EUPLOID DERIVATIVES OF DUPLICATIONS FROM A TRANSLOCATION IN NEUROSPORA[1]

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ABSTRACT

Nontandem terminal chromosome duplications derived from *N. crassa* translocation *T(I→VI)NM103* give rise mitotically to some daughter nuclei which have become euploid by loss of one or the other of the two duplicated segments. Loss of the segment in normal sequence occurs as often as loss of the translocated segment. This is in contrast to all of several other Neurospora duplications that have been studied, where loss of the segment in normal sequence is absent or rare.——*T(NM103)* has the distal two thirds of linkage group IR exchanged with the right tip of VI. Crosses to normal sequence produce a class of morphologically distinct progeny with IR chromosome duplications. For a few days after germination, test crosses of these progeny are barren (make perithecia but few or no spores, as observed commonly with Neurospora duplications). Growing duplication cultures become fertile by accumulating nuclei which have been reduced to either normal sequence (by loss of the segment in translocation sequence) or translocation sequence (by loss of the segment in normal sequence). Both types usually appear within the first week of growth. Naturally formed mixtures or heterokaryons of *NM103* duplication nuclei and their reduced euploid products have been studied by plating and by progeny testing. Determination of nuclear type is based on culture morphology, expression of genetic markers, and crossing behavior. Within the limits of testing, loss is found to begin precisely at the interchange points. The unique finding of frequent breakdown of normal-sequence linkage group I chromosomes is not dependent on the strain from which the chromosome was derived. Many different strains were tested, and for each one evidence was found that nuclei reduced to translocation sequence had been produced from duplication nuclei by loss of the segment in normal sequence.

MANY chromosome rearrangements have been detected in this laboratory by crossing mutagenized cultures to testers of normal chromosome sequence (Normal testers). Strains with rearranged chromosomes usually produce 20% to 50% aborted (white) ascospores in such crosses, in contrast to isosequential crosses, where 90% or more of ascospores are viable and black. The strain studied here, *NM103*, produced 25% white spores, which is typical of several different types of duplication-producing rearrangements (PERKINS 1974). When progeny from *NM103* × Normal were tested, up to one third showed markedly reduced

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fertility. In Neurospora such "barren" progeny are commonly found to have part of the genome duplicated ("partial diploids" or "duplications"). Further analysis of strain *NM103* verified the presence of duplications and showed that a long terminal segment of one chromosome had been translocated to the tip of another.

The present paper includes the first detailed analysis of a translocation to a tip in Neurospora. Previous reports of other duplication-producing rearrangements have described an insertional translocation (PERKINS 1972), and two quasiterminal pericentric inversions (NEWMEYER and TAYLOR 1967; TURNER *et al.* 1969).

Duplications from *T(NM103)* were found to be unstable, and their behavior is described here. Instability of duplications from other Neurosopra rearrangements had previously been found by NEWMEYER and TAYLOR (1967) and by PERKINS (cited briefly in PERKINS and BARRY 1977). Unstable duplications are also known in Aspergillus (ROPER 1973). When the various Neurospora duplications are compared, diverse patterns of instability are found (reported briefly in PERKINS, NEWMEYER and TURNER 1972). Duplications from *T(NM103)* are unique in their pattern of breaking down by deleting with equal frequency, either the translocated segment or the segment in normal position. A preliminary account has been published in abstract (TURNER 1975). The accompanying paper by NEWMEYER and GALEAZZI describes another type of duplication instability and presents a model applicable to both types.

MATERIALS AND METHODS

T(I→VI)NM103 originated as a prototrophic survivor of a filtration-enrichment experiment (see PERKINS and BARRY 1977). In this experiment ultraviolet irradiation killed 40 to 60% of ((multinucleate) conidia from Emerson *a* wild type (PERKINS, personal communication). The original *NM103* stock also carried a morphological mutation that segregated from the aberration and was not carried in the stocks used for this analysis. The FGSC (Fugal Genetics Stock Center) numbers of the *T(103)* standard reference stocks are 2138 (*a*) and 2137 (*A*). The series of crosses from which they were obtained involved several of the standard laboratory strains, so that their genetic background is considerably different from the strains of origin.

