THE MANIFESTATION OF CHROMOSOME REARRANGEMENTS IN UNORDERED ASCI OF NEUROSPORA[1,2]

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ABSTRACT

Rapid, effective techniques have been developed for detecting and characterizing chromosome aberrations in Neurospora by visual inspection of ascospores and asci. Rearrangements that are detectable by the presence of deficient, nonblack ascospores in test crosses make up 5 to 10% of survivors after UV doses giving 10–55% survival. Over 135 rearrangements have been diagnosed by classifying unordered asci according to numbers of defective spores. (These include 15 originally identified or analyzed by other workers.) About 100 reciprocal translocations (RT's) have been confirmed and mapped genetically, involving all combinations of the seven chromosomes. Thirty-three other rearrangements generate viable nontandem duplications in meiosis. These consist of insertional translocations (IT's) (15 confirmed), and of rearrangements that involve a chromosome tip (10 translocations and 3 pericentric inversions). No inversion has been found that does not include the centromere. A reciprocal translocation was found within one population in nature. When pairs of RT's that involve the same two chromosome arms were intercrossed, viable duplications were produced if the breakpoints overlapped in such a way that pairing resembled that of insertional translocations (27 combinations).—The rapid analytical technique depends on the following. Deficiency ascospores are usually nonblack (W: "white") and inviable, while nondeficient ascospores, even those that include duplications, are black (B) and viable. Thus RT's typically produce 50% black spores, and IT's 75% black. Asci are shot spontaneously from ripe perithecia, and can be collected in large numbers as groups of eight ascospores representing unordered tetrads, which fall into five classes: 8B:0W; 6B:2W, 4B:4W, 2B:6B, 0B:8W. In isosequential crosses, 90–95% of tetrads are 8:0. When a rearrangement is heterozygous, the frequencies of tetrad classes are diagnostic of the type of rearrangement, and provide information also on the positions of break points. With RT's, 8:0 (alternate centromere segregation) = 0:8 (adjacent-1), 4:4's require interstitial crosssing over in a centromere-break point interval, and no 6:2's or 2:6's are expected. With IT's, duplications are viable, 8:0 = 4:4, 6:2's are from interstitial crossing over, 0:8's or 2:6's are rare. Tetrads from RT's that involve a chromosome tip resemble those from IT's, as do tetrads from intercrosses between partially overlapping RT's that involve identical chromosome arms.—Because viable duplications and other aneuploid derivatives regularly occur among the offspring of rearrangements such as insertional translocations, care must be taken in selecting stocks, and original strains should be kept for reference.

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THE first studies of chromosome rearrangements in fungi employed ordered asci, representing tetrads of meiotic products in which the number and position of visibly defective spores (containing deficiencies) were shown to provide information that is characteristic for the type of rearrangement. Theoretical expectations were compared with ascus-pattern data from two translocations in Neurospora (McCLINTOCK 1945). SINGLETON (1948) and ST. LAWRENCE (1952) used information of this type in their analysis of several Neurospora rearrangements. HESLOT (1958) deduced the nature of two rearrangements from patterns of aborted spores in ordered asci of Sordaria.

Subsequently, as Neurospora aberrations were studied genetically and cytologically in this laboratory, it was found that most of the needed information could be obtained more rapidly and easily with unordered tetrads, and on this basis quick, effective techniques were developed for detecting and analyzing rearrangements. First, these methods were tested using unordered asci from previously well known rearrangements. Having been so validated, they were then used to detect and analyze new arrangements, and to characterize more fully existing rearrangements found by other workers.

Unordered tetrads are readily obtained in Neurospora, as in many related fungi, because ascospores are ejected forcibly from asci as they mature, and these can readily be collected as unordered groups of eight ascospores, each representing a tetrad. When a rearrangement is heterozygous, the ascospores that contain deficiencies are included among those shot, even though they are recognizably defective. As will be shown, the frequencies of unordered tetrads that contain various numbers of white, deficient spores are diagnostic of the type of rearrangement and they provide information also as to the positions of break points relative to centromeres.

With most rearrangements, it is easier to obtain large unbiased samples of unordered asci than of ordered asci. Therefore, we have routinely used unordered asci for all preliminary analyses of rearrangements. (Ordered tetrad data have been obtained from intact asci only when some special item of information was needed.) An additional advantage of this approach is that the methods of analyzing unordered tetrads are generally applicable to all tetrad organisms, including the many where tetrads are naturally unordered as in yeast, Coprinus, Chlamydomonas and Sphaerocarpus.

The present paper outlines the theoretical basis for using unordered tetrads, describes the techniques, and gives results for representative rearrangements, including some of those previously studied by other workers. Brief preliminary accounts have been published (PERKINS 1966, 1967).

MATERIALS AND TECHNICAL METHODS

Reference strains: Standard wild types were 74-OR23-1A and 74-OR8-1a. The fluffy strains *fl*^{p}*A* and *fl*^{p}*a* were used routinely as testers for mating type (mt, *A*/*a*), fertility, and Aberration *vs.* Normal sequence. These highly fertile fluffy testers are isosequential with the wild types, and are convenient to use because no conidia are produced and ejected ascospores are thus observed more readily.

Source of rearrangements: Several hundred new rearrangements have been obtained from a

variety of sources. Nearly all have come from experiments not designed primarily to find aberrations. Some were first detected in routine crosses, which we have monitored routinely during 15 years for the presence of defective white ascospores that might indicate the presence of structural heterozygosity. Over 6,500 crosses have been made during this period. In some cases the rearrangement appeared to have arisen spontaneously in the cross where it was first detected. In other cases, a newly detected rearrangement proved to be present also in other related stocks, indicating that it may have arisen earlier in the pedigree, perhaps after exposure to a mutagen.

Stock cultures of mutant strains from Stanford (BEADLE and TATUM 1945) and other sources frequently contained aberrations. The largest single source of rearrangements, however, has been the survivors of filtration enrichment or of replica-plating, following UV irradiation, in experiments carried out for the primary purpose of obtaining point mutants (see Table 4). With relatively light UV doses (22–25% survival), approximately 8% of survivors in representative experiments gave rise to new rearrangements that could be detected by the procedure described below.

When this study began, several rearrangements that had been studied by other workers were available for testing the method: *T(I;II)4637* (MC CLINTOCK 1945; HAGERTY 1952; ST. LAWRENCE 1952; HOULAHAN, BEADLE and CALHOUN 1949), *T(I;VII)17084* (HOULAHAN, BEADLE and CALHOUN 1949), *T(IV;VI)45502* (MC CLINTOCK 1945; HOULAHAN, BEADLE and CALHOUN 1949; ST. LAWRENCE 1952), and *T(I→III)4540* (ST. LAWRENCE 1952, 1959). Other available strains which had been initially recognized as aberrant by other workers were examined further using the unordered tetrad methodology: *T(I;V)36703* (A. M. SRB, quoted by SINGLETON 1948), *T(VII→I)5936* (REGNERY 1947; SINGLETON 1948). Several other rearrangements were subsequently analyzed by others in this laboratory: *T(I;II;IV;IV→VII)S1229* (BARRY 1960a, b; originally shown to be aberrant by R. W. BARRATT and L. GARNJOBST); *T(I→V)S1325* (ST. LAWRENCE and SINGLETON 1963); *In(IL→IR)H4250* (NEWMEYER and TAYLOR 1967); *In(IL→IR)NM176, In(IL→IR)AR16* (TURNER *et al.* 1969); *T(I→VI)NM103* (B. C. TURNER, unpublished; *T(II→III)AR18, T(VI;VII)NM124, T(II→I)NM177* (A. KRUSZEWSKA, unpublished).

All mapped rearrangements have been deposited in the Fungal Genetics Stock Center and are listed by BARRATT and OGATA (1974) with limited documentation. Duplication-generating rearrangements were listed and described by PERKINS (1972a appendix).

Steps in identifying and characterizing rearrangements. Step 1: Scoring defectives among random ascospores. Heterozygous reciprocal translocations usually produce 50% defective, nonblack spores, due to deficiencies, while insertional translocations and other types of aberrations that generate viable duplications typically produce 25% nonblack, deficiency ascospores. Such crosses are clearly distinguished from structurally homozygous crosses, where about 95% of ascospores are viable and develop normal black pigment.

Each strain to be tested is crossed to a Normal-sequence tester strain. The tester, usually fluffy strain *fl^{p}A* or *fl^{p}a*, is grown 4 days (*A*) or 5 days (*a*) at 25° in 12 × 75 mm tubes containing Synthetic Cross medium (SC) with 2% sucrose and 2% agar (WESTERGAARD and MITCHELL 1947). Each fluffy culture is then fertilized with dry conidia from one of the strains being tested, using a stiff flat blade to spread conidia over the surface of the slant. The glass wall of the tube opposite the slant is wiped free of fluffy mycelia with a swipe of the blade, at the time of fertilization, in preparation for later observations.

Ten days after fertilization (25°), the glass wall of each tube is examined under 60× magnification with transmitted light from a frosted reflector, and the fraction of black *vs.* white spores is estimated. Tests with approximately 90–95% black spores are classed as Normal (isosequential). Cultures testing with significantly fewer than 90% black ascospores are saved as putative rearrangements.

STEP *2: Scoring unordered asci.* Each putative rearrangement strain which has been identified as described under step 1, is next tested by crossing it on a petri dish to the same Normal-sequence fluffy tester. This allows unordered asci to be collected readily in large numbers by the method of STRICKLAND (1960), with modifications as described below.

The fluffy tester is grown 6 to 7 days at 25° on a petri dish containing SC, then fertilized

with conidia of the culture to be tested, so as to cover thoroughly a central area 4 cm in diameter with the fertilizing inoculum. After fertilization, the plate is inverted and incubated 10 days at 25° in the dark.

Groups of eight ascospores are collected on a 4% agar–water slab (about 3 × 6 cm) placed on a microscope slide under the inverted cross plate. The agar should have a smooth surface, without bubbles or scratches. The collecting surface should be elevated to within 1 mm of the ostioles. This is conveniently done by using a stack of microscope slides (6 or 7 slides may be required if 10 × 100 mm glass petri dishes are used). Ascospore scatter is a function of distance from the ostiole to the collecting surface, and short distance is critical for obtaining closely spaced groups.

The collecting slab is exposed for a period ranging from a few seconds to several minutes, depending on the rate of shooting. Cross plates incubated in the dark shoot asci slowly when first brought into the light. The rate of projection accelerates for the next hour or two, becoming extremely rapid and then falling off to a low level. If plates are then returned to the dark for 24 hours, they are capable of shooting again.

After exposure, the agar collecting-slab is examined using a binocular dissecting microscope at about 40X magnification. A combination of incident and transmitted light is used, from two lamps whose relative intensity can be varied to achieve the balance desired. Defective, white or light brown ascospores can be seen with high contrast, or can be made nearly invisible, depending on the light intensities and angles. Failure to see all-white groups can result from too high a ratio of incident to transmitted light. At the other extreme, defectives might be confused with normal black ascospores.

