AN INSERTIONAL TRANSLOCATION IN NEUROSPORA THAT GENERATES DUPLICATIONS HETEROZYGOUS FOR MATING TYPE[1,2]

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ABSTRACT

In strain *T(I→II)39311* a long interstitial segment is transposed from IL to IIR, where it is inserted in reversed order with respect to the centromere. In crosses of T × T essentially all asci have eight viable, black spores, and all progeny are phenotypically normal. When *T(I→II)39311* is crossed by Normal sequence (N), the expected duplication class is viable while the corresponding deficiency is lethal; 44% of the asci have 8 Black (viable) spores and 0 White (inviable) spores, 41% have 4 Black : 4 White, and 10% have 6 Black : 2 White. These are the ascus types expected from normal centromere disjunction without crossing over (8B:0W and 4B:4W equally probable), and with crossing over between centromere and break point (6B:2W). On germination, 8B:0W asci give rise to only parental types—4 T and 4 N; 4B:4W asci usually give four duplication (Dup) progeny; and 6B:2W asci usually give 2 T, 2 N, 2 Dup. Thus one third of all viable, black ascospores contain duplications.—Recessive markers in the donor chromosome which contributes the translocated segment can be mapped by duplication coverage. Ratios of 2 Dominant : 1 Recessive *vs.* 1 Dominant : 2 Recessive distinguish location in or outside the transposed segment. Eleven loci including mating type have been shown to lie within the segment, and markers at four loci have been transferred into the segment by meiotic recombination. The frequency of marker transfer indicates that the inserted segment usually pairs with its homologue. Ascus types that would result from single exchanges within the insertion are infrequent, as expected if asci containing dicentric bridges usually do not survive.—Duplication ascospores germinate to produce distinctive inhibited colonies. Later these "escape" to grow like wild type, and genes that were initially heterozygous in the duplication segregate when escape occurs. As with duplications from pericentric inversion *In(IL→IR)H4250* (NEWMEYER and TAYLOR 1967), the initial inhibition is attributed to mating-type heterozygosity, and escape to a somatic event that makes mating type homo- or hemizygous.—Twenty additional duplication-generating Neurospora rearrangements are listed and described briefly in an Appendix.

COMPARED to reciprocal translocations, reports of insertional translocations are relatively rare. One insertional translocation has been described in maize (RHOADES 1968), one in the mouse (OHNO and CATTENACH 1962; EICHER 1967;

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CATTENACH and ISAACSON 1967), and one in Aspergillus (BAINBRIDGE 1970). Fewer than 10% of the 800 Drosophila translocations listed by LINDSLEY and GRELL (1967) involve insertions.

Insertional translocations appear to be relatively frequent in the fungus *Neurospora crassa*, where insertionals can be distinguished from reciprocals by simple visual examination of patterns of aborted ascospores. This suggests that insertional translocations may also occur frequently in other eukaryotic organisms, and that the scarcity of recorded examples in species such as maize may reflect difficulties in recognition and recovery rather than failure of occurrence.

Insertional translocations possesses a number of interesting properties, including the recurrent production of viable duplications in crosses between the aberration and normal sequence. The unbalanced duplications and deficiencies produced by insertionals can have marked phenotypic effects. It has been suggested that several abnormal syndromes in man may owe their genesis to insertional translocations (PATAU *et al.* 1961; LEJEUNE and BERGER 1965; BLOOM and GERALD 1968).

Historically, the first translocation ever discovered proved to be an insertional (Pale, *T(2;3)P* in Drosophila—BRIDGES 1923). The possibility of a side-branched chromosome was not excluded for the Pale translocation for many years, until linear insertion was proved by polytene analysis (KOSSIKOV and MULLER 1935; BRIDGES 1935). Other early examples of insertional translocations were described by OLIVER (1930), MULLER and STONE (1930), DOBZHANSKY (1930) and RHOADES (1931), and MULLER (1935). DEMEREC (1940) and SUTTON (1940) published genetic and cytological studies on insertional translocations which were used to study position effects. Duplications and deficiencies from insertional translocations of Drosophila have been valuable for studies of gene action, position effect, recombination, and genetic fine-structure (RATTY 1954; LEFEVRE and MOORE 1968; WELSHONS 1965).

Although these and other Drosophila insertionals have had many useful applications (summarized for Pale by MULLER 1967), there are aspects of insertionals that cannot be studied as effectively in Drosophila as in Neurospora. Among the advantageous features of Neurospora for such studies are haploidy, the possibility of tetrad analysis, the fact that deficiency ascospores can be detected visually because they mature but fail to pigment, the viability of long duplications, the fertility of most rearrangements in homozygous condition, and the possibility of recovering for genetic analysis the mitotic products of somatic recombination or chromosome breakage.

Four insertional translocations have been reported in Neurospora (ST. LAWRENCE 1959; DE SERRES 1957; BARRY 1960a, b; MURRAY 1968, BARRY and PERKINS 1969), but no detail description of these has been published. This paper is concerned with a fifth example, *T(I→II)39311*, which was singled out for study because the duplications it produces are heterozygous for the mating-type alleles *A* and *a*. The morphology of the *A/a* heterozygote is strikingly abnormal as first shown in *A/a* duplications produced meiotically by a pericentric inversion (NEWMEYER and TAYLOR 1967). In both cases the abnormal *A/a* phenotype is

somatically unstable, and phenotypically normal sectors arise that are no longer heterozygous for mating type. The abnormal phenotype is due to the vegetative (heterokaryon) incompatibility that is associated with mating type (NEWMEYER 1970).

This paper presents detailed genetic data on *T(I→II)93911* to illustrate the behavior of insertional translocations in fungi. Additional new insertionals are described briefly in the Appendix. Cytological observations on *T(I→II)39311* are reported in an accompanying paper (BARRY 1972). A preliminary report has been published (PERKINS 1966a).

MATERIALS AND METHODS

Strains: The original 39311 strain was isolated as a nutritional mutant from a cross of *Neurospora crassa* wild types 1A × 25a following exposure of conidia to ultraviolet light (BEADLE and TATUM 1945). The strain required succinic acid or a related substance (LEWIS 1948). The present study began with a derived strain, 39311–2973–5a, obtained from the lyophil collection of E. L. TATUM. Subsequently the rearrangement was separated from the nutritional requirement by crossing over.

Standard wild types were 74-OR23-1A and 74-OR8-1a. Standard testers for mating type (mt, *A/a*) and for presence of the aberration were the fluffy strains *fl*^{P}*A* and *fl*^{P}*a* or *fl(P605)a*; these fluffy testers are isosequential with the wild types, and were used for convenience because no conidia are produced, and ejected ascospores are thus observed more readily. For information on origin, characteristics and scoring of the markers used, see references given by BACHMANN and STRICKLAND (1965) or BARRATT and OGATA (1970).

The meaning of locus symbols is given in the legend of Figure 1. Mutant isolation numbers (allele numbers) were as follows except where specified otherwise in Table 3: *arg-1* B369, *arg-5* 27947, *arom-3* C163, *aur* 34508, cyt-1 C115, *fl* P605 or L, *fr* B110, *hist-2* Y152M14, *leu-3* R156, *lys-4* STL4, *os* B135, *pe* Y8743m, *phen-1* NM160 or H6196, *pyr-4* 36601, *rg* B53 or B187 or R2357, *ser-3* 47903, *sn* C136, *so* B230, *suc* 39311.

Gene order: The normal sequence in linkage groups I and II is shown in Figure 1. This order has been documented by PERKINS *et al.* (1969) for I and II, and by GROSS and FEIN (1960), and GILES *et al.* (1967) for II.

Media and technical methods: Medium N (VOGEL 1964) with 1% sucrose was used as minimal. Glycerol-complete medium (GCP) was used routinely for ascospore isolation (except as noted); this is essentially medium 2 of TATUM *et al.*, (1950). Stocks were preserved in suspended animation on anhydrous silica gel (PERKINS 1962b). Crosses were carried out on synthetic crossing medium (SC) (WESTERGAARD and MITCHELL 1947) containing 2% agar and 2% sucrose, usually in 18 × 150 mm tubes. Mating type, fertility, and presence of the aberration were scored by fertilizing 5-day old fluffy testers in individual 10 × 75 mm tubes of SC at 25°C (TAYLOR 1965). Cultures were scored as aberrant when the ascospores shot from perithecia of these test crosses were about 75% black and 25% white (defective); about 95% of ascospores are black in crosses between strains that are isosequential, either both Normal or both transposed.

Unordered tetrads were obtained by the method of STRICKLAND (1960; see also PERKINS 1966b) using crosses with fluffy testers as protoperithecial parents on petri dishes. Asci are collected about 12 days after fertilization, from an agar surface to which they are shot as unordered groups of eight ascospores.

Crosses involving *hist-2* were isolated to minimal + histidine; *arom-1* to minimal + shikimic acid; *arom-3* to minimal plus L-phenylalanine (40 μg/ml), L-tryptophan (40 μg), *p*-aminobenzoic acid (0.25 μg), and tyrosine (40 μg). GCP was supplemented with L-arginine (0.3 mg/ml) when *arg-1* was present. *eth-1* was scored by inability to grow at 39°C.

Nomenclature: The symbol *T(I→II)39311* serves to distinguish insertional translocation 39311 from a typical reciprocal translocation such as *T(I;II)4637*. It is proposed to use an arrow

in this manner to symbolize not only insertional translocations but also other rearrangements that resemble insertionals in being capable of generating viable duplications. These include interstitial transpositions within the same chromosome, as well as certain reciprocal two-break rearrangements where one of the break points is so close to a chromosome tip that no essential gene is located distal to the break. Numerous duplication-generating tip-base rearrangements of this type are known in Neurospora (see Appendix). For example, in the translocation *T(I→V)AR190* the right arm of linkage group I is translocated to the satellite at the left end of V, while in the pericentric inversion *In(IL→IR)H4250* a long terminal segment of the left arm of linkage group I is attached to the right tip. In all three examples, the arrow signifies that the segmental transfer is either unidirectional or is so asymmetrical as to be effectively unidirectional. The arrow indicates that viable duplications are produced, and its direction indicates which component is the donor and which the recipient. The proposed symbol does not distinguish whether the transferred segment is interstitial or terminal, or whether insertions are inverted.

The arrowed symbol departs from ordinary usage in Drosophila (LINDSLEY and GRELL 1967), where symbols with semicolons have been used that do not enable an insertional translocation to be distinguished from a reciprocal. Insertional translocations, which regularly generate viable duplications, differ markedly in behavior and properties from reciprocal translocations that do not, and from our experience in Neurospora it seems important to use symbols capable of clearly distinguishing the two.