The wild types used in this study were 70-OR8-1a and 74-OR23-1A and their derivatives. The nonconidiating normal-sequence fluffy strains *fl^{p}a* (FGSC No. 1690) and *fl^{p}A* (FGSC No. 1838) were used as standard testers for scoring chromosome structure and mating type (*mt*, *A* or *a*). Linkage group VI markers were: *lys-5*, lysine (STL7); *rib-1*, riboflavin (51602t); *trp-2*, tryptophan (45302); *ylo-1*, yellow (Y30539y). Linkage group I markers in addition to mating type were: *ad-9*, adenine (Y154M614); *al-1^{y}*, albino-yellow (ALS4); *al-1^{aur}*, aurescent (34508); *al-2*, albino (15300); *arg-13*, arginine (RU3); *cyh-1* (KH52) [the wild type allele, *cyh^{S}*, is sensitive to cycloheximide (actidione), the mutant, *cyh^{R}*, is resistant]; *fr*, frost (B110); *his-3*, histidine (Y175M614); *met-6*, methionine (35809); *nit-1*, nitrate (34547); *R*, Round spore (35408R); *thi-1*, thiamine (56501); *un-18* unknown lesion, heat-sensitive (T54M94). References are given by BARRATT and OGATA (1976). The normal gene order for the two groups is shown in Figure 1 (for documentation see RADFORD 1976. The order *nit-1 cyh-1-al-1* is from unpublished data of the author).

Technical methods: Chromosome structure is scored by crossing to normal sequence *fl* testers on slants of synthetic cross medium (WESTERGAARD and MITCHELL 1947) in 75 mm tubes and

examining at 10 days and about 12 days to score for fertility and percent of black ascospores (spore pattern). Barren test crosses indicate duplications; fertile test crosses indicate euploids, and the latter are scored as Normal (more than 90% black) or Translocation (75% black). Occasionally a duplication becomes fertile very early, so that additional criteria are required for scoring, such as morphology. (See ANALYSIS OF THE REARRANGEMENT for further explanation.)

Conidial platings from duplication stocks were made on a medium that produces colonial growth (carbon source 2% sorbose, .05% fructose, and .05% glucose). A conidial suspension in medium with .75% agar was poured over a 2% agar base. Supplements were added for all biochemical requirements carried by the parents of the duplication. Plates were incubated at 30° for three days, and then transfers were made to 12 × 75 mm slants of supplemented minimal (VOGEL 1964) or glycerol-complete medium.

Cycloheximide resistance is scored by inoculating tubes of medium which have 10 μg/ml cycloheximide (actidione) added before autoclaving. Growth is scored after three days at 25° or two days at 34°. Since cycloheximide resistance is recessive (TURNER 1976), initially sensitive tests are held for seven additional days and then scored for appearance of a cyh^R component that has "escaped" from inhibition.

ANALYSIS OF THE REARRANGEMENT

Initial observations: DAVID PERKINS (unpublished) detected the rearrangement, showed that it produced viable duplications, and found that linkage groups I and VI were involved. As a first step in verifying that the white (aborted) ascospores were caused by a rearrangement, individual groups of eight ascospores, representing unordered tetrads, were collected and classified according to their content of Black:White spores (method of PERKINS 1974). There was some difficulty distinguishing between 4:4 and 6:2 tetrads because some ascospore pairs are intermediate in color. However, only three classes of asci were found in any significant quantity: 8:0, 6:2, and 4:4. This pattern is typical of duplication-producing rearrangements.

