage point of T₂(I;VIII) must be very close to or at the marker sD on linkage group VIII. Figure 5 contains the data from tests for meiotic linkage of T₂(I;VIII) to markers of linkage group I and VIII. In the heterozygous T₂(I;VIII) cross 1300, it was difficult to isolate recombinants of su and gal, indicating that they were meiotically close. When recombinants of various marker arrangements were analyzed in test-diploids, T₂(I;VIII) showed meiotic linkages to su (21% recombination) and gal (23% recombination); a loose linkage to an (39% recombination); and as expected, no linkage to markers on the right arm of I and the other linkage groups (50% recombination) (refer to Fig. 5 for meiotic linkage data). The left arm of linkage group I in Aspergillus nidulans is meiotically very long - marker an being unlinked to both su and gal (Dorn, 1967). It is therefore unlikely that the translocation-break would be distal to the three markers on II and still be meiotically linked to all of them. This indicated that the translocation-break must be between these markers, possibly between gal and an.
Meiotic linkage of T2(I;VIII) to markers of linkage group I & VIII

Meiotic distances not indicated are unlinked (50% recombination) to the breakage point of the translocation.
IV Analysis of "new linkage groups" in diploids homozygous for T2(I;VIII)

In diploids homozygous for the translocation, complete linkage of the two involved linkage groups seen in heterozygous diploids disappears since all haploids now contain the "crossover chromosomal arrangements" and recombinants between I and VIII are genetically balanced and viable. However, patterns of marker segregation in mitotic haploids from such diploids should now show the new linkage relationships caused by the translocation and thus determine the markers which are translocated (Käfer, 1962).

For this purpose, diploids homozygous for T2(I;VIII) with different combinations of relevant markers were synthesized (see diploids 1255, 1256, Table 2). Since efforts to separate the translocation from the allele sD has failed, all diploids were homozygous for sD. Mitotic haploids were selected from complete media with pfp as well and "all - ad" for diploid 1256, while only the latter selective method was used for diploid 1255 which is homozygous for phen, a marker selected against on pfp-media.

Results (Table 2) from both diploids are consistent, hence will be described together. All suppressed mitotic haploids showed the gal phenotype, indicating that markers su and gal were completely linked and segregated as one unit. On the other hand, mitotic suppressed haploids showed two color phenotypes - yellow-fawn or chartreuse from diploid 1255; yellow-fawn or green from diploid 1256. In no case were yellow or yellow-chartreuse haploids recovered, which would be expected from homologue segregation in standard diploids. The present result reveals a different marker arrangement in diploids homozygous for T2(I;VIII) and indicates
TABLE 2

Relevant genotype and analyses of diploids homozygous for T2(I;VIII)

<table>
<thead>
<tr>
<th>Diploid</th>
<th>Selected phenotype</th>
<th>Complete genotype for linkage group I &amp; VIII</th>
<th>Number tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>1256</td>
<td></td>
<td><strong>T2(I;VIII)...sD + ve fac ribo an + + ad</strong></td>
<td>su gal + +</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>T2(I;VIII)...sD fw ve+ + + + paba y ad</strong></td>
<td></td>
</tr>
<tr>
<td>1255</td>
<td></td>
<td><strong>T2(I;VIII)...sD + ve fac ribo cha an + + +</strong></td>
<td>su gal + +</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>T2(I;VIII)...sD fw ve+ + + + paba y ad</strong></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Diploid</th>
<th>Selected phenotype</th>
<th>Complete genotype for linkage group I &amp; VIII</th>
<th>Number tested</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>y fw</td>
<td>sD fw ve+ paba y ad; su gal</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>+ +</td>
<td>sD ve+ fac ribo an ad; su gal</td>
<td>15</td>
</tr>
<tr>
<td>1256</td>
<td>su</td>
<td>sD fw ve+ paba y ad; su gal</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>sD fw ve+ paba y ad; + +</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>y fw</td>
<td>sD ve+ fac ribo an ad; su gal</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>sD ve+ fac ribo an ad; + +</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>y+ fw</td>
<td>sD ve+ fac ribo an ad; su gal</td>
<td></td>
</tr>
<tr>
<td>1255</td>
<td>su</td>
<td>sD fw ve+ paba y ad; su gal</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>cha</td>
<td>sD ve fac ribo cha an ad; su gal</td>
<td>49</td>
</tr>
</tbody>
</table>
that \textit{fw} and \textit{cha} previously on VIII now show complete linkage to \textit{v} on IR suggesting that they are on the same chromosome. Other markers on VIII and I confirmed this new linkage in mitotic haploids. The new linkage group I consisted of markers \textit{sD fw ve fac ribo cha} previously on chromosome VIII in addition to \textit{an papa v ad} of chromosome I. This new linkage group I nevertheless segregated independently from \textit{su} and \textit{gal}, since mitotic haploids of both "linkage groups" in the four expected chromosomal arrangements were recovered. It is interpreted that the piece of chromosome VIII carrying markers \textit{sD fw ve fac ribo cha} must have been translocated to chromosome I. Since \textit{gal} and \textit{an} segregated independently, the breakage points of the translocation are confirmed as being between \textit{gal} and \textit{an} on the left arm of chromosome I; while the other at \textit{sD} or proximal to \textit{sD}, with all markers of chromosome VIII used in these experiments distal to \textit{sD}. These results are consistent with the meiotic mapping data and in agreement with the revised map order of markers on VIII (Cove, unpublished; see Fig.2).

The most probable topology of \textit{T2(I;VIII)} can therefore be deduced. The two translocation-breaks must be on linkage group VIII near \textit{sD}, and between \textit{gal} and \textit{an} on linkage group I. The large segment carrying all markers of VIII, \textit{sD fw ve fac ribo cha}, is attached distal to \textit{an}, while the small segment of I carrying markers \textit{su} and \textit{gal} is reciprocally translocated to VIII proximally to all known markers (Fig.6). Mitotic recombination in diploids homozygous for \textit{T2(I;VIII)} recently confirmed this topology (Käfer, personal communication). Green suppressed diploid segregants selected from diploids homozygous for \textit{T2(I;VIII)}, but heterozygous for \textit{su} and \textit{gal} showed both \textit{gal} phenotypes, i.e. \textit{gal} and \textit{gal}⁺; but none of
FIGURE 6 Proposed topology of T₂(I;VIII) deduced from T₂(I;VIII) homozygous diploids (⁎)

<table>
<thead>
<tr>
<th>Linkage group I</th>
<th>Linkage group VIII</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Standard arrangement</strong></td>
<td><strong>Marker arrangement as in diploid 1256</strong></td>
</tr>
<tr>
<td><strong>Breakage of homologues at indicated positions</strong></td>
<td><strong>Breakage point of T₂(I;VIII)</strong></td>
</tr>
<tr>
<td><strong>Rejoining of broken ends giving the translocation</strong></td>
<td></td>
</tr>
</tbody>
</table>

*Marker arrangement as in diploid 1256*

• **Breakage point of T₂(I;VIII)**
them segregated for an when su and an were in the coupling arrangement.
The former confirmed that su is distal to gal; the latter confirmed that the markers su gal are no longer on linkage group I, and presumably on VIII.
In diploids homozygous for T2(I;VIII) and sD, but heterozygous for an fw fac ribo cha, one selected homozygous chartreuse (cha/cha) diploid segregant was homozygous for fac and ribo as well; while another was not. Selected homozygous fawn (fw/fw) diploid segregants were homozygous for fac ribo and presumably cha though its phenotype was masked. This established cha to be distal to fac ribo, which are in turn distal to fw.

2. Effects of T2(I;VIII) on mitotic recombination in heterozygous diploids

The presence of a heterozygous translocation in a diploid has pronounced effects on mitotic crossing over in the arms involved in the translocation, previously described for T1(I;VII) and T1(I;VIII) (Köfer, 1962, 1965) (see Discussion). In brief, selection for mitotic crossing over in one of the arms of involved in a reciprocal translocation results in trisomy for one of the translocation-arms and monosomy for the other translocation-arm. These near-diploid mitotic crossover segregants are genetically unbalanced, as reflected by their abnormal growth and reduced viability. Hence, such abnormal phenotypes resulting from the selection for mitotic crossing over in a chromosome arm indicates that the selected arm must be involved in a translocation. Any second order mitotic event in these segregants resulting in a balanced genome would restore normal morphology, viability and growth rate. This second order mitotic compensating event may be mitotic nondisjunction or mitotic crossing over in the nonselected translocation-arm. In Aspergillus nidulans, the
selective advantage of a balanced genome enabled them to appear as stable normal sectors. Presently, stable sectors is used to refer to any patch of faster growing, normal looking conidia arising from abnormal unbalanced segregants. Hence a sectoring phenotype confirms genomic imbalance. Further, the patterns of marker segregation in the selected first order event, and the second order spontaneous event reveal the marker-arrangements, thus confirm the topology of the translocation.

