THE INSTABILITY OF NEUROSPORA DUPLICATION *Dp(IL→IR)H4250*, AND ITS GENETIC CONTROL[1]

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ABSTRACT

Previous work (NEWMEYER and TAYLOR 1967) showed that a nontandem duplication, *Dp(IL→IR)H4250*, is regularly produced by recombination in crosses heterozygous for the effectively terminal pericentric inversion *In(IL→IR)H4250*. The duplications initially have strongly inhibited growth because they are heterozygous for mating type, which behaves like a vegetative-incompatibility (*het*) locus. Such cultures "escape" from the inhibition as a result of events that eliminate the mating-type heterozygosity. The product of a given escape event may be barren or fertile. (Neurospora duplications are characteristically barren; that is, when crossed, they make many perithecia but few ascospores.)—The present paper reports on a genetic analysis of the instability of *Dp(IL→IR)H4250*. Most of the barren escape products behave as if due either to mitotic crossovers, which make mating type and distal markers homozygous, or to very long deletions which uncover mating type and all distal markers; presumably the latter would retain enough duplicated material to render them barren. It is difficult to distinguish between these two possibilities, but homozygosis seems more probable and has been clearly demonstrated in one case. Only a few barren escapes could be due to short deletions or to changes at the mating-type locus.—The fertile escape products appear to be euploid. Most of these behave as if they arose by precise deletion of one or the other duplicated segment, thus restoring one of the parental sequences. A large majority of the precise deletions restore normal sequence; only a few restore inversion sequence. Preferential restoration of the normal sequence has also been found by other workers for Neurospora duplications from several other rearrangements. A hypothesis is presented to explain these findings; it is posulated that the precise deletions result from mitotic crossing over in homologous material located at chromosome tips and tip-breakpoints.—There is a smaller group of fertile escapes that are unlike either parental sequence; at least one of these involves a chromosome break outside the duplicated region.—Duplications in which the vegetative incompatibility is suppressed by the unlinked modifier *tol* are extremely barren; they only rarely lose a duplicated segment so as to become fertile.—The instability of *Dp(IL→IR)H4250*, with and without *tol*, is markedly altered by factors in the genetic background. The two factors studied in detail have qualitatively different effects.

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A pericentric inversion in *Neurospora crassa*, called *In(IL→IR)H4250*, produces duplication progeny by meiotic recombination when it is crossed with a strain having the normal sequence (NEWMEYER and TAYLOR 1967). One inver-

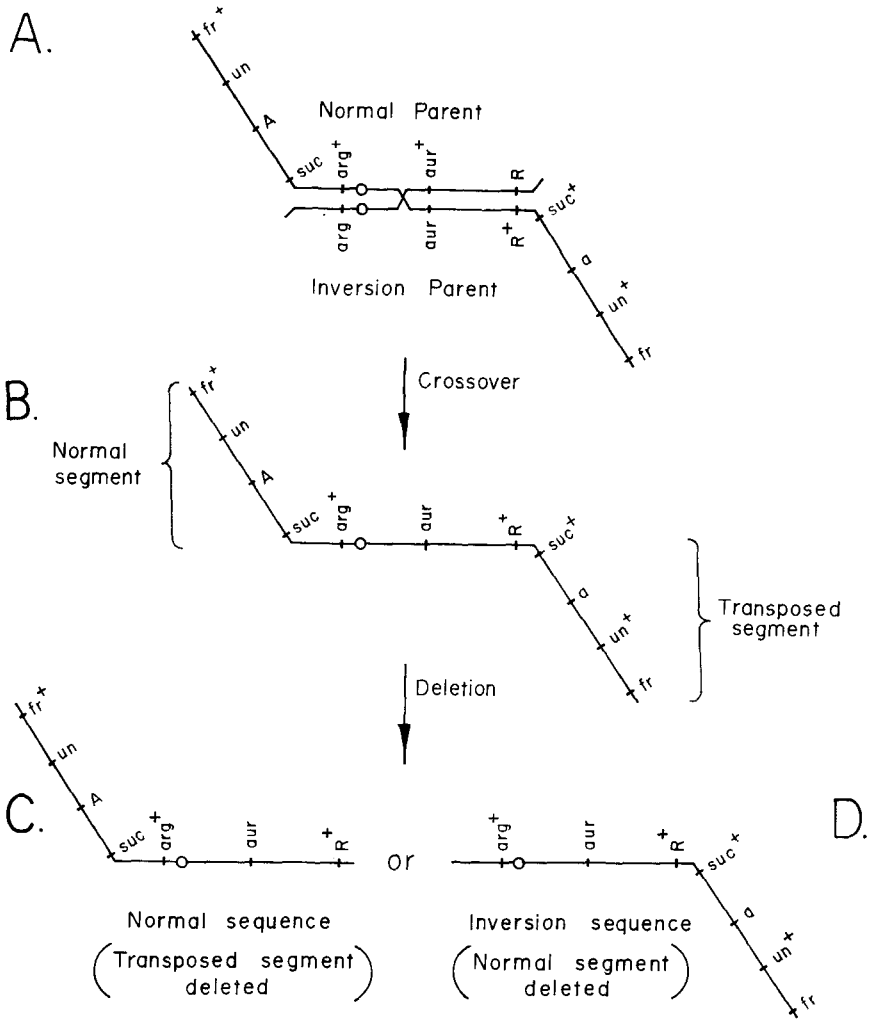


FIGURE 1.—A. Linkage group I at meiotic prophase in a cross of Normal sequence × *In(IL→IR)H4250*. For simplicity, in this and most other figures the diagrams are drawn with only the inverted region synapsed, and only two of the four strands are shown. Angles indicate the inversion break points. Distances are not to scale. There is no evidence that the inversion is subterminal rather than terminal; it is drawn as subterminal solely because evidence from higher organisms indicates that broken ends do not attach to unbroken ends.

B. The viable duplication produced by the meiotic crossover shown in A. The duplicated region includes most of the left arm of linkage group I. (The reciprocal crossover product is deficient for most of IL and therefore lethal.)

C. and D. Normal and inversion sequence derivatives produced by complete deletion of one or the other of the duplicate segments.

sion break point is essentially terminal, so that when a crossover occurs in the inversion loop, one of the resulting duplication-deficiency products lacks no essential genes and is therefore viable (Figure 1). This product is called *Dp(IL→IR)H4250*. Such duplications are usually unstable. The present paper concerns the nature of the instability and its genetic control.

Since the initial report, many other Neurospora rearrangements have been found to produce unstable nontandem duplications (PERKINS 1972, 1974; TURNER 1975; PERKINS and BARRY 1977). Similar unstable duplications have been studied extensively in Aspergillus (see ROPER 1973 for review).

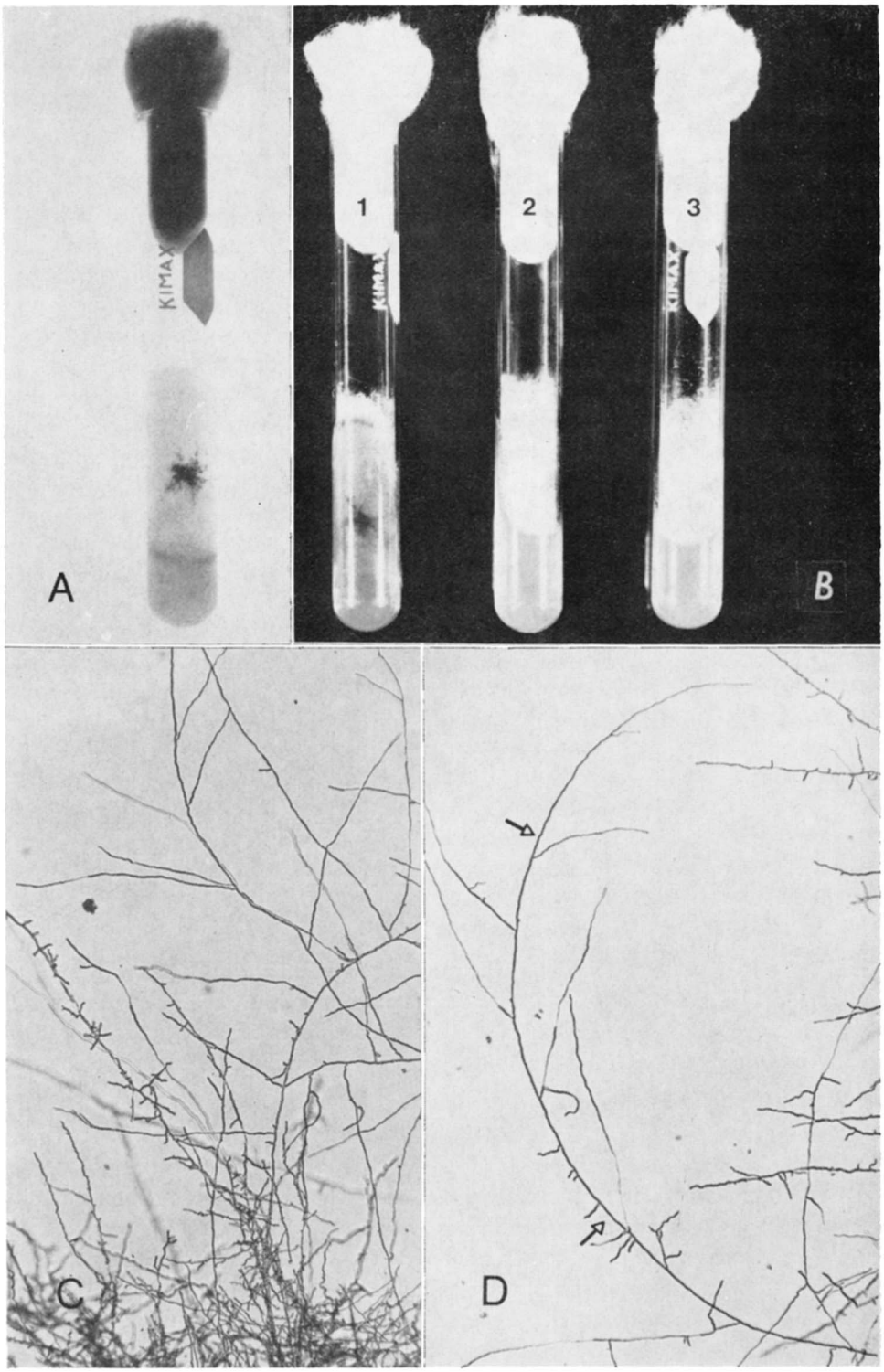
In Neurospora, duplications from different rearrangements differ markedly both in the degree and the type of their instability. In many cases, one of the duplicate segments is deleted precisely so as to restore the euploid condition. It should be possible to delete either duplicate segment so as to restore either normal or rearrangement sequence (see Figure 1). Actually, only one case has been found in which the two events are equally frequent (see accompanying paper by TURNER). In all other cases studied, including the one described here, the normal sequence predominates. These findings have been reported so far only in abstract (PERKINS, NEWMEYER, and TURNER 1972).