Reciprocal to the 25%-deficient spores, it is expected that another 25% of spores (one third of the black, viable spores) carry a duplication—that portion of the genome that is missing from the aborted spores. In crosses of *NM103* × Normal about one third of the progeny are barren, a phenotype often found for Neurospora duplications. Barren progeny typically make abundant perithecia when crossed to the Normal testers, but no spores, or relatively few spores, are produced.

As a rule the barren (duplication) progeny from NM103 are retarded in growth at 25° and have a distinctive vegetative morphology when grown up at 34°. Growth is concentrated at the top of the slant and has a compact appearance. When combined with mutants that affect morphology or growth rate, the duplication-mutant class sometimes can be scored by growing transfers at both 25° and 34°. The type of medium used has not had a noticeable effect on morphology.

Genetic mapping of T(I→VI)NM103: Mapping was done in two ways, by conventional meiotic recombination and by duplication coverage (by the method of PERKINS *et al.* 1969). Table 1 gives the results of crosses from which the rearrangement sequence in Figure 1b was derived.

Crosses 1, 2, and 3 show the location of the interchange points, and cross 4 shows that the most distal IR marker is covered by the duplication.

In brief, for Group VI markers, barrens usually carry the alleles that entered with the *NM103* parent. For markers in Group I that are not covered, the barrens usually carry the alleles that entered with the Normal parent. For the covered markers in Group I, the barrens have the dominant phenotype regardless of the coupling phase.

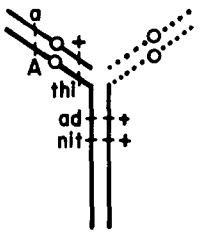
An occasional exception arises because, when parents are heterozygous for a covered marker, meiotic crossing over can produce some duplications that are homozygous recessive.

Cross 7 is an isosequential cross of two *NM103* parents. All progeny are fertile translocations, and the map order is the one inferred from the structurally heterozygous crosses. As predicted from the proposed structure, *mt* is not linked to the other markers in cross 7. A well marked isosequential cross provides, of course, a definitive demonstration of the structure of a rearrange-

TABLE 1

Crosses related to structural analysis

CROSS 1



T (a) X N thi ad nit (A)

Genotype* of cross	Non-duplications: Progeny types thi	structure#	ad	nit	No.	Duplications†: Progeny types thi	ad	nit	No.
	-	N	-	-	33§				
	+	T	+	+	33	+	+	+	1
	-	T	+	+	3	-	+	+	12
	-	N	+	+	1				

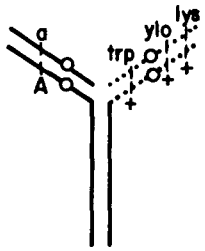
The phenotype of the duplications can be explained only by placing interchange point between thi-1 and ad-9 so that thi-1 is not covered but ad-9 and nit-1 are. Recombinants among the fertiles are consistent with this order.

CROSS 2

Genotype of cross	Non-duplications: Progeny types structure	met	No.	Duplications: Progeny types met	No.
	N	-	14	-	0
T (a) X N met (A)	not tested	+	15	+	19

Interchange point was located between thi-1 and the next known marker met-6 by scoring duplications for met-6 coverage.

CROSS 3

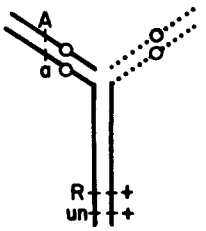


T (a) X N lys ylo trp (A)

Genotype of cross	Non-duplications: Progeny types lys	ylo	trp	structure	No.	Duplications: Progeny types lys	ylo	trp	No.
	+	+	+	T	11	+	+	+	9
	-	-	-	N	13				
	-	+	+	T	3	-	+	+	1
	+	-	-	N	4				
	-	-	+	T	5	-	-	+	1
	+	+	-	N	5				
	+	+	+	N	2				
	-	-	-	T	4	-	-	-	2
	+	-	+	T	1				
	-	+	-	N	0				

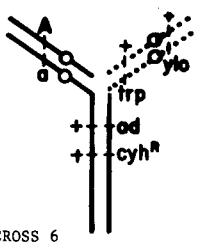
Map relations from the non-duplications are: lys 17 ylo 23 trp 13 T. Duplications follow the same distribution as T(NM103) progeny, showing no indication of coverage.