I Confirmation of a translocation-break on IL

To confirm that the left arm of linkage group I is involved in the translocation T2(I;VIII), two selective markers were used - suAladE20 (su) and yAl (y) (refer to Table 1 for a complete description). Both are located distal of most other markers: su is on the left arm of linkage group I, while y is on the right arm. Diploids heterozygous for the translocation T2(I;VIII) and the selective markers were synthesized. It was desirable to have the translocation and selective markers in both coupling and repulsion arrangements since the relative viability of the unbalanced segregants from the two types of arrangements would indicate the direction and the relative size of the pieces involved in the translocation. The relevant genotype of diploids used in this experiment and their coupling or repulsion relationships of T2(I;VIII) to the selective markers su and y are shown in Table 3. The obtained results indicate that from four diploids with T2(I;VIII) in coupling or repulsion to the marker y, all yellow mitotic diploid segregants (y/y) isolated were phenotypically normal (Plate 1). Under a light microscope these apparently normal mitotic segregants showed arrangements and shapes of conidial heads typical of normal diploid Aspergillus nidulans. It is concluded that mitotic crossing over in the
PLATE 1

Morphology of first order mitotic segregants

selected from diploids heterozygous for $T_2(I;VIII)$
a) selected "yellow" 1st order mitotic segregants

b) selected "suppressed" 1st order mitotic segregants

e) close up of Abnormal "suppressed" near-diploid segregants

c) selected Abnormal "suppressed" near-diploid mitotic segregants

d) selected Abnormal "suppressed" near-diploid mitotic segregants
right arm of linkage group I did not upset genomic balance, therefore the translocation $T_2(I;VIII)$ is not likely to involve the right arm of linkage group I. This provides indirect evidence that $T_2(I;VIII)$ affects the opposite arm ($IL$) of linkage group I.

The following experiment was performed to provide direct evidence that the left arm of linkage group I is involved in $T_2(I;VIII)$. Diploids heterozygous for $T_2(I;VIII)$ and $su$, carrying $su$ in coupling with $T_2(I;VIII)$ or in repulsion to it (called "coupling" and "repulsion" diploids for short), were plated on "all-ad" to select for phenotypically "suppressed", near-diploid mitotic segregants. The relevant genotypes and analyses of these diploids are shown in Table 3.

Results showed that all near-diploid "suppressed" segregants obtained from both "coupling" and "repulsion" diploids have an aconidial phenotype (see Plate 1), and under the microscope revealed small conidial heads typical of abnormal unbalanced conidia (Plate 1e). Their genomic imbalance was confirmed by isolating these unbalanced "suppressed" segregants by the nonselective technique of needle plating onto complete media. On complete media, normally conidiating sectors of improved morphology arose spontaneously and frequently (details of these sectoring events is given in the following section). Since the selection for mitotic crossing over involving markers of the left arm, but not the right arm, of linkage group I led to genetic imbalance, it is confirmed that the left arm of I is involved in the translocation $T_2(I;VIII)$.

II Patterns of marker segregation in "coupling" or "repulsion" diploids

Since the selected mutant $su$ is recessive, selection for its phenotypic expression from a diploid originally heterozygous for it ($su/+)$
<table>
<thead>
<tr>
<th>GENOTYPE ON RELEVANT LINKAGE GROUPS</th>
<th>ARRANGEMENT OF T2(I;VIII) WITH RESPECT TO</th>
<th>PHENOTYPE OF MITOTIC CROSSOVER SEGREGANTS AND NUMBER TESTED</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>y</td>
<td>su</td>
</tr>
<tr>
<td>+ paba  y  +  +</td>
<td>T2(I;VIII) ... sD +</td>
<td>coupling repulsion normal 15</td>
</tr>
<tr>
<td>su  +  +  ad  bi</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>+ paba  +  +  bi</td>
<td>T2(I;VIII) ... sD +</td>
<td>repulsion repulsion normal 12</td>
</tr>
<tr>
<td>su  +  y  ad  +</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>su  +  +  y  ad</td>
<td>T2(I;VIII) ... sD +</td>
<td>repulsion normal 15</td>
</tr>
<tr>
<td>+ paba  y  ad  +</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>su  +  gal  an  paba  y  ad  +</td>
<td>T2(I;VIII) ... sD + ve + ve + + + pal +</td>
<td>coupling coupling normal 5</td>
</tr>
<tr>
<td>+  +  +  +  +  +  ad  bi</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>+ an  paba  y  ad  +</td>
<td>T2(I;VIII) ... sD fac ribo</td>
<td>coupling repulsion normal 5</td>
</tr>
<tr>
<td>su  +  +  +  +  ad  bi</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>+ an  paba  +  +  ad</td>
<td>T2(I;VIII) ... sD + ve + fac ribo cha</td>
<td>repulsion repulsion normal 5</td>
</tr>
<tr>
<td>su  +  +  y  ad</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
calls for the loss of its wild type allele during selection, resulting in either homozygosis or hemizygosis for the recessive marker. If the assumed topology of $T_2(I;VIII)$ is correct, then selection for "suppressed" diploid segregants in "coupling" or "repulsion" diploids results in trisomy for the translocated segment of VIII and monosomy for the translocated segment of I (Fig. 7, 8, & 9). A second event of mitotic segregation leading to the duplication of the monosomic segment and the loss of a trisomic segment would restore normal disomy, hence normal morphology and viability in these near-diploid, unbalanced, "suppressed" segregants. Genotypes of the "coupling" and "repulsion" diploids analyzed are shown in Table 4. These diploids were plated on "all - ad" and abnormal looking, presumably "suppressed" segregants were chosen under a light microscope and conidia from these were needle plated onto complete media. About 45 independently arisen segregants from each diploid were needle plated. This was achieved by taking abnormal conidia from different diploid colonies or by taking abnormal segregants that are far apart on the same diploid colony. However, these abnormal "suppressed" segregants were not easy to recognize and other types of abnormal segregants were often obtained which will not be considered. Only the abnormal "suppressed", near-diploid segregants will be discussed in the following analyses.

All the "suppressed" abnormal near-diploid segregants from a single diploid were identical in their abnormal morphology. From each independent needle plating of these segregants, 5 to 20 abnormal looking and sectoring colonies were usually obtained. These were presumably identical since they originated from the same abnormal segregant. Normally, 2 to 4 of the sectoring colonies were further analyzed. The
center of the abnormal colony, which presumably is the near-diploid, abnormal "suppressed" segregant from "all - ad", were analyzed by needle plating onto the appropriate test media. From these, stable sectors arose frequently and spontaneously; and in some cases concomitant with the expression of recessive color phenotype. This is presumably coincidental with segregation of other recessive markers as reflected by their nutritional requirements. To establish the genotype of the stable sectors, all stable sectors from each abnormal sectoring colony were characterized by standard techniques. From both "coupling" and "repulsion" diploids, most of the stable sectors were shown to be diploid, though occasional haploid stable sectors did arise. Each stable sector represents an independent second order mitotic event restoring genomic balance in the near-diploid abnormal "suppressed" segregant. Depending on density of the plating, hence the space available, the number of stable sectors may vary from 15 to 40. Their size and position reflect the time at which the second event occurs - large sectors originating close to the center indicates early events. Genotypes of all stable sectors from a single plating of abnormal "suppressed" segregant reveal the pattern of marker segregation in this segregant. Such patterns may be used to deduce the genotype of the abnormal "suppressed" segregant. Genotypic analysis of the abnormal "suppressed" segregants by needle plating of the abnormal center of a sectoring colony were more difficult. Due to the poor conidiation of some of these segregants they were difficult to collect and often contaminated with second order segregants when the plating density was slightly high (above 8). On test media, a sectoring phenotype suggests that neither the abnormal segregant or its stable sectors were
nutritionally requiring. Growth of the abnormal segregant, but failure of it to sector on test media reveals that only the stable sectors were deficient. Failure to grow indicates that both were nutritionally mutant.

a) Analysis of "coupling" diploid (1238, Fig. 7)

Assuming the proposed topology for T2(I;VIII), in diploid 1238 with su in coupling to the translocation, selection for hemizygous or homozygous "suppressed" near-diploid segregants (i.e. the loss of su+) calls for a mitotic event on I, since the wildtype allele su+ is carried on the left arm of the normal homologue I. Most frequently this will be a mitotic crossing over on the left arm of linkage group I, proximal to the translocation-break, but either distal or proximal to an. This results in the loss of su+ and gal+, and in some cases also an+, with homozygosis for the parts of II and the attached translocation-segment of VIII that are distal to the point of exchange. This segment of VIII becomes trisomic, while the translocation-segment of I that attaches to VIII, and carrying the markers su and gal, now becomes monosomic. If mitotic crossing over is distal to an (in region 2, Fig.7), the resulting abnormal "hemizygous suppressed", near-diploid segregant should remain heterozygous for an, that is phenotypically an+; if proximal to an, the resulting segregant should be homozygous for an, hence of mutant phenotype (region 1, Fig.7).