The symbol *T(I→II)39311* should immediately alert the user to anticipate viable duplications and the consequent off-ratios among the progeny, and to assure that care is taken to select a strain with the balanced transposition (I^{Del},II^{Ins}) when new stocks are established, rather than one with the duplication (I^{N},II^{Ins}), which might inadvertently be kept if the aberration were thought to be a simple reciprocal translocation.

A derived strain containing a normal linkage group I and a II bearing the insertion will be symbolized *Dp(I→II)39311*. The complementary deficiency type *Df(I)39311* is inviable. The four chromosomes concerned will be called I^{N}, I^{Del}, II^{N}, and II^{Ins}.

Inverted insertion is used in the sense of dyscentric, i.e. inverted with respect to the centromere (DARLINGTON 1936).

RESULTS

Crosses homozygous for *T(I→II)39311* will be presented first to establish the orientation of the inserted sequence. Structurally heterozygous crosses will then be considered.

Crosses of *T(I→II)39311* × *T(I→II)39311:* When two *T(I→II)39311* strains are crossed, the results are as expected from an isosequential cross—90% of spores are black, germination is good, and no duplications or other unbalanced aneuploid classes appear among the progeny.

Recombination data from appropriately marked, structurally homozygous crosses (Table 1) show that a long segment has been removed from linkage group IL and inserted into IIR. The inserted segment is inverted relative to the centromere (Figure 1). Those group I markers that are now carried in II^{Ins} segregate independently of the untransposed markers that remain in I^{Del}.

Crosses of T(I→II)39311 × Normal. Description of classes: Strains containing the 39311 rearrangement are phenotypically indistinguishable from wild type. When *T(I→II)39311* is crossed to wild type, about one fourth of the ascospores are white and inviable because they contain deficiencies. About one third of the viable, black ascospores give rise to characteristic inhibited colonies called Dark Agars (symbol DA). These are *A/a* duplications.

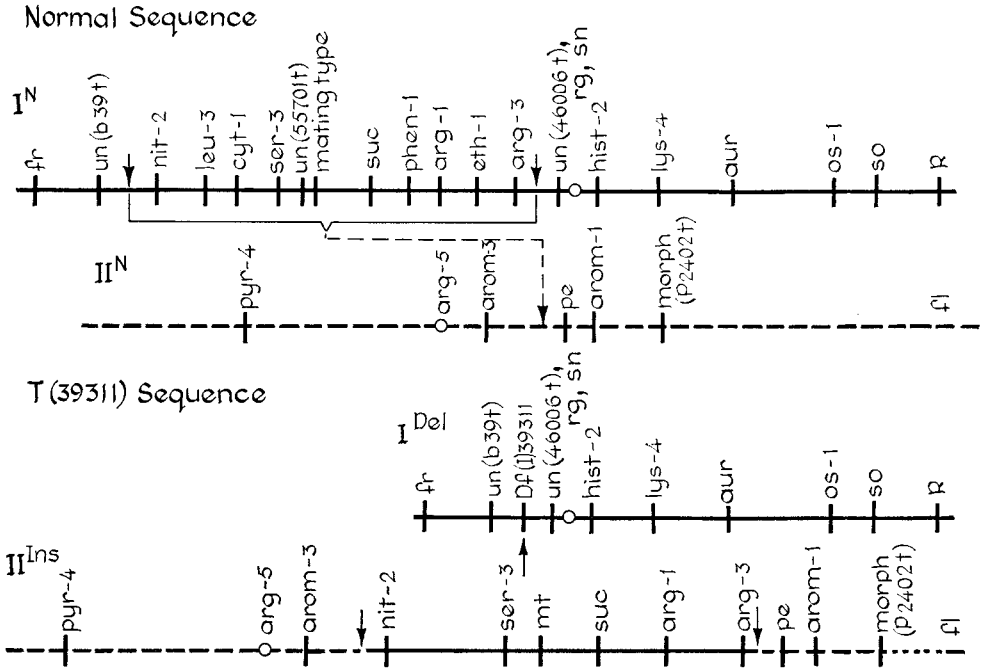

FIGURE 1.—Location of markers in Normal sequence (above) and *T(I→II)39311* translocation sequence (below). Arrows indicate the break points of *T(I→II)39311*. Roman numerals signify linkage groups. In this and following figures, chromosome segments normally in I are drawn with solid lines and those in II are drawn with broken lines. There are no standard map lengths in Neurospora because of variability in crossing over. Distances in IL from a Normal × Normal control cross were *fr* (12) *un(b39t)* (30) *mt* (3) *suc* (5) *hist-2* (1) *lys-4*. Representative values for IR and II are *hist-2* (55) *aur* (30) *os-1* (17) R, and *pyr-4* (30) *arg-5* (2) *arom-3* (8) *pe* (3) *arom-1* (25) *fl*. Meaning of locus symbols: *arg*–arginine (requirement), *arom*–aromatic amino acids + *p*-aminobenzoic acid, *aur*–aurescent pigment, *cyt*–cytochrome deficient, *eth*–ethionine resistant, *fl*–fluffy morphology, *fr*–frost morphology, *hist*–histidine, *leu*–leucine, *lys*–lysine, *morph*–altered morphology, *mt*–mating type (*A* or *a*), *nit*–nitrate nonutilizer, *os*–sensitive to high osmotic pressures, *pe*–peach morphology, *phen*–phenylalanine, *R*–Round ascospore, *rg*–ragged morphology, *ser*–serine, *sn*–snowflake morphology, *so*–soft morphology, *suc*–succinate.

Duplications generated by a variety of different insertional translocations and other rearrangements have generally proved viable in Neurospora (ST. LAWRENCE 1959; DE SERRES 1957; BARRY 1960, 1961; NEWMEYER and TAYLOR 1967; TURNER *et al.* 1969), and usually the duplication strains are nearly wild-type in morphology and growth. Duplications from *T(I→II)39311* are typical in showing good viability, but they are atypical in being morphologically abnormal when they originally grow up from ascospores. Unlike the complementary duplication-deficiency classes from heterozygous reciprocal translocations, the deficiency class from an insertional translocation contains no duplication and the duplication contains no deficiency. The abnormal, DA phenotype of duplications from *T(I→II)39311* cannot therefore be attributed to deficiency. Nor is the inhibition due to the duplication itself. All evidence indicates that the abnormality

TABLE 1

Results of crosses with T(I→II)39311 *homozygous, supporting the gene orders: fr un(b39t) hist-2 lys-4;* and *pyr-4 arg-5 arom-3 ser-3 mt suc arg-1 pe morph(P2402t)*

Zygote genotype and recombination %	Parentals	Single crossovers				Double crossovers						Triples
		1	2	3	4	1,2	1,3	1,4	2,3	2,4	3,4	2,3,4
+ a + pe / pyr-4 A suc + (31.4 2.0 3.9)	30	13	0	4		0	0		0			
	34	19	2	0		0	0		0			
+ ser-3 (a) + pe / pyr-4 + (A) suc + (26.1 5.4 6.2)	44	15	2^{a}	1		1^{A}	0		0			
	41	15	4^{A}	4		0	3^{a}		0			
+ A + / pyr-4 a suc (19.6 3.1)	39	7	2			0						
	37	11	0			1						
+ arom-3 (A)suc + + / arg-5 + (a) + pe morph* (2.6 37.2 14.1 15.4)	18	0	$14^{\pm}$	4	3	0	0	1	0	0	0	2
	13	1	$12^{\S}$	4	4	0	0	0	0	1	1	0
+ a suc + / arg-5 A + pe (12.6 4.7 6.3)	54	5	1	2		1	0		0			
	49	6	2	2		1	3		1			
+ ser-3 (a) + / arg-5 + (A) pe (27.3 10.2)	26	13^{A}	4^{a}			0						
	29	11^{a}	5^{A}			0						
+ (a) suc + / arg-5 (A) + pe (13.9 1.9)	68	13^{+}	1^{a}			0						
	65	9^{a}	2^{A}			0						
+ a + pe / arom-3 A suc + (15.6 3.9 5.2)	24	6	2	1		0	2		0			
	36	4	1	1		0	0		0			
+ arg-1 pe / ser-3 + + (7.0 10.5)	47	4	6			0						
	..	..	..			..						
+ un(b39) hist-2 + A / fr + + lys-4 a (6.2 2.5 6.2)												
A:	54	1	0	2		0	0		0			
	43	1	3	8		0	0		0			
a:	53	4	1	2		1	1		0			
	56	7	1	2		0	0		0			
+ + / fr lys-4 (7.1)	..	..										
	13	1										
+ + / fr ser-3 (41%)	20	..										
	..	14										
+ + / un(b39) ser-3 (60%)	10	..										
	..	15										
+ + / rg ser-3 (57%)	6	..										
	..	8										
+ + / hist-2 ser-3 (63%)	5	..										
	..	9										
+ hist-2 + / un(b39) + ser-3 (4.9 (41%))	46	..	..			2						
	..	2	31			..						

results simply from the presence of both *A* and *a* mating alleles in the same nucleus.

The Dark Agar duplication progeny (DAs) from *T(I→II)39311* are very similar in appearance and behavior to those from *In(IL→IR)H4250*, which were described and illustrated by NEWMEYER and TAYLOR (1967). DAs grow slowly after germination, with sparse, aconidiate spidery outgrowths on and into the agar medium, in contrast to their normal siblings, which fill the tube and produce bright orange conidia in 3 or 4 days at 25°C. Dark pigment appears in and around the main hyphae of DAs at about 4 days and diffuses into the surrounding medium, either on complete medium or on minimal medium supplemented with tyrosine and pheylalanine. (No brown pigment appears on unsupplemented Minimal, but the abnormal morphology is the same as on Complete.) DA colonies achieve a diameter of 5 to 10 mm after 5 to 8 days at 25°C; they then "escape." Growth that is essentially wild-type in rate and morphology originates at one or more discrete points and overgrows the culture, producing abundant aerial mycelia and conidia. Possible mechanisms underlying the somatic instability will be considered in the Discussion.

The presence of a morphological mutant outside the duplication commonly alters the appearance of DA progeny before escape occurs. This is true, for example, of *pe*, *fr*, *rg*, *sn*, *morph(P1798)*, and *morph(D5)*.

