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# Meiotic recombination in a duplication strain of *Aspergillus nidulans*

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SUMMARY

The (I; II) duplication strain of *Aspergillus nidulans* contains a terminal segment of chromosome I twice, one copy being in the normal position, and the other one translocated to a tip of chromosome II. We show that in selfed cleistothecia (homozygous for the duplication) crossing-over between the two segments of the duplication followed by the appropriate disjunction and distribution of the meiotic chromosomes, results in segregation of recessive markers in the duplication. Alternative segregation mechanisms like the deletion mechanism responsible for mitotic non-conformity, cannot explain the observations. The average segregation frequency of the marker *yA2* is 11 %. Using the mathematical model outlined in the Appendix to this paper, we estimated the recombination frequency between the translocation breakpoint and the *yA* locus from the segregation frequency. The essential feature of the model is the computation of the probabilities of combinations of different tetrad types in quadrivalents. Application of the model leads to the qualitative conclusion that the meiotic recombination frequency within the duplication is increased over the normal level. Elsewhere in the genome the frequencies are either unchanged or decreased. Also in crosses heterozygous for the duplication, an increase by at least a factor of two is found. As judged from the appearance of certain recombinant classes, the quadrivalent frequency in homozygous and heterozygous crosses is 2/3 (random pairing) or slightly higher. The increased recombination is attributed to the same lesions that are responsible for the occurrence of deletions in duplication strains of *A. nidulans*.

## 1. INTRODUCTION

Partially diploid systems like aneuploids and duplication strains, have been exploited in microorganisms in order to isolate recombination-deficient (*rec*) mutants (e.g. Van den Ende & Symonds, 1972; Rodarte-Ramon & Mortimer, 1972; Roth & Fogel, 1971; Parag & Parag, 1975), mainly because these systems allow expression of most induced mutations whether recessive or not. The actual procedure includes (1) making the diploid segment heterozygous for a recessive marker, (2) the measurement of the frequency with which the recessive marker segregates among the progeny and (3) the isolation of mutants with aberrant segregation frequencies. If the system indeed segregates through recombination,

there is a good chance of finding *rec* mutants in this way. As we are interested in genetic recombination of *A. nidulans*, we have explored the possibilities of a duplication strain of this fungus for obtaining mutants with aberrant frequencies of meiotic crossing-over. This paper deals with the analysis of the system. The properties of a set of isolated segregation-deficient (*seg*) mutants will be published separately.

The duplication strain used in this study has been isolated by Pritchard (1956) and was subsequently employed in recombination studies (Pritchard & Siddiqi, unpubl., quoted in Pritchard, 1960). The terminal segment of the right arm of chromosome I is present twice, one copy being in the normal position, and the other copy translocated to the tip of the right arm of chromosome II. The duplication is not stable: by a not yet understood process, called mitotic non-conformity (Nga & Roper, 1969), the duplication occasionally gains or loses genetic material during vegetative growth (Nga & Roper, 1968), giving rise to addition and deletion mutants which appear as sectors (mitotic segregants) in the colonies.

As *A. nidulans* is a homothallic species, cleistothecia will arise in monospore colonies of the duplication strain. If the strain is heterozygous for a recessive marker on the duplicated chromosome segment, ascospores may arise which segregate the marker, although most ascospores will remain heterozygous. One may ask if the meiotic segregants arise through recombination, or rather through deletion of the dominant allele of the recessive marker by a meiotic process similar to mitotic non-conformity. In this paper we show that the meiotic segregants arise through crossing-over between the duplication segments. We have found no indication for the frequent occurrence of deletions during meiosis. Additionally we have found that the duplication itself affects the frequency of crossing-over, this frequency apparently being increased within the duplication. Any statements about recombination frequencies within the duplication must rest on a mathematical function which allows these frequencies to be computed from the observed segregation frequencies. This function is complex at least in the case of homozygous crosses, in particular if different modes of genetic interference have to be accounted for. The Appendix to this paper deals with this problem.

## 2. MATERIALS AND METHODS

(*a*) *Strains*. The strains used are described in Table 1. The crinkled phenotype of the duplication strains (Bainbridge & Roper, 1966) shows best if the colonies are grown for at least three days at 37 °C on Petri plates containing a thick layer of complete medium additionally supplemented with arginine and riboflavin. Besides the typical crinkles the phenotype includes a rough surface, bad sporulation, reduced growth rate and an increased colony height. If the colony has started from a multi-spore inoculum, the crinkles do not form, but the roughness increases and poorly sporulating aerial mycelium is formed centrally.

(*b*) *Media*. The minimal and complete media used, and the concentrations of the supplements in these media, have been described by Jansen (1970). Presently, however, also 1 mg/l $CuSO_4.5H_2O$ is added.

(c) *Techniques.* General techniques were those of Pontecorvo *et al.* (1953). In some cases the arginine-limitation technique (Bainbridge, 1974) was used for sexual crosses. Ascus analysis was performed according to Strickland (1958*a*), by means of a Singer micromanipulator. We obtained the asci from very mature cleistothecia (at least four weeks old) by opening the cleistothecia and rolling them on the surface of a freshly poured agar plate. From the trail left behind suitably located asci were isolated by means of a microloop, and the eight spores were transferred to premarked places on the plate. From there they were moved to an incubation plate by cutting out a block of agar.

Table 1. *Genotype and origin of strains*

| Strain number | Genotype* | Origin |
|---|---|---|
| UT 150 | *biA1; pyroA4* | FGSC 33 |
| UT 173 | *yA2; argB2* | Bainbridge, London |
| UT 175 | *proA1 pabaA6 yA2;* TB1(I; II) *adE20 biA1* | Nga & Roper, 1968 |
| UT 236 | *yA2 adE20; nicB8* | Stock Utrecht |
| UT 251 | *riboA1 anA1 biA1* | Stock Utrecht |
| UT 252 | *riboA1 anA1 proA1 pabaA6 yA2;* TB1(I; II) *adE20 biA1* | 175 × 251 |
| UT 253 | *riboA1 anA1 proA1 pabaA6 yA2* | 175 × 251 |
| UT 312† | *phenA2 galA1 dilA1 sC12 cnxH4* | Stock Utrecht |
| UT 313 | *riboA1 anA1 proA1 pabaA6 yA2;* TB1(I; II) *adE20 biA1* | 252 × 312 |
| UT 314† | TB1(I; II) *adE20 biA1; phenA2 galA1 dilA1 sC12 cnxH4* | 252 × 312 |
| UT 602 | *pabaA124 biA1; argB2* | 173 × 175 |
| UT 675 | *proA1 pabaA6 yA2;* TB1(I; II) *yA2 adE20* | 175 × 236 |
| UT 676 | *adE20 biA1;* TB1(I; II) *yA2 adE20 nicB8* | 175 × 236 |

* Nomenclature conforms to Clutterbuck (1974). TB1(I; II) refers to the translocation breakpoint of the duplication, or rather the new joint linking the translocated I segment to chromosome II.

† These strains harbour a non-reciprocal VI → VII translocation.

(d) *Nomenclature.* As stated in the Introduction a terminal segment of chromosome I is present twice in the duplication strains, one copy in the normal position, and the other one translocated to a tip of chromosome II. We shall refer to these copies as the *normal segment* and the *translocated segment* respectively. Strains not containing the duplication will also be called *normal*, because they possess only the normal segment, and show a *normal* (non-crinkled) morphology.