From this "coupling" diploid, 32/45 independent platings of abnormal conidia from "all - ad" were later confirmed to be the "suppressed" near-diploid type and 19 of these were analyzed. Their sectoring phenotypes are shown in Plate 2. As shown in Table 4, all 19 of the abnormal "suppressed" near-diploid segregants analyzed were unable to utilize galactose for growth when needle plated onto supplemented galactose media. This indicates that
FIGURE 7  First order and second order mitotic events in "coupling" diploid 1238

I

cha pal⁺ ribo fac ni⁺ ve⁺ fw sD an paba y ad +

su⁺ gal + 1

i.e. loss of su⁺

1st order selection of genomically unbalanced "suppressed" near-diploid segregants

an

paba y ad +

cha pal⁺ ribo fac ni⁺ ve⁺ fw sD

1st order mitotic crossover unbalanced segregants (crossing over in region 1, proximal to an)

an

+ + ad bi

cha pal⁺ ribo fac ni⁺ ve⁺ fw sD

an

+ + ad bi

VIII

gal su

sD fw⁺ ve ni fac⁺ ribo⁺ pal cha⁺

2nd order mitotic event restoring genomic balance, i.e. the "duplication" of monosomic segment and "loss" of trisomic segment

gal su

gal su
Table 4  Genotype and analyses of "coupling" and "repulsion" diploids of T2(I;VIII)  

<table>
<thead>
<tr>
<th>Diploid</th>
<th>Colour</th>
<th>Ploidy</th>
<th>Frequency (%)</th>
<th>Visual phenotype</th>
<th>Ploidy</th>
<th>Recessive markers phenotypically expressed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1238</td>
<td>green</td>
<td>2N</td>
<td>10/19</td>
<td>fawn-chartreuse</td>
<td>2N</td>
<td>su gal; sD fw ve⁺ + fac ribo cha</td>
</tr>
<tr>
<td>1322</td>
<td>green</td>
<td>2N</td>
<td>8/19</td>
<td>fawn-chartreuse</td>
<td>2N</td>
<td>su gal an; sD fw ve⁺ + fac ribo cha</td>
</tr>
<tr>
<td>1348</td>
<td>yellow</td>
<td>2N</td>
<td>1/19</td>
<td>yellow</td>
<td>2N</td>
<td>su gal an paba y; sD fw ve⁺ fac ribo cha</td>
</tr>
<tr>
<td>1322</td>
<td>Aconidial</td>
<td>2N</td>
<td>14/45</td>
<td>chartreuse</td>
<td>2N</td>
<td>cha bi; cha</td>
</tr>
<tr>
<td>1348</td>
<td>Aconidial</td>
<td>2N</td>
<td>8/40</td>
<td>compact</td>
<td>2N</td>
<td>co</td>
</tr>
</tbody>
</table>

Analysis of abnormal "suppressed" near-diploid segregants selected from 'all-ad'

Analysis of the corresponding stable sectors of improved morphology
PLATE 2

Sectoring phenotype of the abnormal "suppressed" near-diploid mitotic segregants selected from "coupling" diploid 1238
a) Single colony 1st order mitotic crossover segregants

b) Single colony 1st order mitotic nondisjunctional segregants

c) Medium density plating of 1st order mitotic crossover segregants

d) High density plating of 1st order mitotic crossover segregants
gal did not segregate in the first step among the unbalanced "suppressed" near-diploid segregants, hence confirms that it was translocated with su to VIII as proposed earlier. The majority of the abnormal "suppressed" near-diploid segregants (18/19) were wildtype in color (green), and amongst these eight gave only aneurin-requiring diploid stable sectors, while the other ten gave only diploid stable sectors not requiring aneurin. Hence, an did not segregate in the second step, but apparently segregated in the first step as expected if the first event occurs on IL. The fact that the abnormal first order segregants were identical to their stable sectors in the aneurin phenotype, an or an+, was later confirmed by low density replatings of the abnormal "suppressed" near-diploid segregants onto supplemented media with or without aneurin (Käfer, personal communication). This confirms that selection for "suppressed" segregants in the "coupling" diploids led to the loss of su+ allele by a mitotic crossing over between the centromere of I and the translocation-break of T2(I;VIII) on IL (Fig.7.). The 1/19 of the abnormal "suppressed" near-diploid segregants analyzed which was yellow in color, also showed a requirement for aneurin as well as para-aminobenzoic acid (paba) indicating that it must have arisen by nondisjunction of the translocated homologue of I.

Platings of the abnormal "suppressed" segregant on media testing for sD, fac, ribo, respectively showed that none of these mutants was expressed, as expected since they all were wildtype for the linked color markers fw and cha. This is consistent with the hypothesis that the translocated VIII-segment becomes trisomic after the first order mitotic event on IL, but remain phenotypically wildtype due to presence of the
wildtype alleles on the normal homologue of VIII. Hence, all the tested "suppressed" near-diploid abnormals in the first step segregated only for markers on linkage group I, but not for markers on linkage group VIII. On the other hand, all of the stable sectors from the abnormal "suppressed" near-diploid segregants were fawn, whether arising from green or yellow unstable centers. Most of these were apparently normal diploid segregants as indicated by their heterozygosity for Acr. Standard techniques characterized their phenotype to be fluffy morphology; sulfite and riboflavin requiring; ammonium acetate non-utilizing. Hence, their most likely genotype for markers on VIII is $sd$ $fw$ $ve^+$ $fac$ $ribo$ $cha$. The genotype of two such stable sectors were further determined for each of the two cases $an$ or $an^+$ phenotype. Mitotic haploids were selected on pfp. All mitotic haploids showed the phenotype of the recessive markers on VIII and I expressed in their parental diploid stable sectors, that is, $sd$ $fw$ $ve^+$ $fac$ $ribo$ $cha$ and $an$ when the stable sectors were aneurin-requiring. Mitotic haploids of both the mutant and its corresponding wildtype allele were recovered for all markers carried on the other linkage groups or on the opposite arm of linkage group I (IR). This was also true for $an$ on IL when the stable sector was $an^+$ in phenotype, i.e. the point of mitotic crossing over being distal to $an$ on IL in the first step. The fact that aneurin-non-requiring stable sectors were heterozygous for $an$ though the marker did not segregate among stable sectors, nor among abnormal centers from a single needle plating of unbalanced "suppressed" segregants indicates that $an$ did not segregate in the second order mitotic event. This confirms that $an$ segregated in the first step which presumably occurs on I in the "coupling" diploids. Presence of heterozygous markers in the stable
sectors as evident from mitotic haploid analysis further confirms their diploid nature and suggests that the recessive markers expressed in the stable sectors after the second mitotic event are most likely present in a heterozygous state. In addition, none of the haploids recovered were cnx or pal as expected in these fawn-sectors which are presumably homozygous for the whole arm of VIII spanning sD to cha. This confirms that the second mitotic segregation event occurs on VIII since it results in the loss of wildtype and mutant alleles of markers of VIII carried on the normal homologue of VIII. Simultaneously, genomic balance is recovered: 1) by the loss of a trisomic segment, i.e. the normal arm of VIII with its many wildtype alleles, which permits the expression of the recessive markers on the translocation-arm; 2) presumably by a "duplication" of the monosomic segment carrying su and gal, hence restoring disomy for both translocated segments (Fig.7). These findings give strong support for the topology deduced from the T2(I;VIII)-homozygous diploids, and demonstrate the sequence of mitotic events leading to genomic imbalance and spontaneous restoration of a balanced genome when selection is applied on one of the arms involved in a translocation. Since no markers are available proximal to the translocation-break or on the opposite arm of VIII, it could not be established whether this second order mitotic "compensating" event occurred by mitotic crossing over or by mitotic nondisjunction.