Apart from its particular pattern of instability, the results with *Dp(IL→IR)-H4250* have been particularly interesting for two reasons. One is that factors in the genetic background have been found to alter the instability in quite specific ways, thus permitting the study of the genetic control of somatic events. The other is that most of the duplications are heterozygous for mating type (*A/a*), which acts as a vegetative incompatibility (*het*) locus. The incompatibility causes inhibited growth, so that there is strong selection for the products of somatic events that eliminate the inhibition. This permits detection of any events that make mating type homo- or hemizygous, even if such events do not remove one of the two duplicate segments. The growth inhibition is virtually eliminated by an unlinked suppressor of the vegetative incompatibility (NEWMEYER 1970). Therefore, in a cross heterozygous for the suppressor, instability can be studied both with and without selection against *A/a* heterozygosity, in largely the same genetic background.

The present paper reports on the genetic analysis of the instability in duplications from *In(IL→IR)H4250*, and the ways in which the instability is affected by the genetic background. A hypothesis will be offered in the DISCUSSION to explain the precise deletions, and why they usually regenerate the normal sequence.

ESSENTIAL BACKGROUND INFORMATION

DA's: Duplication strains that are heterozygous for mating type (*A/a*), and in which vegetative incompatibility of the mating-type alleles is not suppressed, initially have a drastically inhibited growth rate with abnormal morphology, and do not form conidia (vegetative spores); on complete medium they produce dark brown pigment. Such strains are called Dark Agars, abbreviated DA's (Figure 2A). Mitotic events causing loss of the *A/a* heterogosity remove the growth


KIMAX
A
1
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inhibition and abnormal morphology, and allow conidiation (Figure 2B1, 2C, 2D). Such events are called "escapes," and cultures that originated as DA's but later escaped are called escaped DA's. The vast majority of cultures grown from single conidia of well-conidiated escaped DA's are either *A* or *a*, and grow at an uninhibited rate. (A few exceptional *A/a* conidial isolates have been found; the two that were tested involved new mutations to suppressors of the *A/a* vegetative incompatibility (NEWMEYER 1970).

(For readers unfamiliar with Neurospora it should be explained that the effect of mating type on vegetative incompatibility is distinct from its effect on crossing. There are several other *het* loci in Neurospora that control vegetative incompatibility in duplications and heterokaryons. Duplications heterozygous for such loci have initial inhibited phenotypes of the same general type as DA's. See PERKINS 1975 and MYLYK 1975. None of these other *het* loci have any effect on crossing.)

Squares: Duplications in which there is no *A/a* vegetative incompatibility are called Squares, because of their morphology (NEWMEYER 1970). Squares have only a slightly reduced growth rate. The morphology is only slightly different from wild type under our usual isolation conditions. The aerial growth looks as if it had been cut off straight across the top of the culture, and the uppermost conidia are sticky (Figure 2B2). See NEWMEYER 1970 for further description. Even under the best conditions, scoring can be complicated by the minor morphological variants that frequently segregate from crosses. Therefore all isolates that could possibly be Square are crossed to testers of both mating types. It has been shown that essentially all Squares can be detected in this way.

The Squares studied here are *A/a* duplications in which the initial inhibition is prevented by the unlinked suppressor, *tol*. The Square phenotype also occurs in duplications that are homozygous for mating type; the occurrence of these is attributed to meiotic crossing over between mating type and break point. These single-mating-type Squares are not included in the present analysis for reasons given in METHODS.

The right tip: If there is any material other than a telomere beyond the right break point in the normal sequence in wild type (Figure 1A), it will be lacking in the duplication, and in the normal- and inversion-sequence products derived from it. If such material exists, it is clearly not essential, because vigorous, fully fertile normal and inversion sequence products can be derived from the duplication strains.

FIGURE 2.—A, B. Four cultures isolated from the same cross, showing the morphologies discussed in the text (10 × 75 mm tubes). A, Dark Agar (DA) after development of dark pigment. B1, Escaped DA; conidiating escaped growth is visible in the upper part of the culture, but the dark area is still visible at the site of the initial inhibited colony. B2 Square. B3, wild type. The cultures shown in A, B2 and B3 were grown for five days at 25°, then refrigerated until the culture shown in B1 was fully grown and ready to photograph. C. Recently escaped DA showing DA morphology at bottom of photograph and escaped, essentially normal morphology at the top. DA morphology is characterized by a great many short irregularly spaced branches extending from the main hyphae at erratic angles (X35). D. DA in the act of escaping. The upper arrow points to escaped growth; the lower arrow points to DA growth (X35). Photographs by DR. N. B. RAJU.

Terminology: Squares and escaped DA's are referred to as duplications, or duplication strains, even though they may be mixed cultures including one or more breakdown products that no longer carry any duplication. The various constituents of such cultures are called "components" or sometimes "escape products" or "products," depending on the context. After purification, a breakdown product is called a "component" or a "derivative."

The duplicated segment in the normal, *i.e.*, old, position is called the Normal segment. The duplicated segment in the transposed, *i.e.*, new, position is called the Transposed segment. Wild-type sequence is called Normal, or normal sequence; the sequence of *In(IL→IR)H4250* is called standard inversion sequence, or simply the inversion. The symbols for this and all other rearrangements, and the duplications obtained from them, are abbreviated after the first mention by omitting the linkage group symbols, *e.g.*, *In(H4250)*.

MATERIALS AND METHODS

Strains: Standard wild types were 74–OR23–1A and 74–OR8–1a. Most other stocks used were inbred to these wild types at least one or two generations, except for the strains mentioned in *Factors affecting the instability of duplications.* Normal-sequence testers for mating type and fertility tests were *fl*^{P}*A* (Fungal Genetics Stock Center No. 1838) and either *fl*^{P}*a* (FGSC 1690) or *fl*(P605)*a* (FGSC 297). The original standard stocks of the inversion *In(H4250)*, FGSC numbers 1160, 1161, 1563, 1564, were described in NEWMEYER and TAYLOR (1967). The suppressor *tol* (tolerant, N83, FGSC 1946) is described in NEWMEYER (1970). Markers were *arg-1*, arginine (B369 or H4250; the latter allele was present in the original inversion stocks but is separable from the inversion); *aur*, aurescent (34508); *fr*, frost (B110); *leu-3*, leucine (R156); *R*, Round ascospore (35408R); *sor-4*, sorbose-resistant (DS); *suc* succinic-requiring (66702); *un-5*, unknown temperature-sensitive (b39). Other strains are described in the text.

Methods: Most methods and media were as described by PERKINS (1959), NEWMEYER and TAYLOR (1967), and PERKINS *et al.* (1969).

Mating type and fertility were routinely scored using *fl* (fluffy) normal sequence testers in 75 mm tubes, prepared as described by TAYLOR (1965). In the initial crosses fertility was estimated only very roughly. In later experiments estimates were much more careful. In tests with up to 20 spores, the exact number of spores was recorded; estimates of whether a cross had less than 1000 spores were made by counting an area of, *e.g.*, 100 spores, and estimating how many similar groups were present. Estimates included both black and white spores. Each duplication was tested by crossing to both *fl A* and *fl a*. Unless otherwise specified, fertility tests of DA's and Squares were performed on the original cultures, without first separating the components. Further details are given in RESULTS.