## 3. RESULTS

### (i) *Segregation by recombination*

In order to detect meiotic segregation in the (I; II) duplication strain we inoculated UT 175 which is heterozygous for *yA2*, *adE20* and *biA1*, on supplemented minimal medium, and collected mature, selfed cleistothecia after at least two weeks of incubation at 37 °C. Each of 13 cleistothecia yielded a certain fraction of yellow colonies among a majority of green ones, upon plating of a sample of ascospores. A total count of 12661 colonies revealed a significant heterogeneity

($P < 1\%$) of the frequencies of *y* segregants among the cleistothecia. These frequencies varied between 8·0 and 14·9 %, the average and the standard deviation being 11·0 and 2·0 % respectively. A symmetrical 95 % confidence interval for the average is 9·9 to 12·1 % (based on the normal distribution). A smaller sample was also scored for the markers *ad* and *bi*. Table 2 shows that the segregation frequency of *ad* is equal to the segregation frequency of *y*, but for *bi* we find a higher value. These results make point mutation and gene conversion unlikely as a major cause of segregation and nicely fit the hypothesis that segregation is

Table 2. *Meiotic segregation to* y, ad *and* bi *in the duplication strain UT 175*

| Segregant phenotype | | | Number |
|---|---|---|---|
| *y* | *ad* | *bi* | observed |
| + | + | + | 366 |
| *y* | + | + | 53 |
| + | *ad* | *bi* | 46 |
| + | + | *bi* | 35 |
| + | *ad* | + | 14 |
| *y* | + | *bi* | 2 |
| *y* | *ad* | + | 1 |
| *y* | *ad* | *bi* | 0 |
| Total | | | 517 |
| 10·8 | 11·8 | 16·0 | segr. freq. (%) |

Frequencies of yellow-spored, adenine- and/or biotin-requiring segregants amongst colonies from ascospores of the duplication strain UT 175, which is heterozygous for *y*, *ad* and *bi*. The ascospores were derived from several cleistothecia; these did not show a significant heterogeneity for the frequencies.

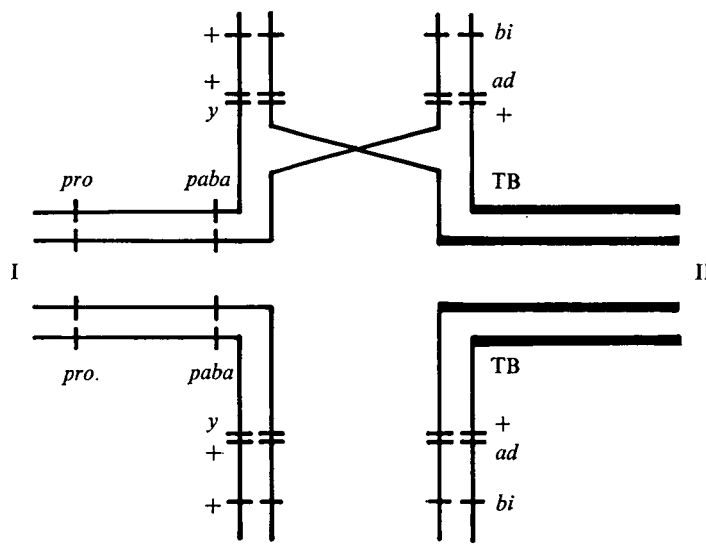


Fig. 1. The duplication in UT 175, in a quadrivalent configuration during meiosis. The combination of a recombinant translocated segment and a parental normal segment yields a *y* segregant. The reciprocal combination yields *ad* and *bi* segregants.

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caused by recombination (crossing-over) in the region between the translocation breakpoint and the marker under study, because *y* and *ad* map at about the same place whereas *bi* is located more distally. Such a crossing-over event can only result in segregation if the chromosomes I and II form a quadrivalent during the meiotic prophase (see Fig. 1).

As already pointed out in the Introduction, we must pay attention to another hypothesis that can explain segregation in the duplication, namely deletion of the dominant allele. We have four arguments which show beyond doubt that the predominant mechanism for meiotic segregation in the duplication is recombination and not deletion.

(*a*) The deletion segregants arising during vegetative growth are typically improved and tend to lose the crinkled phenotype characteristic for the duplication (Nga & Roper, 1968). The meiotic segregants, however, are fully crinkled, which indicates that they still contain the duplication.

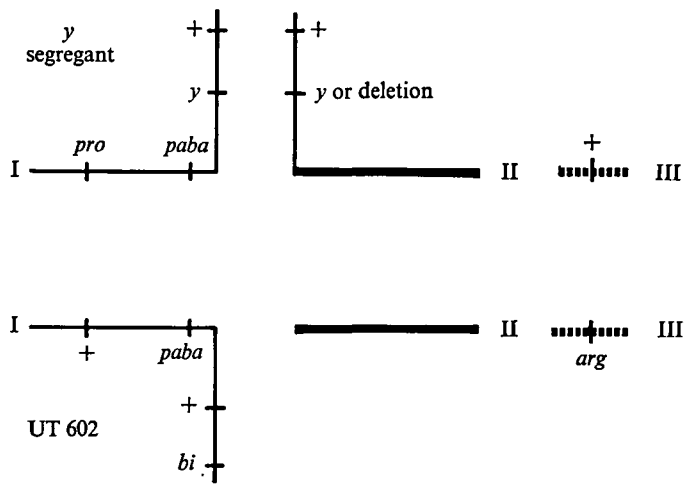


Fig. 2. Diagram showing a cross of UT 602 with a *y* meiotic segregant from UT 175.

(*b*) The markers *y* and *ad* map very close to each other, but in UT 175 *y* is located on the normal segment of the duplication, and *ad* on the translocated segment (see Fig. 1). Viable deletions mainly occur in the translocated segment (Cooke, Roper & Watmough, 1970), and thus give rise to many more *y* than *ad* deletion segregants. In contrast with this there are as many *y* as *ad* meiotic segregants (Table 2). Experiments with strains containing *y* in the translocated segment fully support this view (data not shown). This suggests that the meiotic segregants arise by a process different from that underlying mitotic non-conformity.

(*c*) If the *y* meiotic segregants arise by deletion of the $y^+$ allele, they should be hemizygous for the deleted region. On the other hand recombination should lead to *y*/*y* homozygotes. We isolated 14 independent *y* meiotic segregants from UT

175, and crossed each of them with the green ($y^+$) *arg* strain UT 602 (see Fig. 2) by means of the arginine-limitation technique. Half of the progeny from each cross were green, and about 50% of the green progeny should have contained the duplication and should have been heterozygous $+/y$ if the meiotic segregants had arisen by crossing-over. We tested a number of green progeny (19 to 58) from each cross for heterozygosity at the *y* locus. This can easily be done by looking for yellow meiotic segregants in the selfed cleistothecia formed by this progeny. For that purpose green $arg^+$ progeny (*arg* progeny are sterile) were grown on supplemented MM for two weeks. From each colony one cleistothecium was crushed on the surface of a CM plate, and the spores were spread. After incubation some plates showed many yellow spots against the green background, indicating heterozygosity. In this way we measured the frequency of heterozygosity among the green progeny colonies obtained from the 14 crosses mentioned above. This frequency varied between 21 and 58% for the various crosses, with a median value of 32%. This shows that heterozygosity was frequently found in the progeny of all crosses. From this we infer that all 14 *y* meiotic segregants from UT 175 must have been homozygous $y/y$, which strongly argues in favour of recombination as the main mechanism of meiotic segregation.

On the other hand it is also clear that the observed frequencies of heterozygosity fall short of the expected 50%. However, this fits in with a general experience with crosses between a duplication and a normal strain, which always yield an excess of normal progeny (Nga & Roper, 1968, and below). In this respect it is important that (for the reason explained above) the tested progeny all contained the unlinked $arg^+$ gene derived from the duplication parent. So the excess of normal progeny cannot be explained by selfing of the normal parent (UT 602) but is caused by a lower viability of the duplication progeny.

(*d*) We performed an ascus analysis of the meiotic progeny of UT 175. If it is crossing-over that yields the *y* meiotic segregants, each *y* spore pair should be accompanied by a spore pair containing the reciprocal recombinant, namely an *ad* spore pair. The results of 55 asci generally showed this picture. As only 66% of the spores were viable it was, however, difficult to obtain a firm conclusion based on complete asci. We therefore divided the asci into two groups, one group containing one or more *y* spores per ascus, the other group consisting of asci with $y^+$ spores only. The former group should contain *ad* spores (the reciprocal recombinants) in about 2 out of 8 cases (25%), the latter in a much lower frequency. The observed figures were 17·6% (19/108) and 3·4% (6/176) respectively. The difference between these figures is highly significant ($P < 1\%$), whereas the first does not differ significantly from 25% ($P$ appr. 20%). This strongly points to crossing-over as the mechanism of meiotic segregation.

### (ii) *A model for interpretation of segregation frequencies*

In the previous section we reported an average segregation frequency of 11·0% for *y*. What does this figure mean in terms of the recombination frequency in the duplication, between the translocation breakpoint and the *yA* locus? A general

approach to this problem is given in the Appendix to this paper. In short, the Appendix introduces the following parameters of the system:

(*a*) The frequency $q$ with which the chromosomes I and II pair to form a quadrivalent during the meiotic prophase (recombination in bivalents can never lead to $y$ segregants). In case of random pairing $q = 2/3$.