An escaped DA culture is initially a mixture which can mate and produce perithecia with both *A* and *a* testers. Pure *A* and *a* derivatives can both be recovered vegetatively from the same individual DA after it escapes.

When an escaped DA is crossed with standard testers, abundant perithecia are usually produced, but these do not develop normally. Perithecial beaks (also called necks) are rare, and few or no ascospores are shot. This type of infertility is a general characteristic of many different duplications in Neurospora, and strains showing it are termed *Barren*. It is not known why the presence of a duplication should impair fertility.

A genic suppressor of the mating-type-associated heterokaryon-incompatibility results in *A/a* duplications that are not DA in phenotype but are essentially wild type in growth and morphology. This suppressor does not affect the infertility of the duplications, however (NEWMEYER 1970).

In all these respects, Dark Agar duplications from *T(I→II)39311* are essentially identical to those from *In(IL→IR)H4250*.

Crosses of T(I→II)39311 × Normal. Genetic data: When insertional translocations are crossed with strains of Normal sequence, four equally frequent

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Ascospores were isolated at random.

The top number of each pair of complementary classes represents progeny of the genotype that contains the wild-type allele of the leftmost marker. Regions are numbered from left to right, ignoring genes shown in parentheses. Where dots replace numbers, the corresponding classes of segregants were not scored. Where (A) and (a) are in parentheses, mating type was not scored except as indicated in footnotes, and *mt* was ignored in numbering the regions. In the last five crosses, the broken line indicates nonlinkage, but the progeny classes are tallied using conventions as though they were linked.

None of the crosses in Table 2 produced Dark-Agar progeny or other unbalanced aneuploids.

[A] All *A* mating type.

[a] All *a* mating type.

* *morph(P2402t)*

† 10 *A*, 3 *a*.

‡ 10 *a*, 4 *A*.

§ 10 *A*, 2 *a*. Sequence is thus *arom-3* (29) *mt* (9) *suc*.

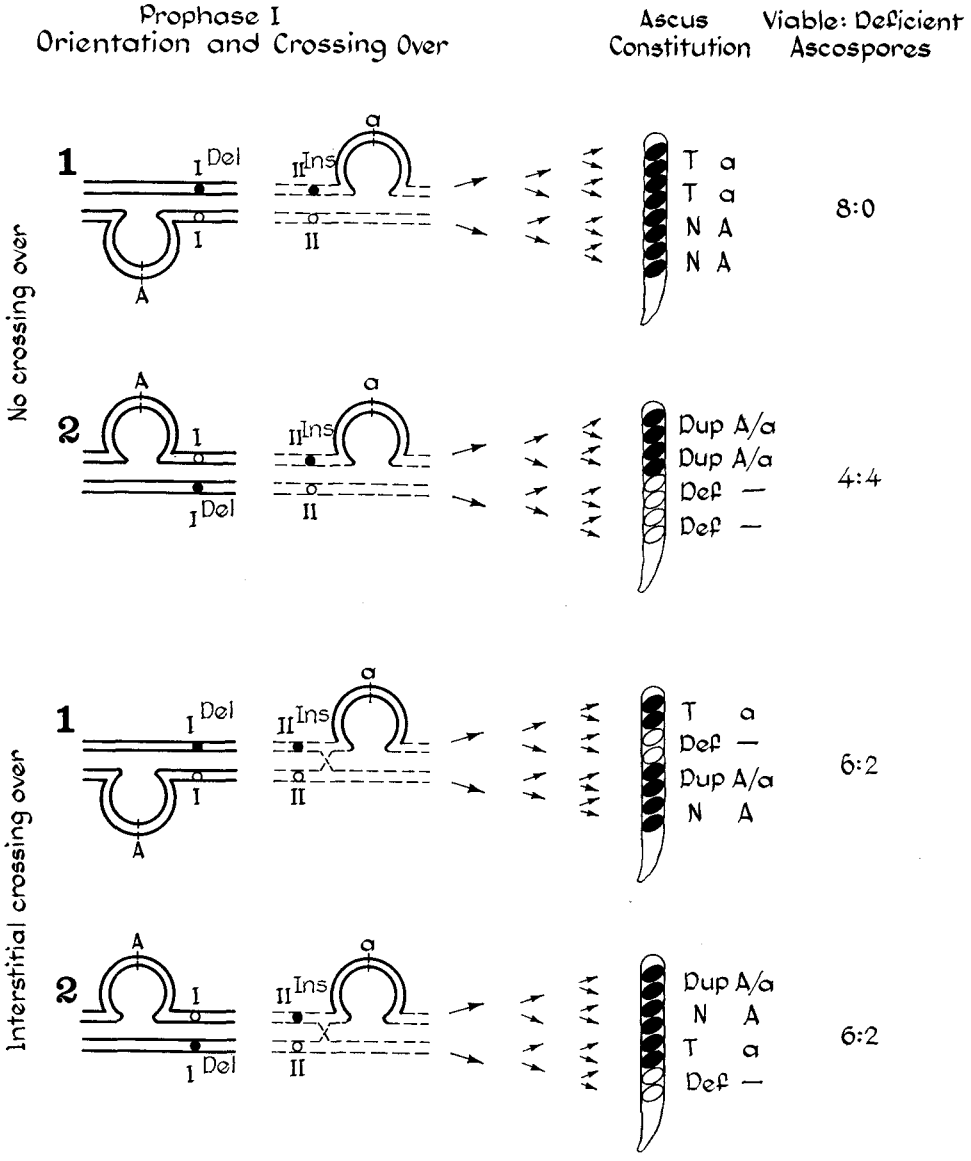


FIGURE 2.—The origin and constitution of asci containing various numbers of deficient spores, from crosses of *T(I→II)39311* × Normal sequence. The consequences of segregation without crossing over are shown in 1 and 2 above. Crossing over between either break point and centromere is expected to produce 6:2 asci, as shown in the bottom two diagrams. Centromere segregation of the two types, 1 and 2, should be equally frequent regardless of crossing over. If parents differ at a locus within the transposed segment, duplications are expected to be heterozygous (*A/a*). Ascospores with deficiencies are white and inviable. Spores with no deficiencies are black and viable. Pairing between the transposed segment and its homologous region is ignored in this diagram.

classes of meiotic products are expected as a simple consequence of random segregation of centromeres (Figure 2). (Consider now only those meioses without crossing over in the transposed segment.) In addition to the parental types, deficiency types and duplication types are each expected to appear with a frequency of one fourth. Only the deficiencies die, while the duplications are fully viable and constitute one third of surviving progeny.

These expectations are realized in the analysis of random ascospores from crosses between *T(I→II)39311* and Normal. (1) One fourth of ascospores are inviable and white, as expected if they are deficient for the long segment of IL. (2) One third of the viable, black ascospores produce Dark Agar cultures. Results from typical crosses are given in Tables 2 and 3 and Figure 3. Thirty-nine crosses gave a combined total of 1998 non-DA and 1024 DA progeny, or 33.9% duplications. After the DAs escape, they can form perithecia when crossed to testers of both mating types, indicating that they originated as duplications heterozygous for mating type. The perithecia are Barren, as expected of duplications.

The nonduplication progeny from crosses of *T(I→II)39311* × Normal show pseudolinkage as expected. For example, *suc* in Table 2 crosses is linked to both linkage groups I (*aur*) and II (*pyr-4*, *pe*, *fl*). Approximately equal numbers of complementary crossover classes are found among the nonduplications.

Results in the duplication class are much more complex. Markers within the transposed segment are expected to be heterozygous in the duplications initially (and either heterozygous, heterokaryotic or mixed after escape). Recessive genes so placed are thus "covered" by the duplication. As a result, complementary classes do not occur in equal numbers among the DAs. An example is provided by the recessive marker *suc* in the first cross in Table 2, which can be most readily interpreted by referring to Figure 3a.

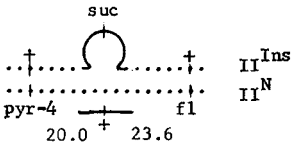
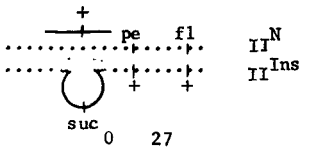
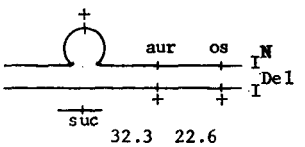
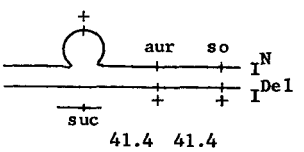
All duplications are suc^+. The most frequent combination of markers among the duplications, pyr^+ suc^+ fl^+ would be mistakenly classified as a double crossover were these not recognized to be duplications; the pseudolinkage might not then be noticed. In fact, the +++ class results from the presence of the two noncrossover chromosomes pyr^+ *suc* fl^+ (II^{Ins}) and suc^+ (I^N).

Heterozygous duplications also have striking effects on allele ratios. When recessive markers of the donor chromosome enter from the Normal parent, the results are dramatically different for loci inside the transposed segment, in contrast to those outside the segment. This can be seen for the loci *nit-2* through *arg-3* in Table 3. These markers are covered by the duplication, and effectively all duplication progeny are dominant (wild type) in phenotype. In contrast, markers that are closely linked outside the duplication show the exact reverse—for them the Duplications are all recessive in phenotype. See, for example, *un(46006t)* in Table 3.

Coverage or noncoverage of markers thus provides a useful method of mapping them with respect to the break points in the donor chromosome. The order from duplication coverage is consistent with that from conventional recombination mapping. Moreover, markers too close to recombine with the arrangement can

TABLE 2

Three-point crosses of T(I→II)39311 suc × *Normal (standard chromosome sequence)*

Zygote genotype and recombination %*	Parental combinations	Recombinations: Singles Region 1	Recombinations: Singles Region 2	Recombinations: Doubles Regions 1 and 2	Total and % germination
T suc X N pyr-4 fl: 					
Non-duplications:	11 21	4 6	5 7	1 0	55
Duplications†‡:	0 0	2 0	0 6	18‡ 0	26 (86%)
T suc X N pe fl: 					
Non-duplications:	22 24	0 0	6 11	0 0	63
Duplications:	§				29 (61%)
T suc X N aur os: 					
Non-duplications:	16 16	8 8	6 4	1 3	62
Duplications†‡:	10 0	14‡ 0	8 0	2 0	34 (96%)
T suc X N aur so: 					
Non-duplications:	6 15	4 9	4 9	4 7	56
Duplications:	§				36 (94%)

Ascospores were isolated at random.