When escaped DA's are tested for fertility, fairly often one of the two fertility tests produces no perithecia, because one escape product has overgrown the culture (NEWMEYER and TAYLOR 1967). All the tests that actually make perithecia are counted when making comparisons of fertility.

On the other hand, when Squares are tested for fertility, the results are counted only if a Square produces perithecia with both mating types. The reason is that there are several possible origins for isolates which are tentatively scored as Square but which react with only one mating type: (1) Scoring Square by morphology is not foolproof, and therefore fertility tests are done on all isolates that could possibly be Square, some of which were probably really euploid. (2) Square duplications that are homozygous for mating type can arise by meiotic crossing over; these can be either *tol* or *tol*+, if *tol* is segregating in the cross that produced the duplications. (3) Conceivably a *tol A/a* duplication could break down very early, with one breakdown product overgrowing the culture; however, we have no evidence that this happens. Because it is often

impossible to know which of these origins is correct, data on Squares that react with only one mating type are not included in the tables.

Normal *vs.* aberration sequence was scored by estimating the percent of white spores in the cross tubes used for fertility tests (see RESULTS). Methods for confirming normal or inversion sequence are described in RESULTS.

For isolation of separate escapes, as they occur, DA's were grown in liquid complete medium at 25°, then ground up by crushing against the wall of the tube with a pipette. Droplets of the resulting suspension were distributed on the surface of media in several plates, to give many colonies of the same DA, and the plates were examined daily under a dissecting microscope. Noninhibited hyphae (escapes) were isolated as soon as they were detected, only the first *fr*+ and *fr*− escapes being isolated from any one colony. Colonies were then excised when necessary to prevent any remaining hyphae from overgrowing the plate, or from conidiating. In the experiment described in RESULTS, the ground-up DA's were plated on complete medium at 25°. In two other experiments, DA's were plated on supplemented minimal containing various concentrations of sorbose, at various temperatures. Sorbose makes Neurospora grow colonially, and was used to prevent the escape of *fr*+ hyphae from overgrowing the plate. However, this means that the escaped products do not grow away from the unescaped tissue. The resulting mixtures must therefore be purified, often repeatedly, by subculturing the isolated material and reisolating escaped sectors from it the next day. This is undesirable because additional escape events may occur during purification. (When the plating is done on complete medium, it is rarely necessary to purify the *fr*+ escapes, and a single purification usually suffices for *fr* escapes.) A second disadvantage of sorbose medium is that *fr* escapes are very much more easily recognized than *fr*+ escapes on sorbose, so the fact that many *fr* were found tells relatively little about the mechanism of escape.

Round spore was scored by vegetative morphology; any uncertain scorings were checked by determining the shape of ascospores produced in progeny tests. The vegetative morphology is similar to the mutants soft and peach, with dense low conidiating growth at the bottom of the slant. This phenotype is not always obvious in cultures grown directly from ascospores; it is best scored in transfers, read after one day.

Shot tetrads were scored as described by PERKINS (1974). Whether or not progeny tests produced DA's was determined by isolating random ascospores to complete medium, or by the plating method described in NEWMEYER, HOWE and GALEAZZI (1973).

RESULTS

We will first discuss instability in DA's, then instability in Squares, and finally, factors affecting instability.

Instability in inhibited duplications (DA's)

Preliminary work (NEWMEYER and TAYLOR 1967) indicated that if we use a marker, *m*, in the duplicated region, and cross *m A Normal* × *m*+*a Inversion* to produce DA's, most escaped DA's are phenotypically m+, and form perithecia with both *A* and *a* testers. On conidial plating these usually prove to be mixtures of phenotypically m+a and m A mycelia, suggesting that they have become homozygous or hemizygous for the markers on one or the other segment of the duplication. Homozygosity could occur by mitotic crossing over proximal to mating type; hemizygosity would result from complete or partial deletion. In some escaped DA's only one allele or the other is recovered for markers distal to mating type; some of these cases could be due to prior meiotic crossing over which made the marker homozygous.

When crossed to euploid testers, most conidial isolates are barren, *i.e.*, they produce many perithecia, and therefore their mating type can be determined, but the perithecia contain few spores. [Barrenness is characteristic of Neurospora duplications (PERKINS and BARRY 1977)]. Other conidial isolates are markedly more fertile. A given phenotype, for example m A, can be either fertile or barren. When a fertile conidial isolate is found, barren isolates of the same phenotype are usually obtained from the same RA. [The barrenness or fertility of conidial isolates is not related to the fact that the RA was initially heterozygous for mating type, because other kinds of duplications, which are not heterozygous for mating type or any other vegetative incompatibility locus, can produce both fertile and barren nuclei (*e.g.*, TURNER 1977)].

Since duplications from many different Neurospora rearrangements are at least initially barren, our working hypothesis has been that the fertile DA derivatives have lost essentially all of the duplication, while the barren derivatives retain all or part of it. Many of the experiments reported below were designed to test this hypothesis and to learn more precisely the nature of the escape events.

DA's that score as barren after escape from inhibition

Conidial platings: Each escaped DA is a mixture of the products of one or more escape events. Conidia from individual escaped DA's were plated in order to isolate the various products, and the data were analyzed in an attempt to distinguish between homozygosis and deletion or mutation.

The most extensive data came from two crosses of Normal *leu-3 a* × *Inversion A*. (*leu-3* is about 15 units distal to mating type.) Assuming no meiotic crossing over, the duplication should have the structure shown in Figure 3. The results show that all the barren *a* escapes must have been due either to homozygosis or to fairly long deletions that removed both *leu-3*+ and mating type; they could not have come from shorter deletions that did not include the *leu-3* locus, or from mutation or conversion at the mating-type locus. The origin of the barren *A* escapes is indeterminate in this type of cross.

Eleven DA's that were definitely *leu*+/*leu* were analyzed by plating conidia (or in four cases by picking sectors), and the resulting isolates were tested for *leu*, mating type, and fertility. Only the barren isolates are discussed here. Ten of the DA's gave *leu a* barren isolates; these could be produced only by homozygosis or by fairly long deletions that removed both *leu*+ and *A*. None of the DA's gave phenotypically *leu*+*a* barren isolates; this class could not be produced by either of the above events, but would be expected if escape resulted from a short deletion that included *A* but not *leu*+, or from mutation or conversion of mating type, or conceivably

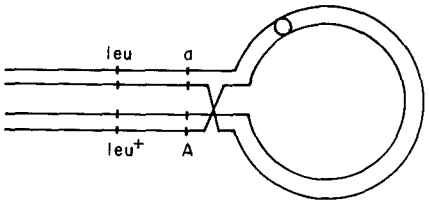


FIGURE 3.—Structure of DA's used for analyzing barren escapes by conidial plating, showing how a mitotic crossover might produce homozygous *leu a* and *leu*+ *A* escape products.

from a double crossover. Ten of the DA's gave *leu+A* barren isolates; these are not diagnostic because they could come from any of the processes mentioned. *leu A* barren isolates are not expected from any single-step process, and none was found.

Analysis of separate escape events: The data from the conidial platings just described are not completely satisfactory because several different escapes can occur in the same DA, and frequently one product tends to overgrow the culture. There is also the problem that the DA might have been initially homozygous as a result of a prior meiotic exchange. Thus, if we find only one allele of a marker, it is uncertain whether it was initially homozygous, or whether the other allele was present but has been overgrown, or whether the escapes really all occurred by events that failed to uncover the marker. Consequently it was impossible to get useful data on more distal markers by this method.

We therefore ground up and plated individual DA's, two days before the earliest escapes, so that we could pick the products of many different escapes from the same DA (see METHODS). Distal markers were used on both duplicate segments. Heterozygotes for the morphological mutant *fr* were used in hope of detecting contiguous fr+ and fr products (twin spots) which would be expected if escapes occurred by homozygosis as a result of mitotic crossing over proximal to mating type.

The phenotypes of the isolates showed clearly that only a few barren escapes could be due to short deletions or to changes at the mating-type locus. The great majority were due either to homozygosis or to very long deletions that included mating type and all distal markers. (Very long deletions could still retain enough of the original duplication to render them barren.) A few twin spots were found, but in most cases it was not possible to determine the presence or absence of twin spots with certainty, for technical reasons. Therefore it was usually impossible to distinguish between homozygosis and very long deletions. Homozygosis seems more likely, because partial deletions would not usually uncover all markers, if the deletions occur at random. However, we cannot exclude the possibility that partial deletions occur nonrandomly. In the unstable duplications of *Aspergillus nidulans*, in which partial deletions are common, it appears that there is nonrandom distribution of deletion break points (ROPER 1973).

The experimental details are as follows.