The collecting slab is scanned and groups are punched into the agar with a short needle as they are scored and tallied. Asci are classed as to number of black and white spores, using the major classes 8:0, 6:2, 4:4, 2:6, 0:8 (Black:White). Only clear groups of eight ascospores are used, that are distinctly separated from other such groups and from odd groups or scattered spores. It is noted whether defective spores in 4:4 groups are all alike, or of two distinct types. Exceptional groups such as 5:3 or 3:5 are noted separately. The same agar slab may sometimes be used for repeated exposures, so long as it is not too cluttered. Figure 1 shows typical groups of ascospores.

Usually collection is continued until tetrads recorded in the major classes total about 100. With some rearrangements, day-to-day variability has been observed (see e.g., TURNER *et al.* 1969). Two or more smaller samples collected on successive days would therefore be preferable to a single large sample. In some cases, shooting still continues for more than a week, but the duration has not been determined systematically.

FIGURE 1.—Photographs of representative groups of ascospores illustrating the unordered-tetrad types expected from a heterozygous rearrangement. The spores were shot spontaneously from perithecia of crosses heterozygous for insertional translocation *T(II→VI)P2869*. Mean size of black ascospores is approximately 29 × 15 μm.

Step 3: Verification. The type of aberration and the position of break points relative to centromeres can be inferred from the frequencies of unordered ascus types as will be described in RESULTS. An appropriately marked tester stock is selected for each putative rearrangement on this basis. Tester strains have been developed specifically for this purpose (PERKINS 1972b, c). Verification crosses are made routinely in 150 mm tubes. Ascospores are isolated at random, and the resulting cultures are scored for markers. The isolates are then crossed to fluffy testers in small slants as described in Step 1, in order to score them for Rearrangement *vs.* Normal on the basis of the incidence of defective spores. Tests with no or few ascospores on the wall of the tube 10 days after fertilization are examined further. If perithecial beaks are absent or rudimentary, these are classed as Barren. (It is characteristic of many duplications in Neurospora that crosses involving them are Barren.) In this way, the rearrangement is confirmed, linkage groups are identified, break points are located, and it is determined whether some of the viable progeny are likely to contain duplications.

Cytological verification is usually more laborious than genetic verification, for technical reasons. Thus, cytological information complementary to the genetic information has usually been obtained only when there is some special reason for doing so.

Conventions and nomenclature: In specifying unordered ascus types, as, for example, 8:0 or 6:2, the number of black spores is given first. In contrast to the normal black ascospores, non-black deficiency spores will often be called white (W) for brevity and convenience, even though enough pigment may sometimes be formed so that they are actually light brown or grey.

Reciprocal translocations are symbolized as in Drosophila (LINDSEY and GRELL 1967). For example, *T(I;II)4637* is a reciprocal translocation involving interchange between linkage groups I and II.

A special symbol is used for insertional translocations and other rearrangements that produce viable duplication progeny when they are crossed by Normal sequence. For example, in the insertional translocation *T(I→II)39311*, the arrow signifies that a segment of linkage group I has been inserted into II, so that the duplication progeny are expected to possess the group I segment in two doses. In the quasi-terminal translocation *T(IR→VIR)NM103*, a large distal segment of IR has been interchanged with the right tip of VI, and duplication progeny which contain the IR segment in two doses are viable. Similarly, with pericentric inversion *In(IL→IR)H4250* in which one break is at the right tip of I, the arrow signifies that a long terminal segment of IL is interchanged with the IR tip (see Figure 9), and that crossover progeny which are duplicated for IL survive.

RESULTS

Asci from structurally homozygous crosses

Figure 2 shows the frequencies of unordered asci classified according to numbers of Black:White ascospores, from crosses homozygous for Normal sequence and for reciprocal and insertional translocations. In most asci, all eight ascospores are black and viable.

Asci from structurally heterozygous crosses

1. *Reciprocal translocations:* Asci from crosses heterozygous for typical reciprocal translocations show a distinctive distribution of the frequencies of ascus types (Figure 3). Asci having all eight spores defective (0 Black:8 White) are theoretically equal in probability to asci having all eight spores normal (8 Black: 0 White). Asci of the 4:4 type occur with a characteristic frequency for each translocation and may be rare or common, depending on the particular rearrangement.

The meiotic basis of these observations is simple and straightforward (Figure 4). Normal disjunction of the centromeres of the two chromosomes involved in

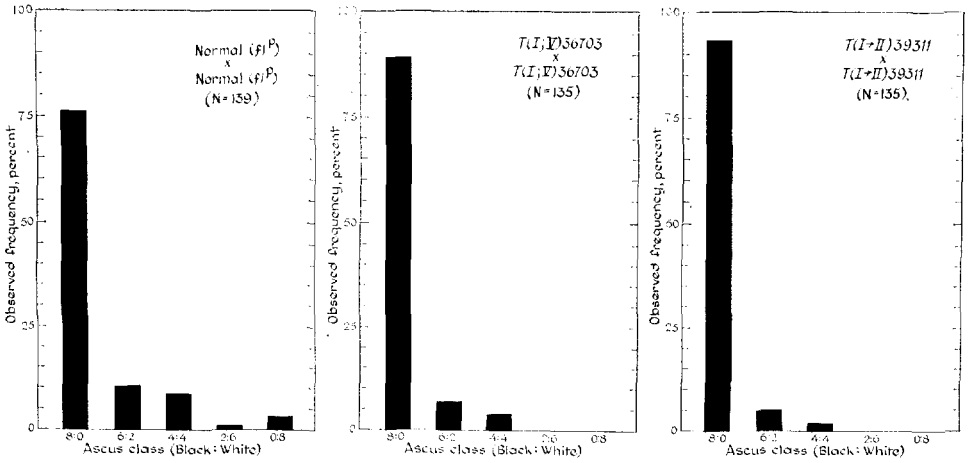

FIGURE 2.—Results of structurally homozygous crosses, showing the frequencies of unordered asci containing various numbers of defective ascospores, when (left to right) parental sequence is wild-type, reciprocal translocation, and insertional translocation. In this and succeeding figures, N is the observed number of asci on which the distribution is based. Asci with odd numbers of defective spores (5:3, 7:1 etc.) were rare, and are not included.

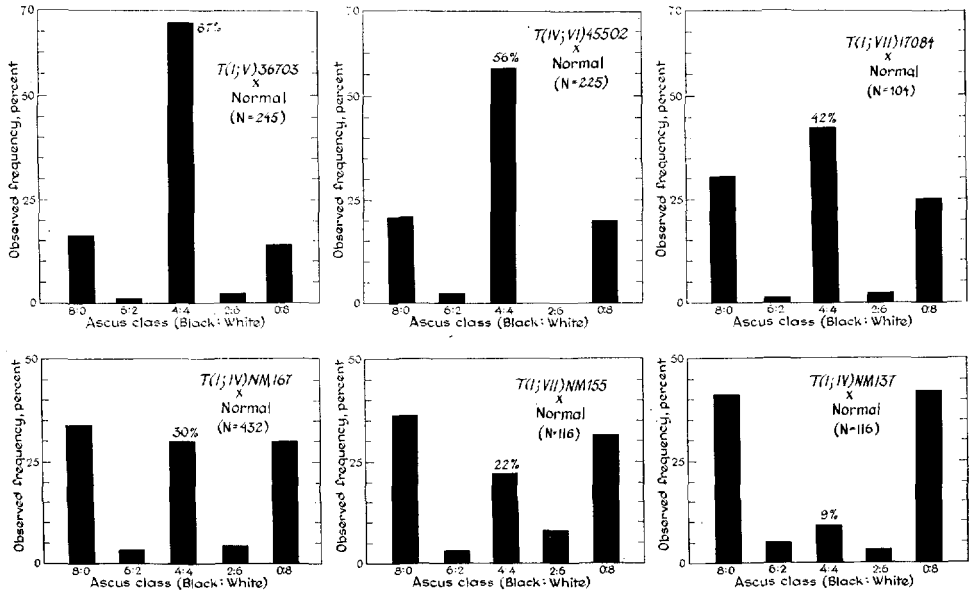

FIGURE 3.—Results of six crosses heterozygous for representative reciprocal translocations. Crosses are presented in decreasing order of the frequency of 4:4 asci, which result from crossing over between centromeres and break points. The Normal-sequence parent in each cross was one of the standard fluffy testers. Each translocation has been verified genetically, and break points have been mapped. The break points of *T(I;V)36703*, *T(IV;VI)45502*, and *T(I;VII)17084* have also been determined cytologically (BARRY 1967 and unpublished; BARRY and PERKINS 1969). Strain 36703 was first recognized as aberrant by A. M. SRB; 17084 and 45502 were recognized as aberrations by HOULAHAN, BEADLE and CALHOUN (1949); and 45502 was studied by MC CLINTOCK (1945) and ST. LAWRENCE (1952).

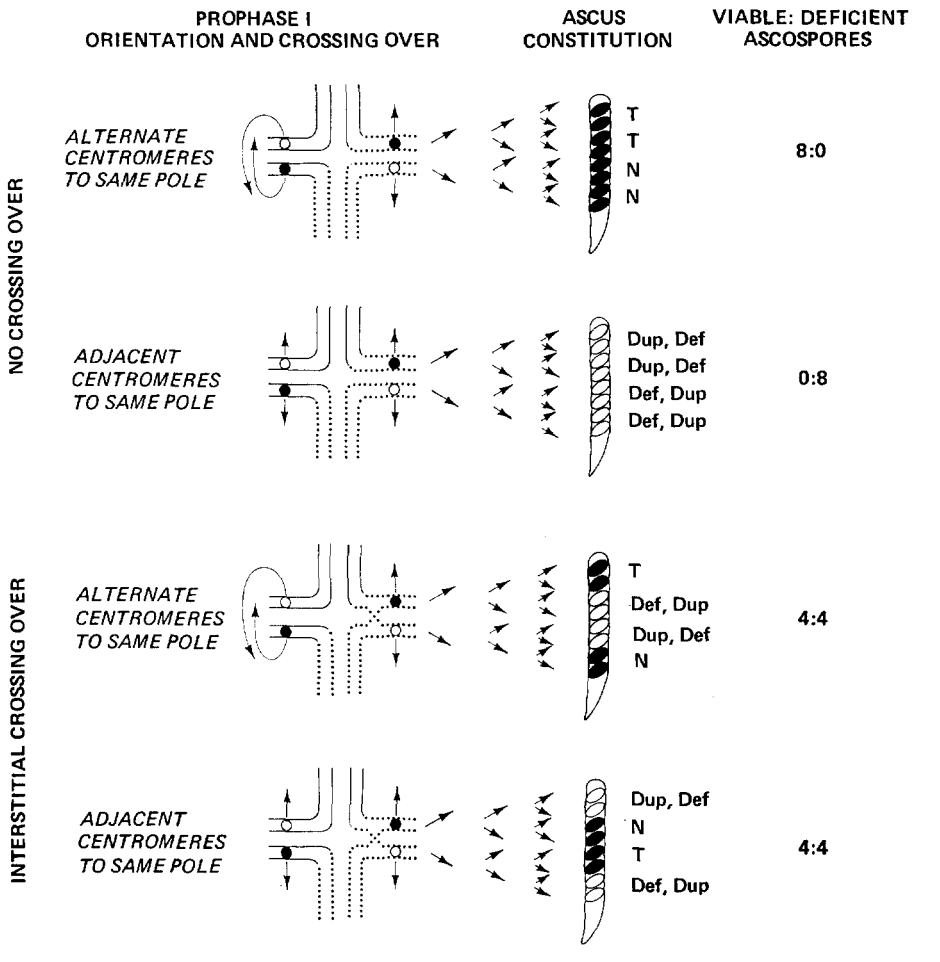

FIGURE 4.—The origin and constitution of asci containing various numbers of deficient spores, from crosses of a reciprocal translocation (black centromeres) × Normal sequence (white centromeres). Segments originally in one of the Normal chromosomes are shown as solid lines, those in the other Normal chromosome as dotted lines. The consequences of segregation without crossing over are shown in the two top diagrams. Crossing over between either break point and centromere is expected to produce 4:4 asci, as shown in the bottom two diagrams. The defective spores are of two types, representing complementary duplication-deficiency classes. These may or may not be recognizably different, depending on the particular translocation. If adjacent-2 segregations occurred (where homologous centromeres failed to disjoin), 0:8 asci would result, with all spores deficient (not shown in Figure).