In all these crosses, *suc*+ was located in I^{N}, *suc* in II^{Ins}. The data are presented, however, as though *suc* (or *suc*+) were simply at a locus in linkage group II (crosses 1 and 2) or in I (crosses

nevertheless be readily mapped to the right or left of the break points on the basis of duplication coverage.

Figure 4 illustrates the effect of duplications on allele ratios. When only the duplication progeny are considered (Figure 4a), the ratios for closely linked markers are either 1 Dominant : 0 Recessive or 0:1, depending on whether the locus is inside or outside the transposed segment. The distorted ratios among total viable progeny are entirely due to the presence of the duplications, in which the covered recessive markers are not expressed. If only nonduplicated progeny are considered (Figure 4b), all markers show allele ratios approximating 1:1, whether or not they are in or near the transposed segment. If all viable progeny are considered, including both duplications and nonduplications (Figure 4c), markers within the transposed segment show allele ratios of approximately 2 Dominant : 1 Recessive. In contrast, markers located just outside the segment and not covered by the duplication give a ratio of approximately 1 Dominant : 2 Recessive. With increasing distance from the break points, markers approach an allele-ratio of 1:1 as a result of crossing over between marker locus and break points. This is seen with *aur* and *os*.

If typical insertional translocation were present that produced phenotypically normal duplications, and if its presence in heterozygous condition went unrecognized in a cross, baffling off-ratios such as those in Figure 4c might unexpectedly be encountered, and closely linked genes on opposite sides of the break points might mysteriously appear to have recombined frequently with one another, e.g. *un(b39t)* with *nit-2*, or *arg-3* with *un(46006t)*. Novel recombination mechanisms need not be invoked to explain deviant results of this type, which can simply be the straightforward consequences of an aberration that generates viable duplications.

In using allele ratios as a criterion of duplication coverage *vs.* noncoverage, the coupling phase is critical. The duplication progeny carry the complete donor linkage group (group I in this case) from the Normal parent. Therefore, linked markers which have the dominant allele in coupling with the Normal parent will produce dominant duplication progeny whether the locus is included in the transposed segment or not. For the test to be meaningful, then, the recessive allele must enter the cross in coupling with the Normal parent so that the duplication progeny will be dominant only if the marker is covered by the transposed segment. A general rule for the donor chromosome is that covered markers give the same result (dominant > recessive progeny) both for *cis* and *trans*, whereas linked markers at loci that are not covered give opposite results (Dom.>Rec. *vs.* Rec.>Dom.) for the two coupling phases. For specific examples

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3 and 4). Regions are numbered accordingly for purposes of tabulating the data. The left-hand number of each pair of complementary classes represents progeny of the genotype that contains the wild-type allele of the leftmost marker.

Material normally in I is shown as a solid line, and material normally in II as a dotted line. Diagrams omit unmarked chromosomes and segments, i.e. I^{Del} in crosses 1 and 2, and II^{N} in crosses 3 and 4. See Figure 3a for a complete diagram.

* Recombination percentages are based on nonduplications only.

† Duplications initially grow with the Dark Agar phenotype. Markers were scored on the mixed culture after escape from inhibition.

‡ Duplication progeny are tabulated according to phenotype only. In fact, they are heterozygous *suc/suc*+. Therefore some of the apparent crossovers are parentals, and certain others are crossover types not corresponding to their appearance.

§ Markers were not scored in these duplications.

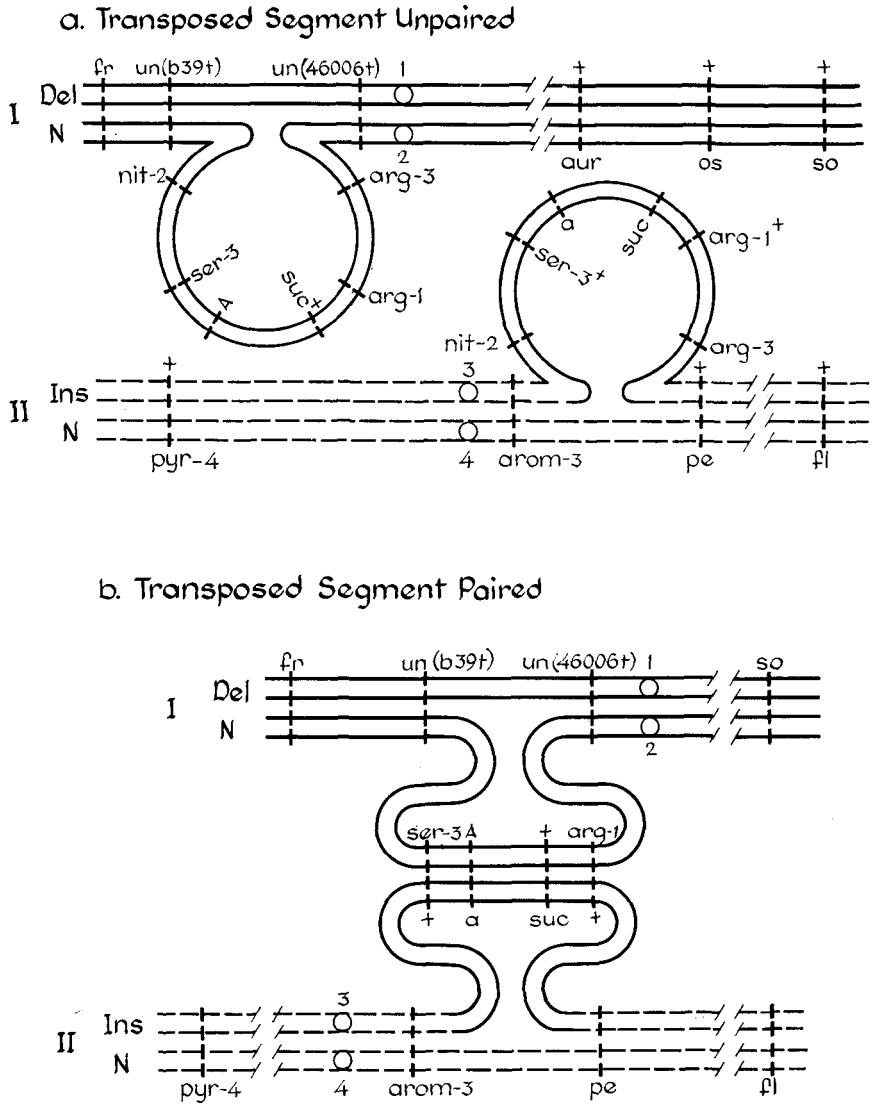

FIGURE 3.—Meiotic pairing in a cross heterozygous for *T(I→II)39311*. Two modes of pairing are shown—with (a) no synapsis and (b) synapsis between the transposed segment and its normal homologue. The coupling phase of markers in (a) corresponds to the crosses in Table 2. If a single exchange occurs between the paired transposed segments and centromeres 2 and 3 go to opposite poles at anaphase I, a dicentric bridge and acentric fragment will result. If centromeres 2 and 3 go to the same pole, no anaphase I bridge will be formed, but bridges can be expected at later divisions.

see Table 3 *cis* and *trans* crosses involving *suc*, which is covered, and involving *un(b39t)*, which is not covered.

The donor (Del) linkage group of an insertional translocation can be identified unambiguously if a linked recessive marker entering the cross in *trans* to the translocation shows recessives in excess over dominants among duplication progeny (and hence among total progeny). *In such*

TABLE 3

Representative singly marked crosses of T(I→II)39311 × *Normal (standard chromosome sequence) showing coverage of markers in the duplication (DA) progeny. The results for these and 14 other loci are summarized graphically in Figure 4*

Marker[a] and isolation number	Phenotype with respect to marker: Non-DA progeny Dominant	Non-DA progeny Recessive	DA progeny[b] Dominant	DA progeny[b] Recessive	Total and % germination	Fraction of isolates carrying dominant allele: DA	non-DA	Combined total
Linkage Group I markers:								
fr B110	65	59	15 (+2)[c]	68	207 69%	.18	.52	.39
un b39t	TRANS: T X N un 48	38	0	33	120 80%	0	.56	.40
un b39t	CIS[d]: T un X N 33	26	21	1	81 68%	.95	.56	.67
nit-2 nr37	26	31	33	0	90 90%	1	.46	.66
suc 66702	TRANS: T X N suc 18	19	20	0	57 95%	1	.49	.67
suc 39311	CIS[d]: T suc X N 64	53	60	0	177 91%	1[f]	.55	.70
arg-3 30300	23 (+3)[c]	30	30	1	87 87%	.97	.43	.63
un 46006t	21	19	0	15	55 92%	0	.52	.38
hist-2 Y152M14	51	44	1	47	143 67%	.02	.54	.36
aur 34508	55	65	32	38	190 95%	.46	.46	.46
os B135	33	29	22	12	96 96%	.65	.53	.57
Linkage Group II markers:								
pyr-4 36601	TRANS: T X N pyr-4 21	34	20	6	81 86%	.88	.38	.51
arom-3 C163	CIS: T arom-3 X N 27	21	0	21	69 69%	0	.56	.39
arom-1 Y7655	CIS: T arom-1 X N 39[c]		1	12	52 26%	.08		
arom-1 Y7655	TRANS: T X N arom-1 17	26	30	0	73 89%	1	.40	.64

Ascospores were isolated at random.

[a] Unless noted to the contrary, the recessive marker entered all crosses in the Normal sequence, in *trans* to *T(I→II)39311*.

[b] Scored after escape.

[c] Scored for DA phenotype but not for the marker.

[d] Crosses in this coupling phase are not critical for distinguishing coverage or noncoverage by the duplication. In order for tests of coverage to be meaningful, recessive markers in the donor chromosome must enter the cross in *trans* to T.

