# GENETIC ANALYSIS OF THE RECIPROCAL TRANSLOCATION *T2(I;VIII)* OF ASPERGILLUS USING THE TECHNIQUE OF MITOTIC MAPPING IN HOMOZYGOUS TRANSLOCATION DIPLOIDS[1]

GLORIA C. L. MA* AND ETTA KÄFER

*Department of Biology, McGill University, Montreal, Canada*

Manuscript received July 23, 1973
Revised copy received January 17, 1974

ABSTRACT

A UV-induced sulphite-requiring mutant (*sD*50) consistently shows mitotic linkage to groups I and VIII in haploids from heterozygous mapping diploids. This linkage was found to be due to a reciprocal translocation *T2(I;VIII)* which could not be separated from the sulphite requirement in about 100 tested progeny from heterozygous crosses, and both may well have been induced by the same mutational event. *T2(I;VIII)* is the first case of a reciprocal translocation in Aspergillus which showed meiotic linkages between markers of different linkage groups, and, in addition, involved chromosome arms containing markers suitable for complete mapping by the technique of mitotic recombination in homozygous translocation diploids.—Using various selective markers, haploid segregants and diploid crossovers of all possible types were isolated from homozygous translocation diploids. (1) Haploid segregants showed new linkage relationships in T/T diploids: all available markers of VIII now segregated as a group with the majority of the markers of I, except for the markers of the left tip of I. These formed a separate linkage group and are presumably translocated to VIII. (2) Diploid mitotic crossovers confirmed this information and showed that the orientation of the translocated segments was unchanged. These findings conclusively demonstrate that *T2(I;VIII)* is a reciprocal translocation due to an exchange of the left tip of group I with the long right arm of group VIII.——Since the position of the break on VIIIR was found to be at *sD*50 this marker could be used to map the break on IL by meiotic recombination in heterozygous crosses. In addition, such crosses showed reduced recombination around the breaks, so that it was possible to sequence markers which normally are barely linked.

IN many organisms chromosomal translocations can be detected and mapped by genetic analysis. Such investigations are usually based either on meiotic linkages between markers of the two chromosomes involved in the translocation (e.g., as first found in Drosophila: PAINTER and MULLER 1929) or on the occurrence of inviable meiotic products from translocation heterozygotes (e.g., in corn: BRINK 1927; BRINK and BURNHAM 1929). In all cases such evidence is supported and confirmed by cytological analysis which in the most favorable systems contributes to the detailed mapping (e.g., as in the Drosophila salivaries: PAINTER

[1] Supported by operating grant No. A2564 (to E.K.) from the National Research Council of Canada.

and MULLER 1929). Cytological confirmation of reciprocal translocations has been obtained even in Neurospora where chromosomes are very small and detection as well as mapping is carried out by genetic analysis, based on ascospore patterns and interchromosomal meiotic linkages in heterozygous crosses (e.g., BARRY and PERKINS 1969). In *Aspergillus nidulans* on the other hand, cytological analysis is impractical since the chromosomes are even smaller; in addition, ascospore patterns cannot be reliably observed, and meiotic linkages are extremely difficult to detect since markers of the same chromosome often are meiotically unlinked. However, two special features of Aspergillus, namely mitotic recombination in diploids and phenotypic specificity of aneuploids (e.g., translocation disomics), provide conclusive evidence for reciprocal translocations and permit their mapping by a number of different techniques even in the absence of meiotic linkages (KÄFER 1962, 1974). The situation is somewhat different for the mapping of unidirectional translocations for which the generally applicable method of identifying heterozygous markers in duplication progeny from translocation crosses can be used (e.g., in Aspergillus: BAINBRIDGE 1970; CLUTTERBUCK 1970; or in Neurospora: PERKINS 1972).

The present investigation represents the first case of a completely mapped reciprocal translocation of *Aspergillus nidulans*. This translocation, *T2(I;VIII)*, was chosen for analysis because the linkage groups involved contain not only many markers useful for genetic mapping, but in addition several selective markers which are indispensable for the isolation of mitotic crossovers from well-marked test diploids. It was, therefore, possible to map the translocation in detail making use, solely, of results from the mitotic analysis of homozygous translocation diploids. In the absence of meiotic linkages this method is the easiest of the various mitotic mapping techniques, since it does not require familiarity with the different aneuploid phenotypes, all segregants being balanced haploids or diploids. In the case of *T2(I;VIII)*, meiotic linkages in heterozygous crosses made it possible to confirm part of the mitotic mapping of the translocation breaks, thus validating the mitotic mapping technique.

## MATERIALS AND METHODS

All strains used in these experiments are descendants of the same haploid wild-type strain of *Aspergillus nidulans* used by PONTECORVO and co-workers (1953). Further details of the genetic markers employed here (origin, methods of testing, etc.) can be found in the three map papers—by KÄFER (1958), DORN (1967), and CLUTTERBUCK and COVE (1974)—and the relevant references quoted there. The only previously undescribed mutants used here are *fpaA*91, a new UV-induced allele of *fpaA*, and *sD*157, a translocation-free allele of *sD* induced by nitrosoguanidine (GRAVEL *et al.* 1970). The approximate map positions of the genes referred to in this work are shown in Figure 1, and the mutant alleles used are indicated in the legend (terminology follows recently adopted proposals by CLUTTERBUCK 1974).