In summary, results from "coupling" diploid 1238 confirm that: 1) the first mitotic crossing over event when unbalanced "suppressed" segregants are selected occurs on the left arm of I, proximal to the translocation-break, but may be distal or proximal to an; 2) the second
order spontaneous mitotic event restoring genomic balance and normal phenotype occurs on VIIIR either by mitotic nondisjunction or crossing over; 3) all results confirm the topology of T2(I;VIII) proposed earlier as deduced from meiotic and mitotic mapping data; 4) the selected unbalanced near-diploid "suppressed" segregants are most likely trisomic for the VIII-segment translocated to I and monosomic for the I-segment translocated to VIII.

b) Analysis of "repulsion" diploids (1322, Fig.8; 1348, Fig.9)

In the "repulsion" diploids, the wildtype allele su+ is carried on the translocated I-segment attached to the remaining piece of homologue VIII. Selection for a "suppressed" phenotype, in this case, calls for a mitotic event on VIII proximal to the translocation-break on the right arm. This results in the loss of su+, with homozygosis for the corresponding markers on the normal homologue of VIII. This segment of VIIIR, distal to the translocation-break becomes trisomic; while the segment of normal homologue I corresponding to the translocated I-segment and carrying su, becomes monosomic. Any second mitotic event restoring normal disomy of both translocated segments results in the loss of a trisomic VIII-segment and the "duplication" of the monosomic I-segment and is expected to occur on I in the "repulsion" diploids. Hence, the sequence of homologues affected during selection for genomic imbalance and spontaneous restoration of a balanced complement is the reciprocal of that in the "coupling" diploids. Results from the two "repulsion" diploids analyzed were the same and as expected for the proposed topology of T2(I;VIII).

From the "repulsion" diploid 1322 (refer to Table 4 for genotype), abnormal, but likely "suppressed" conidia on "all - ad" were chosen from
FIGURE 8 First order and second order mitotic events in "repulsion" diploid 1322

Selection for 1st order abnormal "suppressed" near-diploid mitotic segregants, i.e. loss of su+

2nd order mitotic nondisjunction

2nd order mitotic crossing over in region 1
FIGURE 9  First order and second order mitotic events in "repulsion" diploid 1348

1. cha ribo fac ve+ fw co+ SD                  an  paba + ad  
   su                     +                   + y ad

2nd order mitotic crossing over at region2

2. su an+  paba + ad  
   su an+  + y ad

2nd order mitotic nondisjunction

VIII

su+

sD+ co fw+ ve fac+ ribo+ cha+

1st order selection for unbalanced "suppressed" near-diploid mitotic segregant, i.e. loss of su+

sD+ co fw+ ve fac+ ribo+ cha+

2nd order mitotic 2nd order mitotic

su an+  paba + y ad  

su an+  paba + y ad
45 independent areas and needle plated onto complete media as described (p. 39). 14/45 of such replatings were later confirmed to be unbalanced "suppressed" near-diploid segregants. Their aconidal morphology giving rise to spontaneous normal sectors on complete media are shown on Plate 3. As shown in Table 4, all 14 of the unbalanced "suppressed" near-diploid segregants analyzed were extremely aconidal even on complete media, and for this reason their color was difficult to establish. All of them either sectored or grew as abnormal centers on minimal media, indicating that they were wildtype in nutritional requirement, hence still heterozygous for their recessive nutritional markers. This is expected if selection for a "suppressed" phenotype calls for a mitotic event on VIII resulting in the loss of \( su^+ \) carried on the translocated I-segment. After the first order mitotic event, the segment of normal homologue I, corresponding to the translocated I-segment and carrying \( su \), becomes monosomic; while the translocation-segment of VIII becomes trisomic (Fig. 8) - a genomic imbalance effectively similar to that recovered from selection in the "coupling" diploids previously described. Unfortunately, conclusive evidence for the first mitotic event occurring on VIIIR is lacking since all markers of VIII used and known so far are distal to the translocation-break of T2(I;VIII). Therefore, VIIIR-markers could not be shown to segregate in the first order mitotic event. Further, no VIIIL-markers are available, hence it could not be established whether the first event occurs by mitotic crossing over or nondisjunction.

As expected, the recessive marker carried on the right arm of the normal homologue VIII made homozygous after the first mitotic event, i.e. \( cha \) in diploid 1322, was not expressed due to the presence of its
PLATE 3

Sectoring phenotype of the abnormal "suppressed" near-diploid mitotic segregants selected from "repulsion" diploid 1322
a) Low density plating

b) High density plating
wildtype allele cha+ on the translocated VIII-segment. However, inspection of the stable sectors from unbalanced "suppressed" near-diploid segregants showed that they were mainly chartreuse (Plate 3). The stable sectors were shown to be diploid by the presence of heterozygous markers, especially for Acr. This suggests that the stable sectors are most likely homozygous for cha. Stable sectors arising from the same abnormal center, or from the same needle plating of unbalanced "suppressed" near-diploid segregants were either biotin-requiring (bi) or wildtype in nutritional requirement (bi+). This indicated that the unbalanced, "hemizygous suppressed" near-diploid segregants are still heterozygous for bi on the right arm of linkage group I. Hence, unlike the "coupling" diploids, markers of I did not segregate in the first step among abnormal segregants, but rather, in the second mitotic event among stable sectors. This provides indirect evidence that the first mitotic event occurs on VIII, and the second event on I. The genotype of these diploid, chartreuse stable sectors were further analyzed; three for the bi phenotype and two for the bi+ phenotype. From both bi or bi+ sectors, mitotic haploids of the sD fac ribo phenotype were never recovered. Further, except when the epistatic marker w was present, all haploids recovered were chartreuse in phenotype, but never green (i.e. cha+). This indicates that the translocated VIII-segment carrying the markers sD fac ribo cha+, and attached to IL, must have been lost during the second mitotic event restoring genomic balance and normal morphology. This confirms that the second event occurs on I. For the biotin-requiring stable sectors, all mitotic haploids recovered were biotin-requiring suggesting that the parental diploid stable sector is likely homozygous for bi. No mitotic haploids of the
genotype an paba y were recovered in these bi sectors indicating that these markers carried on both arms of the translocated homologue of I must have been lost. This is expected if the second mitotic event occurred by mitotic nondisjunction of linkage group I such that both sister chromatids go to the same anaphase pole. The daughter nuclei carrying the two sister chromatids of the normal homologue of I would be genomically balanced since it has effectively "duplicated" the monosomic I-segment and "lost" the trisomic VIII-segment (Fig.8). For the bi+ sectors, mitotic haploids of both the mutant and its corresponding wildtype allele were recovered for the markers paba y bi carried on linkage group I, and for all markers on other linkage groups. However, no aneurin-requiring haploids were recovered from both bi+ sectors analyzed. This indicates that both of them are likely homozygous an+, while heterozygous for markers paba, y, and bi. This is expected if the second mitotic event occurs by a crossing over proximal to an, that is, between the centromere of I and an, such that an, being distal to the point of crossing over would be lost with the translocated VIII-segment carrying ad fac ribo cha+ (region 1, Fig.8). It was further postulated that the second mitotic crossing over could occur distal to an, that is, presumably between an and the translocation-break of T2(I;VIII) on IL. The resulting stable sector thus remaining heterozygous for an. The relative frequency of these two types of second order mitotic segregants may be a function of the distance of the point of exchange from the centromere. Since mitotic crossing over is concentrated around the centromere region, it is not surprising that segregants resulting from mitotic crossing over proximal to an may be more frequent. However, no
actual comparison was made. Though both bi+ sectors analyzed for 1322 did not confirm the possibility of a second mitotic crossing over distal to an, stable sectors from another "repulsion" diploid, 1348, gave evidence for it.