Table 1 continued

CROSS 4 Genotype of cross	Non-duplications: Progeny types structure	R	un	No.	Duplications: Progeny types R	un	No.
	T	+	+	10	+	+	13
	N	-	-	13			
	N	+	+	8			
	T	-	-	5			
	T	+	-	2			
	N	-	+	2	-	+	2

T (A) X N R un (a)

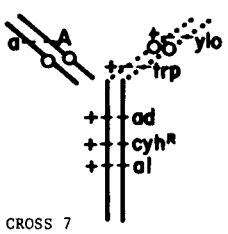
un-18 is the most distal marker known on IR. Map relations from the non-duplications are T 33 R 10 un.

CROSSES 6 and 7 Genotypes of crosses	Progeny types cyh	ad	trp	Cross 6: No. non-duplications	Cross 6: No. duplications	Cross 7: No. (all T)
	S	+	+	27 N	5	37
	R	-	-	34 T		32
	S	-	-	2 T		2
	R	+	+	1 N		2
	S	+	-	6 N	9	5
	R	-	+	5 T		7
	S	-	+	0		1
	R	+	-	0		0

CROSS 6

T ad cyh^R; ylo trp (A) X wild type (a)

Progeny types ad	mt	Cross 6: No. non-duplications	Cross 6: No. duplications	Cross 7: No. (all T)
+	a	29 N	11	23
-	A	36 T		23
+	A	5 N	3	21
-	a	5 T		19

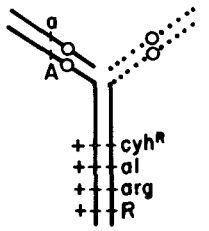


CROSS 7

T ad cyh^R al-1^y; ylo trp (A) X T (a)

The marked T(NM103) parent is crossed to normal sequence in cross 6 and to T(NM103) sequence in cross 7. In cross 6 coverage of cyh-1 and ad-9 is shown for the T(NM103)-mutant coupling phase. Map relations (relevant non-duplications of crosses 6 and 7 pooled) are al 12 cyh 5 ad 14 trp 14 ylo. Moderate linkage between T(NM103) and mt (e.g., mt 13 T, in cross 6) was found in all T X N crosses. In cross 6, mt and ad are linked and, in contrast in cross 7, mt and ad segregate independently, as expected.

Continuation of Table 1

CROSS 8 Genotype of cross		Non-duplications: Progeny types Structure	cyh	al	arg	R	No.
	Non-crossovers	T	R	-	-	-	9
		N	S	+	+	+	13
	Single crossovers:	T	S	+	+	+	5
		N	R	-	-	-	6
		T	R	+	+	+	4
		N	S	-	-	-	1
T cyh^{R} al-1^{y} arg R (a) X		T	R	-	+	+	7
wild type (A)		N	S	+	-	-	8
		T	R	-	-	+	0
		N	S	+	+	-	2
	Double crossovers:	T	S	+	-	-	2
		N	R	-	+	+	1

Crossovers are predominantly single, and reciprocal classes are roughly equal, consistent with the simple structure proposed for T(NM103). Map relations from these non-duplications are T 24 cyh 9 al 31 arg 1 R.

* The map of VI, shown as a dotted line, is inverted in the table in order to show pairing of IR. Conventionally, *trp* is shown at the right end of VI.

† Duplications are usually scored by both morphology and barrenness, but sometimes by only one or the other.

‡ Scored by percent black ascospores in crosses to normal sequence testers. See MATERIALS AND METHODS.