The presence of a I;VIII translocation was first demonstrated by NIKLEWICZ-BORKENHAGEN, who analyzed an "*sD*205" mutant obtained by GRAVEL (GRAVEL *et al.* 1970). Recently it was demonstrated conclusively that "*sD*205" is an isolate of DORN's (1967) *sD*50, which we obtained from the Fungal Genetics Stock Center (# 249) and used extensively for allelism tests of new s-mutants (in agreement with results by NIKLEWICZ-BORKENHAGEN, who found practically no recombination between "*sD*205" and *sD*50). Since DORN (1967) reported *sD*50 to be

free of translocations, the Stock-Center isolate of the original *biA*1;*sD*50 strain and an $F_1$ *sD*50-descendant were analyzed carefully for translocations, not only in heterozygous diploids using standard tester strains (KÄFER 1962, 1965), but also in crosses using the meiotic technique for detection of translocations (UPSHALL and KÄFER 1974). All results agree and indicate that both these *sD*50 strains contain *T2(I;VIII)*. In addition, an increasing number of other *sD*50 descendants used in various crosses and diploids are found to carry this translocation which has not yet been separated from the sulphite requirement. All other strains used here are shown to be translocation free.

Standard media for *Aspergillus nidulans* were used; the general meiotic techniques are those of PONTECORVO *et al.* (1953). The mitotic techniques are described by PONTECORVO and KÄFER (1958). To select segregants homozygous for *fpaA* or *fpaB* from partially resistant heterozygous diploids, conidia were transferred to complete medium containing high concentrations of p-fluorophenylalanine (up to 0.18 mg/ml).

## EXPERIMENTS AND RESULTS

In addition to meiotic mapping, two basically different mitotic methods have been used for the detailed genetic mapping of the reciprocal translocation *T2(I;VIII)*. These are based on the two independent processes of mitotic segregation in diploids—namely, mitotic nondisjunction or chromosome loss which produces haploid segregants, and mitotic crossing over (KÄFER 1961). Results from the three methods of analysis were complementary and confirmed one another: (1) Meiotic linkages between the translocation and adjacent markers permitted mapping of the exact position of the translocation breaks in linkage groups I and VIII. (2) Haploid segregants from diploids homozygous for the translocation were selected to identify the translocated markers in their new linkage groups and, thus, to determine the extent of the translocated segments; these results also established the reciprocality of the translocation but not the order of the markers. (3) Mitotic crossovers were selected from diploids homozygous for the translocation and heterozygous for markers in the affected chromosome arms. This identified the translocated pieces as terminal segments which retained their orientation with respect to the new centromeres, as expected for a reciprocal translocation.

### 1) *Meiotic mapping of the breakage points of* T2(I;VIII)

The translocation *T2(I;VIII)* was induced by UV treatment in the *biA* strain at the same time as the sulphite-requiring mutant *sD*50. To map the translocation breaks this translocation strain was first crossed to standard strains and $F_2$ segregants were then used in three crosses with distal and proximal markers of group VIII. An *sD*-recombinant carrying five other markers of group VIII was obtained in this way and used in two successive crosses heterozygous in repulsion and coupling for six markers of group I. From these five well-marked crosses 54 *sD*50 and 31 $sD^+$ segregants were isolated and checked for the translocation in heterozygous test diploids. In all 85 cases the translocation was found only if the mutant *sD*50 was present, indicating close or complete linkage of the translocation to the mutant *sD*50 (MA 1972). Therefore, the translocation breaks could be mapped accurately and easily by measuring meiotic recombination of *sD*50 with markers of group I as well as with markers on the proximal part of VIII (linkage of *sD*50

with the latter first found by DORN 1967, 1972). The results from several heterozygous crosses (Figure 1) showed decreasing linkages of *sD*50 to the distal markers on the left arm of group I, namely *suAadE*, *galD* and *fpaB* as well as to the more proximal markers of IL, *sulA*, *riboA* and *anA*. In addition, *sD*50 showed decreasing linkages with other proximal markers on VIIIR *coA*, *fwA*, *facC* and *cnxB*, as shown in Figure 1 (details of the meiotic results in homo- and heterozygous crosses of this and other translocations will be published elsewhere).

In conclusion, the meiotic analysis indicates that the most likely positions of the translocation breaks of *T2(I;VIII)* are very close to, or at, *sD* in linkage group VIII, and between *sulA* and *suAadE*, close to the latter in linkage group I. Heterozygous translocation crosses clearly establish the order of the markers on IL as *anA–riboA–sulA–suAadE–galD–fpaB* at increasing distances from the centromere, and the sequence *sD–coA–fwA–facC–cnxB* on VIIIR. Results from homozygous crosses support these conclusions.