Table 4 contains the genotype and analyses of unbalanced near-diploid "suppressed" segregants from "repulsion" diploid 1348. The sectoring morphology of the selected unbalanced "suppressed" segregants on complete media is shown on Plate 4. Eight out of forty independent needle platings of likely "suppressed", abnormal conidia from "all - ad" were later confirmed to be unbalanced "suppressed" near-diploid segregants. All of them were nutritionally wildtype and gave diploid stable sectors of compact morphology (co/co). These results are consistent with those established for "repulsion" diploid 1322. Stable sectors arising from the same abnormal center or the same independent needle plating of unbalanced "suppressed" near-diploid segregant were either yellow (y) in color or green (y+). The genotype of these compact stable sectors were further analyzed; one for the y phenotype and two for the y+ phenotype. As expected, from both y or y+ stable sectors, no mitotic haploids of the sD fw ve+ fac ribo cha phenotype were recovered, confirming that the translocated VIII-segment carrying these markers were lost in the second mitotic event. In the case of y sectors, no mitotic haploids of an and paba were recovered suggesting that these sectors arise by mitotic nondisjunction of linkage group I as described for 1322. The two y+ sectors analyzed both segregated for paba and y in the mitotic haploids. However, one gave only an+ haploids, while the other gave both an and an+ haploids. This indicates that the former
PLATE 4

Sectoring phenotype of the abnormal "suppressed" near-diploid mitotic segregants selected from "repulsion" diploid 1348
stable sector arises by a mitotic crossing over proximal to an, while the latter distal to an as postulated (Fig. 9).

Amongst all stable sectors recovered from both "repulsion" diploids, nondisjunctional mitotic segregants appeared to be more frequent than the crossover ones - 69% (198/284) for diploid 1322 and 89% (100/122) for diploid 1348. This finding was unexpected.

In summary, results from the "repulsion" diploids confirm that:

1) the first mitotic event during selection for unbalanced "suppressed" segregants most likely occurs on VIII; 2) the second order spontaneous mitotic event restoring genomic balance and normal phenotype occurs on I, either by mitotic crossing over or nondisjunction, the latter event seems to be the more frequent one in the "repulsion" diploids; 3) the sequence of homologues affected during selection for genomic imbalance and restoration of a balanced genome is reciprocal of that in the "coupling" diploids; 4) all data obtained are consistent with those from the "coupling" diploid and together with them, confirmed the proposed topology of T2(I;VIII).

3. Meiotic recombination in crosses heterozygous and homozygous for T2(I;VIII)

For this experiment, sD-T2(I;VIII) strains were crossed to a standard strain (cross 1300, Fig.3 & 4) or to another T2(I;VIII) strain (Table 2, strains of diploid 1256) to set up crosses heterozygous and homozygous for T2(I;VIII) respectively. Table 5 contains map distances established from crosses heterozygous for T2(I;VIII), while Table 6 those from crosses homozygous for T2(I;VIII). Since efforts to separate sD from the translocation T2(I;VIII) had failed, the control
Table 5  Meiotic distances established from T₂ (I;VIII) heterozygous crosses.

Total number of colonies tested = 1215

<table>
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<th>Linkage group I</th>
<th>Linkage group VIII</th>
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<tr>
<td>su</td>
<td>sD</td>
</tr>
<tr>
<td>gal</td>
<td>fw</td>
</tr>
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<tr>
<td>paba</td>
<td>fac</td>
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<tr>
<td>y</td>
<td>ribo</td>
</tr>
<tr>
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</tr>
<tr>
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<td>49.1</td>
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</tbody>
</table>

* Total number of colonies tested = 635
Table 6  Meiotic distances established from T₂ (I;VIII) homozygous crosses.

Total number of colonies tested = 1086

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<th>Linkage group VIII</th>
</tr>
</thead>
<tbody>
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<td></td>
</tr>
<tr>
<td>paba</td>
<td></td>
</tr>
<tr>
<td>y</td>
<td></td>
</tr>
<tr>
<td>7.3 47.5 50.1 42.4</td>
<td>47.6 51.2 51.2</td>
</tr>
<tr>
<td>47.5 50.4 41.7</td>
<td>47.8 50.7 50.8</td>
</tr>
<tr>
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<td>50.1 48.8 48.1</td>
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<tr>
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<tr>
<td></td>
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cross (translocation-free) heterozygous for sD was not possible.

Published map distances (Dorn, 1967; Clutterbuck, 1970; Sinha, 1970) were used as comparison instead (Fig. 10). From crosses heterozygous for T2(I;VIII), it can be seen that meiotic crossing over between gal and su on linkage group I, and in the region sD fw ve on linkage group VIII has been greatly reduced. Further, there was an apparent meiotic linkage between sD of VIII and su or gal of I - about 3% recombination. Meiotic crossing over in other regions normally meiotically unlinked to areas of the translocation-breaks of T2(I;VIII) appeared normal, i.e. not reduced compared to published data. In crosses homozygous for T2(I;VIII) meiotic distance between su and gal did not return to the normal published value, but remained close to the value established from heterozygous T2(I;VIII) crosses instead (Table 6).
FIGURE 10

Comparison of meiotic distances of markers on linkage group I and VIII

* Meiotic distances established from $T_2(I;VIII)$-heterozygous crosses

** Meiotic distances established from $T_2(I;VIII)$-homozygous crosses

*** Published meiotic distances, Dorn, 1967

Meiotic distances not indicated are unlinked

Otherwise indicated, values shown are established from crosses heterozygous for $T_2(I;VIII)$
DISCUSSION

In *Aspergillus nidulans*, complete genetic mapping of a translocation involved its detection and establishing meiotic breakage points, chromosome arms involved and markers translocated. The principle and procedures of these analyses were described in Experiments and Results. Both types of translocations have been identified and analyzed in *A. nidulans*. Complete genetic analysis has been achieved for the insertional translocation T1(III;VIII), first identified by Käfer (1958) who later also reported meiotic linkage of *sCl2* of linkage group III to *cha* of VIII in crosses heterozygous for T1(III;VIII) (1962). Meiotic analysis of T1(III;VIII) utilizes the changed phenotype of their duplicated progenies (Bainbridge and Roper, 1966; Bainbridge, 1970). In crosses heterozygous for the insertional translocation T1(III;VIII), in absence of crossing over, the two crossover arrangement of the translocated chromosomes would result in genetically unbalanced meiotic products - one duplicated for the translocated segment; the other deficient for it. In *Aspergillus nidulans*, while deficient progenies are usually inviable and not recovered; the duplicated ones, though viable, show characteristic abnormal phenotypes. They are aconidial with reduced viability, and easily identified. In the case of T1(III;VIII), these duplicated progenies have been called "crinkles". Thus, in crosses heterozygous for T1(III;VIII), there is a regular and characteristic segregation of 2 normal : 1 "crinkle" progenies. Subsequently, such segregation ratios from crosses of phenotypically normal strains to standard tester strains may be used to indicate the presence of T1(III;VIII).
Thus, meiotic mapping of breakage points of the translocation may be simplified by detection of meiotic linkage between "crinkle" morphology and genetic markers in crosses homozygous and heterozygous for T1(III;VIII). By this method, sC13 on linkage group III was shown to give 17% recombination from the breakage point of T1(III;VIII). Further analyses showed that "crinkles" were duplication progenies for chromosome III; thus, the direction of translocation was deduced to be from chromosome III to chromosome VIII. Recently, the markers of III translocated and the point of insertion on chromosome VIII were mapped.

Apart from their easily identified, characteristic abnormal phenotype, these duplicated, near-haploid, meiotic progenies of T1(III;VIII) also show an interesting consequence of genetic imbalance. They undergo spontaneous chromosome losses of either duplicated segments, subsequently leading to a balanced genome and normal morphology. This phenomenon has been known as "vegetative instability" or "mitotic nonconformity", the mechanism of which has not been established conclusively (Nga and Roper, 1968, 1969).

Genetic analyses of reciprocal translocations are slightly different from that of the insertional ones, utilizing both meiotic and mitotic techniques. So far, no complete genetic analysis of a reciprocal translocation has been achieved in Aspergillus nidulans. Meiotic mapping of breakage points is extremely laborious in the case of reciprocal translocations since to detect meiotic linkage of the translocation to relevant markers, progenies from a cross heterozygous for the translocation have to be put into test-diploids for mitotic haploid analyses. To date, one of the meiotic breakage points of T1(I;VII) has been mapped 4 cmo units
from suAladE20 (su) on the left arm of linkage group I (Käfer, 1962). However, no meiotic linkage of T1(I;VII) to any marker of linkage group VII has been identified. Similarly, no markers on linkage group VI or VII has been identified linked to T(VI;VII) (Käfer, unpublished). The very unequal translocation T1(I;VIII) has been reported linked to \( \chi \) on the right arm of linkage group I (Käfer, 1965).