The most reliable separate escape experiment involved the cross *un-5 suc Normal A* × *fr arg-1 aur In a*. The map locations and approximate distances between markers are *fr* 20 *un* 25 *mt* 8 *suc* < 1 break point 4 *arg* 50 *aur* (Figure 1). The data are given in Table 1. It is clear that all four DA's were initially heterozygous for *fr* and *un*. It is also clear that the usual mechanism of escape was not interstitial deletion unless the deletions were very large. The 21 fr+un escapes cannot still be heterozygous for *un*, and the 11 fr un+ escapes cannot still be heterozygous for *fr*, so that if escape is due to simple interstitial deletions, the deletions must extend from mating type past one or these distant markers. Only the two fr+un+ isolates could be short interstitial deletions.

In the three DA's with adequate data, the results indicate that there was no meiotic crossover, and that escape almost always involved either homozygosis, or deletion of mating type and all distal markers. (The fr+un A suc+ could be due to homozygosity via mitotic crossovers between mating type and *suc*, or to very long deletions that began distal to *suc* and extended

past *un.*) Fertility was scored on six fr+un A suc, seven fr+un A suc+ and seven fr un+a suc+. All were barren except one fr+un A suc.

Attempts to demonstrate homozygosis by finding contiguous fr+ and fr "twin spots" were less successful. When escapes are isolated on complete medium, as in Table 1, the fr+ escapes grow so fast that they often must be picked, and the colony excised, before the fr are detectable; hence the smaller number of fr isolates. (Isolating from sorbose medium presents somewhat the reverse difficulty. See METHODS.) Nevertheless, four cases were found of a large fr+ escape with a very small fr near its origin, strongly suggesting twin spots. All but one of the remaining fr escapes isolated from complete medium had an fr+ escape in the same patch of tissue, but the two were not contiguous. These could be twin spots, in which the fr+ and fr products of a mitotic crossover failed to free themselves of unescaped nuclei simultaneously; alternatively, the fr+ and fr could have come from independent deletion events.

Two other separate-escape experiments, in which escapes were isolated from sorbose medium, led to the same conclusions. Most escapes behaved as if due to homozygosis or to deletion of the whole marked region. However, one barren escape was found with the phenotype expected if escape was due to a change at the mating-type locus, or to a short deletion that did not extend as far as *leu-3*.

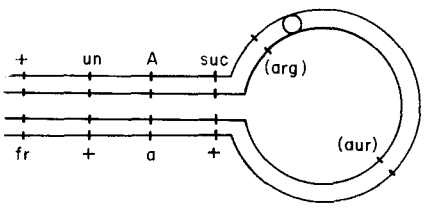
We have been unable to make a reliable distinction between homozygosis and long deletions on the basis of morphology. Most barren escapes tend to look Square and most fertile escapes look wild type (as is expected if the barrens carry the intact duplication and the fertiles are euploid), but exceptions and intermediate phenotypes have been found. Also it is not clear whether all or only part of the duplication is necessary for the Square phenotype. No consistent difference has been found between fertile and barren isolates with respect to colony size and morphology on sorbose media; this is unlike the situation with *Dp(NM103)* reported by TURNER (1977).

TABLE 1

Separate escapes isolated from four DA's from the cross un-5 suc
Normal A × fr arg-1 aur *Inversion* a

Phenotype	DA No. 50	DA No. 51	DA No. 52	DA No. 53*
fr+ un A suc	2	3	1	0
fr+ un A suc+	2	6	2	0
fr+ un§ suc+	2	2	0	1
fr+ un+ A suc+	1	0	0	1
fr un+ a suc+	4†	3	3	0
fr un+§ suc+	1	0	0	0

The structure of the DA's before escape is as follows: (The diagram is drawn assuming no prior meiotic crossover. Duplicate segments are drawn as if somatically paired.)



* Plus 6 *fr+* and 2 *fr* escapes not isolated.
† Three of these were isolated from sorbose. All other escapes in this table were isolated from complete medium. See METHODS.
§ Not tested for mating type.

One clear case of homozygosis: In one case it was clearly demonstrated that a barren escape was due to mitotic crossing over and homozygosis, rather than to a long deletion that uncovered all markers. This was done by analyzing the progeny that were obtained after the barren escape product eventually broke down and became fertile.

One of the *fr+un A suc aur barren* escapes (No. 51–4d) from the single escape experiment in Table 1 was shown to be pure by conidial plating, and one of the plating isolates was then crossed to *leu-3 Normal a*. Although initially barren, the cross eventually produced many spores. (Post-fertilization loss leading to delayed fertility is common in Neurospora duplications. PERKINS and BARRY 1977; also see later in this paper.) Analysis of 71 of these progeny indicated that after fertilization the duplication underwent deletion events, producing fertile Normal *un A suc aur* nuclei and also a few fertile Inversion *un A suc aur* nuclei; and that both types of nuclei participated in the cross.

Among eight apparently euploid *un A suc aur* progeny, six behaved like normal sequence and two like inversion. The remaining 63 progeny included three typical H4250 duplications. Extensive testing of one of the fertile inversion isolates confirmed that it was standard inversion sequence.

The only reasonable explanation of these results is that the duplication parent (No. 51–4d) escaped from the original DA by a mitotic crossover in the short interval between *suc* and break point, making it homozygous for *fr+un A suc*, so that subsequent breaks could produce fertile Normal and fertile inversion nuclei, both carrying *suc*.

Alternative explanations seem highly unlikely. The original escape product could not have gone through the cross intact, because no barren class was found that resembled the original escape. If the original escape had been a partial deletion, it might have undergone further deletion to become fertile and still retained a tiny piece of the Transposed segment proximal to *suc*. Then, in the cross to *leu-3 Normal a*, meiotic crossovers involving this piece of Transposed segment could have produced inversions or typical H4250 duplications. But the data require at least five such meiotic crossovers out of 71 progeny whereas one would expect that such a tiny piece in the transposed position would only extremely rarely participate in pairing when two long normal-sequence pairing partners were present. In contrast, the postulated mitotic crossover occurred under strongly selective conditions, during escape, and involved pairing of two long duplicate segments.

DA's that score as fertile after escape from inhibition

In most cases, escaped DA's were tested for fertility without first plating conidia to isolate the individual escape products. Fertility was tested by crossing each escaped DA to both *A* and *a* normal-sequence testers. Such tests frequently contained a mixture of fertile and barren perithecia, as is expected if the escaped DA consisted of a mixture of fertile and barren escape products.

Scoring was based on the number of ascospores shot to the wall of the cross tube. Barren escaped DA's produce from zero to a few hundred spores per 75 mm tube, compared to far more than 10,000 for a euploid cross. Fertile escaped DA's produce markedly more spores than barrens, although rarely as many as a euploid cross; the ejected spores are often clustered as if derived from very few perithecia. There is no sharp cutoff point that would distinguish between barren and fertile, so any test with more than about 1000 spores is arbitrarily called fertile. In some cases a further distinction is made between "highly fertile" (at least several thousand spores) and "barely fertile" (only one or two thousand spores).

Frequency of fertile escaped DA's: Early in this work, about 20% of the escaped DA's from any one cross were fertile in test-crosses to one (or, rarely, both) mating types, so that about 10% of total tests were fertile. In later experiments the frequency of fertility was often much higher, up to 87% of escaped DA's and 71% of total tests being fertile. The major reason for this change is that fertility is drastically increased in some of the genetic backgrounds used in the later crosses. The genetic factors responsible are called "instability factors" and will be discussed in a later section, under "Factors affecting the instability of duplications." In all other parts of this paper, no obvious instability factors were present unless specifically mentioned.

A second reason for the increased fertility in later crosses was that, once it became clear that escaped DA's often contain both fertile and barren nuclei, we began taking inocula from many parts of the culture, to increase the chance of including a rare fertile patch.

Lastly, we learned that, while some fertile DA's shoot spores as early as wild type, others do so only after a lag. The size of this effect varied according to the cross from which the DA's were obtained. In the most extreme case, the percent of escaped DA's that scored as fertile increased from 43% at 14 days after fertilization to 78% at 21 days after fertilization. Once this effect was noticed, we began scoring all fertility tests at both 14 and 21 days after fertilization in order to detect those that shoot late, keeping the racks of cross tubes in plastic bags to prevent dehydration of the crosses during aging.

Time of occurrence of events leading to fertility: In those cases where spore shooting is delayed, it seems likely that the fertility-producing events occur after the escaped DA's are used to fertilize the Normal testers. On the other hand, it is clear that most of the events leading to fertility at the 14-day reading occur in vegetative cells, before the test crosses are fertilized. Conidial platings show that many escaped DA's contain both fertile and barren nuclei, and that the proportion of fertile nuclei is usually much higher in those escaped DA's that were originally scored as fertile. It is usually possible to obtain a pure culture of the fertile component by replating. These results are not compatible with the alternative hypothesis that fertile escaped DA's are simply prone to post-fertilization loss.