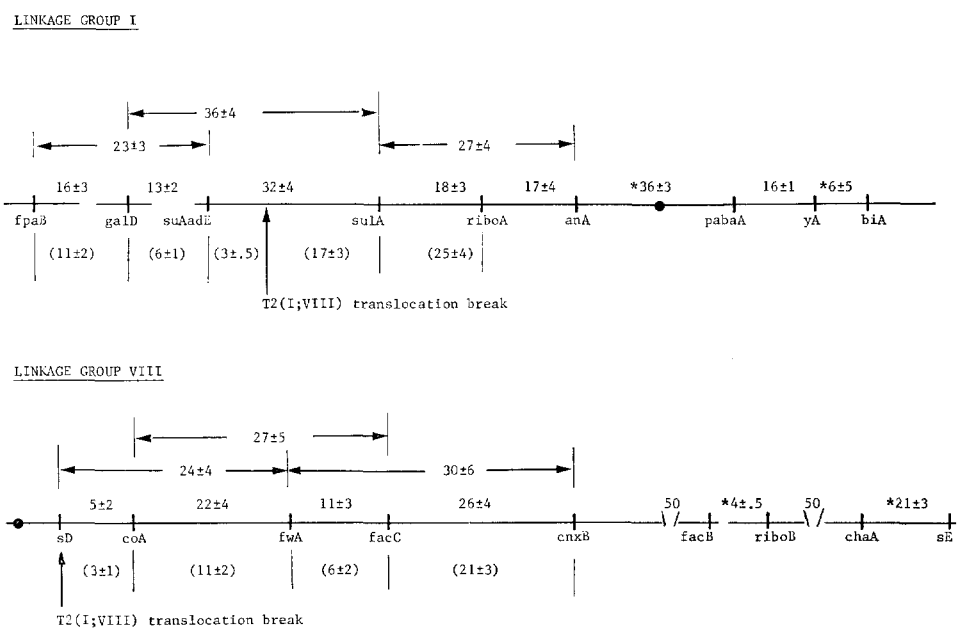


FIGURE 1.—Genetic map of linkage groups I and VIII, and meiotic recombination frequencies (%) as obtained from four standard crosses (recombination frequencies given only if significantly less than 50%; * values from earlier work). Corresponding values from heterozygous translocation crosses in brackets underneath for comparison. Markers used: conidial colors: *chaA*(1) = chartreuse, *fwA*(1 or 2) = fawn, *yA*(2) = yellow; resistance markers: *fpaA*(91) and *fpaB*(37) to p-fluorophenylalanine, *sulA*(1) to sulfonilamide; suppressor of *adE*(20) = *suA*(1)*adE;* morphological marker *coA*(1) = compact colony; nutritional markers: *adE*(20) = adenine, *anA*(1) = aneurin (=thiamine), *biA*(1) = biotin-requiring; *cnxB*(2) = nitrate, *facB* (101) and *facC*(102) = acetate and *galD* = galactose-nonutilizing; *pabaA*(1) = p-aminobenzoic acid, *riboA*(1) and *riboB*(2) = riboflavine, *sD*(50) [associated with *T2(I;VIII)*], *sD*(157) and *sE*(15) = sulphite-requiring.

2) *Mitotic haploids from homozygous translocation diploids*

When haploid mitotic segregants are isolated from homozygous T/T diploids the linkage of all markers of the two involved linkage groups which is found in heterozygous translocation diploids disappears, since all haploids now contain the same rearranged chromosomes; therefore, four types of haploids can be isolated with respect to markers of these groups. These now reflect the new linkage arrangements and hence indicate which markers are translocated (provided at least one centromere position is known).

Eight homozygous translocation diploids, heterozygous for many markers, were used for this mitotic analysis (the relevant genotypes for three of them are shown in Table 1). A total of over 550 haploids were isolated from the eight T/T diploids, using various methods of selection. Consistent results were obtained in all cases (results from the three diploids of Table 1 are given in detail in Table 2).

All mitotic haploids from the T/T diploids showed complete linkage of *suAadE* and *galD* which are present in coupling in all diploids. Therefore, these two markers of linkage group I were still completely linked with each other and were also found linked to *fpaB* (in diploid 1833, Tables 1 and 2). However, these three markers now showed free recombination to all other markers of group I, segregating about 1:1 for the alleles of the two homologs (allowing for known differences in viability). On the other hand, all markers of linkage group VIII (namely *fwA1*, *facC*, *cnxB*, *facB*, *riboB* and *chaA1*) now showed complete linkage to the untranslocated markers of group I, as expected if the chromosome segment carrying all these group VIII markers has been translocated to chromosome "I".

In summary, two new mitotic linkage groups are identified in T/T diploids and are found to be of very unequal size. One contains the markers *suAadE*, *galD* and *fpaB* normally located on the left arm of linkage group I (CLUTTERBUCK and COVE 1974) and presumably the centromere of VIII. The other contains all the remaining markers (and therefore presumably also the centromere) of linkage group I in addition to all the markers of VIII used in this investigation (as illustrated in Table 1 and Figure 2). These results locate the breakage point in group I proximal to *suAadE* but distal to *sulA*, *fpaA* and *riboA*, in agreement with the meiotic mapping. The other translocation break must be proximal to all markers, except *sD*, on VIII. Considering the close meiotic linkage of the translocation to *sD*, it can be concluded that *sD* must be the most proximal of the markers used here.