an interchange is expected to result in 8:0 and 0:8 asci with equal probability, when there is no crossing over in the interstitial segments proximal to the two break points (top half of Figure 4). Occurrence of interstitial crossing over is expected to result in 4:4 asci, regardless of whether alternate or adjacent centromeres go to the same pole at Anaphase I (bottom half of Figure 4). It follows that 4:4 asci will be rare if interchange points are close to their respective centromeres, whereas 4:4's will be frequent if one or both break points are far out

in a chromosome arm. The break points of the six translocations in Figure 3 have all been mapped genetically, and their locations are consistent with these expectations.

2. *Insertional translocations:* Asci from crosses heterozygous for insertional translocations show a distribution of frequencies of ascus types that is distinctively different from reciprocal translocations. Few or no asci occur that have all eight spores defective, and 4:4 asci are approximately equal in frequency with 8:0's. Asci of the 6:2 type occur with a characteristic frequency for each translocation, and may be rare or common, depending on the particular rearrangement (Figure 5).

The rationale for this is shown in Figure 6. The duplication products generated meiotically by an insertional translocation are not deficient for another segment, and our experience is that ascospores containing the duplication are usually black and viable. With insertionals, normal centromere disjunction is expected to result in 8:0 and 4:4 asci with equal probability, when there is no interstitial crossing over (top half of Figure 6). Interstitial crossing over will produce 6:2 asci (bottom half of Figure 6). Thus 6:2 asci will be rare if the proximal break

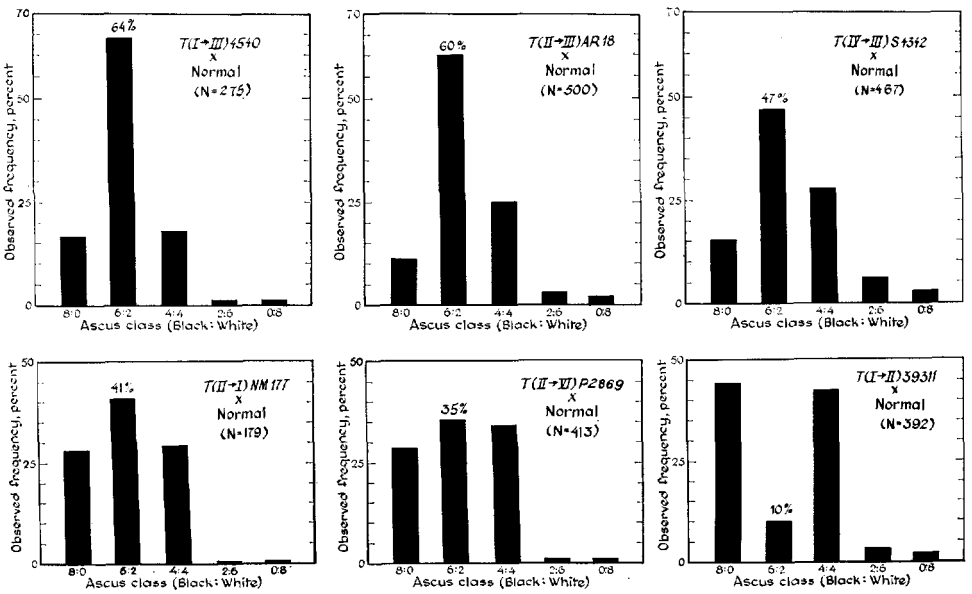

FIGURE 5.—Results of six crosses heterozygous for representative insertional translocations. Crosses are presented in decreasing order of the frequency of 6:2 asci, which result from crossing over between centromeres and break points. Each translocation has been verified genetically, break points have been mapped, and loci within the transposed segment have been identified by coverage of recessive alleles in heterozygous duplication progeny. The break points of *T(I→II) 39311* have also been determined cytologically (BARRY 1972). The insertions are inverted in 39311, S4342, and P2869. Insertions in 4540, AR18, and NM177 are probably too short for their orientation to be determined readily. *T(I→III)4540* was discovered, mapped, and identified as an insertional translocation by ST. LAWRENCE (1952, 1959). *T(II→III)AR18* was mapped by ANNA KRUSZEWSKA, who also obtained much of the data on *T(II→I)NM177*.

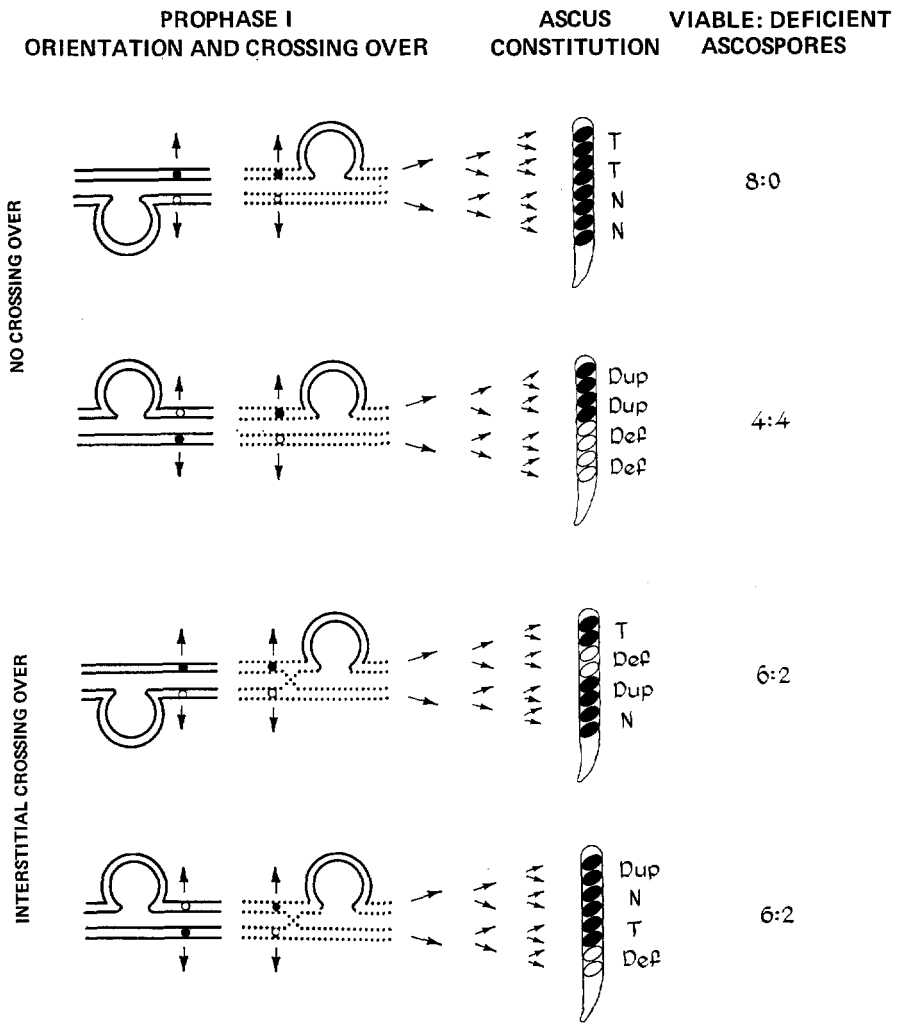

FIGURE 6.—The origin and constitution of asci containing various numbers of deficient spores, from crosses of an insertional translocation (black centromeres) × Normal (white centromeres). The consequences of segregation without crossing over are shown in the two top diagrams. Crossing over between either break point and centromere is expected to produce 6:2 asci, as shown in the bottom two diagrams. The defective spores are all identical, containing the same deficiency and no duplication. Pairing and crossing over between the translocated segment and its normal homolog are not shown in the diagram.

points are close to their respective centromeres, and frequent if one or both of the interstitial regions are long. The break points of the insertional translocations used as examples in Figure 5 have been mapped genetically, and their locations are consistent with the ascus distributions.

Some of the examples in Figure 5 show an unexplained excess of 4:4 over 8:0 asci. Two phenomena which might contribute preferentially to the 4:4 class are

nondisjunction leading to 3:1 segregations, or misscoring of potential 6:2's as 4:4's (if duplications darken more slowly than euploids).

In addition to the prophase I configurations shown in Figure 6, pairing and crossing over might also occur between the translocated inserted segment and its homolog, both of which are shown as unpaired loops in the figure. The consequences of such crossing over would differ for noninverted (eucentric) and inverted (dyscentric) insertions. With noninverted insertions, crossing over could result in viable duplications having chromosome segments interchanged that are distal to the insertion. This situation probably occurs but has not been demonstrated yet in Neurospora. Crossing over in the reverse-pairing loop of inverted insertional translocations will be considered later. Evidence will be given that this does not usually change the ratio of types among surviving asci. For a detailed study of an inverted insertional translocation, and for references to insertionals in other organisms, see PERKINS (1972a).