Further analyses of these translocations to identify arms involved and markers translocated gave the following results (Käfer, 1958 and 1962). Diploids heterozygous for T1(I;VII) and su when plated onto media lacking adenine, practically gave no conidiating "suppressed" sectors. Similarly, in diploids heterozygous for T1(I;VIII) and \( \chi \), selection for yellow diploid mitotic segregants gave an abnormal phenotype. This confirmed that the left arm of linkage group I being involved in T1(I;VII), and the right arm of linkage group I being involved in T1(I;VIII). Further, the relative viability of the first order, unbalanced mitotic segregants selected from "coupling" or "repulsion" diploids of the translocation gave an indication of the relative size of the translocated segments and the direction of translocation. The more unequal the translocation, the greater the difference in viability. In T1(I;VII), the selected unbalanced "suppressed" mitotic segregants from either "coupling" or "repulsion" diploids showed no drastic difference in viability. Hence, the relative size of the translocated pieces were not drastically different. The translocation T1(I;VII), therefore, is likely a reciprocal translocation. However, a different situation exists in T1(I;VIII). In this case, when the translocation was in coupling to \( \chi \), yellow mitotic segregants showed almost normal viability; but when
the translocation was in repulsion to y, they were so inviable that they were rarely recovered. From this, it was inferred that T₁(I;VIII) is probably a sizable unidirectional translocation from chromosome VIII to the right arm of chromosome I. For T(VI;VII), identification of chromosome arms by mitotic crossing over was not possible since no selective markers were available on either linkage group VI or VII, and the position of their centromeres was not known. The topology of the translocation: and markers translocated may be determined in diploids homozygous for the translocation provided if known markers are translocated and other known markers not. In all the translocations analyzed so far, this condition was not fulfilled. In the case of T(VI;VII), homozygous diploids showed all known markers of the two affected linkage groups to behave as one set with their centromeres indistinguishable from one another. For T₁(I;VII), the topology in homozygous diploids has not been established either. In this case, if the translocation-break is distal to su on the left arm of linkage group I, other further distal marker would have to be used so that the translocation-segment of I could be identified (Käfer, personal communication).

Similar to the clinical conditions manifested in genomically unbalanced human progenies, duplicated in presence of a translocation, the unbalanced near-diploid mitotic segregants selected from diploids heterozygous for a translocation in Aspergillus nidulans show remarkably characteristic abnormal phenotypes. In addition, genomic imbalance in A. nidulans has quite unique consequences in that it is capable of inducing events that restore genomic balance, easily recognized by spontaneous sectoring of normal conidia. This is true for the near-haploid
duplicated progenies of T₁(III;VIII) - "mitotic nonconformity" in the "crinkles", though the events leading to spontaneous chromosome losses have not been fully understood. Similarly, the near-diploid unbalanced mitotic segregants of reciprocal translocations are capable of restoring genomic balance by second step compensating events. Unbalanced mitotic segregants may be selected if selective markers are available on one or both of the affected chromosome arms. However, unless both chromosome arms can be easily selected for, the reciprocal types of unbalanced segregants will not be available for investigation. On the other hand, all the expected types of unbalanced segregants may be isolated from diploids heterozygous for the translocation without selective pressure, i.e. by nonselective techniques. Though extremely laborious, this has been achieved for \( T(VI;VII) \) and provided evidence for their genomic imbalance by spontaneous sectoring (Käfer, 1961 and unpublished). One of the four types has been also selected regularly from diploids heterozygous for \( T₁(I;VII) \) (Käfer, 1962). These experiments provided partial evidence for the deduction that in diploids heterozygous for a reciprocal translocation, selection for homozygosis in one of the arms involved in the translocation leads to genomic imbalance. However, inspite of these, the exact events leading to the production of unbalanced near-diploid mitotic segregants and their recovery of a balanced genome have not been clearly demonstrated since for both translocations \( T(VI;VII) \) and \( T₁(I;VII) \), the exact topology was not known. Success in such analysis requires: 1) selective markers on at least one of the translocation arms, but preferably on both; 2) other markers on the opposite arm of the affected chromosome, and on both sides of the translocation-breaks; and
3) recognition and exclusion of non-relevant mitotic segregants from analysis; this, however, has to be justified and can only be done confidently with lots of experience. Except for one minor point, the translocation T2(I;VIII) fulfills most of these requirements. Selective markers are available on both translocation-arms - su on the left arm of I; fw and cha on the right arm of VIII. On chromosome I, markers on both chromosome arms, as well as on both sides of the translocation-break are known. However, on chromosome VIII, only markers on one chromosome arm (VIIIR) and distal to the translocation-break are available. The last condition means that the reciprocal transfer of the translocation-segment of I to chromosome VIII is only deduced and not proven by its new linkage to markers of chromosome VIII. However, since majority of the diploids used in this thesis are marked on all linkage groups, involvement of another linkage group would have been identified if it was present. This was not found, therefore the deduction of a reciprocal translocation is justified.

The present efforts represent the first successful and complete genetic analysis of a reciprocal translocation. Meiotic linkage of T2(I;VIII) to su (21% recombination), gal (23% recombination) and an (39% recombination) of linkage group I; and sD (no recombination) of linkage group VIII was detected. Amongst the 80 recombinants from five different crosses heterozygous for T2(I;VIII) isolated for meiotic analysis (refer to Fig.4), none were recombinants between sD and T2(I;VIII). This gave the breakage point of T2(I;VIII) less than 2% recombination away from the mutant sD205, a remarkably close meiotic linkage. Since the translocation T2(I;VIII) was inferred to originate
with the induction of \textit{sd205} by treatment with nitrosoguanidine, a mutagen known to cause chromosome breaks, an interesting notion would be that the mutant phenotype arise as a consequence of a break within the \textit{sd} gene due to the translocation. If this is true, \textit{T2(I;VIII)} could never be separated from \textit{sd205}, and would represent the first of its kind in \textit{Aspergillus nidulans}.

The topology of \textit{T2(I;VIII)} was deduced utilizing the principles and corollaries of genomic imbalance in diploid strains of \textit{A. nidulans} heterozygous for a reciprocal translocation. These results established the two corollaries to hold true, thus, demonstrated the hypothesis that complete linkage of two independent linkage groups in mitotic haploids is due to the presence of a heterozygous reciprocal translocation in parental diploid strains to be correct.

Detailed analysis of \textit{T2(I;VIII)} gave the following results. In diploids homozygous for \textit{T2(I;VIII)}, two new linkage relationships appeared for linkage group I and VIII in mitotic haploids - \textit{su} and \textit{gal} segregated as one group and independent of other markers on linkage group I; all markers of VIII presently used segregated with the untranslocated markers of I. This mapped one of the breakage point of \textit{T2(I;VIII)} between \textit{gal} and \textit{am} on the left arm of linkage group I; the other proximal to all markers used on the right arm of VIII. This incidentally mapped the centromere of VIII proximal to \textit{sd} and \textit{ve}. Mitotic recombination in diploids homozygous or heterozygous for \textit{T2(I;VIII)} further confirmed such marker arrangements and the topology of \textit{T2(I;VIII)} to be as follows. The translocation-segment of VIII attached onto the left arm of linkage group I distal to \textit{am}, with orientation \textit{sd} proximal and \textit{cha} distal to the centromere.
of linkage group I; the translocation-segment of I most likely attached onto the tiny, remaining right arm of VIII, with orientation $gal$ proximal and $su$ distal to the centromere of VIII. The marker $sd$ was established to be proximal to all markers of VIII presently known; The arrangement from proximal to distal on the right arm of linkage group VIII being $sd$ $fw$ $ve$ $fac$ $ribo$ $cha$. This order is consistent with the new meiotic order established by Cove (personnal communication).

In diploids heterozygous for the reciprocal translocation $T_2(I;VIII)$, selection for homozygosis of one of the translocation-arms gave progenies trisomic for the selected translocation-arm and monosomic for the other. The present experiments demonstrated that such genomic imbalance did indeed induce second mitotic events that restore disomy in both translocation-arms, seen as spontaneous sectors of normal morphology. Patterns of marker segregation in these first order selected events and second order spontaneous events further confirmed the topology of $T_2(I;VIII)$. Using diploids heterozygous for $T_2(I;VIII)$, the sequence of mitotic events during selection for genomic imbalance and recovery of genomic balance could be followed for the first time, and their nature demonstrated. The position of selective marker used with respect to the translocation-break is very important in following the sequence of homologues affected in these two mitotic events. If the recessive selective marker used is proximal to the translocation-break, it will be carried on the unaffected region of the homologue; both the mutant and its wildtype allele will be carried in their normal position irrespective of heterozygosity for the translocation. Selection for a recessive phenotype, in this case, results in homozygosis for the selective marker, as expected for the
normal complement. This appears to be the case for the translocation T_{L}(I;VII) on selection for a "suppressed" phenotype, since the breakage point of T_{L}(I;VII) is likely slightly distal to \textit{su} (Käfer, personal communication). If the recessive selective marker used is distal to the translocation-break, it will be carried on the translocated segment. In diploids heterozygous for such a translocation and selective marker, selection for a recessive phenotype has quite different consequences.