3) *Mitotic crossing over in diploids homozygous for* T2(I;VIII)

Analysis by mitotic crossing over completely depends on the availability of selective markers for the isolation of the rare crossover segregants. Selected crossovers give information on the position of any markers proximal to the point of selection and identify simultaneously segregating distal markers.

For the translocation analyzed here, two main types of selection were relatively easy and were employed extensively to isolate diploid mitotic crossovers, namely (a) selection for homozygous "suppressed" (*suAadE/suAadE*) segregants on supplemented minimal media lacking adenine; and (b) selection for resistance to p-fluorophenylalanine from diploids heterozygous for *fpaA* or *fpaB*

TABLE 1

*Relevant genotypes of control diploids and homozygous translocation diploids*

| Diploid numbers | Control diploids | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Standard linkage group I | | | | | | | | | | | Standard linkage group VIII | | | | | | |
| 1279 | + | *galD* | *suAadE* | + | + | + | *anA* | • | + | *yA* | *adE* | *biA* | • | + | + | + | + | + | + |
| | + | + | + | + | + | + | + | | + | + | *adE* | + | | + | *fwA1* | + | *facB* | *riboB* | *chaA* |
| 1832 | + | *galD* | *suAadE* | + | + | *riboA* | + | • | + | + | *adE* | *biA* | • | *sD157* | *fwA2* | + | *facB* | + | + |
| | + | + | + | + | fpaA | + | *anA* | | *pabaA* | *yA* | *adE* | + | | + | + | *cnxB* | + | + | *chaA* |
| 1834 | + | *galD* | *suAadE* | + | + | *riboA* | + | ○ | + | + | *adE* | *biA* | • | *sD157* | *fwA2* | + | *facB* | + | + |
| | fpaB | + | + | *sulA* | + | + | *anA* | | *pabaA* | *yA* | *adE* | *biA* | | + | + | + | + | + | *chaA* |
| Diploid numbers | Homozygous translocation diploids | | | | | | | | | | | | | | | Translocated linkage group VIII′ | | |
| | Translocated linkage group I′ | | | | | | | | | | | | | | | | | |
| 1830 | + | + | + | + | *facC* | *fwA2* | *sD50* | + | + | *riboA* | + | • | + | + | *adE* | *biA* | • | *suAadE* | *galD* | + |
| | *chaA* | + | + | *cnxB* | + | + | *sD50* | + | fpaA | + | *anA* | | *pabaA* | *yA* | *adE* | + | | + | + | + |
| 1833 | + | + | *facB* | + | + | *fwA2* | *sD50* | + | + | + | *anA* | • | *pabaA* | + | *adE* | *biA* | • | *suAadE* | *galD* | + |
| | + | + | + | *cnxB* | + | + | *sD50* | *sulA* | + | *riboA* | + | | + | *yA* | *adE* | + | | + | + | fpaB |
| 1253 | + | + | + | + | + | + | *sD50* | + | + | + | *anA* | • | *pabaA* | *yA* | *adE* | + | • | *suAadE* | *galD* | + |
| | *chaA* | *riboB* | *facB* | + | + | *fwA1* | *sD50* | + | + | + | + | | + | + | + | *biA* | | + | + | + |

TABLE 2

*Segregation of markers in mitotic haploids from control and homozygous translocation diploids*

| Test diploid number | Selected allele | Haploid segregants Genotypes | | | | | | | | | | | | | | | Numbers Each type | Numbers Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Linkage group I | | | | | Linkage group VIII | | | | | | | | | | | |
| Control | | | | | | | | | | | | | | | | | | |
| (1279) | *suAadE* | .. | *galD* | *suAadE* | .. | .. | *anA* | .. | *yA*† | *biA* | .. | *fwA* | .. | *facB* | *riboB* | *chaA* | 21 | 48 |
| | | .. | *galD* | *suAadE* | .. | .. | *anA* | .. | *yA* | *biA* | .. | + | .. | + | + | + | 27 | |
| (1832) | fpaA | .. | + | + | fpaA | + | *anA* | *pabaA* | *yA* | + | + | + | *cnxB* | + | .. | *chaA* | 64 | 136 |
| | | .. | + | + | fpaA | + | *anA* | *pabaA* | *yA* | + | *sD* | *fwA* | + | *facB* | .. | + | 72 | |
| (1834) | fpaB | fpaB | + | + | .. | + | *anA* | *pabaA* | *yA* | (*biA*) | + | + | .. | + | .. | *chaA* | 35 | 55 |
| | | fpaB | + | + | .. | + | *anA* | *pabaA* | *yA* | (*biA*) | *sD* | *fwA* | .. | *facB* | .. | + | 20 | |
| | | | | | | | | | | | | | | | | | Total | 239 |
| Homozygous *T2(I;VIII)* | | | | | | | | | | | | | | | | | | |
| (1830) | fpaA | .. | *galD* | *suAadE* | fpaA | + | *anA* | *pabaA* | *yA* | + | (*sD*) | + | *cnxB* | .. | .. | *chaA* | 41 | 105 |
| | | .. | + | + | fpaA | + | *anA* | *pabaA* | *yA* | + | (*sD*) | + | *cnxB* | .. | .. | *chaA* | 64 | |
| (1833) | fpaB | fpaB | + | + | .. | *riboA* | + | + | *yA* | + | (*sD*) | + | *cnxB* | + | .. | .. | 52 | 141 |
| | | fpaB | + | + | .. | + | *anA* | *pabaA* | + | *biA* | (*sD*) | *fwA* | + | *facB* | .. | .. | 89 | |
| (1253) | *yA** | .. | *galD* | *suAadE* | + | + | *anA* | *pabaA* | *yA*† | + | (*sD*) | + | .. | + | + | + | 31 | |
| | | .. | + | + | .. | .. | *anA* | *pabaA* | *yA* | + | (*sD*) | + | .. | + | + | + | 11 | |
| | *fwA** | .. | *galD* | *suAadE*§ | .. | .. | + | + | + | *biA* | (*sD*) | *fwA* | .. | *facB* | *riboB* | *chaA*§ | 46 | 127 |
| | | .. | + | + | .. | .. | + | + | + | *biA* | (*sD*) | *fwA* | .. | *facB* | *riboB* | *chaA*§ | 39 | |
| | | | | | | | | | | | | | | | | | Total | 373 |