§ Two additional isolates were fertile with patches of N and T spore patterns on the cross tube. It is presumed they were duplications which broke down early.

ment. Before such a cross can be made, however, it is necessary to find out what regions to mark and to recombine markers into the rearrangement sequence. If the earlier steps are correct, the isosequential cross provides confirmation and proof of structure, but little new information on marker sequence. Since it sometimes proves impossible to obtain recombinants between break points and closely linked loci, the duplication-coverage data provided by crosses of Translocation × Normal are critical for mapping them.

The origin of *T(NM103)* from normal sequence can most simply be explained as a simple interchange between the right end of I and the right (dispensable) tip of VI. More complex structures were rejected because of experimental evidence in the nonduplication progeny. If the translocated piece of I were not functionally terminal, or if it were inserted into VI proximal to indispensable material, then the reciprocal crossover classes would not be equal. If both these conditions were true, single crossovers would be lethal in both directions making double crossovers the predominant type of exchange. In cross 8, which is well marked, there are only three doubles but 33 singles. The reciprocal single crossover classes are equal—16 in translocation sequence and 17 in normal sequence.

RESULTS

The structure of *T(I→VI)NM103* is shown in Figure 1b, and evidence for it has been given in the preceding section. The duplication cultures obtained by crossing *T(NM103)* × Normal will be symbolized *Dp(NM103)*, or will be called *NM103* duplications, or simply duplications where this is not ambiguous.

NM103 *duplications produce both barren and fertile perithecia in crosses*: When young cultures of duplications are crossed back to either normal or translocation sequence they produce many perithecia but few ascospores. As with most other Neurospora duplications, the perithecia are typically barren. However, in individual cross tubes testing *NM103* duplications, it was observed that among the much larger number of rounded perithecia, there commonly appeared one or more fully fertile perithecia that developed beaks and produced ascospores without any delay. This observation raised the question whether the nonbarren perithecia might originate from fertilization by exceptional nuclei that had lost all or part of the duplicated material. Such a reduction from duplication to euploid appears to be the explanation.

Vegetative cultures of Dp(NM103) *include reduced nuclei*: Insight into the timing of nuclear reduction was obtained by chance when a basket of cultures that had been at room temperature for several weeks needed to be retested. The duplications no longer were scored as barren in the repeat test crosses. This observation strongly suggested that the occasional fertile perithecia produced by younger duplication cultures resulted from events taking place during vegetative growth prior to fertilization, and that aging provided increased opportunity for such events to take place. This was verified directly by plating conidia (asexual spores) from duplications and analyzing the cultures derived from single conidia.

Conidial platings: Nineteen duplications from five crosses were analyzed. Their genotypes are shown in Table 2. Thousands of individual colonies were observed on the plates, and representatives of every type seen were sampled to test tubes and tested for genetic markers. In addition to those which were scored only for the markers, 495 were also crossed to normal sequence testers and scored for fertility and spore pattern (percent of black ascospores).

The major finding is that almost every duplication that was plated produced two kinds of reduced nuclei, either normal or translocation sequence (as shown in diagrams b and c in the heading of Table 2). A number of the duplications in Table 2 were plated repeatedly, at different ages, and they were found to have a higher proportion of reduced nuclei after aging. The reduced nuclei are considered euploid because the cultures arising from them are fully fertile, and all alleles that mark one of the segments of the duplication have been lost.

The colonies were of four types: intact duplication, derived normal sequence, derived translocation sequence, and mixed. Six exceptional cultures were also obtained (one of these is discussed under somatic recombination and shown in Figure 3d).

Intact duplications produce large, diffuse, nonconidiating colonies on sorbose medium. Reduced nuclei produce smaller, conidiating colonies. For the duplica-

TABLE 2

NM103 duplications of varied genotype and genetic background used for conidial platings Conidial isolates from duplication cultures consistently made colonies of three classes

a. Duplication structure b. Reduced to normal structure c. Reduced to T(NM103) structure