(*b*) The degree of genetic interference $k$ *within* each of the two tetrads constituted by the duplication segments in the quadrivalent. The value of $k$ for no interference is 1, but in most genetic systems positive interference is the rule ($k < 1$).

(*c*) The degree of genetic interference $\rho$ *between* the two tetrads mentioned. The value for no interference is 0, but negative interference is suspected ($0 < \rho < 1$).

(*d*) The frequencies with which the quadrivalents show the various possible combinations of two tetrad types, each tetrad being either parental, tetratype or non-parental. These frequencies can be computed once the map distance and $q$, $k$ and $\rho$ are known.

(*e*) The frequencies with which each of these combinations segregates $y/y$ homozygotes. These frequencies are fixed by the basic features of chromosome disjunction and distribution.

Experimentally we only tried to obtain some information about the real value of $q$. Regarding the value of $k$, reports of chiasma interference during meiosis in *A. nidulans* are conflicting (Käfer, 1958; Strickland, 1958*b*; Elliott, 1960; Dorn, 1972), but they agree that interference, whether positive or negative, is slight. Therefore we have chosen $k = 1$, though the real value may be different. Fortunately it appears from the calculations in the Appendix that the variation of $k$ has no great impact on the predicted frequencies. This is different for $\rho$. Here we first tried to see what conclusions would still be justified even if major fluctuations of $\rho$ are permitted. Secondly, we have compared the system with a different system (a cross heterozygous for the duplication) in which $\rho$-type interference plays no role.

### (iii) *Crosses heterozygous for the duplication*

We have tried to determine the value of $q$ in a cross heterozygous for the duplication (duplication × normal) and in a cross homozygous for it (duplication × duplication). As we are interested in meiotic segregation in selfed cleistothecia of the duplication strain, and as selfed cleistothecia constitute a homozygous cross, only this type of cross is relevant to our problem. However, the heterozygous cross serves as a basis for comparison, and as an easier version of the problem. The normal progeny of a heterozygous cross can be used directly to calculate a recombination frequency, as the recessive markers cannot be masked by their dominant alleles. Also, $\rho$-type interference plays no role, because there is one tetrad only. Moreover, we can compare the results with those of Pritchard & Siddiqi (unpubl., quoted in Pritchard, 1960) who performed the same type of cross. Our findings fully support their conclusions. The diagram of the cross is given in Fig. 3; in fact we used two heterozygous crosses (UT 150 × UT 175 and

UT 150 × UT 675) which differ in the genetic labelling of the translocated segment. The figure shows only one cross (UT 150 × UT 175) in the three possible pairing configurations (two quadrivalents and one pair of bivalents). It is evident from the figure that certain recombinant classes amongst the normal progeny can only arise from quadrivalents (e.g. the *paba* $y^+$ *ad* class), whereas others predominantly arise from bivalents (e.g. the *paba* $y^+$ $ad^+$ class).

An analysis of a total of 6 cleistothecia from the two crosses is shown in Table 3. The results of the two crosses do not differ significantly and from their combined data it is clear that, for the region *pabaA–yA*, as many quadrivalent recombinants

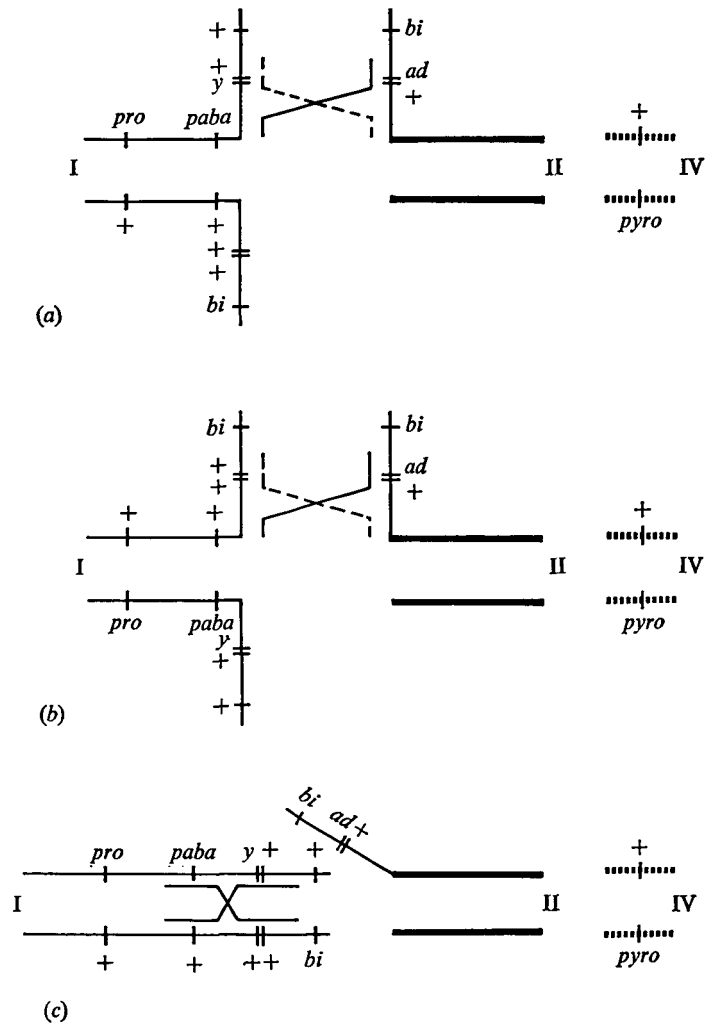


Fig. 3. Diagram of the cross UT 150 × UT 175, heterozygous for the duplication. The figure shows the three principal meiotic configurations of chromosomes I and II: in (*a*) and (*b*) quadrivalents have been formed, either with the normal segment of UT 175 (*a*) or with that of UT 150 (*b*). In (*c*) two bivalents have been formed, and the translocated segment is unpaired.

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(98) as bivalent recombinants (95) were found amongst the normal progeny. It is evident from Fig. 3 that in quadrivalents only one of the two normal segments will engage in pairing and recombination. The results therefore mean that there

Table 3. *Analysis of* normal *progeny from crosses heterozygous for the duplication: recombination between* pabaA *and* yA

| Normal progeny from cross: Genotypes | | | | UT 150 × UT 175 Cleistothecium no. | | | UT 150 × UT 675 Cleistothecium no. | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *paba* | *y* | *ad* | Origin | 1 | 2 | 3 | 4 | 5 | 6 | Total |
| + | + | + | Parental | 66 | 145 | 146 | 198 | 91 | 146 | — |
| *paba* | *y* | + | Chromosome rec. | 14 | 137 | 112 | 50 | 65 | 2 | — |
| + | +* | *ad* | Recombination | 2 | 15 | 11 | 3 | 4 | 0 | 98 |
| *paba* | +* | *ad* | in quadrivalent | 1 | 21 | 21 | 12 | 8 | 0 | |
| + | *y* | + | Recombination | 3 | 21 | 9 | 5 | 10 | 0 | 95 |
| *paba* | + | + | in bivalent | 1 | 17 | 18 | 4 | 7 | 0 | |
| Total | | | | 87 | 356 | 317 | 272 | 185 | 148 | 1365 |
| % *paba*$^+$ *pyro* progeny | | | | n.d. | n.d. | n.d. | 65·4 | 24·9 | 98·6 | — |
| Total, corrected† | | | | 16 | 356 | 151 | 94 | 185 | 2 | 804 |
| Quadrivalent recombinants, corr.† | | | | 1 | 36 | 21 | 14 | 12 | 0 | 84 |
| Frequency (%) | | | | — | 10·1 | 13·9 | 14·9 | 6·5 | — | 10·4 |
| Bivalent recombinants, corr.† | | | | 1 | 38 | 18 | 6 | 17 | 0 | 80 |
| Frequency (%) | | | | — | 10·7 | 11·9 | 6·4 | 9·2 | — | 10·0 |
| Total recombinants, corr.† | | | | 2 | 74 | 39 | 20 | 29 | 0 | 164 |
| Freq. (%) for paired segments‡ | | | | — | 30·9 | 39·7 | 35·2 | 22·2 | — | 30·8 |

See text for explanation, and Fig. 3 for a diagram of the cross UT 150 × UT 175. The other cross, UT 150 × UT 675, differs in the genetic labelling of the translocated segment.