* solated from complete medium supplemented with p-fluorophenylalanine.

† *adE* (omitted in all cases) usually homozygous, otherwise deduced from conidial color because closely linked to *yA*; other homozygous mutants in parentheses.

§ Likely allele.

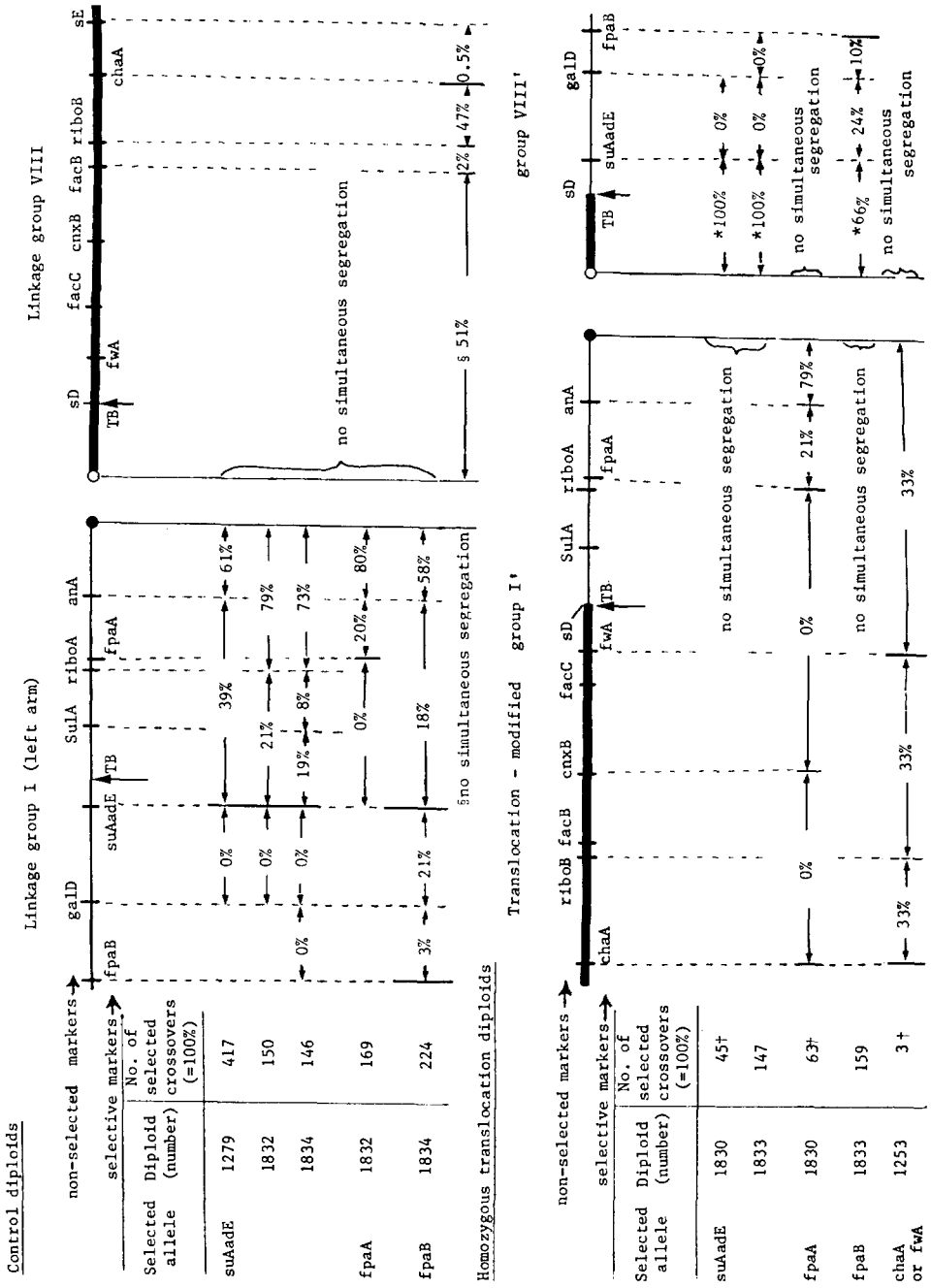


FIGURE 2.—Mitotic crossing over (in percent) in various intervals among selected crossover segregants from normal and homozygous *T2(I;VIII)* diploids (for genotypes see Table 1). ↑ = Selective marker; 0% indicates complete linkage of distal markers. * Values for *suAadE/suAadE* segregants from T/T diploids include nondisjunctional types (see text); † results include samples of small sizes and are only used to deduce order, not relative distance of markers; § data of NIKLEWICZ (1970), total of 182 chartreuse (*chaA/chaA*) crossovers selected from two heterozygous diploids (one double crossover obtained with an exchange between *chaA* and *sE*).