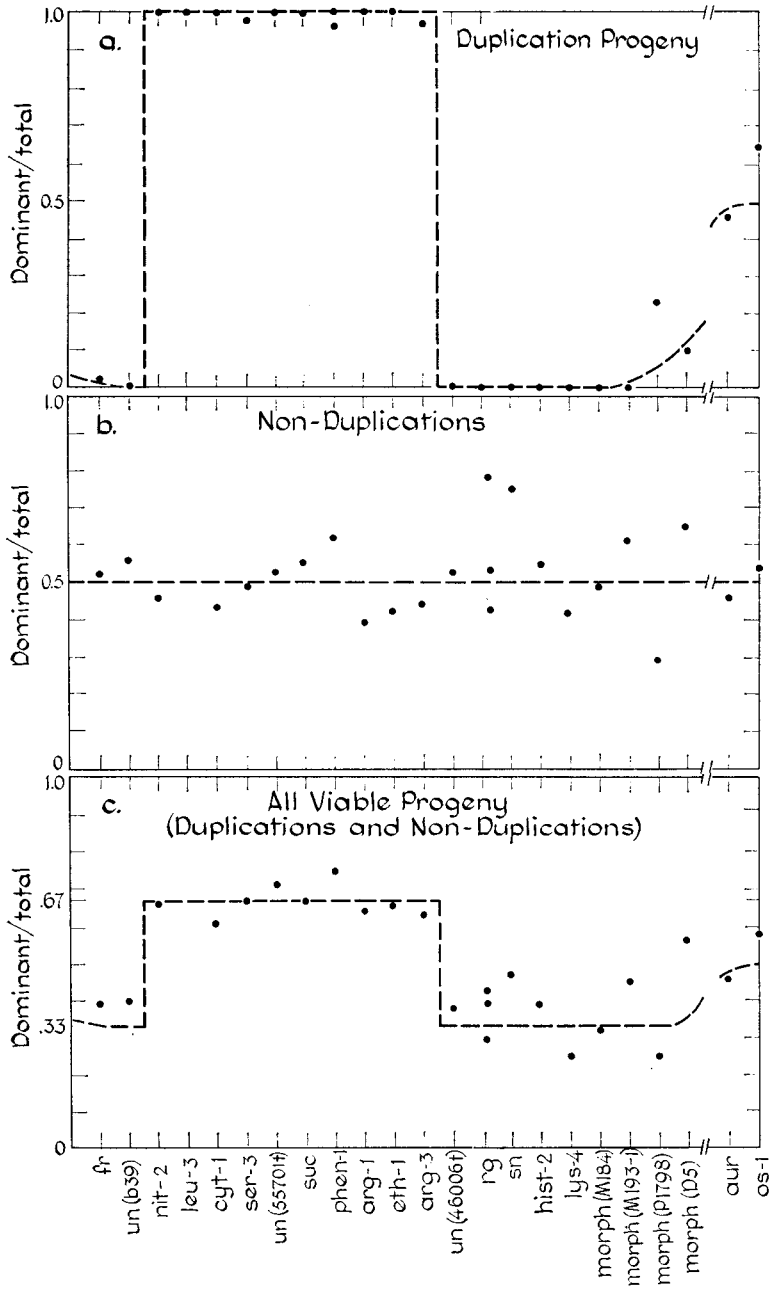

FIGURE 4.—The proportion of progeny that are dominant with respect to markers in linkage group I, from crosses where the recessive marker was *trans* to *T(I→II) 39311*. The fraction of progeny that are dominant is shown for (a) duplications only, (b) nonduplications only, and (c) all viable progeny, including duplications and nonduplications. Values are from Table 3 and from 19 similar crosses. The broken lines indicate values expected if the duplicated segment ends between *un(b39t)* and *nit-2* on the left, and between *arg-3* and *un(46006t)* on the right. The loci

a case, the marker can only be situated in the donor chromosome, and outside the transposed segment.

Because of duplication coverage, mapping the two break points in the donor chromosome of an insertional translocation is far easier and more accurate that mapping the break in the recipient chromosome, or than mapping the break points of reciprocal translocations.

Meiotic recombination within the inserted segment in crosses of T(I→II)39311 by Normal: So far it has been assumed that pairing occurs as in Figure 3a, where the transposed segment remains unpaired, precluding transfer of included markers by crossing over. Figure 3b shows an alternative where pairing does occur between the inserted segment and its normally placed homologue. Transfer of markers into and out of the insertion would then be possible by means of a 2-strand double exchange. Most exchanges, including singles, are expected to result in dicentric bridges and acentric fragments because the 39311 insertion is inverted with respect to the centromere. BARRY (1972) has confirmed cytologically that bridges and fragments are frequent.

With *T(I→II)39311* × Normal, marker transfer has been accomplished meiotically for four markers—*arg-1*, *suc*, *mt*, and *ser-3*. Thus meiotic pairing must sometimes occur between the inserted segment and its homologue as in Figure 3b, and double crossing over takes place when the two are so paired. The frequency with which loops pair with one another as in Figure 3b can be estimated roughly from the frequency with which markers are transferred into the segment. Observed transfer frequencies among nonduplication progeny from heterozygous T/N crosses were 1/37 for *ser-3*, 0/71 and 1/15 for mating type (simultaneous with *suc*), 2/15 for *suc*, and 1/104 for *arg-1*—a total of four marker transfers in 227 random strands tested, or 2.8% among surviving strands. This provides a minimal estimate of the frequency of 2-strand double exchanges because a particular marker is not always included between the two exchanges.

The inserted segment is about 40 map units long in its normal IL position, and considerably less than this when homozygous in its transposed IIR position (Table 1). The tetrad frequencies of 0, 1, 2, etc. exchanges are known for representative intervals of various lengths in Neurospora (PERKINS 1962a). These can be used to estimate the expected frequency of double crossovers among surviving strands for the T/N heterozygote, on various assumptions about the frequency with which the inserted segment pairs with its untransposed homologue, and about its effective genetic length when so paired. (Because the insertion is inverted, single crossover strands are expected to be lost as a result of dicentric bridges being formed.)

A. Assume pairing in 100% of tetrads and the following three interval lengths. 1. Effective length 43 units: The expected frequency of double crossovers among surviving strands in nonduplication progeny would be 4.3% if exchanges in the paired loops were distributed as in the 43-unit interval *cr-aur*, Table 3 of PERKINS (1962a). 2. Effective length 36 units (as *cr-nit-1* in the 1962 paper): 2.8% double crossovers would be expected (this is the actual value observed).

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are shown in map order, so far as order is known, with no indication of spacing except that *aur* and *os-1* are located far to the right of the other markers. *morph(D5)* and *un(46006t)* are known to be recessive in heterozygous duplications from *In(IL→IR)H4250* or *In(IL→IR)NM176;* morphological mutants M193-1, M184, P1798, *rg*, and *sn* are assumed to be recessive. From recombination evidence, *rg*, *sn*, *morph(M184)* and *morph (M193-1)* are close to *hist-2*, *morph (P1798)* is right of *hist-2*, and *morph(D5)* is well right of *lys-4* (PERKINS *et al.*, 1969).

3. Effective length 23 units (as *thi-1-nit-1*, Table 2 of PERKINS 1962a): 1.6% double crossovers would be expected.

B. Assume pairing in 50% of tetrads, and the same three effective interval lengths when pairing occurred: 1. 43 units: 1.6% double crossovers expected. 2. 36 units: 1.1% double crossovers expected. 3. 23 units: 0.7% double crossovers expected.

C. The above calculations assume that noncrossover strands survive when the insertion pairs, whether or not it undergoes crossing over with its partner. However, if pairing of the transposed segment precluded pairing of the nontransposed portions of the same two chromosomes, then nondisjunction might follow when no chiasma was formed, and this would alter the expectations. Assume as many as three fourths of such noncrossover strands to be lost by nondisjunction. The frequencies of double crossovers among surviving strands would increase to 15.2%, 10.2%, 6.0% for 100% pairing, and 2.25%, 1.54%, and 1.0% for 50% pairing.

In all the above calculations it is assumed that when a single exchange or 3-strand double occurs, the resulting dicentric kills only two of the products, while the remaining two survive. If instead such asci aborted completely so as to leave no surviving spores, the calculated values would not be changed enough to affect conclusions about the frequency of pairing.

Even though several assumptions must be made, the observed 2.8% frequency of marker transfer suggests that the transposed segment usually pairs with its homologue as in Figure 3b rather than remaining unpaired as in Figure 3a.

T(I→II)39311 × *Normal. Unordered tetrads:* A number of predictions can be arrived at from Figure 2, where pairing between the insertion and its homologue is ignored.

(1) If nonhomologous centromeres segregate independently, asci with 8 Black : 0 White ascospores and those with 4 Black : 4 White are expected to be equally frequent. This is approximately true for *T(I→II)39311* (Figure 5). There are few 0B:8W asci (2%); therefore homologous centromeres apparently disjoin normally and adjacent-2 segregations, which would produce 0:8 asci, are rare or absent.

(2) The frequency of asci with 6 Black : 2 White spores reflects crossing over

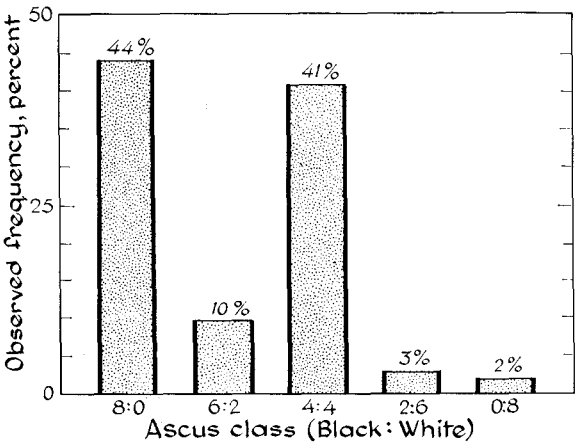


FIGURE 5.—Frequency of unordered tetrads of various types from crosses of *T(I→II)39311* × Normal sequence, based on 392 asci. As shown in Figure 2, 6:2 asci are the result of crossing over between centromere and a break point, whereas the 8:0 and 4:4 classes result from segregations where such interstitial crossing over has not occurred.

between centromeres and break points, and with *T(I→II)39311* this should be low, because both interstitial regions are only a few map units long. As expected, 6B:2W asci are infrequent (10%—Figure 5).

(3) Asci having 2B:6W spores are not expected to result from ordinary disjunction, but would require special combinations of centromere disjunction and crossing over within the insertion, as detailed in the next section. Asci of this type are in fact infrequent in crosses with *T(I→II)39311* (3%—Figure 5).

When ascospores from tetrads are germinated, additional predictions can be made:

(1) Asci having 8 Black : 0 White spores are not expected to give rise to any duplications, because crossover products complementary to duplications would be deficiencies, resulting in white spores. With *T(I→II)39311* × Normal, 70 of 71 8:0 tetrads gave all four spore-pairs non-DA, as expected. The one exception, with 7 non-DA : 1 DA, is attributed to technical error.