Cross No.	Parent genotypes Normal X Translocation	Duplication No.	Original genotype of duplication*
8	N wild type (A) X T cyh^R al-1^y arg R (a)	8-1, 8-2, 8-3$^+$	A + + + + / cyh^R al-1^y arg R
9	N ad al-1^{aur} R (A) X T cyh^R al-1^y (a)	9-1	A ad + al-1^{aur} R / + cyh^R al-1^y +
10	N ad (A) X T cyh^R al-1^y arg R (a)	10-1	A ad + + + + / + cyh^R al-1^y arg R
11	N al-2 (A) X T cyh^R al-1^y arg (a)	11-1 to 11-8	A al-2 + / + al-1^y
		11-9	a al-2 + / + al-1^y
		11-10	a + + al-1^y arg / cyh^R al-2 + +
12	N met al-2 R (a) X T ad cyh^R al-1^y; trp ylo (A)	12-1	a met + + al-2 + R / + ad cyh^R + al-1^y +
		12-4	A met + + al-2 + + / + ad cyh^R + al-1^y +
		12-6	A met + cyh^R + al-1^y + / + ad + al-2 + +
		12-7	a met ad cyh^R + al-1^y + / + + + al-2 + R

* Upper line shows linkage group I normal sequence. Lower line shows translocated segment of the duplication. Linkage group VI is omitted. The wild type *cyh*^{S} allele is symbolized by a plus sign. Genotypes in this column were obtained by scoring the reduced derivatives shown in diagrams b and c. (Except that in cross 8, plated cultures were not tested for *cyh* or *arg*. *cyh*^{R} cultures were obtained from the original heterokaryotic cultures of Dp 8-1, 8-2 and 8-3 by transfer to cycloheximide medium and were scored for all markers and crossed to testers.) (Among duplications, meiotic recombination of covered markers is roughly comparable to that found in

tions in Table 2, one or both of the reduced classes express an albino allele which allows for visual selection. When a duplication carries *al-2* on one segment and *al-1^{v}* on the other, the intact duplication colonies are the orange wild-type color because of complementation, and the colonies from reduced nuclei are white and yellow, respectively.

A gene that is especially useful for a study of this type is *cyh-1*. Mixtures or heterokaryons growing on cycloheximide-supplemented medium will tolerate only a small fraction of nuclei carrying the wild-type sensitive allele (symbolized + or *cyh^{S}*). The sensitive allele is dominant in intact heterozygous duplications. However, heterozygous *NM103* duplications transferred to cycloheximide medium usually start to grow after a lag. The cultures that finally grow up contain a majority of nuclei that have lost the *cyh^{S}* allele and the alleles of the other markers that were coupled to it. Therefore, in addition to scoring the reduced cultures as either resistant or sensitive, it is also possible to score the intact duplication cultures as heterozygous. When a mixed culture is transferred to cycloheximide medium, the transfer culture is almost always a pure enough sample of the *cyh^{R}* component to allow scoring of the other markers and spore pattern of that component.

Conidial isolates with intact duplication nuclei (Table 2, diagram a) were found for all plated duplications except 12–1. However an early test cross of the original 12–1 culture had many barren perithecia, so it is assumed that intact duplication nuclei were present, but in such small numbers that none happened to be present in the sample plated. Many of the duplication type subcultures obtained from various platings were further analyzed by observing their breakdown products in crosses and in transfers to test media.

The derived normal sequence cultures (Table 2, diagram b) gave 90 to 95% black ascospores when crossed to Normal testers. These isolates, derived by loss of the segment in translocation sequence, were found for all duplications except 8–1 and 11–3. These two, however, also produced normal sequence derivatives in a different type of analysis.

In test crosses of the original cultures, Dp 8–1 produced both round and nonround spores. As described later, this has been shown to be the result of crossing with a culture that contains both types of reduced nuclei, one carrying *R* and the other *R+*. Progeny from Dp 11–3 × wild type included some that were normal sequence, *al-1^{v} arg*. These progeny must have been produced by a nucleus which retained the markers in the segment in normal position and which had become fertile by losing the segment in translocation position.