(see genotypes, Table 1). Since the data obtained in this way—combined with the results of the previous sections—gave all the evidence needed for a consistent map of *T2(I;VIII)*, no systematic selection of color segregants was attempted. This third method of selection is rather difficult and time consuming. However, a few homozygous *chaA* or *fwA* crossovers were obtained incidentally and their analysis provided some additional confirming evidence. The results from all these segregants are summarized in Figure 2 and are described in detail below.

a) *Mitotic crossovers homozygous for* suAadE*:* "Suppressed" diploid mitotic segregants were isolated from several diploids heterozygous for *suAadE* and homozygous for *adE*. These diploids were all heterozygous for a proximal marker on the left arm of group I, either *anA* or *riboA*, in coupling to *suAadE*. It was found that none of the 883 *suAadE/suAadE* segregants from four T/T diploids were homozygous for a proximal marker of IL, while "suppressed" crossover segregants from controls regularly are homozygous for such proximal markers part of the time, indicative of the distribution of crossing over in the various intervals (Figure 2). On the other hand, all these *suAadE/suAadE* segregants, whether from T/T or control diploids, showed the phenotype of the mutant *galD* (total tested 1300), indicating that *galD* is distal to *suAadE* in both positions, on IL and when translocated to VIIIR. Incidentally, the "suppressed" diploid recombinants from the T/T diploids are the only group of selected diploid segregants which includes not only diploid crossovers but also diploid nondisjunctional segregants, since these cannot be distinguished by any test. Nondisjunctional segregants have been identified in all other cases and are not included in Figure 2. Generally, nondisjunctional types are found among selected diploid segregants from structurally homozygous diploids with frequencies ranging from 5–15%, depending on markers used for selection.

b) *Mitotic crossovers selected for maximum resistance to p-fluorophenylalanine (homozygous for* fpaA *or* fpaB*):* Two diploids heterozygous for *fpaA* were used, one homozygous for the translocation, the other without *T2(I;VIII)* as a control (1830 and 1832, Table 1). Both diploids carried the markers *anA* of linkage group I and *cnxB* and *chaA* of group VIII in coupling, while *suAadE* was present in repulsion. The results obtained show that in T/T diploids and in controls, selected *fpaA/fpaA* crossovers have become homozygous for *anA* with the same frequency (see Figure 2). This indicates that these markers must be on the same arm, namely IL, in both cases. On the other hand, homozygous *fpaA*-segregants from T/T diploids do not become homozygous for *suAadE*+ like those from controls, but become homozygous for markers of VIII instead. These results confirm that in *T2(I;VIII) fpaA* is not translocated, but all markers of VIII used here are translocated to IL distal to *fpaA*; in addition, *suAadE*, normally on the left tip of linkage group I, is now independent of *fpaA* and the centromere of I.

Diploids heterozygous for *fpaB* gave results similar to those obtained with *suAadE*. When resistant homozygous *fpaB* segregants were selected from the T/T diploid (1833, Table 1), *galD* and *suAadE* were the only markers that segregated. In contrast to these results, *fpaB/fpaB* crossovers from the control diploid (1834) showed segregation of all other markers of IL as expected if these are all proximal to *fpaB*. The distribution of the exchanges in the various marked intervals from both these diploids are shown in Figure 2.

Since homozygous *fpaB* segregants from T/T as well as control diploids were found to be homozygous for *galD*+ and *suAadE*+ only in a fraction of the cases, these results establish that in both cases *fpaB* is the most distal of the three translocated markers. Considering that the "suppressed" *suAadE/suAadE* crossovers establish *suAadE* as the most proximal of the three markers, their order on IL, as well as on VIIIR, must be *suAadE–galD–fpaB*, at increasing distances from the centromeres.

c) *Mitotic crossover segregants homozygous for conidial color mutants* chaA *or* fwA*:* A few chartreuse and fawn color segregants were obtained from the translocation diploid 1253 (Table 1). The complete genotype of three of these segregants was determined by selecting and testing a sample of mitotic haploids. Two of these segregants were *chaA/chaA*, one of them was also homozygous for *riboB* and *facB* but heterozygous for *fwA*, while the other was heterozygous

for all markers other than *chaA*. An *fwA/fwA* segregant was homozygous for all group VIII markers, but heterozygous for all markers of I. This establishes the order and orientation of the markers translocated from group VIII to the distal part of IL, *chaA* being distal to *riboB* and *facB*, and these in turn distal to *fwA*.