3. *Quasi-terminal rearrangements that produce viable nontandem duplications:* Insertional translocations are not the only rearrangement type that is capable of generating viable duplications as a result of meiotic recombination. Duplications can also be generated by rearrangements that involve a chromosome tip and are thus effectively unilateral (see e.g., BRINK and COOPER 1932; BURNHAM 1932; CLUTTERBUCK 1970; DUTRILLAUX *et al.* 1973; examples in MULLER and HERSKOWITZ 1954, WHITE 1973, and BURNHAM 1962). Figure 7 shows the results of

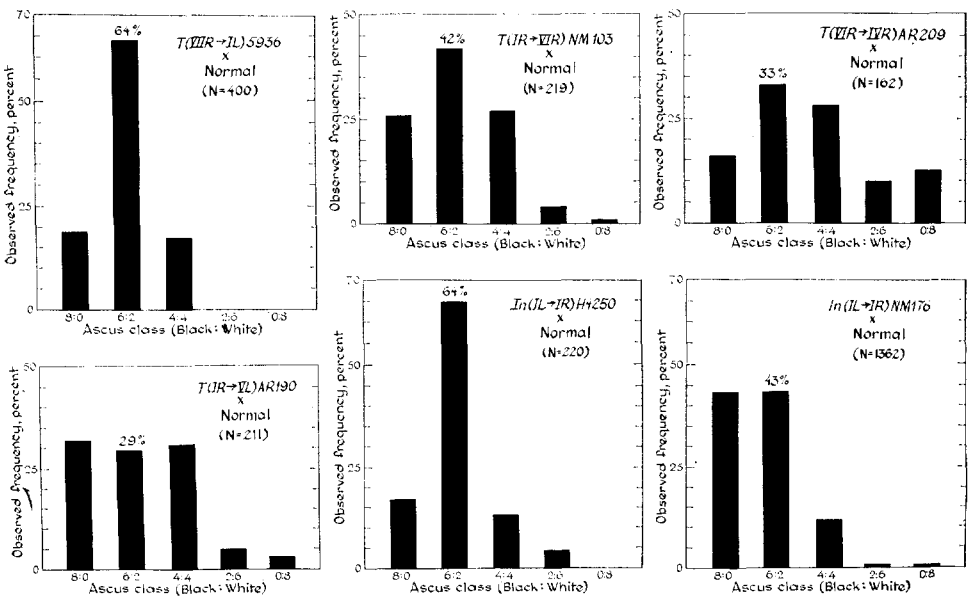

FIGURE 7.—Results of crosses heterozygous for rearrangements involving a chromosome tip. Four are examples of apparent reciprocal translocations which have one break point effectively terminal. The remaining examples are pericentric inversions in which a substantial segment of the left arm of I is transferred to the right tip. *T(VIIR→IL)5936* was recognized as aberrant by REGNERY (1947), and *In(IL→IR)H4250* by NEWMEYER. In this Figure, the data on H4250 are taken from NEWMEYER and TAYLOR (1967), and those on NM176 from TURNER *et al.* (1969).

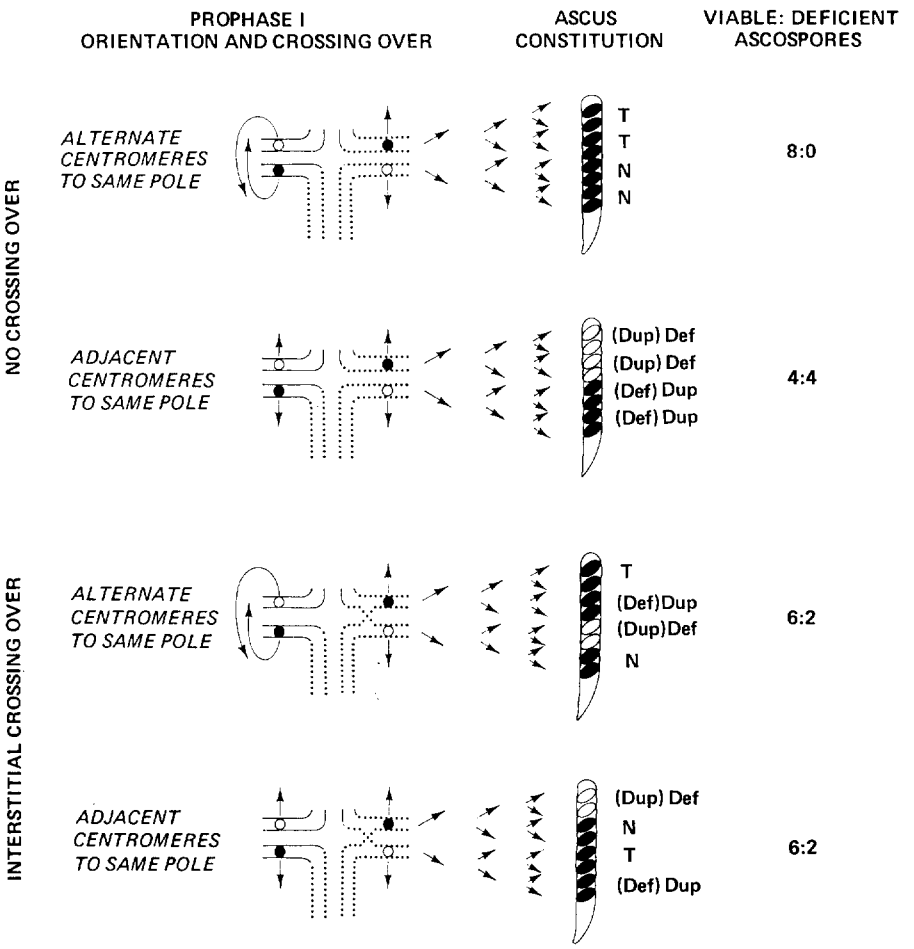

FIGURE 8.—The origin and constitution of asci containing various numbers of inviable spores, from crosses between Normal and a reciprocal translocation in which one break point is effectively terminal. Because one of the duplication-deficiency classes is viable, the asci resemble those from an insertional translocation (Figures 5, 6) rather than from an ordinary reciprocal translocation (Figures 3, 4).

crosses heterozygous for duplication-generating rearrangements of this type. Four of the examples involve reciprocal translocations and two involve pericentric inversions that have one break point effectively terminal. It is assumed that the break points are subterminal rather than strictly terminal, and that the tip is translocated, but there is no direct evidence for this assumption.

Distributions of ascus types from translocations of this type resemble those obtained from insertionals (Figures 5, 6) rather than from typical reciprocal translocations (Figures 3, 4). The rationale for this is diagrammed in Figure 8. The surviving duplication class is in reality a duplication-deficiency, but the missing material is the tip segment, which cannot be essential for survival.

The ascus distributions for tip–nontip pericentric inversions are more complex

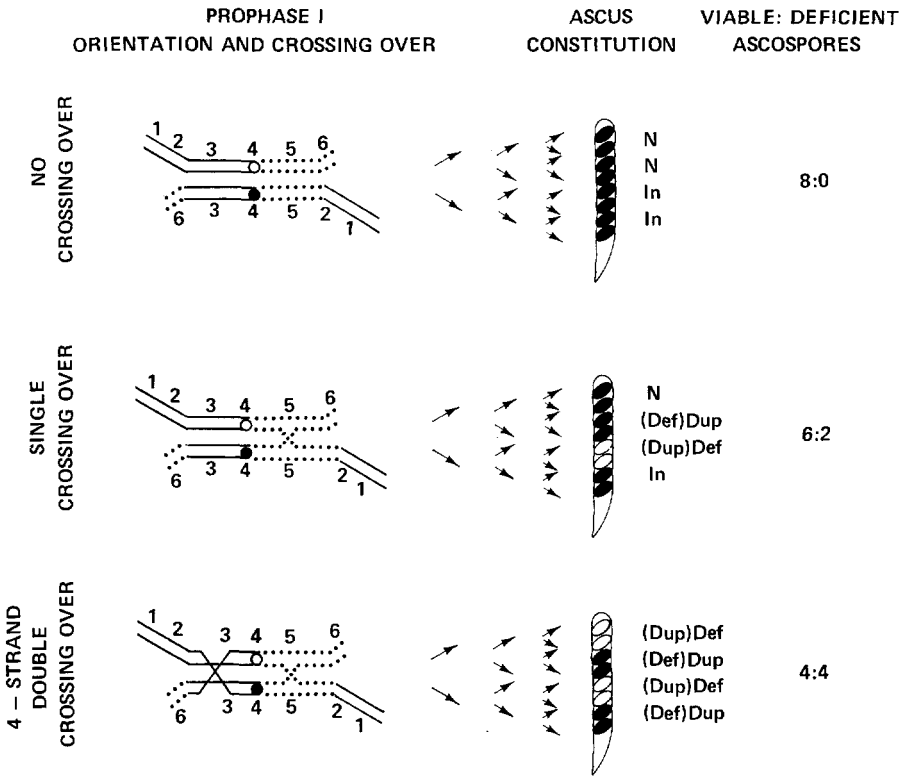

FIGURE 9.—The origin and constitution of asci from crosses of Normal sequence (white centromeres) × a pericentric inversion (black centromeres) in which one break point is effectively terminal. For clarity, terminal segments are shown unpaired rather than synapsed to form a typical inversion loop. Segments originally in the left arm are shown as solid lines, those in the right arm as dotted lines. Duplications and deficiencies occur only when crossing over occurs within the paired, inverted segment. Single exchanges and 3-strand doubles result in 6:2 asci, while 4-strand doubles result in 4:4 asci.

in their origin than those of the translocations, because all types other than 8:0's depend on crossing over within the long inverted segment. 6:2's are mainly the result of singles and 3-strand doubles, 4:4's of 4-strand doubles, and 8:0's result from noncrossovers and 2-strand doubles. The meiotic origin of duplications from inversions of this type is diagrammed in Figure 9. For data and a fuller discussion of theory regarding such inversions, see NEWMEYER and TAYLOR (1967) and TURNER *et al.* (1969).

It might be expected for within-chromosome duplication-generators that the ratio of 8:0's to 4:4's might vary for the same rearrangement, because of differences in crossing-over frequency between and within crosses, depending on genetic and environmental variables, including age. High variability has in fact been documented by TURNER *et al.* (1969) with *In(IL→IR)NM176*, both for different crosses and for asci shot from the same cross at different times.

Some apparently terminal rearrangements could in fact be insertions. When

the transposed piece is not inverted, and there are no distal markers in either the donor or the recipient chromosome arm, the distinction of interstitial from terminal becomes difficult. The two alternatives might even then be distinguished if there were appropriate markers within the transposed segment, because frequencies of complementary crossover products should be equal for a terminal but not for an insertional. It may not be possible to distinguish the alternatives by any of these methods. For simplicity, duplication-generating rearrangements in this situation are assumed to be terminal (two breaks required) rather than insertional (three breaks), until proved otherwise.

4. *Partially overlapping rearrangements:* Viable nontandem duplications can also be obtained from crosses between two reciprocal translocations whose break points involve the same two chromosome arms (BLAKESLEE, BERGNER and AVERY 1936; MULLER and PROKOFYEVA 1935; GOPINATH and BURNHAM 1956; HAGBERG 1962). A condition for the production of viable duplications is that the translocations must overlap so that each has one break point distal and one proximal, relative to the other, or one break point in common and the other break points in the same chromosome arm. If either of these conditions obtains, synapsis in the intercross between the two translocations (Figure 10) resembles that of an insertional translocation × Normal sequence, and one third of the viable progeny are duplicated for the segments between break points. As with insertional translocations, the Duplication progeny from partially overlapping reciprocals contain no deficiencies, and ascospores containing such duplications are viable and black.

When tetrads are examined from a Neurospora cross between two partially overlapping reciprocal translocations, the results (rightmost frames of Figure 11) resemble those from insertional translocations, as expected. 6:2 asci are produced as the result of interstitial crossing over, and 0:8 asci are absent. Twenty-seven such duplication-generating pairs of reciprocal translocations have already been identified in Neurospora (PERKINS 1971a and unpublished). KOWLES (1972) has reported similar results from intercrosses involving several pairs of reciprocal translocations in Neurospora.

With combinations of reciprocal translocations where one translocation has both break points proximal to those of the other, or where break points are in opposite arms of one of the shared chromosomes, all duplications are inviable because they contain deficiencies, and this is reflected in the distributions of ascus types in Table 1. These are qualitatively different from those of Figure 11.