* *y* in UT 150 × UT 675.

† All *paba*$^+$ progeny from the cleistothecia 1 and 3 excluded, and all *paba*$^+$ *pyro* progeny from 4 and 6, in order to correct for the presence of selfed asci in these cleistothecia.

‡ Twice the frequency of quadrivalent recombination, plus the frequency of bivalent recombination.

are two quadrivalents for every pair of bivalents, in other words $q = 2/3$. The assumptions underlying this conclusion will be given in the Discussion.

Table 3 also shows that the cleistothecia are quite heterogeneous for the extent to which their ascospores are of selfed origin. This can be seen from the excess of the parental over the chromosome recombinant class and, in the case of UT 150 × UT 675, more specifically from the frequency of the parental *paba*$^+$ *pyro* progeny, in which *pyro* is an unlinked marker (in the other cross *pyro* was not scored). This frequency should be 25 %, but is much higher in cleistothecia 4 and 6. It is a general rule that crosses heterozygous for the duplication suffer to a varying extent from selfing of the normal parent. This phenomenon, although also contributing to the excess of normal progeny, is quite different from the decreased viability of duplication progeny described in section (i).

The frequencies of quadrivalent and bivalent recombinants, between *pabaA* and *yA*, and corrected for selfing where necessary (cleistothecia 1, 3, 4 and 6), are also shown in Table 3. In this respect there is no significant heterogeneity amongst the cleistothecia ($P > 5\%$). The combined results then show a frequency

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of 10·4% for quadrivalent and 10·0% for bivalent recombination. A meaningful estimate of the total recombination frequency can be obtained if we realize again that in quadrivalents only one of the two normal segments will engage in pairing and recombination, which leads to a corrected quadrivalent recombination frequency of 20·8%. The total recombination frequency between *pabaA* and *yA* then becomes 20·8 + 10·0% = 30·8%. If there is a lack of pairing near the switch point in the quadrivalent, the recombination frequency is still higher. As the standard recombination frequency between *pabaA* and *yA* is only 15% (Clutterbuck, 1974), we conclude that the presence of the duplication raises the recombination frequency with at least a factor of two.

Table 4. *Analysis of* normal *progeny from the cross UT 150 × UT 175, heterozygous for the duplication: recombination between* yA *and* biA

| UT 150 × UT 175, progeny genotypes | | | Number of normal progeny | |
|---|---|---|---|---|
| *y* | *ad* | *bi* | | |
| + | + | + | 4 | 162 |
| + | + | *bi* | 158 | |
| *y* | + | *bi* | 24 | 296 |
| *y* | + | + | 272 | |
| + | *ad* | + | 6 | 71 |
| + | *ad* | *bi* | 65 | |

$$\text{Recombination frequency} = \frac{4 \times 3 + 24 \times \frac{3}{2} + 6 \times 3}{162 + 296 + 71} = 12{\cdot}5\%$$

See text for explanation, and Fig. 3 for a diagram of the cross. The normal progeny analysed in this table are the combined progeny from the cleistothecia 1, 2 and 3 of the cross UT 150 × UT 175, analysed in Table 3. These progeny were also scored for *bi*. Correction for selfing was obtained by excluding the $y^+$ $ad^+$ class from cleistothecia 1 and 3.

The increase in recombination is also found in the adjoining region *yA–biA*. The *bi* marker was scored in the cross UT 150 × UT 175, and the recombinant classes can be identified from Fig. 3. Table 4 presents the relevant data and the way in which the recombination frequency was calculated. This calculation is based upon the consideration that in the three equally frequent situations shown in Fig. 3, *y* $ad^+$ *bi* recombinants have two chances of arising (*a* and *c*), but $y^+$ *ad* $bi^+$ and $y^+$ $ad^+$ $bi^+$ recombinants only one (*a* and *c*, respectively). Also the $y^+$ $ad^+$ class is the only class possibly suffering from selfing. The calculation then leads to a recombination frequency of 12·5% which is a factor of 2 higher than the standard value of 6% (Clutterbuck, 1974).

### (iv) *Crosses homozygous for the duplication*

The aim of these crosses was to estimate the frequency of quadrivalent formation at meiosis in nuclei homozygous for the duplication. Homozygous crosses are difficult to analyse because in the progeny wildtype alleles located on one segment

of the duplication mask the expression of recessive markers on the other segment. A full analysis is therefore practically impossible. The application of a mathematical model (see Appendix) requires the knowledge of several parameters, and cannot serve our present aim of gaining insight into the actual value of one of them ($q$). Therefore we made a detour through a cross shown in Fig. 4, which is

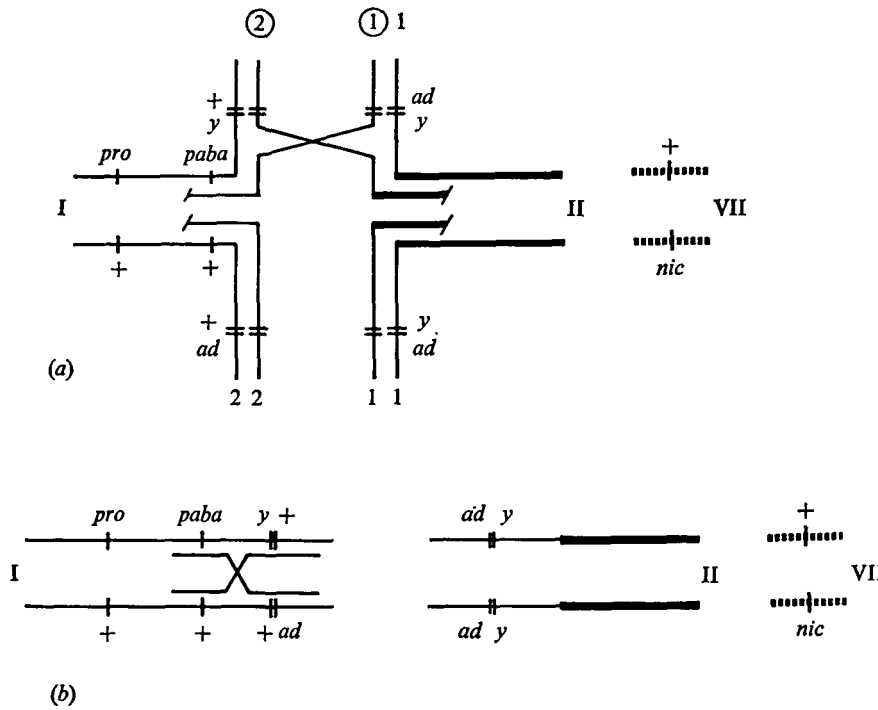


Fig. 4. Diagram of the cross UT 675 × UT 676, homozygous for the duplication. The figure shows two meiotic configurations of chromosomes I and II: a quadrivalent in (*a*) and two bivalents in (*b*) (random frequency 2/3 and 1/3 respectively). Part of (*a*) is drawn as a 4-chromatid stage in order to show the consequences of recombination between the duplicated segments of the quadrivalent: recombinant chromatid ① in combination with one out of *three* non-recombinant chromatids 1 yields *y ad* progeny (type 1). The $y^+$ $ad^+$ progeny (type 2) is derived from the reciprocal recombinant chromatid ② but only in combination with one out of *two* non-recombinant chromatids 2.

homozygous for the duplication and has been devised specifically to measure bivalent recombination. Quadrivalent recombinants can also be recognized but again require the model for further calculations. A comparison with Fig. 3 (the heterozygous cross) will show that in principle the bivalent recombination frequencies should be the same in the two crosses, if at least the bivalent frequencies are the same. The results of the homozygous cross UT 675 × UT 676 are shown in Table 5. The essential features of the table are the bivalent recombination frequencies for the six cleistothecia analysed. These are not significantly heterogeneous ($P$ appr. 10 %), so the results can be combined to a total frequency of 6·6 %. This frequency appears to be lower than the frequency of 10·0 % in the