In conclusion, *mitotic crossing over* in diploids *homozygous for T2(I;VIII)* further established the following orientation of markers, even of meiotically unlinked ones, in the translocated arrangement: on the translocation chromosome I′ all markers of VIII distal to *sD*50 are now distal to *fpaA* with an order from the translocation break, in a proximal to distal direction, of *fwA–facB–riboB–chaA*; on the translocation chromosome VIII′, the translocated markers of I show the order of *suAadE*, *galD* and *fpaB*, proximal to distal from the translocation break. Therefore, as expected in reciprocal translocations, the orientation of markers relative to the centromeres remained unchanged when the translocated segments became part of the new linkage groups.

## DISCUSSION

The present investigation represents the first complete genetic analysis of a reciprocal translocation in Aspergillus. While many such translocations have been postulated (KÄFER 1965), in no case were suitable genetic markers on both chromosome arms available for meiotic or mitotic analysis.

On the other hand, linkage groups I and VIII contain such markers and complete mapping of *T2(I;VIII)* was possible using the simple genetic technique based on mitotic recombination in homozygous translocation diploids. In addition meiotic linkages confirmed the mitotic results. The general procedure of the mitotic mapping technique—applicable in any fungus which has meiotic and mitotic recombination—is the following: After a reciprocal translocation has been detected and the two linkage groups involved have been identified in heterzygous diploids, or crosses (UPSHALL and KÄFER 1974), the translocation is crossed to strains containing a selection of markers of these two linkage groups. Recombinants with an assortment of markers are checked for the translocation. Strains carrying the translocation are combined into homozygous T/T diploids, heterozygous for as many markers as possible. As a first step of analysis mitotic haploid segregants are isolated by any of the standard techniques. The new linkages found in these haploids identify the markers of the translocated distal segments, provided the centromere positions are known. This is true even if only one centromere region is identified, and in such cases it is possible to deduce that all markers which are found to be translocated together must be located on the same chromosome arm. This contributes to the mapping of centromeres and is especially useful if the markers are meiotically unlinked, as found in the case of *T2(I;VIII)* for *fwA*, *riboB* and *chaA*. The untranslocated markers of this same group are then either proximal to the break or on the other arm. Such information is very helpful for the construction of genetic maps in organisms with meiotically long chromosomes.

The second step of analysis depends on the suitable location of markers which can be used for selection of mitotic crossovers from homozygous translocation diploids. Such markers have by now been mapped on nine chromosome arms of

Aspergillus (in which two of the chromosomes definitely have two arms, two others probably only one—giving a minimum of ten and maximum of fourteen arms; CLUTTERBUCK and COVE 1974; KÄFER unpublished). When selective markers are located on chromosome arms involved in the translocation, they can be used to confirm the position of the breaks and to identify translocated markers and their orientation. In addition, if one of them is translocated, it may permit mapping by mitotic crossing over in a chromosome arm which normally does not contain such a marker.

Once the approximate position of a break is determined by mitotic analysis, crossing of the translocation to markers of the identified chromosome parts often produces meiotic linkage information. Such results may help in the sequencing of markers, since linkage relationships may become clearer when meiotic recombination is reduced, because of the heterozygosis for the translocation.

In the present case reduced recombination was observed close to both breaks in several heterozygous crosses (ca. 2000 segregants tested) and it was possible to deduce unambiguously the order of the markers on the left arm of group I, as well as those on the proximal part of VIIIR. The deduced order also was consistent with the less conclusive data from controls (ca. 1500 tested) and amply confirmed by the results from the mitotic analysis. The order of markers on IL obtained here (*anA–riboA–sulA–suAadE–galD–fpaB*) does not agree completely with the preferred one shown in the map of DORN (1967) and of CLUTTERBUCK and COVE (1974), which place *galD* proximal to *suAadE*, based on results by ROBERTS (1963). However, the results of ROBERTS are somewhat aberrant, the crucial recombinant class showing a large excess of prototrophs over the complementary mutant types (total of 201 tested). It therefore appears likely that a few of the excess prototrophs actually were diploids (or possibly heterokaryotic mixtures), which eliminates the disagreement with our results. The order of the markers proximal on VIIIR (*sD–coA–fwA–facC–cnxB*) agrees with the recent map quoted above, which is based on unpublished data of COVE. The map order preferred by DORN is slightly different, but his results produce one pair of values inconsistent with the preferred sequence (DORN 1967, 1972). The published recombination values actually fit equally well with the order obtained here, since the samples analyzed were so small that neither of the three crucial values is significantly different from the other two. In addition, it is very likely that all *sD*50-strains in circulation contain *T2(I;VIII)*, and inconsistent linkage relationships are to be expected if heterozygous translocation crosses are compared with standard ones. Indeed, because of the complete linkage of *sD*50 to the translocation it was not possible to map *sD* on VIII with certainty until the translocation-free allele *sD*157 became available (GRAVEL *et al.* 1970). A similar situation exists for two other mutants which have not yet been separated from their translocations (KÄFER 1965, 1974). In all three cases the mutant was induced by UV treatment at the same time as the translocation. These mutants may therefore be due to position effects or small deletions caused by inaccurate joining or repair during the formation of the translocated arrangement (as has been demonstrated for some of the X-ray-induced translocations in Drosophila; e.g., MULLER 1940).