In selection for a recessive phenotype one is in effect selecting for the loss of its wildtype allele which permits expression of the recessive marker. But due to heterozygosity for the translocation, the recessive marker and its wildtype allele are carried on different homologues. Thus, selection for a mitotic event leading to the loss of the wildtype allele would no longer lead to homozygosis for the recessive marker, since it is translocated to another homologue. Instead, it results in hemizygosis for the recessive marker and homozygosis for the reciprocally translocated segment of the other affected homologue now distal to the point of exchange by virtue of the rearrangement. Therefore, the first order mitotic event selected for occurs on the homologue carrying the wildtype allele, not on the homologue carrying its recessive marker. This is the case for T_{2}(I;VIII) when the selective marker used is either \textit{su} on the left arm of linkage group I; \textit{fw} or \textit{cha} on linkage group VIII - the right arm. In the present thesis, only one type of unbalanced segregants was selected and studied from either "coupling" or "repulsion" diploids of T_{2}(I;VIII), chosen for its ease of selection. They were the near-diploid "suppressed" segregants unbalanced because of trisomy for the VIII-segment translocated to I and monosomy for the \textit{su-gal} segment of I now translocated to VIII. Since
they were phenotypically suppressed, but near-diploid, such segregants of T2(I;VIII) were referred to as unbalanced "suppressed" near-diploid segregants in this thesis to distinguish them from the unbalanced "homozygous suppressed" segregants of T1(I;VII) (Küfer, 1962). The reciprocal unbalanced type, i.e. trisomic for the I-segment and monosomic for the VIII-segment, requires selection of fawn or chartreuse abnormal conidia amongst normal diploid conidia, a task quite time consuming and difficult, hence was not attempted. In the "coupling" diploids, su is carried on the translocation-segment of I attached to the centromere of chromosome VIII. Results demonstrated that during selection for a "suppressed" phenotype, markers of linkage group I segregated. This confirmed that the first order mitotic event occurred on linkage group I, the homologue carrying the wildtype allele su+ in the "coupling" diploids. In the "repulsion" diploids, the reciprocal arrangement is found, i.e. su is now carried on the normal homologue of I and in its normal position. Since only the centromere of VIII was available, direct demonstration of a first order mitotic event on VIII was not possible. However, results from these diploids demonstrated that markers of linkage group I segregated in the second mitotic event amongst stable sectors, and not in the first event amongst "suppressed" near-diploid segregants as in the "coupling" diploids. This confirmed the sequence of homologues affected in the "repulsion" diploids to be first on VIII and second on I, reciprocal of that in the "coupling" diploids.

As postulated, the unbalanced "suppressed" near-diploid segregants of T2(I;VIII) selected from either "coupling" or "repulsion" diploids showed an unstable phenotype accompanied by a high frequency of mitotic
recombination. Further, it appeared that a single mitotic event of a type known to be relatively frequent, i.e. mitotic crossing over or mitotic nondisjunction, can restore genomic balance in these unbalanced segregants, easily identified by spontaneous formation of normal sectors. This single mitotic event must simultaneously restore disomy for both the translocation-segments, hence must have occurred on the homologue carrying the structurally heterozygous arms. Other events requiring more than one mitotic event could presumably occur during recovery of genomic balance in these unbalanced near-diploid segregants. Such segregants have been identified in the present analysis but since they were demonstrated to be either not suppressed, or not giving spontaneous normal sectors, they have not been analyzed. Other unbalanced "suppressed" segregants giving only haploid normal sectors have also been identified in both "coupling" and "repulsion" diploids of T2(I;VIII). They have not been discussed because the unbalanced "suppressed" segregants were likely to be near-haploid, similar to the duplicated progenies of T1(III;VIII). The present analysis only focused on single step, second order, mitotic events that restore the balanced diploid complement in the unbalanced, near-diploid, "suppressed" segregants of T2(I;VIII). In the "coupling" diploids, this second step event presumably occurs on VIII. Though there was no marker on VIII to confirm this it was inferred from the segregation patterns of markers on VIIIR during the first and second order mitotic events. It was demonstrated that the segment of the normal homologue of VIII was lost by a single step, second order, mitotic event; hence, provided evidence for a second step on chromosome VIII in the "coupling" diploids. As described earlier, untranslocated
markers on linkage group I segregated in the second order mitotic events in the "repulsion" diploids of T2(I;VIII); hence, demonstrated chromosome I being involved in the second step. These results confirmed that single step restoration of the balanced diploid complement involves the pair of homologues carrying structurally heterozygous chromosome arms.

When markers are available on both chromosome arms, segregants arising by mitotic crossing over or mitotic nondisjunction can be conveniently distinguished. Mitotic crossover segregants are homozygous for markers distal to the point of exchange; markers proximal to the point of crossing over or on the opposite chromosome arm remain heterozygous. Mitotic nondisjunctional segregants, on the other hand, are simultaneously homozygous for all markers carried on both arms of the chromosome. However, since only linkage group I has markers available on both chromosome arms, only mitotic events occurring on I can be distinguished for crossing over or nondisjunction. Mitotic events occurring on chromosome VIII cannot be determined since no marker on the left arm of linkage group VIII has been identified so far. From the established sequence of homologues affected, it follows that only the first order mitotic events can be studied in the "coupling" diploids, and the second order events in the "repulsion" diploids. An unexpected result was found during the analysis of the "repulsion" diploids: the relative frequency of the two types of mitotic segregants arising from second order mitotic compensating events was not equal; the nondisjunctional segregants being the more frequent type. The present results established slightly over 2/3 of all second order mitotic segregants from "repulsion" diploids were of the nondisjunctional type. Compared to the frequency
of mitotic nondisjunctonal segregants from normal diploids (1/10) (Pontecorvo and Kühler, 1958), this represents approximately a five-fold increase in the frequency of mitotic nondisjunction above the normal frequency. Increased frequencies of nondisjunctonal mitotic segregants were also found in diploids heterozygous for a deletion on the left arm of linkage group I. This increase may be explained by the fact that the chromosome arm is shorter on account of the deletion; hence the affected chromosomes are possibly not paired in all of their homologous stretches (Kühler, personal communication). Previous experiments also established crossing over between the centromere of I and an to be relatively frequent in normal translocation-free diploids - about 1/2 to 2/3 of all mitotic crossover segregants represented crossing over in this interval (Pontecorvo and Kühler, 1958). Since the breakage point of T2(I;VIII) is mapped distal to an, mitotic crossing over between an and its centromere should be comparable to the established frequency during the second order compensating events in the "repulsion" diploids if the translocation T2(I;VIII) bears little influence on it. However, the present analysis showed that less than 1/3 of all second order mitotic segregants recovered from "repulsion" diploids represented mitotic crossing over between the breakage point of T2(I;VIII) and the centromere of I. This indicates a reduction in the frequency of mitotic crossover segregants compared to that of the normal diploids. Thus, a relationship between mitotic nondisjunction and crossing over similar to that found in meiotic situations is presently indicated in the "repulsion" diploids, i.e. structural heterozygosity of a chromosome arm reduces the frequency of mitotic crossover segregants, but apparently increases that of mitotic nondisjunctional segregants.
Further, these results suggest that, in addition to the influence of centromere distance on the relative frequency of mitotic crossing over, and nondisjunction as found in normal diploids, the relative size of the translocation-segments may come into play when these events involve structurally heterozygous chromosome arms. The relationship between mitotic crossing over and nondisjunction may be explained as follows.