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Table 5. *Analysis of progeny from the cross UT 675 × UT 676, homozygous for the duplication: recombination between* pabaA *and* yA

| Phenotypes | | | | UT 675 × UT 676 cleistothecium no. | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *paba* | *y* | *ad* | Origin | 1 | 2 | 3 | 4 | 5 | 6 | Total |
| *paba* | *y* | + | Parental | 244 | 99 | 88 | 120 | 119 | 121 | 791 |
| + | + | *ad* | | 231 | 56 | 59 | 65 | 70 | 71 | 552 |
| *paba* | *y* | *ad* | Recomb. in | 23 | 8 | 7 | 7 | 4 | 3 | 52 |
| + | *y* | *ad* | quadr., type 1 | 29 | 11 | 9 | 8 | 5 | 5 | 67 |
| *paba* | + | + | Recomb. in | 21 | 8 | 4 | 4 | 5 | 4 | 46 |
| + | + | + | quadr., type 2 | 13 | 6 | 10 | 6 | 8 | 10 | 53 |
| *paba* | + | *ad* | Recombination | 22 | 4 | 5 | 2 | 5 | 2 | 40 |
| + | *y* | + | in bivalent | 17 | 8 | 18 | 10 | 9 | 9 | 71 |
| Total | | | | 600 | 200 | 200 | 222 | 225 | 225 | 1672 |
| *nic* amongst *paba* (%) | | | | 47 | 62 | 57 | 53 | 48 | 52 | — |
| *nic*$^+$ amongst *paba*$^+$ (%) | | | | 50 | 47 | 44 | 55 | 46 | 46 | — |
| *ad* (%) | | | | 51 | 40 | 40 | 37 | 37 | 36 | — |
| Quadr. rec. freq., type 1 (%) | | | | 8·7 | 9·5 | 8·0 | 6·8 | 4·0 | 3·6 | — |
| Quadr. rec. freq., type 2 (%) | | | | 5·7 | 7·0 | 7·0 | 4·5 | 5·8 | 6·2 | 5·9 |
| Bivalent recomb. freq. (%) | | | | 6·5 | 6·0 | 11·5 | 5·4 | 6·2 | 4·9 | 6·6 |

See text for explanation, and Fig. 3 for a diagram of the cross. The diagram also shows the two different types (type 1 and type 2) of recombinants that can arise in the cross as a result of crossing-over, in the quadrivalents, between the normal and the translocated segments.

heterozygous cross (Table 3; $\chi^2_1 = 8{\cdot}36$; $P < 1\%$). All other things being equal it follows that also the frequency of bivalents is lower, and the frequency of quadrivalents higher in the homozygous cross. If in the heterozygous cross $q = 0{\cdot}67$, then $q = 0{\cdot}78$ in the homozygous cross (but see Discussion).

Of the other features of the homozygous cross we mention first the absence of any indication for selfing (see the frequency of the unlinked marker *nicB8* in the *paba* and *paba*$^+$ progeny). This is quite different from the heterozygous cross in which selfing (of the normal parent) often occurs (see section iii). Secondly, the cleistothecia are heterogeneous for the frequency of *paba*$^+$ *y*$^+$ *ad* progeny which we believe to result from a variation in the strength of selection against *ad* ascospores (see Table 5). This heterogeneity does not affect the bivalent recombination frequencies, but it does affect the frequencies of the *y ad* quadrivalent recombinants (type 1, Fig. 4*a*), tending to decrease these frequencies in several cleistothecia. This may explain why the observed frequencies (see Table 5, type 1) fall short of the frequency of 11·0% observed for *y* segregants of UT 175 (see section i), though these segregants arise in essentially the same way. A second type of quadrivalent recombinant, the *y*$^+$ *ad*$^+$ class (type 2, Fig. 4*a*), is, as might be expected from the absence of *ad*, about equally frequent in the different cleistothecia. The combined result (5·9%, see Table 5) is also lower than 11·0% but Fig. 4(*a*) shows that type 2 is less likely to occur than type 1. Type 2 can only arise in combination with 2 instead of 3 of the non-recombinant chromatids (if multiple exchanges are neglected), hence the comparable frequency is $3/2 \times 5{\cdot}9\% = 8{\cdot}9\%$ approximately. This frequency is well within the range mentioned in section (i) for the segregation frequencies of *y* in UT 175.

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(v) *Application of the mathematical model to meiotic segregation in UT 175*

The mathematical model outlined in the Appendix can now be applied to the meiotic segregation frequency of *y* in the duplication strain UT 175 (11·0%, see section i). If $q = 0{\cdot}78$ and $k = 1$ we arrive at the following values for the recombination frequency between the translocation breakpoint and *yA*, in the quadrivalents: 17·7, 23·4 and 30·8% for $\rho = -1$, 0 and $+1$ respectively (Fig. 2 of Appendix; $s_q = 0{\cdot}141$). It is difficult to compare these frequencies with a standard value for the recombination frequency between the translocation breakpoint and *yA*, because the exact location of the breakpoint is unknown (the figure of 9 to 10%, given by Nga & Roper, 1968, is unjustified). Published experiments do not even exclude rigorously a location proximal to the *paba A* locus, though there is no real doubt about this. Our own experiments (data not shown) locate the breakpoint between *paba A* and *yA*, proximally to *uvsA* so between 4 and 15% from *yA*. As this range does not overlap the above mentioned range for the calculated recombination frequencies, we conclude that the frequency of recombination within the duplication is definitely increased, as was also found in the heterozygous cross (section iii). Other systems indicate positive values for $\rho$ (see Appendix), so the increase might well be by a factor of 2, as in the heterozygous cross. In that case the standard recombination frequency between the translocation breakpoint and *yA* is 12–15%.

(vi) *Recombination outside the duplication*

In the previous sections we found an increased recombination for two regions located inside the duplication. In this section we ask if these increases also occur outside the duplication. Therefore we measured the recombination frequencies with markers on the chromosomes I and III, in normal crosses, and in crosses heterozygous or homozygous for the duplication. It is clear from Table 6 that the duplication tends to decrease recombination frequencies elsewhere, so the increase inside the duplication is even more significant.

The last line of Table 6 shows free recombination between the unlinked markers *cnxH4* and *riboA1*. Therefore, selfing cannot account for the decreases in recombination. As we could find no significant differences between the heterozygous and the homozygous crosses (except for *proA–pabaA*), the combined results of these crosses were compared with the result of the cross without the duplication. The ratio of the two results is shown in the table, and appears to vary from region to region. The most significant decreases were found in *anA–proA* and *proA–pabaA*, regions adjoining the duplication. We ascribe these effects to pairing difficulties. Elsewhere the decreases are smaller, and not significant in several instances. We have no ready explanation for them. The significant decrease in the homozygous cross compared to the heterozygous cross, in the case of *proA–pabaA*, can be ascribed to the pairing difficulties being greater in homozygous quadrivalents than in heterozygous ones.

Table 6. *Recombination frequencies (%) outside the duplication region in crosses with and without the duplication*

| Region and chromosome | Cross homozygous for duplication* | Cross heterozygous for duplication† | Total of crosses containing duplication | Cross lacking duplication‡ | Ratio with and without duplication | Significance (*P*)§ (%) |
|---|---|---|---|---|---|---|
| *riboA–anA* (I) | 16·9 | 11·8 | 13·9 | 16·6 | 0·84 | 20 |
| *anA–proA* (I) | 25·1 | 27·6 | 26·6 | 40·2 | 0·66 | < 1 |
| *proA–pabaA* (I) | 5·8 | 8·3 | 7·3 | 12·4 | 0·59 | < 1 |
| *proA–pabaA* | 2·9‖ | 6·7¶ | — | — | — | 2–5** |
| *phenA–galA* (III) | 11·1 | 10·5 | 10·7 | 14·5 | 0·74 | 5 |
| *galA–dilA* (III) | 28·8 | 28·2 | 28·4 | 32·5 | 0·87 | 10–20 |
| *dilA–sC* (III) | 30·5 | 35·6 | 33·6 | 43·5 | 0·77 | < 1 |
| *sC–cnxH* (III) | 20·2 | 17·7 | 18·7 | 20·7 | 0·90 | 30–50 |
| *cnxH–riboA* (III; I) | 57·6 | 52·5 | 54·5 | 53·2 | 1·02 | 50–70 |

For explanation, see text.