Unordered ascus distributions can thus be used in favorable cases to determine the relative positions of break points of pairs of reciprocal translocations, and if a particular pair overlaps so as to produce duplications, the break points can be mapped precisely by testing recessive gene markers for coverage or noncoverage in the duplications, as in mapping by duplication coverage with insertional or quasi-terminal rearrangements (PERKINS *et al.* 1969).

Similar considerations apply to intercrosses between partially overlapping inversions (STURTEVANT and BEADLE 1936). Crossing over produces viable progeny that are duplicated for two segments, between the left break points and be-

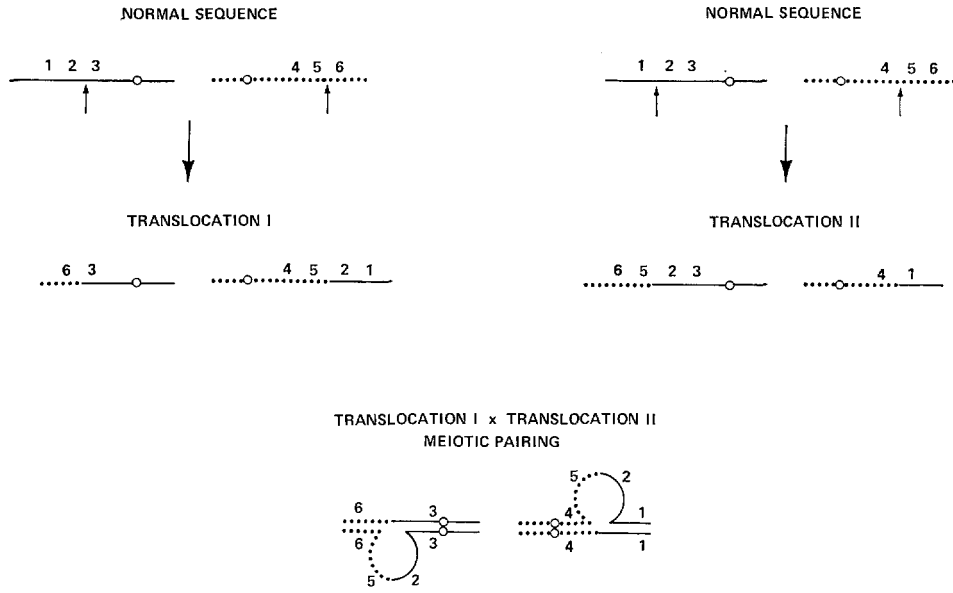

FIGURE 10.—The origin of two partially overlapping reciprocal translocations from the same standard sequence, and meiotic pairing when the two are intercrossed. Meiotic behavior in the intercross is expected to resemble that of an insertional translocation, as in Figure 6. 8:0 and 4:4 asci are equally likely in the absence of crossing over, while interstitial crossing over results in 6:2 asci.

TABLE 1

Unordered tetrad distributions from intercrosses that involve reciprocal translocations which have break points in the same two chromosomes, but which do not overlap in the manner required to produce viable duplications

Cross	Tetrad types*					No. of asci
	8:0	6:2	4:4	2:6	0:8	
T(IR;IIR)4637 × T(IR;IIL)AR216	26	0	44	3	27	261
T(IR;IVR)D304 × T(IR;IVR)NM164	52	2	5	1	40	176
T(I;IV)cut × T(IR;IVR)NM164	42	4	24	0	30	202
T(IR;IV)NM167 × T(IR;IVR)NM164	25	1	45	4	25	110
T(I;VII)S1007 × T(IR;VIIL)17084	55	1	2	0	42	151
T(II;IIIR)AR62 × T(II;III)36703b	41	0	14	4	42	81
T(IV;VII)NM156 × T(IV;VII)NM158	26	1	34	6	32	108

These distributions differ strikingly from those for the overlapping combinations illustrated in the two rightmost frames of Figure 11.

* Black:White.

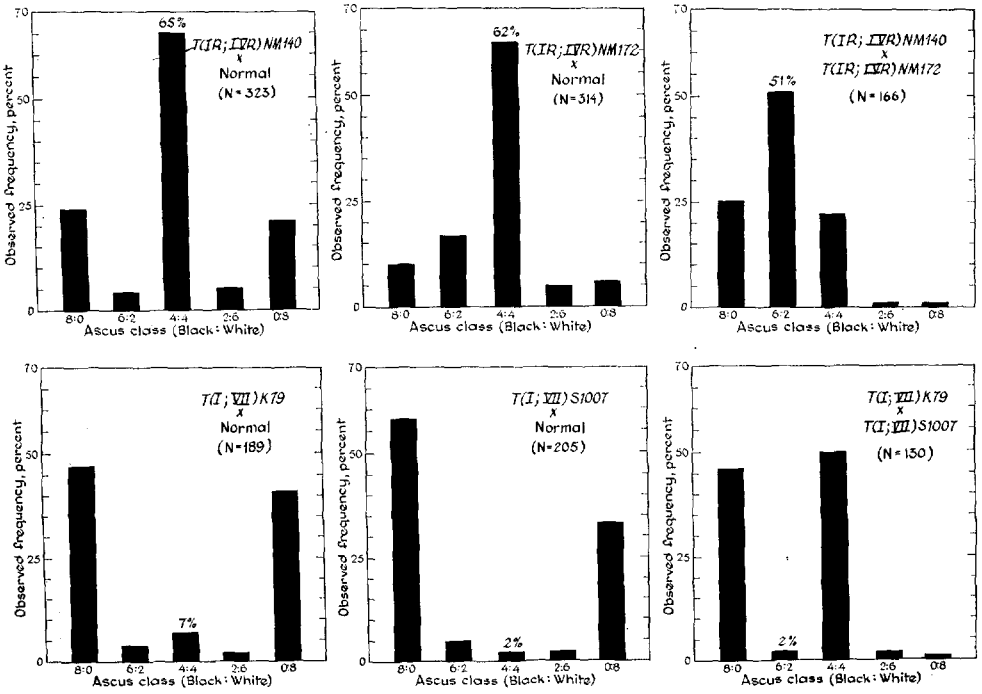

FIGURE 11.—Results of intercrossing reciprocal translocations that have overlapping break points in the same two chromosome arms, as in Figure 10. When each parent translocation is crossed to the Normal-sequence fluffy tester, it produces unordered asci that are typical of a simple reciprocal translocation (left and middle diagrams in each row). Intercrosses between the two translocations of each pair result in the frequency-distributions shown on the right, which are typical of duplication-producing combinations. The frequency of 6:2 asci in the intercrosses reflects the frequency of crossing over between centromeres and the most proximal break points of the two rearrangements involved. Thus, if 4:4 asci are infrequent when each parent is crossed individually × Normal as in the bottom row, then 6:2 asci must be infrequent in the intercross. But two overlapping reciprocals that exhibit high 4:4 frequencies as in the top row may or may not give a high 6:2 frequency when intercrossed, depending on whether one break point of each translocation is near a centromere. *T(I;VII)K79* was discovered and mapped by DR. N. E. MURRAY.

tween the right break points. An intercross between inversions *In(IL→IR)H4250* and *In(IL→IR)NM176* resulted in the ascus frequencies 18% 8B:0W, 64% 6:2, 16% 4:4, 1% 2:6, 0% 0:8 (N = 207). (These two inversions have one break in common at the right tip of I. Thus each of them also makes IL duplications when crossed singly to Normal sequence, as shown in Figure 7).

5. *Inversions that do not include the centromere:* No paracentric inversion has been found among the many Neurospora rearrangements which have been detected and analyzed by our present methods, although strains thought to be likely prospects have been investigated with special care. Speculation as to the reason for this failure will be found in the DISCUSSION, where the validity of reported cases of paracentric inversion in tetrad organisms is examined critically.

TABLE 2

Unordered tetrad distributions that illustrate deviations from the simple expectations predicted for rearrangements undergoing meiotic recombination

Parent genotype*	Ascus type† 8:0	6:2	4:4	2:6	0:8	No. of asci
a. *Gene-determined autonomous ascospore color (structurally homozygous crosses)*						
asco	2	4	94	0	1	128
cys-3	1	1	94	4	1	124
bs	0	8	92	0	0	72
ws-2‡	1	8	82	8	1	219
b. *Ascospore size* vs. *pigmentation*						
T(IV;VI)45502						
Size (Large:small) in unshot asci (McClintock 1945)	25	49	26	0	0§	776
Pigment, in shot asci (DDP)	21	2	56	0	20	225
c. *Inviable black ascospores (deficiencies that blacken)*‖						
T(VI:VII)ALS7	31	21	37	5	7	272
T(IV;VII)AR10	15	52	22	6	4	208
T(II:V)AR30	49	30	19	2	1	177
T(III;IV)T42M36	16	53	23	7	1	100
T(V;VII)AR45	37	25	34	2	2	92
T(I:II)NM168	32	23	35	3	6	99
T(IV:VII)STL384b	23	42	28	3	3	803
T(II:VII)T51M143	28	36	31	3	3	163
201, Sordaria macrospora (Heslot 1958)	13	66	21	0	0	692
d. *Spore-color effect associated with a break point*						
T(I;IV)NM139						
Brown spores classed as defective (incorrect)	0	0	22	53	25	271
Brown spores classed as viable (correct)	22	0	53	0	25	271
e. *Failure to recover 0:8 asci from heterozygous reciprocal translocations*						
T(I:V)ALS111	39	16	35	3	6	148
T(III;IV)AR211	55	5	33	2	5	677
T(I;V)P5166	23	2	70	2	3	230
f. *A gene-determined anomaly of meiosis (structurally homozygous cross)*						
mei-1 × *mei-1*	0	0	18	23	58	141
g. *Probable 3:1 segregations*						
T(I;VI)T51M138 (Excess 4:4's)	20	7	40	9	25	258
T(I;II)NM129 (Aneuploids)	29	5	9	6	51	661

* The second parent in all crosses was Normal sequence. In part a, the second parent was wild-type. Gene symbols: *asco:* ascospores slow to pigment. Allele of lysine-5. Isolation No. 37402. *cys-3:* cysteine-3 (requirement), NM27t. *bs:* brown spore, AR62. *ws-2:* white spore-2, NM122. *mei-1:* meiotic-1.

† Black:White unless size or viability is specified instead of color. Proportions are given in percent.

‡ *ws-2* was first mapped and shown not to be a translocation by Anna Kruszewska.

§ 93 additional asci were seen with tiny degenerate spores. These may be attributable to physiological abortion, or possibly to adjacent-2 segregation.

‖ If classified according to viability, these distributions would become approximately 31:0:21:0:37, 15:0:52:0:22, etc.

We also do not have any established example in Neurospora of an inversion which includes the centromere, other than those having one break at a chromosome tip, as described in NEWMEYER and TAYLOR (1967) and TURNER *et al.* (1969). There is no reason to think that simple pericentrics do not occur, and they should be detectable by present methods.

Complicating factors: sources of error or uncertainty: The examples given so far have conformed well to predictions based on the simplest assumptions. They are indeed typical of a majority of the rearrangements that have been recognized and subjected to analysis. Not all known rearrangements appear to fit as nicely, however. Several metabolic, developmental, or cytogenetic causes of exceptional behavior have been identified. The best understood of these will now be described.