In the "repulsion" diploids, the left arm of linkage group I where the second order mitotic events are to occur is structurally heterozygous — one homologue being normal, the other carrying the translocated segment of VIII (Fig. 7). Since the segment of chromosome VIII translocated is extensively long, it is possible that it competes effectively for pairing with homologous parts on the normal homologue of VIII. This may reduce pairing along homologous stretches on the left arm of the translocated homologue and the normal homologue of I. Since synapsis is required for crossing over, poorly synapsed regions reduce chances for mitotic crossing over. Mitotic nondisjunction, on the other hand, may not require synapsed chromosomal conditions; its frequency is thus unaffected by poorly synapsed regions. This explains the apparent increase in nondisjunctional segregants and the actual reduction in crossover segregants among the second order mitotic segregants recovered from the "repulsion" diploids. Therefore, mitotic recombination may be affected in a similar manner by the presence of a heterozygous translocation as meiotic recombination, which favours a common mechanism in the two processes. Unfortunately, limited by time, the first mitotic events occurring on the left arm of linkage group I in the "coupling" diploids could not be re-analyzed for the above effect. Therefore, the present relationship on mitotic crossing
over and mitotic nondisjunction is only preliminary and requires further confirmation; preferably in "coupling" diploids of T2(I;VIII); with other translocations in Aspergillus nidulans; and possibly with comparable studies on meiotic crossing over and meiotic nondisjunction in crosses heterozygous for T2(I;VIII).

Preliminary efforts to investigate the effects of T2(I;VIII) on meiotic crossing over were undertaken presently. Results indicated that it has a localized effect. In crosses heterozygous for T2(I;VIII), meiotic distance between markers in the neighbourhood of both breakage points were greatly reduced compared to the standard published distances. However, meiotic distances between markers far distal to the translocation-break on chromosome VIII, i.e. \textit{fac ribo che}, were not reduced. Since there were no markers more distal to \textit{su} on the translocated I-segment in these experiments, and both \textit{gal} and \textit{su} were pretty close to the translocation-break on chromosome I, no information on meiotic distances of far distal markers on this translocation-segment could be obtained. Crossing over in the remaining arms of the translocated homologues were not followed, since on VIIIR all markers used and known so far were translocated, while on II ideal markers for this kind of studies have not been established into the strains presently used. These experiments were not ideal to investigate influences of proximal or distal translocation-breaks on crossing over along the entire chromosome arm similar to those studied by Roberts (1970). However, some interesting results did arise from these studies. On the homologue with a proximal translocation-break, even though crossing over between markers far distal to the break was normal, that between markers close to the break was
greatly reduced. Therefore, it cannot be said that crossing over is normal throughout the entire chromosome arm with a proximal break in the present case. A localized reduction of crossover progenies may be explained by inviability of these products, or an actual reduction in the process of crossing over in the areas concerned. The former may be explained by nonhomologous pairing around the translocation-break; thus, crossing over in these regions would result in unbalanced progenies which are inviable. Multiple crossover progenies may be balanced and therefore, viable. Regions further away from the translocation-break may have more chances for multiple crossing over, or may be less likely to participate in nonhomologous pairing. In either case, meiotic distance of markers in these regions would not be reduced by inviable crossover products. An actual suppression of crossing over in the neighbourhood of the translocation-break may be explained by asynaptic or poorly synapsed conditions in these areas. The presence of a translocation-break may impair meiotic pairing in the regions around it. Choice between the two alternatives came from other presently identified influences of T2(I;VIII) on meiotic crossing over (described in the following paragraphs).

In crosses homozygous for T2(I;VIII), the reduced meiotic distance between su and gel, markers close to the translocation-break on I, did not return to the normal published value, but remained the same as that established from crosses heterozygous for T2(I;VIII). It is unfortunate that crossing over around the translocation-break on VIII in crosses homozygous for T2(I;VIII) cannot be studied to confirm the above effects, since all efforts to separate sD and T2(I;VIII) had
failed. The above finding has several implications. First, the true meiotic distance between gal and su is as that established in the present experiments for the particular strains used. Inconsistency between the published data and the present value may be due to differences in some intrinsic property, may be structural abnormality, in the strains concerned. Second, there is an actual reduction of crossing over in this area though it cannot be accounted for by structural heterozygosity, and other explanations for this reduction has to be sought. With the established topology of T2(I;VIII), it is known that the translocation-segment of I carrying the markers su and gal attaches proximally to the tiny, remaining right arm of linkage group VIII. It could be argued that the close-by centromere of VIII or proximal heterochromatin, if such exists in Aspergillus nidulans, impairs meiotic crossing over. Therefore, the meiotic distance between these markers would not be expected to return to normal as long as the rearrangement exists. If this is true, the reduced meiotic distance obtained in heterozygous T2(I;VIII) crosses between markers around the translocation-break on I should return to normal in crosses homozygous for T2(I;VIII), since the translocation-break on I is quite distal, hence away from the influences of its centromere. This cannot be confirmed in the present thesis since all crosses homozygous for T2(I;VIII) were homozygous for sD simultaneously. Another possibility may be that there is an actual reduction of crossing over in homozygous T2(I;VIII) crosses due to impaired pairing around the region of the translocation-break, by virtue of some intrinsic property of the break, not related to structural heterozygosity of homologous chromosomes.

Another result from crosses heterozygous for T2(I;VIII) worth discussion is the apparent meiotic linkage of sD to su and gal (about
3% recombination). With the topology of $T_2(I;VIII)$ confirmed, $sD$ is known to be carried on a different homologue from $su$ and $gal$. Since the only common property between these markers is that they are all in the vicinity of a translocation-break, the simplest and most logical explanation for this apparent meiotic linkage is to assume an actual reduction of crossing over around both the translocation-breaks of $T_2(I;VIII)$. Taken into account all the established influences of $T_2(I;VIII)$ on meiotic crossing over, the present thesis favours the interpretation of an actual reduction in crossing over around the translocation-break by some property of the break that reduces pairing efficiency in these regions. The actual reduction in mitotic crossing over during the second order, single step, mitotic events recovering genomic balance in the "repulsion" diploids of $T_2(I;VIII)$, earlier explained by "pairing competition of the chromosome arms", may also be explained by this impaired pairing around the translocation-break proposed for meiotic crossing over. Though it is not sure whether chromosome pairing in Aspergillus nidulans involves formation of structures like the synaptonemal complexes of higher organisms, one may speculate that chromosome pairing in $A. nidulans$, whether meiotic or somatic, may be "initiated" at specific points on the chromosome arms. Reduced pairing efficiency results if some of these postulated "initiation points" are not available due to the translocation. Since synapsis is required for crossing over, poorly synapsed regions reduce chances for crossing over. This explains the actual reduction of crossing over around the translocation-break in both meiotic and mitotic recombination observed in the present experiments.
As a closing comment, the process of mitotic nondisjunction will be discussed. Earlier attempts to identify any influence of a heterozygous translocation on mitotic nondisjunction gave negative results (Pollard, 1966; Pollard, Käfer and Johnston, 1968). This failure may be explained by their experimental design. The relative frequency of aneuploid progenies of the near-haploid type (N+1, N+2, etc.) recovered from diploids heterozygous for a translocation or normal, translocation-free diploids was compared. However, if one considers chromosomal behavior during meiosis and mitosis, there is a basic difference in the consequence of meiotic and mitotic nondisjunction. Since homologous chromosomes segregate from one another, meiotic nondisjunction would result in aneuploid progenies of the near-haploid type. Mitotic nondisjunction, on the other hand, requires failure of sister chromatids to disjoin. In both previous and the present experiments, it has been shown that genomic imbalance or aneuploidy was not a necessary consequence of mitotic nondisjunction – the nondisjunctional segregants identified by simultaneous homozygosis of both chromosome arms were perfectly balanced and viable. Even if it did, the unbalanced segregants recovered from diploid strains of *A. nidulans* would be most likely of the near-diploid type, and not of the near-haploid type studied in these experiments. Since the term mitotic nondisjunction was first used to describe the situation of stepwise chromosome loss during haploidization via aneuploidy in diploid strains of *Aspergillus nidulans* (Käfer, 1961), it is felt that the above experimental design was really following this process of haploidization instead. Therefore, it is not surprising that this process is not different in normal or structurally heterozygous
diploids. This brings the discussion to what exactly is meant by the "process of mitotic nondisjunction" in terms of chromosomal behavior. Can one visualize a common mechanism in the process leading to the formation of "nondisjunctional diploid segregants" and that of "mitotic haploids"? Though a precise answer is not possible at the present, it is felt that the answer is likely not, since they seemed to be affected differently by structural heterozygosity. Until they are shown to operate on similar principles, it may be wise to keep these terms separate. More precise terms are preferable until it can be demonstrated what the process of "mitotic nondisjunction" entails.
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