* UT 313 × UT 314, total 243 progeny.

† UT 252 × UT 312, total 362 progeny.

‡ UT 253 × UT 312, total 579 progeny.

§ Crosses containing the duplication compared with cross lacking the duplication.

‖ Data from UT 150 × UT 175, total 276 progeny.

¶ Data from UT 675 × UT 676, total 800 progeny.

** Cross homozygous for the duplication compared with cross heterozygous for the duplication.

## 4. DISCUSSION

In this paper we show that an *A. nidulans* duplication which is heterozygous for three recessive markers, segregates these markers during meiosis. This segregation results from meiotic recombination (crossing-over), followed by the appropriate disjunction and distribution of the chromosomes. Of the arguments presented the most specific one comes from ascus analyses, which suggest that each meiotic segregant is accompanied by its reciprocal type in the same ascus. Meiotic segregation, therefore, is clearly different from mitotic segregation, which proceeds through deletion of the dominant allele (Nga & Roper, 1968). These processes are not mutually exclusive: recombination is very infrequent during mitosis, so that minor pathways of segregation like deletion, can become prevalent.

A particular recessive marker, *yA2*, located in the duplication, has been studied in greater detail. The meiotic segregation frequency of *y* is 11·0 % on the average. The cleistothecia (containing the ascospores) appeared to be heterogeneous for this frequency: a range from 8·0 to 14·9 % was noted amongst the analysed cleistothecia. We suggest that age and location of the cleistothecia may influence the recombination proficiency. Varying degrees of selfing cannot account for the variation, as all the analysed cleistothecia are selfed ones taken from colonies of the duplication strain. To our knowledge this is the first reported case of recombination in selfed cleistothecia of *A. nidulans*. This trait, of course, makes

the system highly suitable for the isolation of *rec* mutations: no crossing-technique is needed, and the system becomes automatically homozygous for induced mutations, which is essential for the expression of the recessive ones. The application of the system for the isolation of mutants deficient in meiotic segregation will be the subject of a separate paper.

It is by no means easy to estimate the recombination frequency from the segregation frequency. The duplication strain considered here is a strain in which a terminal segment of chromosome I is present twice: one copy being in its normal position, and the other copy translocated to a tip of chromosome II. Following selfing these chromosomes form either two bivalents or a quadrivalent during meiosis. Only quadrivalents can yield the segregants, and the frequency of quadrivalents has therefore to be determined. Further, one has to consider that each quadrivalent contains two regions ('tetrads') where crossing-over leading to segregation can occur (see Fig. 1 of the Appendix). Within and between these regions genetic interference may take place. Once the recombinant chromatids have been formed, only particular disjunctions and distributions will lead to segregation. The mathematical model described in the Appendix is based on classical formulae for meiotic tetrad types, modified for two types of genetic interference. Disjunction and distribution are supposed to be random but regular. In this respect it is important that the duplication (in particular the translocated segment) is not centromere-linked. Any preferred disjunction of the centromeres in the quadrivalent, will therefore not be followed by the duplication segments.

In order to be able to use the model we had to fix several parameters. One of these, the quadrivalent frequency $q$, was studied by means of genetic crosses, as a cytological approach did not seem to be reliable. In crosses heterozygous for the duplication (section iii) some recombinant classes could mainly arise from bivalents, other classes only from quadrivalents. From their numerical relationship we deduced $q = 0{\cdot}67$, which indicates random pairing of the three interacting duplication segments. The main problem is the interpretation of the classes supposed to have arisen from bivalents. Some of the recombinants between *paba A* and *yA* might have been formed in quadrivalents, but proximally to the switch-point (see Fig. 3). In fact our interpretation requires the translocation breakpoint to be located immediately distally to the *pabaA* locus, and the switch-point should be coincident with the breakpoint. Moreover, the quadrivalent should be fully paired distally to the switch-point. Finally, the recombination proficiencies of quadrivalents and bivalents should be the same. If these requirements are not met, this will lead to an underestimation of $q$, except in the case that the recombination proficiency of bivalents would be greater than that of quadrivalents. In section (vi) we already got an indication for pairing difficulties proximal to the translocation breakpoint, so the prospects look rather grim. However, we used the calculated value of $q$ only for the estimation of the recombination frequency in the region between the translocation breakpoint and the *yA* locus, i.e. for approximately the same region for which $q$ was calculated, and for this purpose the calculated value of $q$ remains fully applicable.

The value of $q$ in a cross homozygous for the duplication (section iv) was obtained by comparing bivalent recombination frequencies in this type of cross with those in the heterozygous cross, because a direct determination is impracticable. Except for some interpretation problems the bivalent recombination frequencies are reliable because the bivalents are probably fully paired in both types of cross. The most debatable point seems to us the recombination proficiency in the two crosses, as the duplication appears to increase recombination frequencies, and it is quite conceivable that this increase would be dose-dependent. The present data indicate a slightly higher quadrivalent frequency in the homozygous cross than in the heterozygous one ($q$ = 0·78 against 0·67). It may be that the heterozygous quadrivalent is less stable owing to the unpaired segment. However it may be, the conclusion that in both crosses most (but certainly not all) meiotic configurations of the chromosomes I and II are quadrivalents, is inescapable.

In the heterozygous cross the total recombination frequency (for paired segments) between *pabaA* and *yA* can be calculated from the observed genotypes of normal progeny (section iii), though additional considerations about pairing difficulties in the quadrivalents lead us to believe that the calculated value is in fact an underestimation. Other considerations, about the location of the translocation breakpoint, are not important as in the calculation the bivalent and quadrivalent recombinants are added together. We concluded that there was an increase in recombination frequency within the duplication by at least a factor of two. This supports observations of Pritchard & Siddiqi (unpubl., quoted in Pritchard, 1960) with the same system. In the selfed cleistothecia of the duplication strain (which constitute a homozygous cross) we had to use the mathematical model outlined in the Appendix. In spite of many uncertainties here too the conclusion is inevitable that the recombination frequency within the duplication is increased. The increase may well be of the same magnitude in both crosses but this is not sure. The increase within the duplication is contrasted with a decrease elsewhere, especially in the region adjacent to the duplication. So we conclude that the duplication is a genetic element increasing recombination (meiotic crossing-over) within its own boundaries. This increase has been noted before in this system and in similar ones (see below), and the literature already contains some answers to the question why this increase may occur.

Pritchard (1960) put forward the *effective pairing* hypothesis which elegantly explained the increase from a limited probability of effective contact between paired homologous chromosomes. If recombination would only occur in regions of effective contact, this would result in an enhancement of recombination as three duplication segments instead of two would generate more such regions. The hypothesis was developed to explain localized negative interference, but since 1960 better explanations have been found for this phenomenon. Therefore the hypothesis has been neglected in recent years, but it still retains its value for the present work. It predicts an enhancement of recombination in all kinds of systems containing extra copies of chromosomes or chromosome segments, like triploids,

aneuploids and tandem duplications. This has indeed been found in yeast (Duck & James, 1976) and in *Drosophila* (data up to 1933 critically reviewed by Rhoades, 1933; Beadle, 1934; Green, 1962).

However, in *Drosophila* marked exceptions have been found. Although short tandem duplications do show the increase (Green, 1962), those of medium length show no or very little change in recombination (Sturtevant, 1925; Kalisch, 1975) and long tandem duplications display decreased recombination frequencies (Roberts, 1966; Kalisch, 1975). In order to explain this length dependence, Kalisch (1975) has proposed that *pairing competition* between the duplication segments provokes the enhancement of recombination whereas this effect is opposed and eventually turned into a decrease by pairing disturbances which become more pronounced as the duplications are longer. The length dependence may be a specific feature of tandem duplications and may not apply to our duplication which probably suffers little from pairing difficulties because it is based on a translocation. As a general explanation, however, the concept of competitive pairing is unsatisfactory as no arguments are known why competitive pairing should increase recombination.

Another type of explanation is derived from recombination theory. Holliday (1964) has proposed that recombination starts from specific points later on called *recombinators* (Holliday, 1968). The properties of some mutants affecting recombination can be explained on the basis of this idea (e.g. Angel *et al.* 1970). It may be hypothesized that in our duplication system the new joint generated by the translocation acts as a potent recombinator, or that the translocated segment has come under the action of such an element located on chromosome II. Though recombination may be affected to some extent by such a factor, it fails to explain the systematic increase, observed in various systems. Moreover, it cannot explain the increase in systems lacking such a new joint, like aneuploids and triploids.