1. *Gene-determined spore pigment differences in the absence of rearrangements.* In early stages of analysis it is possible to confuse spore-color mutants with rearrangements, since both produce defectively pigmented ascospores. However, the two possible origins can usually be distinguished when asci are examined, because all asci will be of the 4:4 type if a pigment gene is responsible which is expressed clearly and autonomously in the ascospores.

Ascus distributions for several spore-color mutants are given in Table 2a. With *cys-3* (MURRAY 1965) and probably *asco* (STADLER 1956), the autonomous pigment effect is apparently a pleiotropic expression of a nutritional requirement. Of the examples shown, only *ws-2* and *bs* originated from experiments where rearrangements were being sought. Ascospore-color mutants were not frequent enough to interfere significantly with efficiency of the method.

Heterozygous chromosome rearrangements could not generate distributions resembling those in Table 2a unless there was an invariant chiasma in one of the interstital regions between centromere and a break point. Such a pattern of interference has been reported among fungi only in *Podospora anserina* (RIZET and ENGLEMANN 1949; KUENEN 1962). 4:4's would not exceed 66.7% in the absence of chiasma interference. Interference of the intensity known in Neurospora might push this up to 80% (PERKINS 1962), but the highest 4:4 frequency yet observed for a Neurospora translocation is 67% for *T(I;V)36703* (Figure 3). There is thus usually no problem distinguishing clearly expressed spore-color genes from rearrangements on the basis of ascus patterns. If ambiguity exists, other criteria are rapidly available to make the distinction.

In practice, genotypes having slight or variable effects on the speed and intensity of pigmentation in euploid ascospores are more bothersome, and can sometimes interfere with the analysis of a rearrangement. Usually the genetic or physiological basis is unknown, but in a few cases the variable or cryptic defect can be attributed to specific loci. For example, with *pan-2*, *lys-5*, and certain *nic* and *cys* genes, pigment of ascospores containing the mutant allele may be pale or variable if heterozygous crosses are made on media containing low levels of the specific required supplement.

Survivors of treatment (in the dark) with the acridine mustard ICR-170 produce large numbers of white spores when crossed by Normal testers, but most of these are apparently not due to chromosome rearrangements (A. RADFORD, personal communication). DE SERRES and BROCKMAN (1968) have shown that ICR-170 administered in the dark fails to induce multilocus deletions. DE SERRES (personal communication) suggests that the white spores might be caused by recessive lethal mutations which are expressed in the ascospore.

2. *Ascospores with ambiguous or shifting pigmentation.* Expectations have all been expressed so far in terms of deficiency spores that are white, and duplication spores that are black. Ascospores do not always conform to these absolutes. Some rearrangements produce aneuploid classes of spores that are slightly pigmented, or that are not quite jet black. Manipulation of lighting can emphasize the distinction, and might even push the same intermediately pigmented class into the blacks or into the whites, depending on the balance between incident and transmitted light. These difficulties no doubt account for some of the minority classes or asymmetries in Figures 3, 5, and 7.

Time of observation may be important. A different kind of ambiguity may result when scoring is done too early, as exemplified by rearrangement *T(IV;VI)45502*. McCLINTOCK (1945) opened perithecia from *T(IV;VI)45502* × Normal at a stage when pigment was still undeveloped, and classified ascospores primarily on the basis of size (first line in Table 2b). On this basis, she suggested that *T(IV;VI)45502* was a quasi-terminal, base–tip translocation. When asci are allowed to shoot, and are then tallied according to pigmentation, the distribution is typical of an ordinary reciprocal translocation (second line, Table 2b). This is probably because the shot ascospores are more mature than those examined by McCLINTOCK, revealing through their failure to pigment that half of the normal sized spores are deficient. In the shot asci, white, defective spores consisted of two size classes—large and small, and both were represented equally in each 0:8 and 4:4 ascus. Genetic analysis shows that *T(IV;VI)45502* is in fact a simple reciprocal translocation having one break point near the centromere of Linkage Group IVR, and the other far out in the right arm of VI. No viable duplications have been found among progeny of *T(IV;VI)45502* × Normal. Cytologically, the break point in VI is definitely not terminal (BARRY, personal communication).

3. *Inviable black ascospores.* If one of the inviable duplication-deficiency classes from a reciprocal translocation developed to the point of forming black pigment, the resulting distribution of unordered ascus types would be visually identical to that of a rearrangement that produced viable duplications. One third of the black spores would fail to germinate, however.

Numerous rearrangements conform to this description (Table 2c). The ascospore distributions based on pigmentation resemble those of an insertional translocation, but based on viability they resemble a reciprocal translocation. Germination falls below two thirds when black ascospores are isolated, and no duplication progeny survive. Such strains have tentatively been classified as reciprocal translocations on the basis of viability rather than spore color.

Rearrangement 201 in *Sordaria macrospora* is another example having ascospore patterns that were interpretable as belonging to a duplication-generating rearrangement, until viability was determined (HESLOT 1958).

A possible alternative exists for examples such as these. They might in fact be insertional translocations in which the duplications were inviable even though they weren't structurally deficient. However, in our experience even very long duplications are viable in Neurospora, including those from *T(I→V)AR190*, where the longest arm of the entire compliment is duplicated in its entirety. Thus Neurospora resembles higher plants rather than Drosophila or man, where large duplications are usually inviable. Conceivably, in special cases a lethal combination of vegetable-incompatibility alleles might be present in heterozygous condition, and might kill the duplications even though they were intrinsically capable of surviving. However, when no viable duplications are found in many different crosses, this explanation becomes unlikely.

In other tetrad organisms where meiotic products are normally unpigmented, the diagnosis of rearrangements by tetrad analysis must necessarily depend on the numbers of viable and nonviable products in individual tetrads rather than on the visual classification that is so convenient in Neurospora. This is illustrated by the last three organisms in Table 6. The examples in Table 2c resemble them in requiring viability tests for reliable diagnosis.

4. *A spore-color effect associated with a rearrangement break point.* One example is known of a translocation inseparable from an autonomously expressed brown-spore trait. The brown spores of *T(I;IV)NM139* are fully viable, similar to those of a known point-mutant, *bs:* brown spore (with which NM139 is not allelic). Initially a visual classification of asci lumped the brown spores with whites as defective, with the anomalous distribution shown in Table 2d. Once it was recognized that the brown spores represent one of the viable parental classes, the ascus distribution was revised, and genetic analyses confirmed *T(I;IV)NM139* to be a typical reciprocal translocation. Cultures from the brown spores are fully viable and homozygous fertile.

5. *Differential survival and recovery of ascus classes.* A few reciprocal translocations are known wherein the 0:8 class is nearly or completely absent among asci that are shot from the perithecium (Table 2e). The mechanism of ascus propulsion is poorly understood. It would not be surprising if asci containing the most abnormal, deficient spores were at a ballistic disadvantage. Alternatively, spores in asci containing only deficiencies might disintegrate in these anomalous cases.

There is also evidence that asci which contain bridges and fragments are usually not shot, and that many such asci disappear in the course of development (PERKINS 1972a; BARRY 1972 and personal communication).

6. *Genes affecting meiosis.* Genes affecting pairing and disjunction would be expected to have profound effects on the frequency of aneuploid meiotic products (for examples see RHOADES and DEMPSEY 1966 in maize; SANDLER *et al.* 1968 in Drosophila; HESLOT 1958 in Sordaria; BRESCH, MÜLLER and EGEL 1968 in Schizosaccharomyces; ESPOSITO *et al.* 1970 in Saccharomyces; SIMONET and ZICKLER 1972 in Podospora). In Neurospora, a recessive gene *mei-1* (meiotic-1) has the effect of skewing the ascus-frequency distribution so that ascospores are predominantly white (Table 2f) and the few viable, black spores include many diploids and aneuploids (SMITH and PERKINS 1972; SMITH 1973).

7. *3:1 segregation in reciprocal translocations.* Nondisjunction of one but not both pairs of homologous centromeres at anaphase-I would result in 3:1 segregation from the complex of four chromosomes in an interchange heterozygote, giving a so-called tertiary disomic product having two chromosome segments in excess, together with the complementary deficiency product. UPSHALL and KÄFER (1974) have shown that disomics of this type occur regularly among progeny of translocation heterozygotes in Aspergillus.

Asci experiencing 3:1 segregation at anaphase-I would most likely fall in the 4B:4W class. Because the 4:4 class also includes asci that have undergone interstitial crossings over and normal disjunction, the occurrence of 3:1 segregation might well go undetected. Other types of evidence should serve to indicate its occurrence in favorable circumstances, however.

a) In a translocation where both breaks have been mapped near centromeres, an excess of 4:4's over the number expected from crossing over should be attributable to 3:1 segregations. The best established case of this type is *T(I;IV)T51M158*, which has break points that map very close to the two centromeres, so that interstitial crossing over should be rare. Nevertheless, 4:4 types make up 40% of all asci (Table 2g), and these are thought to arise from 3:1 segregations. As expected, the four white spores are usually alike.

b) Genes governing vegetative incompatibility produce a characteristic abnormal phenotype when they are heterozygous in the same nucleus. If one or more such genes are present in the interchanged chromosomes, they may signal the occurrence of 3:1 segregations. An example is provided by *T(I;II)NM129*, a reciprocal translocation having both break points near centromeres. In crosses heterozygous for the translocation and for alleles at the *het-c* locus in IIL, a few progeny are recognized to be heterozygous for mating-type (in IL), or for *het-c* alleles, or for both. These are attributed to 3:1 segregations, which are, however, too infrequent to be recognized from their contribution to the 4:4 class when unordered asci are tallied (Table 2g). The excess 0:8 asci suggest that adjacent-2 segregations may also occur.

8. *Adjacent-2 segregation.* Nondisjunction of both pairs of homologous centromeres would render all eight ascospores deficient in asci where it occurred, and would thus augment the 0B:8W class. Whereas 8B:0W and 0B:8W asci should be equally frequent in the absence of adjacent-2 segregation, the proportion would be one 8:0 to two 0:8 if adjacent-1, adjacent-2, and alternate types of segregation were equally probable.

In our experience, most reciprocal translocations do not have an excess of 0:8 asci, and so adjacent-2 segregation cannot be frequent. However, a few rearrangements have been found that show asymmetry in the direction expected from adjacent-2 segregation. There is no independent evidence for this explanation of the excess 0:8's, however.

Compound or complex rearrangements: The array of new arrangements recovered is by no means limited to simple 2-break or 3-break types, even after mild treatment with a mutagen such as ultraviolet light. If a new strain shows an excess of ascus types with more than four defective spores, one likely explanation is that it may be compound or complex. Table 3 gives representative unordered ascus distributions from crosses where complex rearrangements are heterozygous. Also included are ascus distributions from crosses heterozygous for two or more independently segregating rearrangements.