In conclusion, the technique of genetic mapping of reciprocal translocations using mitotic recombination in homozygous diploids is very useful when meiotic linkages are absent and, in addition, may provide results for the construction of linkage maps. At a later stage, when all centromeres are mapped and most markers are sequenced, a few haploids from a homozygous diploid with suitable heterozygous markers will map the breaks very satisfactorily. The disadvantage of this method is that well-marked translocation strains have to be produced first. This is not the case for two other mitotic methods, one based on mitotic crossing over in heterozygous T/+ diploids, the other based on mitotic analysis of translocation disomics from heterozygous crosses (KÄFER 1962, 1974). However, in both these latter cases unbalanced, aneuploid products have to be analyzed and recognized visually, which can only be done safely after much experience. In the present case, some data from heterozygous diploids have been obtained which further confirm the presented results (MA 1972; KÄFER, unpublished). However, it has become clear that the use of mitotic crossing over in heterozygous diploids is complicated by a relative increase of nondisjunctional segregants from unstable intermediates, some of which appear to be trisomics. Further research with well-marked diploids heterozygous for various translocations is planned to identify all the observed types of segregation.

We would like to thank DRS. W. F. GRANT and D. D. PERKINS for helpful comments and criticism of the manuscript. We also appreciate the cooperation of DR. A. J. CLUTTERBUCK, who provided several strains and unpublished mapping data.

## LITERATURE CITED

BAINBRIDGE, B. W., 1970 Genetic analysis of an unequal chromosomal translocation in *Aspergillus nidulans*. Genet. Res. **15**: 317–326.

BARRY, E. G. and D. D. PERKINS, 1969 Position of linkage group V markers in chromosome 2 of *Neurospora crassa*. J. Heredity **60**: 120–125.

BRINK, R. A., 1927 The occurrence of semi-sterility in maize. J. Heredity **18**: 266–270.

BRINK, R. A. and C. R. BURNHAM, 1929 Inheritance of semi-sterility in maize. Am. Naturalist **63**: 301–316.

CLUTTERBUCK, A. J., 1970 A variegated position effect in *Aspergillus nidulans*. Genet. Res. **16**: 303–316. ——, 1974 Gene symbols in *Aspergillus nidulans*. Genet. Res. (In press.)

CLUTTERBUCK, A. J. and D. J. COVE, 1974 The genetic loci of *Aspergillus nidulans*. Handbook of Microbiology, Cleveland, Ohio: The Chemical Rubber Co. (In press.)

DORN, G., 1967 A revised map of the eight linkage groups of *Aspergillus nidulans*. Genetics **56**: 619–631. ——, 1972 Computerized meiotic mapping in *Aspergillus nidulans*. Genetics **72**: 595–605.

GRAVEL, R. A., E. KÄFER, A. NIKLEWICZ-BORKENHAGEN and P. ZAMBRYSKI, 1970 Genetic and accumulation studies in sulphite-requiring mutants of *Aspergillus nidulans*. Can. J. Genet. Cytol. **12**: 831–840.

KÄFER, E., 1958 An eight chromosome map of *Aspergillus nidulans*. Adv. Genet. **9**: 105–145. ——, 1961 The processes of spontaneous recombination in vegetative nuclei of *Aspergillus nidulans*. Genetics **46**: 1581–1609. ———, 1962 Translocations in stock strains of *Aspergillus nidulans*. Genetica **33**: 59–68. ——, 1965 Origins of translocations in *Aspergillus nidulans*. Genetics **52**: 217–232. ——, 1974 Reciprocal translocations and translocation disomics of Aspergillus, and their use for map construction. (submitted for publication).

MA, G. C. L., 1972 Influences of the translocation *T2(I;VIII)* on mitotic and meiotic recombination in *Aspergillus nidulans*. M.S. thesis, McGill University, Montreal.

MULLER, H. J., 1940 An analysis of the process of structural change in chromosomes of Drosophila. J. Genet. **40**: 1–66.

PAINTER, T. S. and H. J. MULLER, 1929 The parallel of cytology and genetics of induced translocations and deletions in Drosophila. J. Heredity **20**: 287–298.

PERKINS, D. D., 1972 An insertional translocation in Neurospora that generates duplications heterozygous for mating type. Genetics **71**: 25–51.

PONTECORVO, G. and E. KÄFER, 1958 Genetic analysis based on mitotic recombination. Advan. Genet. **9**: 71–104.

PONTECORVO, G., J. A. ROPER, L. M. HEMMONS, K. D. MCDONALD and A. W. J. BUFTON, 1953 The genetics of *Aspergillus nidulans*. Advan. Genet. **5**: 141–238.

ROBERTS, C. F., 1963 The genetic analysis of carbohydrate utilization in *Aspergillus nidulans*. J. Gen. Microbiol. **31**: 45–58.

UPSHALL, A. and E. KÄFER, 1974 Detection and identification of translocations by increased specific nondisjunction in Aspergillus. Genetics **76**: 19–31.

Corresponding editor: D. R. STADLER