In our view the best way to explain the observed increase in recombination is by relating it to the phenomenon of *mitotic non-conformity* shown by the duplication present in our strain (see Introduction), as well as by other *Aspergillus* duplications (Bainbridge & Roper, 1966; Burr, 1973). The genetic instability underlying this phenomenon is caused by repairable lesions in the DNA, as shown by the increased instability caused by mutations and chemical substances interfering with repair (Burr, 1973; Roper, Palmer & Watmough, 1972). It could well be that the same lesions are also recombinagenic. It would therefore be worth while to study to what extent recombination and deletion are correlated with each other during mitosis and meiosis. To explain mitotic non-conformity Nga & Roper (1969) have proposed the existence of a limited number of membrane sites where the replication of the DNA of specific chromosome segments can start. In the duplicated segments a competition for these sites is thought to take place, which would provoke the lesions responsible for mitotic non-conformity. Similar lesions could be responsible for the enhancement of meiotic recombination dealt with in this paper.

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## APPENDIX

### The relationship between the recombination frequency and segregation of a recessive marker in a translocation duplication: an approach involving tetrad type frequencies

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A translocation duplication can be defined as a strain in which a terminal chromosome segment is present twice, one copy being in its normal position, and one copy translocated to one of the tips of a different chromosome. The duplication can be made heterozygous for a recessive marker which will not be expressed unless the dominant allele has been replaced by recombination, or removed by deletion, which permits segregation of the marker. In this Appendix we will confine ourselves to recombination (meiotic crossing-over) and how it can lead to homozygosity, and thus to segregation of the recessive marker. The only way in which recombination can do so, is an exchange event between the proximal end of the duplication (the *translocation breakpoint*) and the marker, followed by an appropriate distribution of the chromatids to the meiotic poles (see Fig. 1). Our problem is how to estimate the recombination frequency $r$ in the interval, from an observed segregation frequency $s$ amongst the haploid products of meiosis. We shall assume that the diploid nucleus undergoing meiosis is homozygous for the duplication, and contains the recessive marker on the non-translocated member of the duplicated segments, in both haploid complements. These assumptions arise

from our experiments with a duplication strain of the haploid ascomycete *Aspergillus nidulans* (see paper). In this duplication strain the recessive spore colour marker *yA2* is present in the non-translocated segment, whereas the translocated segment contains the wildtype allele. Upon selfing ascus primordia are formed which contain diploid nuclei homozygous for the duplication. The nuclei undergo meiosis and yield haploid ascospores which, following growth, can be scored for segregants showing the recessive phenotype. Other experimental situations, like crosses heterozygous for a duplication, or crosses containing the recessive marker in only one haploid complement, ask for different assumptions. However, these situations are mostly simpler versions of the same problem.

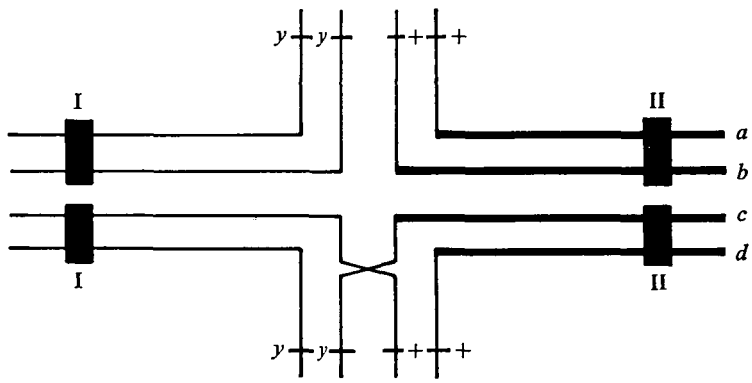


Fig. 1. The duplication during meiosis, in a quadrivalent configuration.

Fig. 1 shows the duplication at pachytene, fully paired in a quadrivalent configuration, with a chiasma in the lower half. The two different chromosomes are designated I and II, and the duplication arose by a non-reciprocal I → II translocation. A *y/y* homozygote will be formed if the recombinant chromatid IIc meets a non-recombinant I chromatid in the same meiotic product. This cannot occur if the homologous chromosomes pair in such a way that two bivalents are formed. Therefore, if $q$ is the frequency of quadrivalents formed by the two chromosomes in meiosis, and $s_q$ the segregation frequency amongst the quadrivalent products, then:

$$s = q.s_q. \tag{1}$$

If the duplication segments pair at random, then $q = 2/3$.

As far as the duplication is concerned, the quadrivalent can be considered to consist of two tetrads, shown in vertical position in the upper and lower halfs of Fig. 1. These tetrads can be of the well-known types: parental ($P$), tetratype ($T$) or non-parental ($N$). In the figure the upper tetrad is $P$, and the lower one $T$. It is essential to know the frequencies of these types and their combinations in the quadrivalents because $s_q$ can be computed from this. Papazian (1952) has already derived the probabilities of $P$, $T$ and $N$ (Prob ($P$), Prob ($T$) and Prob ($N$), respectively) as functions of the map distance $x$ ($= \frac{1}{2}m$ in Papazian's paper). His

functions, however, do not account for genetic interference. In order to better this situation we must exploit a mapping function that accounts for interference and that can also be used to predict the probability $p_n$ of $n$ crossing-over events in the interval under study. This probability distribution must be known in order to derive Prob$(P)$, Prob$(T)$ and Prob$(N)$ (see Fincham & Day, 1971, p. 101, and below). A suitable mapping function has been published by Barratt *et al.* (1954). This function introduces an interference constant $k$, which is $< 1$ for positive interference.

As mentioned in the main paper, we have chosen $k = 1$ (no interference) for our case with *A. nidulans*. Strictly speaking therefore, we could have omitted the treatment of $k$-type interference here. However, the simplicity thus gained would have detracted from the general applicability of the model, as it is known that most other organisms show reasonably strong positive interference (e.g. $k = 0{\cdot}2$ for *Drosophila* and *Neurospora*). Moreover, the duplication may exert its influence on the value of $k$, so it seems wise to at least inquire into the impact of $k$ on predicted frequencies.

In short, the function is based on a Poisson distribution, modified in order to account for positive interference by decreasing the probabilities of multiple events. This has been achieved through multiplication by $k^{n-1}$ for $n > 0$, and correction to bring the total of all probabilities back to 1. The probability $p_o$ is assumed not to change by interference. Then

$$p_o = e^{-2x} \quad \text{and} \quad p_n = \frac{(2xk)^n}{n!} \cdot \frac{1 - e^{-2x}}{e^{2xk} - 1}, \quad n > 0,$$

where $x$ is a dummy variable. For computation we use

$$p_n = \frac{(-k \cdot \ln p_o)^n}{n!} \cdot \frac{(1 - p_o)}{e^{-k \cdot \ln p_o} - 1}. \tag{2}$$

If, for exactly $n$ exchanges,

$$\text{Prob}\,(P/n) = \text{Prob}\,(N/n) = 1/6\{1 + 2(-\tfrac{1}{2})^n\}, \quad n > 0, \tag{3}$$

$$\text{Prob}\,(T/n) = 2/3\{1 - (-\tfrac{1}{2})^n\}, \quad n > 0, \tag{4}$$

$$\text{Prob}\,(P/o) = 1, \tag{5}$$

$$\text{Prob}\,(T/o) = \text{Prob}\,(N/o) = 0, \tag{6}$$

then

$$\text{Prob}\,(P) = p_o + \sum_{n=1}^{\infty} p_n \cdot \text{Prob}\,(P/n) = 1 - \frac{(1 - e^{-2x})(5 - 3 \cdot e^{-2xk} - 2 \cdot e^{-3xk})}{6(1 - e^{-2xk})},$$

$$\text{Prob}\,(T) = \sum_{n=1}^{\infty} p_n \cdot \text{Prob}\,(T/n) = \frac{2(1 - e^{-2x})(1 - e^{-3xk})}{3(1 - e^{-2xk})}$$

and

$$\text{Prob}\,(N) = \sum_{n=1}^{\infty} p^n \cdot \text{Prob}\,(N/n) = \frac{(1 - e^{-2x})(1 - 3 \cdot e^{-2xk} + 2 \cdot e^{-3xk})}{6(1 - e^{-2xk})}$$

which formulae may be used in the case of the simpler versions mentioned above.