Relative frequencies of loss of the Transposed segment vs. *the Normal segment, in fertile escaped DA's without instability factors:* Euploid fertile derivatives of DA's could theoretically be either normal sequence (as a result of complete loss of the Transposed segment) or inversion sequence (as a result of complete loss of the Normal segment) (Figure 1). Normal sequence derivatives should be the same mating type as the Normal parent, and inversion sequence derivatives should be the same mating type as the inversion parent, unless there has been a crossover in the short interval between mating type and break point.

The fertile escaped DA's were scored as Normal or as aberration (not necessarily standard inversion sequence) on the basis of the frequency of white ascospores in crosses to Normal testers. Viable spores in Neurospora are black; inviable spores are white (including deficiencies). Homozygous Normal crosses (and crosses homozygous for a rearrangement) regularly give 5 to 10% white

spores from unknown causes. Any excess above this background level usually indicates heterozygosity for a rearrangement. Crosses of *In(H4250)* × Normal sequence give 20 to 25% whites; reciprocal translocations × Normal give about 50% whites. Analysis of rearrangements by means of the frequencies and distributions of white spores has been described in detail by PERKINS (1974).

The fertile escaped DA's from a large number of crosses were scored for mating type and for white spore frequency. Many of them were tested for their ability to produce DA's when crossed to Normal, and some were subjected to additional tests (see later). The results show clearly that a large majority of all fertile escapes deleted the transposed segment so as to restore Normal sequence.

Well over half the fertile escaped DA's were scored Normal. Furthermore, most escaped DA's that scored as Normal were highly fertile (at least several thousand spores shot), while most of those scored as aberration were barely fertile. The lower fertility of the latter class could mean either that the duplicate segment was not removed completely or that the frequency of fertile nuclei was very low.

Almost all Normals were of the mating type expected if a deletion had occurred so to make normal sequence (Figure 1), and all the Normals that were tested further behaved like normal sequence. (See below.)

In contrast to the Normals, about half of the aberrations were not of the mating type expected if a deletion had occurred so as to produce inversion sequence (Figure 1), implying that they were not standard inversions. This was confirmed in crosses to normal sequence testers, where several fertile aberrations of the unexpected mating type either produced far too many white spores for them to be standard inversions, or else produced few or no DA's. Furthermore, out of seven fertile aberrations of the *expected* mating type which were adequately tested, five proved to be unlike standard inversion. The remaining two proved to be standard inversion sequence, as will be described below; these were both of the expected mating type.

A precise estimate of the relative frequencies of loss of the two duplicate segments is not possible because the data were collected bit by bit from crosses done for other purposes, so that often progeny tests were not made and uncertain scorings were not rechecked. (Uncertain scorings are frequent because often only a few perithecia are fertile, so the spores may be clustered in a part of the tube where visibility is too poor to permit accurate determination of the frequency of white spores.) Also, the estimate varies according to what assumptions are made as to the origin of the fertile aberration products that are unlike standard inversion. From the available data, the ratio *loss of the Transposed segment*: *loss of the Normal segment*, in crosses without instability factors, could be anywhere from 5:1 to 44:1.

Possible origin of fertile aberration escape products that are unlike standard inversion: Fertile aberrations that are unlike standard inversion have usually not been analyzed. Several origins are possible. (1) Those with questionable scorings and the unexpected mating type, and which produced no DA's, could simply be Normals that were misscored as probable aberrations. (2) Some fertile aberration components could have additional breaks superimposed on normal or inversion sequence. The 5:1 ratio given above for *loss of Transposed segment* : *loss of Normal segment* is based on the assumption that all aberrations that are unlike standard inversion belong in this class, except where there are data to the contrary. (One fertile aberration component that was studied further is clearly of this type; it produces complex duplications involving both IL and IR. This new rearrangement appears to be quite different from the kinds of new rearrangements that AZEVEDO and ROPER (1970) found among variants of an Aspergillus duplication.) (3)

Deletion of a duplicate segment could begin slightly proximal to the inversion break point, so as to delete essential genes. Such a product could be maintained in a heterokaryon with other escape products, but, on crossing, it should give deficient white spores. (4) A fertile component might be scored as aberration if a small proximal piece of the Transposed segment remained and sometimes participated in meiotic crossing over. This might explain the one case where a barely fertile aberration made rare DA's. (5) Conceivably, white spores could result if the complete duplication went through meiosis, and if the imbalance caused irregularities in disjunction.

Evidence that fertile components that score as "Normal" are really normal sequence and euploid: There are four lines of evidence. (1) Fertile Normal components that have been purified by plating are pure *A* or pure *a*, and distal linked markers, when present, are uncovered. Thus the complete duplication could not still be present unless there had been a mitotic crossover to make mating type and markers homozygous. (2) No fertile escapes classified as Normal have produced DA's when crossed to Normal testers. This indicates that mating type is no longed present in the Transposed position (see Figure 4). (3) When fertile components are scored definitely Normal, it is because they produced no more than the usual background level of 5 to 10% white (inviable) spores when crossed to a Normal tester. This indicates that there has been no deletion from the Normal segment, as shown in Figure 5. (4) From the foregoing it is clear that there must have been a deletion from the Transposed segment and that this must include mating type (plus any distal markers, unless there was a prior mitotic crossover) plus enough additional material to remove the barren phenotype. Nevertheless a piece of the Transposed segment could remain. This possibility has been eliminated only for the proximal part of the Transposed segment. Here careful genetic tests show that the marker closest to the inversion break point is no longer present in the transposed position in the fertile Normal com-

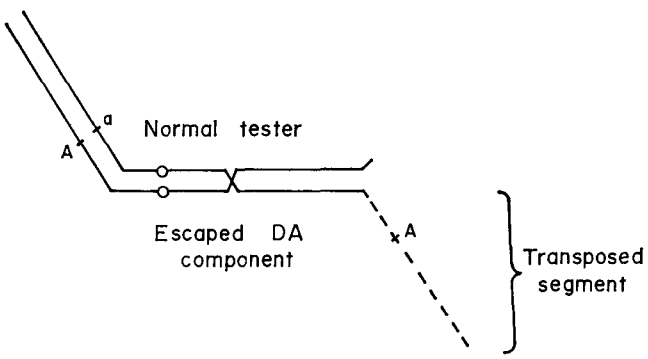


FIGURE 4.—Diagram showing why a fertile escaped DA component that retains mating type on the transposed segment should produce many DA's when it is crossed to a normal sequence tester, as a result of crossing over in the long interval between the two break points. The transposed segment is drawn with a dashed line, because there is no evidence that any of it is present in the fertile escapes that score as Normal. (The escaped DA component is shown as homozygous for mating type because when escaped DA components are isolated they are pure *A* or pure *a*. Therefore if the escaped DA component retained mating type on both duplicate segments, as in the example shown, it must be derived from a mitotic crossover that made mating type homozygous, or from a change at the mating-type locus.)

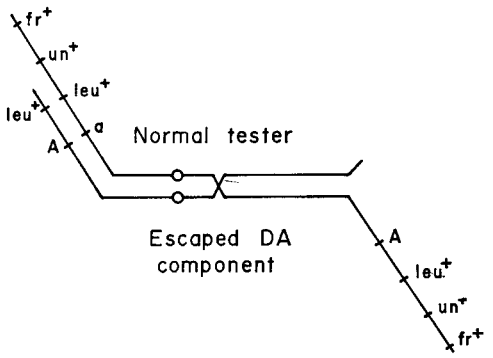


FIGURE 5.—Diagram showing why any deletion from the Normal segment of a DA should result in excess white spores when the product is crossed to a normal sequence tester. In the example shown, crossovers in the long interval between the original inversion break points, or in the remaining part of the Normal segment, will result in deficiencies for the *fr*+*un*+ region, and thus cause inviable white spores.

ponents. These tests utilized *suc*, which is less than one unit distal to the left break point of the inversion. The results indicate that the deletion must begin at or very close to the break point. The tests were as follows:

Six phenotypically fertile Normal suc+ escaped DA's were examined, all of which had arisen from crosses of Normal *suc* × Inversion *suc*+. When conidia from these were plated, three of them gave fertile Normal suc isolates, as expected if the deletion of the transposed arm began proximal to *suc*+ (see Figure 1B,C). The other three escaped DA's gave phenotypically fertile Normal suc+ isolates, which could indicate that the deletions began distal to *suc*+. However, replating showed that the fertile Normal suc+ isolates were actually heterokaryons of fertile Normal suc and barren suc+ of the same mating type. (This problem arises because Neurospora conidia are multinucleate; heterokaryons yield both homo- and heterokaryotic conidia.)

Two other phenotypically fertile Normal suc+ escaped DA's from the same crosses were examined by crossing to Normal and scoring for *suc* and a linked marker. Both gave the results expected if the deletion of the Transposed segment had begun proximal to *suc*+.

It is clear that in all eight cases tested there was no *suc*+ remaining. However, it is possible that in some cases a mitotic crossover could have occurred prior to deletion, so as to make *suc* homozygous. (This clearly occurred in one case described above in this paper, and it could be common.) If this happened, the deletion could begin distal to *suc*, in the transposed position, and our tests would not detect it.