(2) All four of the viable progeny from asci having 4 Black : 4 White spores are expected to be duplications. With *T(I→II)39311* × Normal, 62 of 67 4:4 tetrads gave both spore-pairs DA, as expected. The exceptions consisted of four tetrads having only non-DA's, and one tetrad having one non-DA and one DA pair. These exceptions could represent incompletely ripened 8:8 or 6:2 asci. Alternatively, they could be due to pairing and crossing over in the transposed segment, as discussed below.

(3) Asci having 6 Black : 2 White spores should be due to interstitial crossing over that would produce one deficiency (white) spore-pair, one duplication pair, and one pair of each of the parental types. Ten of fifteen 6:2 asci from *T(I→II)33911* continued one DA (duplication) pair and two non-DA (parental) pairs, as predicted. Five others contained only non-DA's. These probably represent incompletely ripened 8B:0W asci.

Ordered tetrads from T(I→II)39311 × Normal: Because most asci with 4 Black : 4 White spores result from centromere disjunction without interstitial crossing over, duplications should segregate from deficiencies at the first division, and the four black spores (duplications) should usually be located adjacent to one another in the top or in the bottom half of the ascus (Figure 2). This was true of 35 of 36 4:4 asci scored (data of Mrs. BARBARA C. TURNER). It is also predicted for 6 Black : 2 White asci, which originate by interstitial crossing over, that the duplicated spore-pair should lie in the half-ascus opposite the deficient white spore-pair. This has not been tested with *T(I→II)39311.*

Consequences for tetrads of crossing over in the transposition: Until now tetrad predictions have been made only for meioses where the transposition remained unpaired as in Figures 2 and 3a. Additional predictions will now be considered for meioses where pairing and crossing over occur between the transposed segment and its normally placed homologue (Figure 3b). The transfer of markers and the occurrence of dicentric bridges indicate that crossing over is frequent within the long 39311 insertion. Reasons were given earlier for thinking that pairing of the insertion may be the rule rather than the exception.

What is the fate of asci wherein exchanges have resulted in dicentric bridges

and acentric fragments? The insertion is inverted in *T(I→II)39311* and effectively all exchanges within the loop except 2-strand doubles would therefore result in bridges and fragments. The frequency of marker transfer indicates that several percent of surviving asci contained 2-strand doubles. It is reasonable to expect that meioses with 2-strand doubles will be greatly outnumbered by exchanges of the types that produce bridges and fragments—notable singles and 3- and 4-strand doubles.

On the simplest assumptions, a single first-division bridge should kill two of the four meiotic products, resulting in an ascus with 4 Black and 4 White spores. To the extent that they survive, asci containing bridges should thus result in an excess of the 4B:4W class over 8B:0W. No such excess was observed among unordered tetrads (Figure 5). It therefore appears that asci containing bridges and fragments do not often survive, or at least do not remain intact so as to be detected by the techniques employed.

This conjecture can be checked more precisely. Asci of critical types would theoretically result from single crossing over within the transposition. The observed numbers of these types would set an upper limit on the frequency of surviving single-exchange asci.

If a single exchange occurred between the loops paired as in Figure 3b, followed by anaphase I disjunction such that centromeres 1 and 3 went to one pole, 2 and 4 to the other, there should be a first-division bridge and the resulting ordered ascus should contain two black and two white spores in each half of the ascus. Only one out of 36 ordered 4B:4W asci was of this type.

A single exchange within the transposition would just as likely be followed by disjunction of centromeres 1 with 4 and 2 with 3 (Figure 3b). In this case bridge formation would be deferred till a later division and the resulting ascus would contain either 2 Black and 6 White spores, or 0 Black and 8 White spores, depending on when the dicentric bridge occurred. A maximum estimate of the survival frequency of the 2B:6W and 0B:8W types can be obtained from Figure 5. The 3% and 2% asci observed in these classes are comparable to background values from structurally homozygous controls.

The infrequent occurrence of these specific ascus types is thus consistent with the absence of any excess of 4B:4W over 8B:0W types in indicating that few asci survive to maturity among those that contain bridges and fragments. Cytological observations also suggest that usually, but not always, a bridge (or fragment) results in abortion of the entire ascus (BARRY, personal communication).

DISCUSSION

All the genetic data and the observations on patterns of aborted ascospores in unordered and ordered tetrads, taken together with BARRY's (1972) cytological observations of chromosome pairing and behavior in the developing ascus, combine to support the structural arrangement shown in Figure 1. Several new and less extensively studied insertional translocations, listed in the Appendix, give similar results.

Biological significance of duplication-generating aberrations: Rearrangements of this type, exemplified by *T(I→II)39311*, may be thought of as a latent reservoir of variability, perhaps of special significance in a haploid organism. So long as such a rearrangement persists (short of fixation), the characteristic duplication will continue to be fed into the population and exposed to selection. Selection against duplication progeny may be drastic without eliminating the phenotypically normal rearrangement that continues to produce them. Duplications that were disadvantageous on first appearance may be beneficial under changed circumstances, perhaps many generations later. The infertility of Neurospora duplications is not a serious objection to this hypothesis. Infertility is not absolute in Neurospora, and Aspergillus duplications are apparently fully fertile (BAINBRIDGE and ROPER 1966).

Advantages of Neurospora for studying duplications and duplication-generators: Neurospora has proved to be nearly ideal material for identifying and investigating rearrangements that recurrently generate nontandem duplications. Duplication-producing aberrations are signalled in Neurospora by the production of unique patterns of meiotic tetrads, where viability of duplications rescues one of the aneuploid classes and results in diagnostic asci having 6 Black and 2 White spores, that can be detected visually (PERKINS 1967). Methods of detecting duplication-producers in Neurospora are just as effective for short transpositions as for long, and are theoretically capable of recognizing transpositions that involve only a single essential locus. At the other extreme, long duplications are quite viable.

In general, duplications are far more readily identified, analyzed and manipulated genetically when they occur as partial diploids against a haploid background (the situation in Neurospora) than when they occur as partial triploids against a diploid background (the situation in most of the eukaryotes studied by geneticists). [X-linked insertional translocations are a notable exception where higher organisms share some of the technical advantages of haploidy. Insertionals that involve the X-chromosome in Drosophila are easily identified genetically, and are quite frequent (G. LEFEVRE JR., personal communication). However, insertionals involving only autosomes are much more difficult to identify genetically in diploids, and have been found mostly after cytological analysis in Drosophila. Male haploidy in Mormoniella and other Hymenoptera should be favorable for studying rearrangements of this type.]

Partial trisomics arising from duplication gametes are increasingly implicated as a cause of anomalies in man (e.g. PATAU *et al.* 1961). Gross aneuploids, such as trisomics for most of the autosomes in man, are usually lethal early in development. Less gross aneuploids from duplication gametes are known often to survive after birth, but to be seriously defective. Rearrangements such as insertional translocations, that produce partial trisomics as one third of viable progeny from a heterozygote, cannot readily be identified as such by conventional karyotype or pedigree analysis in man, especially if the transposed segment is small. In contrast, such rearrangements can easily be identified and studied in Neurospora, which may thus be valuable as a model.

Expectations for paracentric inversions: From the rarity of certain ascus types in crosses involving *T(I→II)39311* it was inferred that a dicentric bridge usually results in abortion of the entire ascus. Bridge-induced ascus abortion would explain our failure to find paracentric inversions in Neurospora. A persistent search for paracentrics has failed to recover a single example, although over 60 typical reciprocal translocations and 30 duplication-generating rearrangements have in the meantime been obtained and identified unequivocally. The method by which these rearrangements were initially recognized depends on observing a proportion of white, deficiency ascospores from structurally heterozygous crosses (comparable to observing pollen abortion in structurally heterozygous plants). This method would fail to detect any rearrangement where recombination resulted in failure of the entire ascus to survive or mature, and if dicentric bridges usually killed the asci in which they occurred, paracentric inversions would go undetected. There is thus no reason to think that paracentric inversions do not occur in Neurospora.

The fact that a segment is inverted cannot in itself be lethal because both pericentric inversions and inverted (dyscentric) insertional translocations are represented among the identified Neurospora rearrangements. Pericentrics produce deficiencies without forming any bridges and fragments, while inverted-insertionals produce white deficiency spores as a result of centromere disjunction in asci where crossing over has not occurred in the inverted segment. These rearrangements are therefore detected.

If this explanation is correct, paracentric inversions may be common and may be present in many normal-appearing Neurospora strains. Such unrecognized inversions could be responsible in part for the high variability in recombination that is often observed between the same markers in crosses of different parentage.

Meiotic recombination outside the transposed segment of T(I→II)39311: The effect of an insertional translocation on recombination in regions adjoining the exsertion and insertion has been carefully studied in maize by RHOADES (1968). LEFEVRE and MOORE (1968) have also used insertional translocations in studying the effects of deficiencies on recombination in Drosophila. Although some of the crosses in Tables 1–3 could provide similar information for Neurospora, they were carried out for qualitative rather than quantitative purposes. Because crossing over is highly variable in Neurospora for the same intervals in crosses of different parentage, experimental and control crosses would need to be designed and executed with special care in order for reliable quantitative conclusions to be drawn, and it seems premature to speculate about quantitative differences on the basis of the data now available.

Somatic instability: Several mechanisms might contribute to the escape of Dark Agar duplications from inhibition: (1) *Mitotic recombination.* Escape would require a 2-strand double mitotic exchange spanning the mating-type locus so as to produce viable daughter nuclei homozygous for mating type. Mitotic gene conversion of mating type would be equivalent to a 2-strand double exchange. Because the insertion is interstitial and inverted, single mitotic exchanges would be eliminated because of dicentric bridges and acentric fragments.

(2) *Chromosome breakage.* Removal of one of the mating-type alleles by interstitial deletion should result in a viable product capable of escape. A single break proximal to mating type would be lethal because of terminal deletion. (3) *Mutation.* Inactivation of one mating-type allele by mutation, or neutralization of the incompatibility reaction by suppressor mutation, would permit escape.

There is as yet little basis for distinguishing these possibilities in the case of *T(I→II)39311*. Somatic changes have seemed simpler to study using terminal duplications deprived from nontip→tip rearrangements. This has been done extensively using both escape from somatic growth-inhibition, as in *In(IL→IR)H4250* and *T(II→V)NM149*, and changes from Barren to fertile, as in *T(I→VI)NM103*, T*(I→V)AR190* and *T(VI→III)AR209*. The findings will be reported elsewhere.