TABLE 3

Unordered tetrad distributions from crosses heterozygous for complex or compound rearrangements

Rearrangement parent	Ascus type* 8:0	6:2	4:4	2:6	0:8	No. of asci
Complex:						
T(III→[I;II])AR17†	16	11	41	17	15	184
T(I→[II;VII])AR217	46	10	13	5	27	166
T(VI→[I;III])Y16329	6	4	35	25	29	996
T(I;V;I or V→VII)AR173	41	8	14	7	31	212
T(I;II;IV;IV→VII)S1229‡	24	2	27	10	36	704
T(I→V)S1325§	5	0	71	3	21	223
Compound:‖						
T(I;IV)NM162; T(V;VI)NM162b	7	2	30	14	48	243
Components:						
T(I;IV)NM162	34	5	22	6	33	162
T(V;VI)NM162b	15	2	52	5	25	182
T(III;VII)NM169r; T(I→)NM169i	2	0	12	19	67	84
Components:						
T(III;VII)NM169r	34	4	30	1	31	143
T(I→)NM169i	29	54	10	6	1	124
T(II;VI)AR9r; In(IL→IR)AR9i	5	8	43	22	22	60
Components:						
T(II;VII)AR9r	18	0	68	3	11	238
In(IL→IR)AR9i	30	54	11	4	2	169
T(I;VII)S1007; T(V;VI)46802	12	0	27	5	56	211
Components:						
T(I;VII)S1007	58	5	2	2	33	205
T(V;VI)46802	12	2	64	4	18	107
T(I,II)4637; T(III;VI)1;						
T(IV;V)R2355 ("alcoy")	1	0	23	26	50	245
Components:						
T(I;II)4637	20	1	63	2	14	237
T(III;VI)1	15	4	55	7	19	223
T(IV;V)R2355	17	0	65	1	17	155

* Black:White. Proportions are given in percent.
† Rearrangement AR17 was analyzed by BARBARA C. TURNER.
‡ Data of BARRY (1960a).
§ Both cytological observations (E. G. BARRY, personal communication) and the absence of viable duplications suggest that S1325 involves not only the long insertion I→V, but also a small complementary insertion from V→I. If so, a more appropriate symbol would be *T(I⇄V)S1325*.
‖ Separable rearrangements that originated in the same strain are distinguished by adding a suffix such as *b*, *r*, or *i* to the isolation number.

The possibility of encountering a second, unexpected aberration is not limited to experiments where new rearrangements are originally being sought. On several occasions, unexpected complications in the behavior of a recognized rearrangement in later stages of analysis have been traced to a second, unrelated rearrangement that was introduced into the pedigree from an unsuspected source,

having been present in a marker stock. This difficulty can be avoided by careful monitoring of marker stocks for excess white spores, in crosses to fluffy testors. Unsuspected rearrangements have also been encountered that either arose anew in the cross where they were detected, or were present in heterokaryotic condition as a minority component of one of the parental cultures. In one extreme case, a cross thought to involve only a single complex translocation proved to contain three rearrangements—the original, *T(VI→[I;III])Y16329*; a reciprocal translocation present in the second parent which had not been monitored previous to use, *T(I;II)P5390*; and a third aberration that apparently originated during the cross, *T(II→VI)P2869*. Resolution into the three components was eventually accomplished. This required both tests for linkage with known gene markers, and tests for sequence by examining ascospores and asci from crosses with reference strains.

The incidence and relative frequencies of new rearrangements of various types: Table 4 lists six major sources of isolates that have been screened for aberrations in this laboratory, with information on mutagenic treatment and rearrangement frequency. The yield of detectable new rearrangements ranges from none with no treatment to 30% with X-rays and enrichment. KÄFER (1965) has shown that ionizing radiation is even more effective than UV in inducing rearrangements in Aspergillus.

In Table 5, known Neurospora rearrangements are classified according to type of aberration. The summary is not limited to rearrangements from the sources in Table 4.

It would be misleading to attribute excessive significance to the exact frequencies in Tables 4 and 5 because the purpose of the screenings was not to provide a quantitative measure of frequencies, but to recover representative new rearrangements for study. Selection of putative rearrangements has been subjective, and criteria for rejection have varied. The tables are intended only to give a general notion of frequencies that may be expected.

Phenotypic characteristics of the rearrangements: A majority of the rearrangements identified in Neurospora have vegetative phenotypes that are normal or nearly so. A few are inseparable from mutant phenotypes affecting morphology or nutritional requirements. Some of the mutant phenotypes are allelic with known point-mutations (*al-1*, *ad-3*, *arom*, *bis*, *cut*, *hist-3*, *inos*, *me-7*, *nic-2*, *pe*, *thi-1*). Others are not (*T(I;IV)NM139 bs*, *T(I;II;IV;IV→VII)S1229 arg*). Most but not all Neurospora rearrangements are fully fertile when homozygous.

Structural heterozygosity in nature: Natural populations of Neurospora have been sampled in numerous geographical areas to determine the feasibility of population studies and to examine variability within and between populations (PERKINS 1971b). Where possible, seven to ten different clones were collected from each site. Crosses have been made among conspecific strains collected at the same site, using the isolates from about 70 localities. One of these populations (Leuwi Malang, Java) contains *N. intermedia* individuals of two types, that appear to differ by a simple reciprocal translocation (Table 6).

TABLE 4

Frequency of detection and verification of new rearrangements from representative sources

Treatment and percent conidial survival	Strain of origin and source of isolates tested*	Isolates tested by examining random spores	Saved as putative rearrangements	Indicated to be rearrangements by means of unordered tetrads†	Break points mapped genetically	Estimated incidence of detectable rearrangements
1. UV + filtration-enrichment (22–55%)	Em a 1964 mutant hunts of N. E. MURRAY	1035	86	68 (of 86 tested)	57‡	5%
2. UV + filtration-enrichment (10–50%)	OR23–1A and others, 1967 mutant hunts of A. RADFORD	417	69	28 (of 38 tested)	28§	10%
3. UV + replica-plating (0.5–2%)	*rg cr* 1967 mutant hunts of A. L. SCHROEDER	406	95	..	6	..
4. UV + filtration-enrichment (10–50%)	ST74A INOUE and ISHIKAWA (1970)	50	13 (+1 Barren)‖	11	7	22%
5. X-rays + filtration-enrichment (70–80%)	ST74A INOUE and ISHIKAWA (1970)	10	3	3	3	30%
6. Untreated¶	*uvs-3*+	300	0	..	..	..
	uvs-3	291	4 (+41 Barren)‖	..	..	..

The experiments were designed to select particular mutant types: methionine (1), pyrimidine (2), UV-sensitive (3), and temperature-sensitive (4,5). In 1–3, nonmutant survivors were screened for aberrations. In 4 and 5, only the irreparable temperature-sensitive mutants were screened for aberrations.

* All tests for aberrations in 1–5 were carried out by PERKINS, using isolates from the sources indicated.

† Isolates were classed as not verified if ascus patterns by the Normal tester were Normal or nearly so, or if the defective ascospores could be attributed to genic defects in ripening or pigmentation of spores, or to genes affecting meiotic disjunction.

‡ One translocation was found twice, one three times, and one six times, among these isolates. The number of different rearrangements is thus 49.

§ The same inversion was found in four of these isolates. The number of different rearrangements is thus 25.

‖ Possible duplications. Not analyzed further.

¶ Data of SCHROEDER (1970).

DISCUSSION

Chromosome rearrangements in other haploid eukaryotes: The methods described here for Neurospora are directly applicable to any organism producing tetrads of meiotic products, where pigmentation or another recognizable trait is expressed autonomously in each product. Examples are given in Table 6. HESLOT (1958) using *Sordaria macrospora*, identified one clear reciprocal translocation, and a second rearrangement which he interpreted as an unequal translocation until viability was determined. In *Sordaria brevicollis*, strain ABW-1 has been interpreted as a paracentric inversion (AHMAD 1970). In other tetrad organisms

TABLE 5

Summary of aberration strains

I. *Reciprocal translocations*	
Confirmed genetically by mapping break points	103
Not mapped, but ordered ascus patterns indicate simple reciprocal translocation	21
Total reciprocals*	124
II. *Rearrangements that generate viable duplications*	
Confirmed genetically:	
Insertional translocations (simple)	10
Terminal pericentric inversions	3
Terminal translocations	10
Complex rearrangements (with insertions)	5
Genetic analysis incomplete	5
Total duplication-generators	33
III. *Complex rearrangements that do not generate viable duplications*†	4
IV. *Analysis suspended because unpromising*‡	~50

Fifteen rearrangements analyzed by other workers are included in this tabulation (see MATERIALS).

* In addition to the reciprocals listed, at least 50 strains are probably reciprocal translocations on the basis of producing 50% whites among random ascospores in tests by Normal. These have not been analyzed further. Since all strains that originally produced 75% white spores were followed up with ascus analyses, the proportion of reciprocals and duplication-generators shown in the table is too low, and 175:33 would be more nearly correct.—Where the same rearrangement was found in several isolates from an experiment, as happened several times, it is counted only once.

† One apparently reciprocally inserted insertional translocation and three multiple-group translocations are included. Multiple rearrangements that were resolvable into their simple components are not included under III. Instead, each component is listed separately in the appropriate category.

‡ Included are isolates that proved to be Normal sequence or nearly so, or from which a rearrangement was not recovered among progeny. Most of the strains in this category were originally classed as producing 75–85% black spores, or just less than 90%. In many of them, the nonblack ascospores may have been genic in origin: spores were often variable in color and frequency, darkening with age, and asci were difficult to classify, skewing toward 8:0. Only two exceptional strains (*bs* and *ws-2*) contained genes that were sufficiently clear-cut to be saved as autonomously expressed spore-color markers.

where it is impossible to distinguish inviable haploid products visually, a similar analysis can be done using inviability to identify the deficiency products. Rearrangements have been diagnosed in this way in Chlamydomonas by McBRIDE and GOWANS (1969), in yeast by McKEY (1967), and in Coprinus by BRYGOO (1972).

In *Aspergillus nidulans* ascus analysis is difficult, and other methods have been used to detect translocations, such as mitotic haploidization of marked diploids (KÄFER 1958) or a characteristic increased frequency of disomic progeny (UPSHALL and KÄFER 1974). Eight translocations were identified and described by KÄFER (1965), including a probable insertional studied by BAINBRIDGE (1970). A probable terminal translocation was described by CLUTTERBUCK (1970). Duplications from the former strain, and other duplications, have been studied by

TABLE 6

Unordered tetrad distributions from rearrangements in organisms other than Neurospora crassa

Cross	Tetrad type* 4:0	3:1	2:2	1:3	0:4	No. of tetrads
Neurospora intermedia						
P168 × P170†	18	0	67	0	14	338
Sordaria macrospora (Heslot 1958)						
T-1 × wild type	17	0	65	0	19	858
201 × wild type‡	13	66	21	0	0	692
(Esser and Straub 1958)						
cr × wild type	10	0	73–75	0	15–17	. .
Sordaria brevicollis (Ahmad 1970)						
ABW-1 × wild type	55	0	39	0	6	519
Chlamydomonas eugametos (McBride and Gowans 1969)						
wild type × wild type	98	1	1	0	0	95
T1 × wild type	55	6	4	0	35	78
T5 × wild type	30	5	34	2	29	86
T4 × wild type	20	3	50	6	21	211
T2 × wild type	16	3	55	11	15	174
Saccharomyces cerevisiae (McKey 1967)						
1375 × wild type	23	3	54	5	15	483
1375 × 1375	89	7	5	0	0	42
Coprinus radiatus (Brygoo 1972)						
wild type × nic_3 B_2 (Normal)	44	3	0	1	0	48
wild type × nic_3 B_1 (Translocation)	26	2	33	3	14	78

* Black:White or Viable:Inviable. Proportions are given in percent. In *N. intermedia* and Sordaria, asci are 8-spored and each chromatid of the tetrad is represented by two identical sister spores.