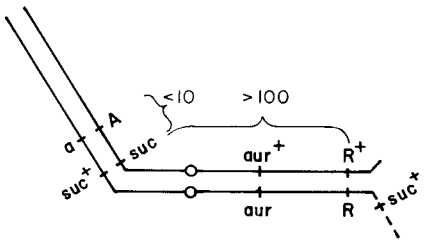


FIGURE 6.—Type of cross used for testing whether deletion of the Transposed segment occurs proximal or distal to *suc*. The upper chromosome is the Normal tester. The lower chromosome is the fertile Normal component of an escaped DA, where the dashed segment containing *suc*+ may or may not be present. See Table 2 for explanation.

This possibility was eliminated by starting with fertile Normal escaped DA's that came from a *suc*+ × *suc*+ cross and that carried the marker *R*, proximal to the right break point. Three such escaped DA's were crossed to *suc Normal A* (Figure 6).

If *suc*+ remained in the transposed position, we would expect most *R* progeny to be *suc*+ (about equal numbers of *A* and *a*). If *suc*+ were no longer present in the transposed position, the *R* progeny should include about equal numbers of *suc* (usually *A*) and *suc*+ (usually *a*), because *R* and *suc* (in its Normal position) show very little linkage. Data for the three crosses are shown in Table 2. The selection against *suc* is found frequently in euploid crosses and can therefore be disregarded. Despite this complication, it is clear that in all three cases no *suc*+ remains in the transposed position.

It is concluded that deletion events that produce fertile Normal products ordinarily begin proximal to the *suc* locus, and therefore the deletion must begin at or very close to the original inversion break point.

Evidence that a few fertile aberration DA components are really inversion sequence and euploid: In addition to the two bona fide inversion components derived from crosses without instability factors, two more inversion components were found among the escaped DA's from crosses with the Adiopodoumé and N193 instability factors. The evidence that these four components are standard inversion sequence is as follows. (1) Two of them were purified by conidial plating and shown to be a single mating type; thus the complete duplication cannot still be present unless there was a mitotic crossover to make mating type homozygous. (2) All four fertile components produced DA's when crossed to normal sequence, showing that mating type is present in the transposed position. (3) All four produced no more than the usual background level of 5 to 10% white spores when crossed to standard Inversion, indicating that there was no deletion from the Transposed segment. (4) The three that were tested produced no DA's when crossed to standard inversion, indicating that mating type is no longer present in the Normal position. (5) One of the two not involving an instability factor was the fertile inversion derivative described earlier in *One clear case of homozygosis.* This was tested further by crossing to a multiply marked

TABLE 2

Evidence that deletion of the transposed segment begins proximal to suc, *in the fertile Normal components of three escaped DA's*

Progeny class	Numbers of test-cross progeny		
	DA No. 7	DA No. 9	DA No. 10
suc R	22	16	17
suc+ *R*	29	30 (26 a, 4 A)	30 (all a)
suc R+	20	17	22
suc+ *R*+	23	29	21

Data are from three test-crosses of the type shown in Figure 6. None of the progeny were DA's. All three test-crosses produced only phenotypically round spores. The column labeled Progeny Class indicates the genotype for *R* (Round spore, determined as in METHODS) and the phenotype for *suc*.

If any of the three fertile Normal components retained *suc*+ in the transposed position, there would be a large excess of *suc*+ *R* (including many *A*), and there would be very few *suc R*.

inversion strain. No DA's were produced, and all markers segregated entirely as predicted for a structurally homozygous inversion cross.

We have not excluded the possibility that a piece of the Normal segment remains. Except for this qualification, it appears that all four apparent inversion components are standard inversion sequence.

Instability of noninhibited duplications (Squares)

The Squares studied here are *A/a* duplications in which the heterokaryon incompatibility is suppressed by *tol*. (See ESSENTIAL BACKGROUND INFORMATION.) Euploid variants that occur in squares do not form visible sectors, because Squares grow almost as well as euploids, and because Neurospora grows in such a way that the various constituents of a culture tend to be mixed together. Square cultures that include euploid variants are still visibly Square. However, when the squares are fertility tested, those with euploid components make ascospores, and the euploid components are therefore detected. If necessary, the euploid components can then be obtaind in pure culture by plating or streaking conidia.

Squares are much less fertile than escaped DA's, even when obtained from the same crosses (Table 3). At least part of the greater fertility of escaped DA's can be ascribed to the selective advantage of breakdown products, compared to the initial inhibited growth. It could also be that *tol* inhibits breakdown of duplications. This has not been tested. Because of their lower fertility, Squares provide a much cleaner background than DA's for detecting factors that make duplications become fertile, and they are used for that reason in the following experiments.

Factors affecting the instability of duplications

Early in this work some variability was noticed in the fertility of escaped DA's and also in the speed with which DA's escaped from the initial inhibition. Later, when inversion strains containing the *tol* suppressor were crossed to normal se-

TABLE 3

Fertility of Squares and escaped DA's from the same crosses

Type of duplication	Days after fertilization	Number of tests with		
		$\leq$ 20 ascospores*	21–1000 ascospores	> 1000 ascospores
Square (*tol*)	14	79	1	0
	21	70	8	2
Escaped DA (*tol*+)	14	38	23	19
	21	31	23	26

Pooled data from three crosses of Inversion × Normal sequence without obvious instability factors. Each cross was heterozygous for *tol* and therefore produced both DA's and Squares. Each Square or escaped DA was tested for fertility by crossing it to both normal sequence *A* and normal sequence *a*; the same tests were scored at both 14 and 21 days. Only *A/a* Squares were used.

* Escaped DA tests in this class often have a few spores visible. Square tests in this class rarely have any spores visible.

quence strains from many different genetic backgrounds, these crosses showed marked variations in DA escape speed, and in the fertility of Squares and escaped DA's.

In the following discussion of these strains, it is important to keep the methodology in mind. Usually about 20 duplications were isolated from each cross of Normal × Inversion. If the cross is heterozygous for *tol*, about half the duplications are DA's and about half are Squares. Each duplication is fertility-tested by crossing to both normal sequence *a* and normal sequence *A* testers. Thus there are two fertility tests per duplication and a total of about 40 fertility tests for the duplications from a given cross of Normal × Inversion, if both Squares and escaped DA's are tested. The number of fertile tests indicates the minimum number of independent events that result in fertility; it does not indicate repeated tests on the same clone.

From among the strains having different genetic backgrounds, the two with the most striking effects on escape and fertility were chosen for study. The factors responsible for these effects are named *mei-3* and Adiopodoumé. [These factors were originally called "breakers" (NEWMEYER and GALEAZZI 1974). They are now called "instability factors" (or simply "factors") because it is likely that at least one of them does not actually break off the duplicated segment but deletes it by another mechanism. (See DISCUSSION.)]

The *mei-3* factor was originally found in a laboratory strain of Lindegren background (*inl cot-2 a*, FGSC No. 1358). Its effects on fertility and escape speed are due to a single gene, designated *mei-3* because it also blocks meiosis. The Adiopodoumé instability factor (or possibly factors) was found in crosses of Adiopodoumé A (a wild strain from Africa, FGSC No. 430) × *In(H4250); tol* (N329-11)*a*. The instability effects are ascribed to the Adiopodoumé parent because similar results were not obtained in crosses of the inversion parent to other wild types, but the genetic basis has not been determined. For simplicity, it is discussed here as if a single factor is involved.

Information on the Adiopodoumé factor is limited to data from the original cross and from a reciprocal cross which behaved in the same way, together with related progeny tests. Much more extensive data have been obtained on *mei-3* in the course of determining its genetic basis and studying its other properties; these will be reported elsewhere (NEWMEYER and GALEAZZI, in preparation).

The effects of both factors on the stability of *Dp(H4250)* are summarized in Table 4. Numerical data on the major cases of altered fertility are given in Table 5. Numerical data on the escape speed of *mei-3* DA's will be given in NEWMEYER and GALEAZZI (in preparation).

It will be noted that the two instability factors are qualitatively different. They differ not only in whether they increase fertility chiefly in Squares or in escaped DA's and in which duplicate segment is affected the most, but also possibly in the type of breaks or deletions that occur. The data on Adiopodoumé are not extensive enough to be conclusive, but it appears that Adiopodoumé makes random breaks and often does not remove the duplication precisely. The *mei-3*

TABLE 4

Summary of the effects of the mei-3 *and Adiopodoumé instability factors on the breakdown of duplications, relative to breakdown in control crosses without these factors*

Instability factor	Type of duplication	Duplication components with mating type of inversion parent		Duplication components with mating type of normal-sequence parent		Speed of DA escape
		Fertility	Constitution of fertile components‡	Fertility	Constitution of fertile components‡‡	
mei-3	Squares (*tol*)	Marked increase	Most are standard inversion sequence*	Great increase	Most are normal sequence**	——
	Escaped DA's (*tol*+)	No significant increase	——	No significant increase	——	1 to 2 days faster
Adiopodoumé	Squares (*tol*)	Slight increase§	——	Slight increase§	——	——
	Escaped DA's (*tol*+)	Marked increase	Most are not exactly standard inversion sequence†	Probably no significant increase	4 out of 17 carry new rearrangements††	No significant change

‡ These are expected to be inversion sequence if they are due to simple loss of an entire duplicate segment, and if there has been no meiotic crossover between mating type and break point.