The mechanisms responsible for somatic escape of Dark Agar duplications are probably similar for both *T(I→II)39311* and *In(IL→IR)H4250*, even though duplications are interstitial in the first and terminal in the second. The time required for escape to occur spontaneously is comparable in the two (6 to 10 days *vs.* 5 to 8 days at 25°C), and escape of both is accelerated to an equal extent by the recessive gene *uvs-3*, which affects sensitivity to ultraviolet light by altering dark-repair mechanisms (SCHROEDER 1970).

A suppressor of the Dark Agar phenotype discovered by NEWMEYER (1970) is equally effective on *A/a* duplications from both *T(I→II)3911* and *In(IL→IR)H4250*.

In *Aspergillus nidulans*, duplications characteristically have abnormal morphology and reduced growth rate (evidently not due to heterokaryon incompatibility), and fast-growing sectors usually arise as a result of interstitial deletions (NGA and ROPER 1968; ROPER 1968). Our evidence so far indicates that small somatic deletions are not the usual mechanism of escape of inhibited duplications in Neurospora.

I am especially indebted to PATRICIA ST. LAWRENCE, who originally introduced me to the study of rearrangements in Neurospora, and who first worked out the properties of insertional translocations in fungi, in a pioneering analysis of *T(I→III)4540 nic-2*. DOROTHY NEWMEYER first recognized that duplication heterozygosity was responsible for an abnormal phenotype in Neurospora in her study of *In(IL→IR)H4250*. Without this precedent the significance of similar duplication types in strain 39311 would probably not have been recognized, nor the present study undertaken. I am grateful to EDWARD G. BARRY, DOROTHY NEWMEYER, and BARBARA C. TURNER for discussions and critical suggestions, to CECILE W. TAYLOR and DIANE C. BENNETT for assistance, and to Mrs. TURNER for contributing her observations on spore arrangements in ordered asci. Drs. BRIAN TURNER and GEORGE LEFEVRE, JR., have provided me with useful information on insertionals in man and in Drosophila. This paper was prepared at the Research School of Biological Sciences, Australian National University, Canberra, and the hospitality of Professor D. G. CATCHESIDE is greatly appreciated.

LITERATURE CITED

BACHMANN, B. J. and W. N. STRICKLAND, 1965 *Neurospora Bibliography and Index*. Yale University Press, New Haven, Conn.

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

BAINBRIDGE, B. W. and J. A. ROPER, 1966 Observations on the effects of a chromosome duplication in *Aspergillus nidulans*. J. Gen. Microbiol. **42**: 417–424.

BARRATT, R. W. and W. N. OGATA, 1970 Neurospora stock list. Fifth revision (June 1970). Neurospora Newsl. **16**: 23–123.

BARRY, E. G., 1960a A complex chromosome rearrangement in *Neurospora crassa*. Ph.D. Thesis, Stanford, Calif. (Abstracted in Diss. Abstr. **21**: 3233–3234, 1961). ——, 1960b Genetic analysis of an insertional translocation in *Neurospora crassa*. (Abstr.) Genetics **45**: 974. ——, 1972 Meiotic chromosome behavior of an inverted insertional translocation in Neurospora. Genetics **71**: 53–62.

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

BEADLE, G. W. and E. L. TATUM, 1945 Neurospora. II. Methods of producing and detecting mutations concerned with nutritional requirements. Am. J. Botany **32**: 678–686.

BLOOM, G. E. and P. S. GERALD, 1968 Localization of genes on chromosome 13: analysis of two kindreds. Am. J. Human Genet. **20**: 495–511.

BRIDGES, C. B., 1923 The translocation of a section of chromosome-II upon chromosome-III in Drosophila. (Abstr.) Anat. Record **24**: 426–427. ——, 1935 Demonstrations (1) of the first translocation in *Drosophila melanogaster* and (2) of normal repeats in chromosomes. (Abstr.) Records Genet. Soc. Am. **4**: 58; also printed in Am. Naturalist **70**: 41 (1936).

CATTANACH, B. M. and J. H. ISAACSON, 1967 Controlling elements in the mouse X chromosome. Genetics **57**: 331–346.

DARLINGTON, C. D., 1936 Crossing over and its mechanical relationships in Chorthippus and Stauroderus. J. Genet. **33**: 465–500.

DEMEREC, M., 1940 The genetic behavior of euchromatic segments inserted into heterochromatin. Genetics **25**: 618–627.

DE SERRES, F. J., JR., 1957 A genetic analysis of an insertional translocation involving the *ad-3* region in *Neurospora crassa*. (Abstr.) Genetics **42**: 366–367.

DOBZHANSKY, TH., 1930 Cytological map of the second chromosome of *Drosophila melanogaster*. Biol. Zbl. **50**: 671–685.

EICHER, E. M., 1967 The genetic extent of the insertion involved in the flecked translocation of the mouse. Genetics **55**: 203–212.

GILES, N. H., M. E. CASE, C. W. H. PARTRIDGE and S. I. AHMED, 1967 A gene cluster in *Neurospora crassa* coding for an aggregate of five aromatic synthetic enzymes. Proc. Natl. Acad. Sci. U.S. **58**: 1453–1460.

GROSS, S. R. and A. FEIN, 1960 Linkage and function in Neurospora. Genetics **45**: 885–904.

KOSSIKOV, K. V. and H. J. MULLER, 1935 Invalidation of the genetic evidence for branched chromonemas in the case of the Pale translocation in Drosophila. J. Heredity **26**: 305–317.

LEFEVRE, G., JR. and L. B. MOORE, 1968 Recombination in regions adjacent to deletions in the *X* chromosome of *Drosophila melanogaster*. Genetics **58**: 557–571.

LEJEUNE, J. and R. BERGER, 1965 Sur deux observations familiales de translocations complexes. Ann. Genet. **8**: 21–30.

LEWIS, R. W., 1948 Mutants of Neurospora requiring succinic acid or a biochemically related acid for growth. Am. J. Botany **35**: 292–295.

LINDSLEY, D. L. and E. H. GRELL, 1967 Genetic variations of *Drosophila melanogaster*. Carnegie Inst. Wash. Publ. **627.**

MULLER, H. J., 1935 The origination of chromatin deficiencies as minute deletions subject to insertion elsewhere. Genetica **17**: 237–252. ——, 1967 Translocational Pale Drosophila and Snaker mice, a semicentennial parallel. Mutation Res. **4**: 201–205.

MULLER, H. J. and W. S. STONE, 1930 Analysis of several induced gene-rearrangements involving the X-chromosome of Drosophila. (Abstr.) Anat. Record **47**: 393–394.

MURRAY, N. E., 1968 Polarized intragenic recombination in chromosome rearrangements of Neurospora. Genetics **58**: 181–191.

NEWMEYER, D., 1970 A suppressor of the heterokaryon-incompatibility associated with mating type in *Neurospora crassa*. Can. J. Genet. Cytol. **12**: 914–926.

NEWMEYER, D. and C. W. TAYLOR, 1967 A pericentric inversion in Neurospora, with unstable duplication progeny. Genetics **56**: 771–791.

NGA, B. H. and J. A. ROPER, 1968 Quantitative intrachromosomal changes arising at mitosis in *Aspergillus nidulans*. Genetics **58**: 193–209.

OHNO, S. and B. M. CATTANACH, 1962 Cytological study of an X-autosomal translocation in *Mus musculus*. Cytogenetics **1**: 129–140.

OLIVER, C. P., 1930 Complex gene rearrangements induced by X-rays. (Abstr.) Anat. Record **47**: 385.

PATAU, K., E. THERMAN, S. L. INHORN, D. W. SMITH and A. L. RUESS, 1961 Partial trisomy syndromes. II. An insertion as cause of the OFD syndrome in mother and daughter. Chromosoma **12**: 573–584.

PERKINS, D. D., 1962a Crossing over and interference in a multiply marked chromosome arm of Neurospora. Genetics **47**: 1254–1274. ——, 1962b Preservation of Neurospora stock cultures with anhydrous silica gel. Canad. J. Microbiol. **8**: 591–594. ——, 1966a An insertional translocation in Neurospora that generates duplications heterozygous for mating type. (Abstr.) Genetics **54**: 354. ——, 1966b Details for collection of asci as unordered groups of eight ascospores. Neurospora Newsl. **9**: 11. ——, 1967 Manifestation of reciprocal and insertional translocations in unordered tetrads, and their frequency in Neurospora (Abstr.) Genetics **56**: 580–581. ——, 1968 Heterozygosity for the *C/c* heterokaryon-compatibility alleles in duplications generated by a translocation in Neurospora. (Abstr.) Proc. 12th Intern. Congr. Genet. **1**: 67. ——, 1969 Multiple alleles at a heterokaryon-compatibility locus in Neurospora. (Abstr.) Genetics **61**: s47. ——, 1971 Viable duplications from intercrossing partially overlapping reciprocal translocations in Neurospora. (Abstr.) Genetics **68**: s50.

PERKINS, D. D., D. NEWMEYER, C. W. TAYLOR and D. C. BENNETT, 1969 New markers and map sequences in *Neurospora crassa*, with a description of mapping by duplication coverage, and of multiple translocation stocks for testing linkage. Genetics **40**: 247–278.

PERKINS, D. D., C. W. TAYLOR, D. C. BENNETT and B. C. TURNER, 1969 New morphological mutants that have been localized to linkage group. Neurospora Newsl. **14**: 13–18.

RATTY, F. J., 1954 Gene action and position effect in duplications in *Drosophila melanogaster*. Genetics **39**: 513–528.

RHOADES, M. M., 1931 A new type of translocation in *Drosophila melanogaster*. Genetics **16**: 490–504. ——, 1968, Studies on the cytological basis of crossing over. pp. 229–241. In: *Replication and Recombination in Genetic material*. Edited by W. J. PEACOCK and R. D. BROCK. Australian Acad. Sci., Canberra.

ROPER, J. A., 1968 Spontaneous deletions and tandem duplications at mitosis in *Aspergillus nidulans*. (Abstr.) Proc. 12th Intern. Congr. Genet. **1**: 68.

SCHROEDER, A. L., 1970 Ultraviolet-sensitive mutants in Neurospora. I. Genetic basis and effect on recombination. Mol. Gen. Genet. **107**: 291–304.

ST. LAWRENCE, P., 1959 Gene conversion at the *nic-2* locus of *Neurospora crassa* in crosses between strains with normal chromosomes and a strain carrying a translocation at the locus. (Abstr.) Genetics **44:** 532.