T(NM103) sequence cultures (Table 2, diagram c), derived by loss of the segment in normal sequence, were found in all platings. They were scored indirectly by their pattern of making about 25% white spores with the normal sequence testers. Additionally, conidial isolates from four different duplications were also tested by crossing to *T(NM103)* testers. Eighteen of the 20 individual tests gave more than 95% black ascospores, and the other two gave slightly less than 90%.

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nonduplication progeny, but *cyh*, *R* and the albino loci were exploited in some cases to preselect nonrecombinants for plating.)

† Four class *a* (intact duplication) conidial isolates obtained from the original plating of Dp 8–3 were themselves plated. All gave isolates of the three classes.

Additional evidence that derived translocation cultures are functionally equivalent to the *T(NM103)* parent comes from progeny analysis of crosses involving the original, still mixed, duplication cultures. Some of the progeny can only be explained as coming from nuclei which retained the markers and structure of the segment in translocation sequence, and which became fertile by losing the segment in normal sequence.

Mixed or heterokaryotic colonies are commonly found in any plating that produces significant numbers of two or more different types of pure colonies, simply because Neurospora conidia are multinucleate. Conidial isolates that were intermediate in color between albino and wild type were automatically considered to be heterokaryons, but a few of them were sampled when one of the components was needed for analysis. In several of the platings, especially those from cross 11, colonies that looked at all different from the three main classes were sampled and tested. In each plating where there was a sizeable class of colonies that had nonduplication morphology but wild-type phenotype for the covered markers, a few of these colonies were sampled. At least one of the components was detected or extracted in order to verify that the colonies were really heterokaryons.

All together, complete tests were done on 69 isolates with intact duplication nuclei, 130 with derived normal sequence, 238 with derived translocation sequence, and 58 mixed cultures, and the six exceptional cultures previously mentioned, which will be described below. The relative numbers of the different types of colonies have no significance with regard to the relative or absolute frequency of loss of the two segments. During the study, platings were made for different purposes, and usually colonies were sampled selectively. Furthermore, even when all colonies were sampled, the frequencies were not reliable. For example, in the original plating of duplication 8–3, approximately 60% of the colonies contained nuclei that were reduced to normal sequence (sometimes mixed with other types of nuclei). Four of the conidial subcultures that behaved like intact duplications were aged and then plated individually. Among these four subcultures, genetically identical to each other and to the original Dp 8–3, the frequency of subcultures of normal sequence varied from 0.7 to 25%. (The proportion of subcultures with translocation sequence also varied widely.) These variations are not surprising, for if reduction to normal sequence occurs early in the growth of the culture, the proportion of normal conidia will be much higher than if the same event occurs much later.

Partially reduced nuclei are not found. No cultures tested showed loss of one marker without loss of all the other markers on the arm. This includes the tested components of mixed cultures and the reduced products of duplication cultures grown on selective media. The distal markers closest to the interchange point, *met* and *ad*, which are found in repulsion in duplications 12–1, 12–4 and 12–6, were scored in 93 nonduplication isolates from these three platings (all 93 were classified as nonduplication by morphology and by expression of *al-1* or *al-2*, and 59 of them were also crossed to testers and found to be fertile). A total of 40 were

met$^+$, and all of these were *ad*, showing that the *ad*$^+$ in normal sequence was deleted. The other 53 nonduplication isolates were *ad*$^+$ *met*, showing that the *met*$^+$ in translocation sequence was deleted. The *met* locus is very close to the interchange point (no recombination has been observed) and *ad* is the first known marker distal to *met* (about 9% recombination between the two markers was found in a Normal × Normal cross, by PERKINS *et al.*, 1969, and less than 1% has been found in Normal × Translocation crosses in this study).

Almost all cultures that had lost markers also showed full fertility, which is taken to mean the loss of substantially all of the duplicated material. However, in the platings of four different duplications, a total of five exceptional cultures were found:

Dp(NM103)8–3: Among *al* isolates tested, 85 were fertile *R*, as expected, and one was barren.