‡‡ These are expected to be normal sequence, if they are due to simple loss of an entire duplicate segment, and if there has been no meiotic crossover between mating type and break point.

§ Reduced frequency of extreme barrens ($\leq$ 20 spores) but no increased frequency of fertiles ($\geq$ 1000 spores).

*, **, †, †† See footnotes with same symbols in Table 5.

TABLE 5

Numerical data summarized in Table 4, on the major effects of the mei-3 *and Adiopodoumé instability factors on the breakdown of duplications*

	Type of duplication	Duplication components with mating type of inversion parent			Duplication components with mating type of Normal parent		
		Barren§§	Barely fertile	Highly fertile	Barren§§	Barely fertile	Highly fertile
mei-3	Squares (*tol*)	5	7*	1*	0	1**	12**
wild-type control	Squares (*tol*)	12	1	0	13	0	0
Adiopodoumé	Escaped DA's (tol^+)	6	7†	6†	6	7††	10††
wild-type control	Escaped DA's (tol^+)	12	1	1	6	8††	4††

The duplications were derived from the following crosses:
Line 1: Three crosses of *mei-3 cot-2 inl a* × *In tol A*.
Line 2: Standard wild type (OR8–1a) × the same *In tol A* strain as in line 1.
Line 3: Two crosses of Adiopodoumé *A* × *In tol a*.
Line 4: Standard wild type (OR23–1A) × the same *In tol a* strain as in line 3.

Amount of fertility is based on crossing a group of Squares or escaped DA's to Normal sequence testers of both mating types, without prior purification of the fertile components. Numbers in the body of the table indicate the number of duplications that score as barren, barely fertile, or highly fertile, when crossed to the tester of the appropriate mating type. Scorings for Normal *vs.* Aberration are based on the frequency of white spores in the crosses to Normal sequence testers. Fertile components were sometimes purified by streaking conidia before further testing. For *mei-3*, data in the body of the table are given only for initial crosses where *cot-2* and *inl* were also segregating. These results were confirmed by numerous later crosses without *cot-2* and *inl*.

§§ Less than 1000 spores.

* Five fertiles were tested, using the same tests described earlier in the paper for determining whether escaped DA components were standard inversion sequence. Four clearly behaved like standard inversion. The fifth did so only after purification. (Before purification it produced slightly more than the expected background level of white spores when crossed to the standard inversion tester).

** Twelve out of 13 scored as Normal. Later, an experiment with *suc* and *R*, identical to the one previously performed on DA's (Table 2, Figure 6), was performed on four *mei-3* Squares from a cross of *mei-3 inl a* × *In(H4250) R tol A*. The results were similar to those in Table 2, showing that the deletion of the transposed segment began proximal to *suc* in all four cases—*mei-3* maps near centromere on Group I so that most of the duplications carry it. This explains why more than half the duplications from *mei-3* crosses score as fertile.

† Five fertiles were tested, using the same tests described earlier in the paper for determining whether escaped DA components were standard inversion sequence. One clearly behaved like standard inversion. One clearly did not. The remaining three behaved like standard inversion except that they produced slightly more than the expected background level of white spores when crossed to the standard inversion tester. See DISCUSSION.

†† Only seven fertiles from Adiopodoumé and eight from the control scored as Normal.—Five from Adiopodoumé and four from the control scored as questionable fertile Aberrations that produced few or no DA's; a few fertile components like this have been found in escaped DA's from many crosses.—The remaining four Adiopodoumé fertiles carry new rearrangements. They were all highly fertile, definitely scored as Aberrations, and produced no DA's. None like this were found in the control cross, so it is probable that they are due to the Adiopodoumé parent. Further data, including data from shot tetrads, indicate that all four new rearrangements are different from each other.

factor (when acting on Squares) appears usually to remove the duplication precisely, although a few random breaks also occur.

Several fertile components (both normal and inversion sequence) have been purified from *mei-3* Squares by streaking conidia; this shows that the events that

cause fertility often occur during vegetative growth. The effect of *mei-3* on fertility cannot simply be due to counteracting a postulated stabilizing effect of *tol*, because *mei-3* also increases the fertility of duplications from some other rearrangements, where *tol* is not present (NEWMEYER and GALEAZZI, in preparation).

The *mei-3* factor also markedly increases the speed with which DA's escape from the initial inhibition. This may sometimes be due to a mechanism other than complete deletion, as the escaped DA's that were tested were not significantly more fertile than *mei-3*$^+$ *DA's*.

Other possible instability factors: Several other cases of variability between crosses have been found, but they have not been retested or shown to be due to genetic factors. For example:

(1) Probably a factor or factors in the wild types Lein *7A* (FGSC No. 847), Chilton, *a* (FGSC No. 1691), and Em *A* (Catcheside's) cause DA's to escape unusually slowly. (2) Possibly a factor from Lindegren wild type makes Squares somewhat less stable. (3) The "N193 factor": Cross N193, a cross of *In(H4250)A* × *sor-4*(N176–4)*a*, produced escaped DA's with an unusually high frequency of fertile aberration components. One of these was well tested and appeared to be standard inversion sequence. Less extensive testing indicated that the others might also be standard inversion sequence. A control cross indicated the N193 effect was not due to the *sor-4* mutant. There is some reason to suspect that the factor or factors responsible for the effect might be environmental rather than genetic.

DISCUSSION

The hypothesis that fertility depends on complete loss of a duplicate segment: On the whole, our results are consistent with this hypothesis. This is true for all types of escapes with the possible exception of one minor class.

The fertile components which behaved like normal sequence or standard inversion sequence are entirely compatible with the hypothesis, and it is clear that, in the cases tested, deletion of the duplicate segment began at or very close to the original inversion break point. However, we were unable to prove genetically that the fertile derivative did not retain an unmarked distal piece of the duplicate segment; also it was conceivable that the duplicate segment was permanently inactivated rather than deleted. Both of these possibilities have been eliminated by BARRY, for the one strain he examined cytologically. One of the fertile inversion sequence derivatives which we had purified vegetatively after it arose in a Square in the presence of *mei-3* (Table 5), was crossed to a standard inversion tester. In a particularly well-spread pachytene figure from this cross (reproduced as Figure 4 of PERKINS and BARRY 1977), chromosome *1* (which equals Linkage Group I) was completely paired throughout its length, with no loop, and no unpaired material was visible at either end. This indicates that the Normal segment has been removed completely, within the limits of visibility. (It also indicates that there is no visible material between break point and telomere in the original inversion, because any such material should have appeared as an unpaired tip in BARRY's figure.)

The barren escapes, in which all markers are exposed, are compatible with the hypothesis, because they could be due to homozygosis or to interstitial deletions that failed to remove a distal piece of the duplicate segment beyond the last marker.

Still to be considered are the fertile components that scored as aberration but

were unlike the standard inversion. The only members of this group that might be incompatible with the hypothesis are the few that made slightly more than the usual background level of 10% white spores when crossed to inversion-sequence testers. (Most of these were from crosses involving the Adiopodoumé factor.) It is possible that these fertile components retain a small piece of the Normal segment, and that this piece occasionally participates in crossing over, thus producing deficient spores. Alternatively these fertile components could be mixtures of two different fertile components, or it could be that occasionally aneuploids can go through meiosis if a euploid is present. Although a few members of this group were scored "highly fertile," only one was as fertile as wild type, and that was not obtained in pure condition. Thus it is possible that a component can be partly fertile even though it retains a small piece of the duplicate segment, but this is far from being proved.

Comparisons with other duplications in Neurospora and Aspergillus: In addition to duplications from *In(H4250)*, terminal duplications have been studied from 13 other rearrangements in Neurospora (PERKINS and BARRY 1977). Most of these duplications are unstable. Duplications from seven of these rearrangements have been studied with respect to the kinds of fertile derivatives they produce—*T(IR→VIR)NM103*, studied by TURNER (1977), and *T(IR→VL)AR190*, *T(VIR→IVR)AR209*, *In(IL→IR)NM176*, *In(IL→IR)AR16*, *T(IIR→VL)ALS176*, and *T(III→ ;III;IV)D305*, all studied by PERKINS (see brief accounts in PERKINS, NEWMEYER and TURNER 1972 and PERKINS and BARRY 1977).

In all of these, the fertility appears to result from complete and precise loss of one of the duplicate segments, as is usually true for duplications from *In(H4250)*. The instability of duplications from most of these rearrangements was studied only by progeny testing, and so the precision might be accounted for by the fact that the crossing process was used to select the fertile variants. However, in the case of *Dp(NM103)* it was shown that the fertile variants often arise during vegetative growth, as in *Dp(H4250)*, and in *Dp(NM103)* this occurs in the absence of any heterokaryon-incompatibility inhibition that might cause selection for breakdown. It seems unlikely that a random-break mechanism could account for the occurrence of so many precise losses.