STRICKLAND, W. N. 1960 A rapid method of obtaining unordered Neurospora tetrads. J. Gen. Microbiol. **22:** 583–588.

SUTTON, E., 1940 Structure of salivary gland chromosomes of *Drosophila melanogaster* in exchanges between euchromatin and heterochromatin. Genetics **25:** 534–540.

TATUM, E. L., R. W. BARRATT, N. FRIES and D. BONNER, 1950 Biochemical mutant strains of Neurospora produced by physical and chemical treatment. Am. J. Botany **37:** 38–46.

TAYLOR, C. W., 1965 A more efficient procedure for scoring mating type and aberrations. Neurospora Newsl. **8:** 21.

TURNER, B. C., C. W. TAYLOR, D. D. PERKINS and D. NEWMEYER, 1969 New duplication-generating inversions in Neurospora. Can. J. Genet. Cytol. **11:** 622–638.

VOGEL, H. J., 1964 Distribution of lysine pathways among fungi: evolutionary implications. Am. Naturalist **98:** 435–446.

WELSHONS, W. J., 1965 Analysis of a gene in Drosophila. Science **150:** 1122–1129.

WESTERGAARD, M. and H. K. MITCHELL, 1947 Neurospora. V. A synthetic medium favouring sexual reproduction. Am. J. Botany **34:** 573–577.

APPENDIX

OTHER NEUROSPORA REARRANGEMENTS THAT GENERATE NONTANDEM DUPLICATIONS

T(I→II)39311 is representative of an important but neglected class of rearrangements—those that regularly produce nontandem duplications in meiosis. The best known Neurospora aberrations that do so are listed in Appendix Table 1. There are three main types—insertional translocations such as *T(I→II)39311*, reciprocal translocations having one break point effectively terminal, and pericentric inversions similarly having one break point terminal. Each of these strains can be used predictably to produce progeny that are duplicated and heterozygous for a specific segment of known marker content, as indicated in column 2 of the table, and in Appendix Figure 1.

Rearrangement *T(IR→VR)S1325* is not listed although it is unquestionably an insertional translocation (MURRAY 1968). The reason for its omission is that duplication progeny do not survive, possibly because a short piece of VR may have been inserted simultaneously into IR (BARRY, personal communication).

At present any locus in at least one third of the genome can be obtained in heterozygous condition in a partial diploid from one or another of the strains listed.

Duplications for defined segments can also be produced by intercrossing partially overlapping aberrations. This has been done extensively in Neurospora using pairs of reciprocal translocations that involve the same two chromosome arms (PERKINS 1971).

APPENDIX TABLE 1

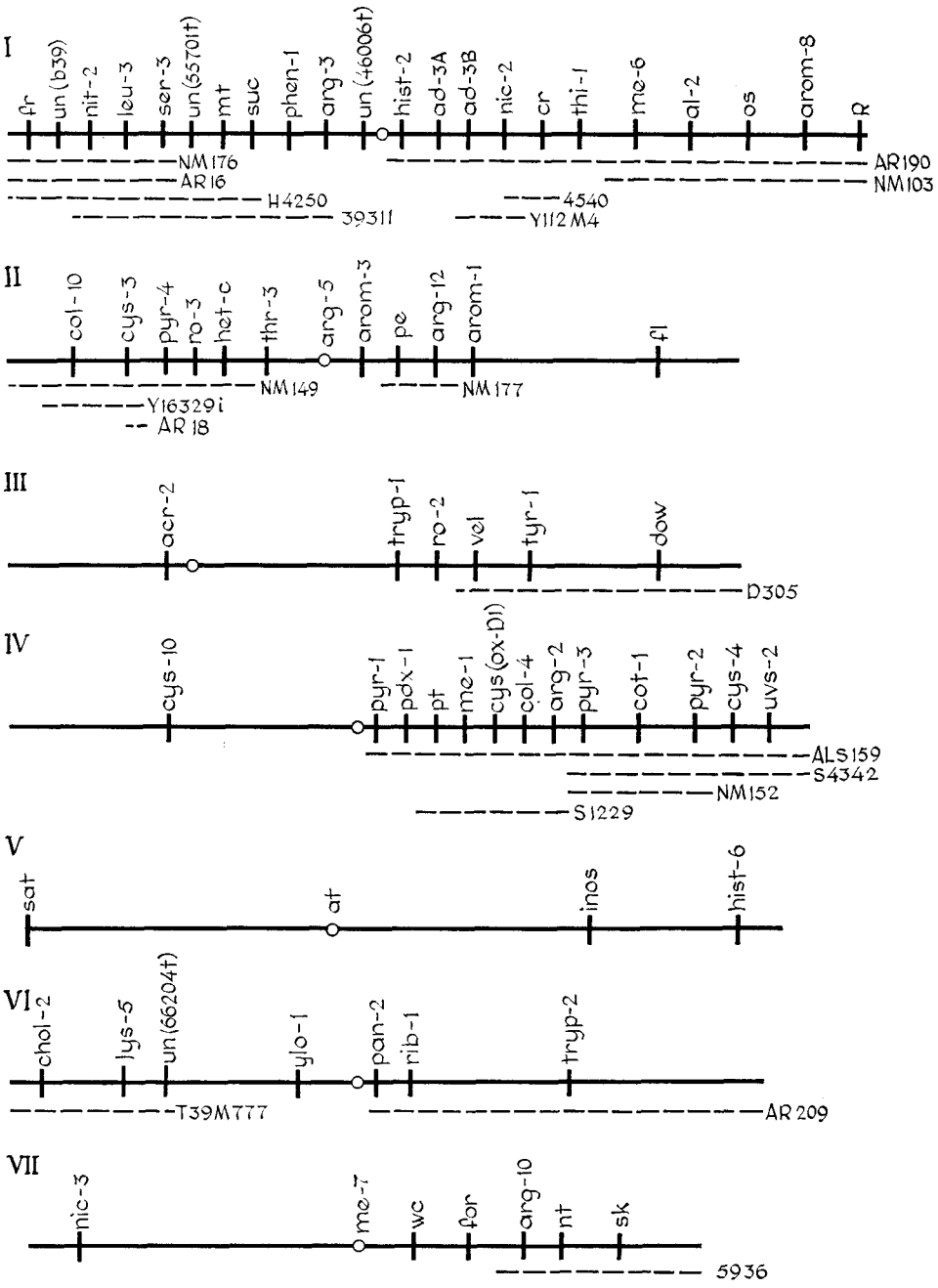
Duplication-generating rearrangements in Neurospora

Rearrangement*	Extent of duplication†	Site of transposition	Reference‡
Insertional translocations:			
T(IIL→IIIR)AR18	Unmarked IIL segment between *col-10* and *pyr-4*	Distal to *dow*	A. KRUSZEWSKA and PERKINS
T(IR→IIIR)Y112M4 ad-3B	*ad-3A ad-3B* **nic-2** *thi-1*	IIIR (P. ST. LAWRENCE, personal communication)	DE SERRES 1957
T(IVR→I)NM152	*col-4* **pyr-3 cot-1 pyr-2** *cys-4*	10 units right of *arg-3*	PERKINS and RADFORD
T(IIR→IL)NM177	*arom-3* **pe arg-12** *arom-1*	Between *leu-3* and *mt*	KRUSZEWSKA and PERKINS
T(I;II;IV;IVR→VIIR)S1229 arg	*pdx-1* **pt me-1 cys(ox-D1) col-4 arg-2** *pyr-3*	Between *me-7* and *nt*	BARRY 1960a, b
T(IVR→IIIR)S4342	*arg-2* **pyr-3 rib-2 . . . uvs-2**	Near *tryp-1*	. . . . .
T(IR→IIIR)4540 nic-2	*nic-2* **cr** *thi-1*	Between *vel* and *tyr-1*	ST. LAWRENCE 1959 and unpubl.
T(IIL→VI)Y16329i	**col-10 cys-3** *pyr-4 ro-3*	Near *ylo-1*	. . . . .
T(IL→IIR)39311	*un(b39t)* **nit-2 mt arg-3** *un(46006t)*	Between *arom-3* and *pe*	(This paper)
Translocations having one break effectively terminal:			
T(IR→VIR)NM103	*thi-1* **me-6 nit-1 . . . R** (*me-6* and markers right of it)	Distal to *tryp-2*	TURNER and PERKINS
T(IIL→VR)NM149	**col-10 . . . het-c ro-3** *thr-3* (*ro-3* and markers left of it)	Near *hist-6*	PERKINS 1968, 1969

APPENDIX TABLE 1—Continued

T(IVR→VIR)ALS159	**pyr-1 pdx pyr-3 cys-4 uvs-2** (all IVR markers)	Distal to *tryp-2*	.....
T(IR→VL)AR190	*un(46006t) sn* **hist-2 . . . R** (all IR markers)	Distal to nucleolus organizer	BARRY and PERKINS 1969
T(VIR→IVR)AR209	*ylo-1* **pan-2 rib-1 tryp-2** (all VIR markers)	Distal to *uvs-2*	.....
T(IIIR→VIL)D305	*ro-2* **vel phen-2 tyr-1 . . . dow**	Distal to *chol-2*	.....
T(VIIR→IL)5936	*for* **arg-10 nt sk**	Distal to *fr*	.....
T(VIL→IR)T39M777	**chol-2 lys-5 un(66204t)** *cys-1 ylo-1*	Near *R*	.....
Pericentric inversions having one break effectively terminal:			
In(IL→IR)AR16 (also AR9i, AR11, AR15)	**fr . . . ser-3** *un(55701t) mt* (*ser-3* and markers left of it)	Distal to *R*	TURNER *et al.* 1969
In(IL→IR)NM176	**fr . . . ser-3** *un(55701t) mt* (*ser-3* and markers left of it)	Distal to *R*	TURNER *et al.* 1969
In(IL→IR)H4250	**fr . . . mt suc** *phen-1 ad-5 arg-1* (*suc* and markers left of it)	Distal to *R*	NEWMEYER and TAYLOR 1967

• Arrows indicate the donor and recipient chromosome arms. R = right arm, L = left arm.
† Loci in boldface type are included in the duplicated segment.
‡ Where no date appears the results are unpublished.



APPENDIX FIGURE 1.—Intervals covered by the duplications listed in Appendix Table 1. Linkage groups are designated by Roman Numerals, and only relevant markers are shown. A broken line under the donor linkage group indicates the segment that is involved in the duplication produced when each designated rearrangement is crossed by Normal sequence. Column 3 of the table gives the new location of each segment in rearranged sequence.