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The next step is to derive the frequencies of the combinations of $P$, $T$ and $N$ for the two tetrads in the quadrivalent. If no further interference occurs, these frequencies can be simply obtained through multiplication, e.g. Prob $(PT)$ = Prob $(TP)$ = Prob $(P)$. Prob $(T)$. However, we must be suspicious of a negative between-tetrad-interference, examples of this having been found in rye (Sybenga, 1970; Sybenga *et al.* 1973). Therefore we wish to introduce here a constant $\rho$, measuring to some extent the degree of correlation between the numbers $i$ and $j$ of crossing-over events in the two tetrads. In the case of negative interference we would expect those situations to be more frequent in which *both* or *none* of the tetrads show crossing-over. Then the following approximations will hold, if $p_o$ is not small, for the probabilities of a combination of exchanges in the two tetrads:

$$p_{oo} = p_o^2(1+\alpha), \tag{7}$$

$$p_{oj} = p_o . p_j(1-\rho), \tag{8}$$

$$p_{io} = p_i . p_o(1-\rho) \tag{9}$$

and

$$p_{ij} = p_i . p_j(1+\beta) \quad (\text{for } i \text{ and } j > 0). \tag{10}$$

If we restrict the correlation to these four general classes (and thus neglect the influence on interference of the actual number of crossing-over events) the probabilities of events in the two tetrads can be given as in Table 1. From this table it can be deduced that

$$\beta = \rho \frac{p_o}{1-p_o}. \tag{11}$$

The correlation coefficient in the table is

$$\rho = \alpha \frac{p_o}{1-p_o}.$$

Therefore

$$\alpha = \rho \frac{1-p_o}{p_o}. \tag{12}$$

We can now compute $p_{ij}$, Prob $(P/i)$, Prob $(T/i)$, Prob $(N/i)$, Prob $(P/j)$, Prob $(T/j)$ and Prob $(N/j)$ from $p_o$ for any $i$ and $j$ (including 0) through the equations (2)–(12) if we fix $k$ and $\rho$.

The next step is to compute the probabilities of combinations of tetrad types for the two tetrads in the quadrivalent. Having corrected for chiasma interference, we assume that for any set of $i$ and $j$ the frequencies of these combinations can be found by simple multiplication, e.g.

$$\text{Prob}\,(PT/i,j) = \text{Prob}\,(P/i).\text{Prob}\,(T/j).$$

The probability Prob $(PT)$ of the combination $PT$ amongst all quadrivalents can be obtained through summation for all possible sets of $i$ and $j$ (in practice limited to a symmetric block with a total probability exceeding an arbitrary value

just below unity). The probabilities of the other combinations can be obtained in the same way.

There are nine possible combinations, which all lead to predictable segregation frequencies to homozygosity of the marker *y* (see Table 2). The segregation frequencies listed in the table are based on the assumption of random disjunction and distribution during meiosis. In particular it is assumed that the segments of

Table 1. *Probabilities of combinations of crossing-over events in the two tetrads of the quadrivalent*

| Number of crossing-over events in the other tetrad | Number of crossing-over events in one tetrad | | |
|---|---|---|---|
| | 0 | > 0 | total |
| 0 | $p_o^2(1+\alpha)$ | $p_o(1-p_o)(1-\rho)$ | $p_o$ |
| > 0 | $(1-p_o)p_o(1-\rho)$ | $(1-p_o)^2(1+\beta)$ | $1-p_o$ |
| Total | $p_o$ | $1-p_o$ | 1 |

Table 2. *Segregation frequencies for tetrad type combinations in the quadrivalent*

| Tetrad type combination | Segregation frequencies* |
|---|---|
| *PP, NN* | 0 |
| *PT, TP, TN, NT* | 3/16 |
| *PN, NP, TT* | 1/4 |

* The probabilities of the marker *yA2* becoming homozygous amongst the meiotic progeny from quadrivalents with the indicated combination of tetrad types.

Table 3. *Segregation frequencies for different values of* k

| $k$ | $s_q$ ($r = 0{\cdot}05; \rho = -1$) | $s_q$ ($r = 0{\cdot}05; \rho = 1$) | $s_q$ ($r = 0{\cdot}35; \rho = -1$) | $s_q$ ($r = 0{\cdot}35; \rho = 1$) |
|---|---|---|---|---|
| 0·2 | 0·0474 | 0·0249 | 0·2225 | 0·1700 |
| 0·4 | 0·0474 | 0·0249 | 0·2184 | 0·1659 |
| 0·6 | 0·0473 | 0·0248 | 0·2150 | 0·1625 |
| 0·8 | 0·0472 | 0·0247 | 0·2121 | 0·1596 |
| 1·0 | 0·0471 | 0·0246 | 0·2095 | 0·1570 |

the duplication do not follow any alternate:adjacent I = 2:1 segregation of the centromeres in the quadrivalent. Such a 2:1 segregation might occur if the duplication is linked to the centromeres of both chromosomes (see review by Sybenga, 1975). $s_q$ can now be computed from the contributions of individual combinations in the following way

$$\begin{aligned} s_q &= 3/16.\{\text{Prob}(PT)+\text{Prob}(TP)+\text{Prob}(TN)+\text{Prob}(NT)\} \\ &\quad + 1/4.\{\text{Prob}(PN)+\text{Prob}(NP)+\text{Prob}(TT)\} \\ &= 3/8.\{\text{Prob}(PT)+\text{Prob}(TN)\}+1/4\{2.\text{Prob}(PN)+\text{Prob}(TT)\}. \end{aligned}$$

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Moreover $r$ can be computed from $p_o$ as follows:

$$r = \tfrac{1}{2}(1-p_o)$$

(assuming absence of chromatid interference; Emerson & Rhoades, 1933). Then, for any value of $p_o$, $r$ and $s_q$ can be computed. The relationship between $r$ and $sq$ is given in Fig. 2, on the basis of calculations for 11 values of $p_o$, for $k = 1$ and

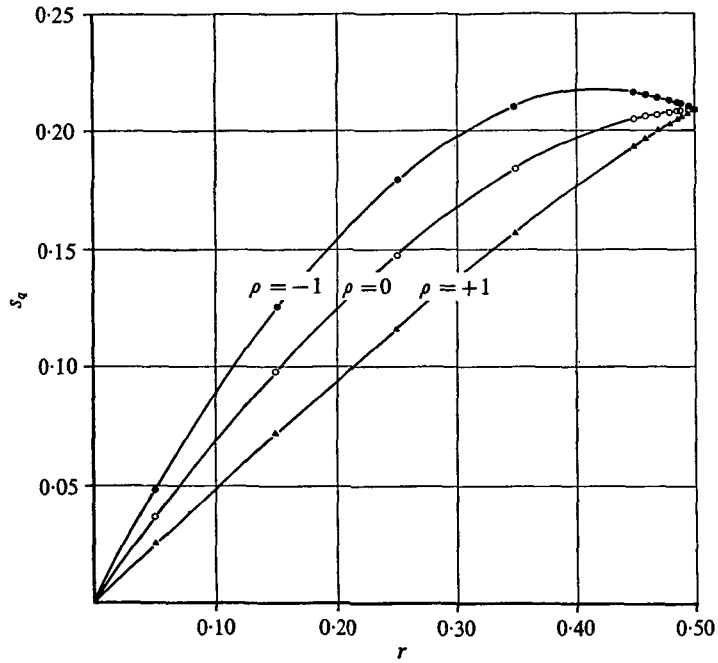


Fig. 2. The segregation frequency $s_q$ amongst progeny from meiotic quadrivalents, as a function of the recombination frequency $r$. $k = 1$ for within-tetrad-interference, $\rho = -1$, 0 or 1 for between-tetrad-interference.

three different values of $\rho$. It can be seen that $\rho$ has a big impact, and that $s_q$ is sometimes maximal for values of $r$ slightly below 0·50. The latter property might be an artefact of the model which is not designed for small values of $p_o$. We also exploited different values of $k$. It then appears that the influence of $k$ is small (Table 3).

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