† This is an intercross between isolates of two structural types found in a population collected from burnt grass at Leuwi Malang, near Bogor, Indonesia.

‡ The distribution shown is based on spore color. If classified according to viability, the distribution becomes 13:0:66:0:21 (Heslot 1958, page 88).

Roper and his co-workers (see, for example, Nga and Roper 1969). Tector and Käfer (1962) showed that new aberrations occur in Aspergillus with an alarmingly high frequency following exposure to ionizing radiation.

A rationale for our failure to detect paracentric inversions. No paracentric inversion has been found during this study. It is difficult to believe that Neurospora differs so much from other eukaryotes that paracentric inversions fail to occur while other types of rearrangements are common. Apparently paracentrics are not detected by our screening procedure, which depends upon the continued development of those asci that contain defective products.

A clue is perhaps provided by inverted insertional translocations. These resemble paracentric inversions in that crossing over between the inverted inserted segment and its normally placed homolog produces dicentric bridges, which have been demonstrated cytologically (St. Lawrence and Singleton 1963; Barry 1972). There is evidence that such bridges are usually lethal for the

entire ascus in which they occur (PERKINS 1972a, pp. 42, 44). If dicentric bridges from inversions behave in a similar way, then heterozygous paracentric inversions are expected to have no or little visible effect on the appearance of surviving asci. Visual inspection of mature asci would then fail completely to distinguish paracentric inversion heterozygotes from structurally normal homozygotes.

If this view is correct, cryptic paracentric inversions may in fact be common, and may be present in many apparently normal Neurospora strains where their presence has been unsuspected. Their detection will require methods that do not depend on the maturation of asci with defective spores. Discovery of a *bona fide* paracentric inversion in fungi and proof of its structure, would provide a much needed model.

A critique of the evidence for paracentric inversions in fungi. There have been three reports suggesting paracentric inversions in Neurospora. ST. LAWRENCE and SINGLETON (1963), showed that the behavior of strain S1325 in heterozygous condition was in many respects that expected of a paracentric inversion. Genetically, crossing over was drastically reduced in a long segment of IR between *nic-2* and *lys-3*, in crosses heterozygous for the rearrangement. Only 2-strand double crossovers were recovered within the segment. No viable duplication progeny were found. Cytologically, a bridge was frequently seen at anaphase I, and a fragment or fragments at interphase II or III. The only detail that did not conform to simple exceptions was an excess of anaphase-II bridges.

Results with the rearrangement in homozygous condition were not consistent with a paracentric inversion, however, and it was shown that the IR segment in S1325 is inserted in inverted order into VR (MURRAY 1968; BARRY and PERKINS 1969). The critical demonstration employed multiple markers both inside and outside the insertion, in crosses homozygous for the rearrangement. The inserted IR markers were linked in V and segregated independently of other markers of linkage group I. Chromosome pairing at pachytene confirmed the genetic evidence.

If S1325 is an inverted insertional translocation, frequent anaphase-II bridges are no longer unexpected (BARRY 1972). Only the absence of viable duplications is atypical of an insertional translocation, and this is reflected in the atypical ascospore patterns in unordered tetrads (Table 3). A possible explanation is given by unpublished cytological observations of BARRY, which suggest that a short interstitial piece of VR may have been inserted into IR, simultaneously with the I→V insertion. If the reciprocally inserted segment contains one or more essential genes, all products containing a duplication would also simultaneously contain a lethal deficiency.

It is clear from the results with *T(I→V)S1325* that suppression of crossing over and the occurrence of bridges and fragments in structurally heterozygous crosses are in themselves an insufficient basis for establishing that a paracentric inversion is involved. Any long, inverted insertional translocation also possesses these properties. Insertional translocations are relatively common in Neurospora (see Table 10), and as expected, about half of them are inverted relative to the centromere, judging from the production of bridges and fragments in meiosis. The most convincing genetic proof that an aberrant strain contains a paracentric inversion rather than an inverted insertional translocation would come from homozygous inversion-sequence crosses showing that the genes whose order is inverted occupy map positions between noninverted flanking markers in the original linkage group. A clear reverse-pairing inversion loop at pachytene would provide convincing cytological evidence, but anaphase bridges and acentric fragments are not in themselves adequate for distinguishing between the alternatives.

Another rearranged Neurospora strain, Y112M15, was briefly described by GRIFFITHS (1970) to have the same genetic characteristics that led ST. LAWRENCE and SINGLETON to suggest originally that *T(I→V)S1325* was a paracentric inversion. Subsequent tests showed, however, that two linkage groups were involved (GRIFFITHS, personal communication).

The remaining report of a possible paracentric inversion in Neurospora was by RIFAAT (1958), who suggested an inversion on the basis of a seemingly reversed order in two 3-point

crosses involving intervals of grossly dissimiliar length. Inasmuch as crossover suppression is expected rather than an inverted gene order when a paracentric inversion is heterozygous, and the results can be explained in terms of frequent double crossovers in normal sequence, it seems unnecessary to invoke an inversion. There was no suggestion of an abnormal sequence when shorter intervals were marked.

Among other fungi, an aberration that has been called a paracentric inversion has been reported in *Sordaria brevicollis* (AHMAD 1970, cited by AHMAD, BOND and WHITEHOUSE 1972). Ascus patterns were as shown in Table 6. The aberration was tested for recombination with single markers on each of the seven linkage groups, and showed linkage to only one of them. Meiotic bridges were reported. Both parental types were usually found among the viable spores of 4B:4W asci from crosses with the linked marker, showing that the rearrangement is not an insertional translocation. It is thus possible that this Sordaria rearrangement is a paracentric inversion. However, because only one marker on each linkage group was tested, the possibility cannot be ruled out that it is a pericentric or a reciprocal translocation in which the 0B:8W asci don't survive, as with the examples in Table 2e. Meiotic bridges are not in themselves reliable evidence for a paracentric inversion or inverted insertional translocation, because spontaneous chromosome breakage may occur during meiosis (LEWIS and JOHN 1966).

Paracentric inversions have sometimes been suggested to explain anomalous linkage results in other fungi (e.g., DAY and ANDERSON 1961 in Coprinus), but no serious attempt has been made to test the hypothesis. Theoretical expectations for asci from heterogygous paracentric inversions were derived by HESLOT (1958, p. 85) on the assumption that asci containing a dicentric bridge survive and form 4:4 asci. The Sordaria rearrangements actually analyzed by HESLOT both proved to be translocations, however.

Autonomy in ascus differentiation. It is remarkable that the presence of aneuploid meiotic products does not usually prevent ascospores from being formed or asci from being shot. Ascospore differentiation usually proceeds on schedule until after spore-wall formation even though the spores contain large deficiencies. (This may be attributed to the absence of cross-walls in the developing ascus, so that all meiotic products share the same pool of genetic information. It could reflect also an autonomous role of organelles such as centriolar plaques.) Clearly, the spore-differentiating apparatus in the Neurospora ascus is remarkably well regulated and independent of the chromosome content of the individual meiotic products. In extreme cases, eight ascospores may be cut out normally even when some of the spores are completely devoid of chromosomal material (P. ST. LAWRENCE, E. G. BARRY, personal communication).

Aneuploid derivatives. Care must be taken to assure that progeny selected as rearrangement stocks represent the original, euploid rearrangement sequence, rather than an aneuploid derivative. This is especially important in the case of insertional translocations and other duplication-generating rearrangements, but even simple reciprocal translocations may undergo 3:1 segregation so as to produce tertiary disomics. It is especially important to test for aneuploidy when rare recombinants have been selected, as when attempting to introduce closely linked markers into the rearrangement sequence.

With insertional translocations, potential aneuploidy will differ depending on whether the insertion is inverted with respect to centromere. With noninverted insertions, crossing over between the insertion and its homologous segment in normal sequence generates an entirely new aneuploid configuration which may be viable.

Duplications from some rearrangements may be cryptic or unstable. Duplications from rearrangements that involve a tip are capable of undergoing somatic breakage so as to remove one of the duplicated terminal segments and simulate one of the euploid parents (PERKINS, NEWMEYER and TURNER 1972). Such breakage does not always coincide precisely with the original break point, however.

The possibility that meiotic derivatives of the original rearrangement are aneuploids can best be examined by a back cross to the original rearrangement strain, or to a *bona fide* derivative that is known to be isosequential with the original. Aneuploidy is indicated in most cases by reduced fertility, poor ascospore production, and/or the presence of excess defective, white spores.

Because of the possibility of encountering aneuploidy, it is important that original strains of each rearrangement be kept in stock permanently, to serve as references.

Uses of ordered tetrads. I have already described the advantages of unordered tetrads (compared to ordered) for analyzing rearrangements. With point mutants, the chief advantage of ordered tetrads is to provide information on centromere distances. With rearrangements such as reciprocal translocations, however, this advantage disappears, because centromere-break point distances can be obtained directly from unordered tetrads.

Some special applications remain for which ordered asci are necessary and useful, however. For example, ordered tetrad data would enable the following predictions to be tested:

a) Arrangement of black (B) and white (W) spores in 4:4 asci should be distinctively different for insertional translocations than for reciprocals. With insertionals, ordered 4:4's are expected to be predominantly of the types B B W W or W W B B, and 4:4 orders other than these would result only from one fourth of double exchanges in the two interstitial regions. In contrast, most of the 4:4 asci from ordinary reciprocal translocations are expected to be of arrangement types other than B B W W or W W B B, which would result only from one half of double exchanges in the two interstitial regions.

b) In 6:2 asci from duplication-generating rearrangements, the duplication product should usually be located in the half of the ascus that does not contain white spores.

c) If noncrossover products survive when a paracentric inversion is heterozygous, an excess of B W W B over W B B W would suggest a chromatid-tie mechanism comparable to that found with paracentric inversion heterozygotes in female Diptera (STURTEVANT and BEADLE 1936; CARSON 1946) and in plant megasporogenesis (MCCLINTOCK 1938; DARLINGTON and LA COUR 1941).

Of the three predictions, (a) is well documented for insertional translocation *T(I→II)33911* (PERKINS 1972a) compared to a reciprocal translocation such as T-1 in Sordaria (HESLOT 1958). Prediction (b) was confirmed by NEWMEYER and TAYLOR (1967) for *In(IL→IR)H4250*. Rearrangements with 4:4 asci conforming to (c) have been sought unsuccessfully in this laboratory as a possible way to identify paracentric inversions.

With a typical new rearrangement it seems most efficient to begin using random shot spores, to proceed next using unordered asci for all the information they will give, and finally to use ordered tetrads only if some special need is seen to exist.

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