The duplications from six of the other rearrangements also resemble those from *In(H4250)* in that it is usually the Transposed segment that is lost. Duplications from five of the rearrangements have never been found to lose the Normal segment. One, *Dp(NM176)*, loses it rarely, as does *Dp(H4250)*. Only *Dp(NM103)* loses the Normal and Transposed segments with approximate equal frequency. No duplication has been found in which it is the Normal segment that is almost always lost. The preferential loss of the Transposed segment in duplications from seven out of eight rearrangements can hardly be due to chance. However, the limited data obtained with the Adiopodoumé and N193 instability factors suggest that such factors can change the proportions of loss of the two segments to some extent.

The total frequency of fertility seems to vary markedly for duplications from different rearrangements, but these have not been systematically compared

under identical conditions, nor has it been excluded that some of the observed differences are due to the slightly slower growth of some of the duplications.

Nontandem duplications have also been studied in *Aspergillus nidulans* (BAINBRIDGE and ROPER 1966; BALL 1967; NGA and ROPER 1968; CLUTTERBUCK 1970. See review by ROPER 1973). These resemble Neurospora duplications in being unstable, but they are not barren. In the best-studied Aspergillus system, it has been shown that the Transposed segment is lost preferentially (NGA and ROPER 1968). However, it is not at all clear that the situation in Aspergillus is comparable to that in Neurospora. It appears that in Aspergillus the loss is usually imprecise, leaving a small proximal piece of the Transposed segment which can occasionally participate in meiotic crossing over to regenerate the complete duplication (NGA and ROPER 1968; BAINBRIDGE and ROPER 1966).

The foregoing comparisons apply to complete or nearly complete deletions of a duplicate segment. Partial deletions and homozygosis cannot readily be detected in duplications from the other Neurospora rearrangements. However, such events can readily be detected as barren escaped DA's from Neurospora *Dp(H-4250)* and as visible sectors in the Aspergillus duplication studied by NGA and ROPER. Here we find that Aspergillus differs from Neurospora in having far more partial deletions that fail to uncover all markers, and far fewer cases that could be ascribed to homozygosis.

A possible mechanism for the precise deletions: We are faced with four problems: (1) Why do duplications, from almost all of the rearrangements studied, lose the Transposed segment of the duplication far more frequently than they lose the Normal segment? (2) Why does the magnitude of the inequity differ for duplications from different rearrangements? (3) How can the duplicate segment be deleted so precisely? (4) How can a terminal segment be completely deleted without leaving a broken end? All these problems could be solved if deletion of the duplicate segment resulted from mitotic crossing over between homologous material present both at a chromosome tip and at the break point. The following model was developed after an initial discussion with EDWARD BARRY and BARBARA TURNER.

We assume that many or all chromosome tips in Neurospora contain repetitive homologous DNA, like the 5S rDNA at the chromosome tips in Xenopus (PARDUE, BROWN and BIRNSTIEL 1973) or the Giemsa staining material at the chromosome tips in Allium (STACK and CLARKE 1973), and that this material is always oriented in the same direction relative to the tip. Rearrangements that produce viable terminal duplications are assumed to originate as two-break rearrangements having one break point within the repetitive terminal DNA, so that some of the repetitive material remains proximal to the break point. Crossing over between this material and the homologous material at the tip of either duplicate segment (or any other chromosome tip containing the repetitive DNA) could delete the Transposed segment and restore the normal chromosome sequence without leaving a broken end. One way of doing this is diagrammed in Figure 7 for *Dp(H4250)*; the mechanism works equally well for terminal duplications produced by quasiterminal translocations. The ease of deleting the dupli-

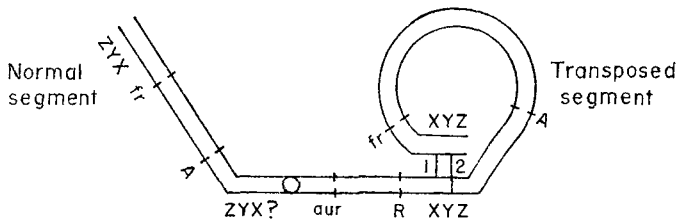


FIGURE 7.—Deletion of the transposed segment of *Dp(H4250)* by the crossover mechanism suggested in the DISCUSSION. (Similar considerations apply to duplications produced by quasi-terminal translocations.) The original inversion break points are indicated by angles. Marker loci are included only for orientation. Repetitive DNA is indicated by XYZ where the letters serve only to indicate direction; there are presumably many copies at each tip, and a smaller number of copies at the tip break point (near *R*). The crossover labeled "1" gives a normal chromosome and a triplication. The crossover labeled "2" gives a normal chromosome and an acentric ring. The transposed segment could also be deleted by a crossover between the repetitive DNA at the tip break point (near *R*) and the repetitive DNA at the tip of the normal segment, or at the tip of a nonhomologous chromosome.

The Normal segment could be deleted by a similar mechanism only if there is also properly oriented repetitive material, labeled ZYX?, at the nontip break point.

cate segment would probably depend on the amount of repetitive material remaining proximal to the break point and on other factors affecting the probability of crossing over, and should therefore vary for duplications from different rearrangements.

If the repetitive materal occurs only at chromosome tips when in the normal sequence, then the crossover mechanism could delete only the Transposed segment. Deletion of the Normal segment could not occur by this method unless some of the repetitive material were also located interstitially, at the nontip break point, labeled ZYX? in Figure 7. Thus the observations could be explained if *Dp(NM103)* had a large block of repetitive material at the nontip break point, if *Dp(NM176)* and *Dp(H4250)* had a small block of repetitive material there, and if the other kinds of Neurospora duplications had little or none. Alternatively, *Dp(NM176)* and *Dp(H4250)* might lose only the Transposed segment by the crossover mechanism, and their infrequent losses of the Normal segment might occur in other ways.

The repetitive DNA would have to be different from, and proximal to, the palindromic telomere sequence postulated by CAVALIER-SMITH (1974), since a rearrangement with a break in the telomere sequence should be lethal.

Mitotic crossing over has been shown to occur in Neurospora disomics (PITTINGER and COYLE 1963), and it has been shown by COYLE and PITTINGER (1965) and SMITH (1974) that almost all cultures produced by disomic ascospores contain at least a few recombinant nuclei. Thus (assuming an equally high crossover frequency in duplications) the frequency of mitotic crossing over might well be high enough to permit the loss of duplicate segments by the mechanism postulated here. [Mitotic crossing over also occurs in Aspergillus disomics (UPSHALL, cited by PARAG and PARAG 1975).]

If precise deletions really occur by mitotic crossing over involving chromosome tips, and if most of our barren escapes are really due to mitotic crossing over between the two duplicate segments, as seems most likely, then a factor that increases mitotic crossing over should speed the occurrence both of barren escapes and of precise deletions. This could explain much of the behavior of *mei-3*. This suggestion is supported by the fact that, in Aspergillus, a single mutation (*uvsB*) increases both mitotic crossing over and duplication instability (BURR, PALMER and ROPER 1971; JANSEN 1970; PARAG and PARAG 1975). However these published accounts do not say whether the duplicate segments were ever lost completely and precisely, as is the case with *mei-3* in Neurospora. We cannot make a direct test of the effect of *mei-3* on the frequency of mitotic crossing over in heteroallelic diploids, as is often done in yeast, Ustilago, and Aspergillus, because stable diploids cannot be isolated in Neurospora. However, it should soon be possible to do a comparable test by using heteroallelic duplication systems now being developed by E. G. BARRY and H. W. SHEW, and by D. D. PERKINS (personal communications).

The crossover model is proposed to explain only the precise deletion of whole duplicate segments. Presumably there are other mechanisms to explain the apparent cases of imprecise deletion, and the evidence suggesting that instability factors may change the relative frequencies of loss of the Normal and Transposed segments. The Adiopodoumé factor might affect one of these other processes.

Other instability factors: In addition to the factors described in this paper, other genes are known to affect duplication instability in Neurospora. SCHROEDER (1970, 1974, 1975) has shown that the ultraviolet-sensitive mutants *uvs-3* and *usv-6* markedly speed the escape of DA's, while *uvs-2*, *uvs-4*, and *uvs-5* do not. These mutants will be discussed elsewhere in connection with *mei-3*. MYLYK (1972) has reported a variant that delays the escape of Neurospora duplication *Dp(II→V)NM149* from the inhibition caused by heterozygosity for *het-c/het-c^{AD}*. In Aspergillus, changes in the stability of duplications have been reported to result from the *uvsB* mutants cited above, from a deletion, a translocation, and a factor on group VIII (PARAG and ROPER 1975; AZEVEDO 1975), and from the use of caffeine and trypan blue (ROPER, PALMER, and WATMOUGH 1972). These last authors have discussed the effects of caffeine and *uvs* mutants, in terms of failure to repair lesions in the DNA. Such a mechanism might account for the imprecise or partial deletions.

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