Dp(NM103)10–1: Two of the ten *ad*+ *cyh*^{R} *al arg* isolates differed from the rest in two ways: their morphology was not *R* (nor exactly wild type), and they were completely barren.

For each of the duplications of cross 11, about 150 conidia were plated. The 1500 colonies were searched for any that might be atypical, especially ones with duplication morphology but with albino phenotype, showing loss of one of the complementing *al*+ alleles. Most of the 70 isolates that were sampled and tested were one or another of the three main types or a mixture of these types, but two of the duplications produced single exceptional isolates:

Dp(NM103)11–7: There were only seven colonies that were not wild type in color. One was *al-1*^{y} Translocation. One was *al-2* (white) with duplication morphology, definitely barren, stably *cyh*^{S}, and after it was aged it made a fertile normal cross. This exceptional component may have been present in some of the other five colonies, all of which appeared to be mixtures containing a high proportion of *al-2* Normal nuclei.

Dp(NM103)11–8: One albino isolate had a nonduplication colonial morphology, but the transfer to complete medium looked like a duplication. Test cross was barren, and a later cross after aging was still barren.

Some or all of these exceptions could be due to deletion beginning between the *NM103* interchange point and the first marker, leaving a short unmarked segment still duplicated. The only reason for postulating incomplete loss in these cases is the general observation that many duplications, including very short ones, are barren. Another reasonable hypothesis is that there was somatic recombination resulting in a nucleus with a complete *NM103* duplication but with the segments homozygous. Such a new duplication might be expected to accumulate derived euploid nuclei just as the original duplication cultures do. Three of the exceptional barren cultures were retested after being aged for several weeks, and one of the three was fertile, the other two still completely barren.

Fertility can result from loss of either segment of the NM103 *duplication.* There is no tendency for breakdown to occur by loss of the translocated segment in preference to the segment in normal sequence, as has been found for duplications from other rearrangements such as *T(IR→VL)AR190* or *T(VR→IIIR)AR209* (PERKINS, NEWMEYER and TURNER 1972). Crosses with a number of wild types and with marked Normal I testers have consistently produced *NM103* duplications that give subcultures missing the Normal-linked alleles. That is, no Normal stock has been found whose IR arm will not readily lose covered markers

when it is introduced into *Dp(NM103)*. The previously described gene *cyh-1* has been very useful in this part of the study. Duplications were made by crossing the Normal stock to be tested by *T(NM103)* stocks carrying the mutant, resistant allele. Because the intact duplications will not grow on cycloheximide medium, even a very small number of nuclei that have lost the sensitive allele carried by the segment in normal sequence can be recovered from platings or transfers to this medium. (When tested by other methods, these same duplications are found to lose the segment in translocation sequence with approximately equal frequency, just like duplications not involving *cyh-1*).

Some of the crosses used for mapping *T(NM103)* included the distal IR marker Round spore (*R*) (see Figure 1). Not only did this marker greatly facilitate the initial observations, but it also became a keystone for the method that was eventually designed to analyze the behavior of *NM103* duplications in crosses.

In the ascus, *R* is dominant to its wild-type allele, and all ascospores, regardless of genotype, are round (in some crosses rare nonround spores are found) (MITCHELL 1966). This is not a maternal effect; in fact, *R* is female sterile. (*R* also causes a subtle change in culture morphology, but this vegetative effect is recessive. Another translocation, *T(I→V)AR190*, which produces duplications with wild-type growth, was used to verify the recessiveness of the *R* morphology because *NM103* duplications are not suitable for scoring subtle morphological variations.)

Heterozygous R/R^+ *NM103* duplications were tested by crossing to normal sequence R^+ testers. Among the tests that produced spores, some had round spores, some had nonround, and many individual tests included both round and nonround spores. This was due to the presence, in the mixed cultures, of euploid derivatives that had lost either the segment of duplication carrying *R* or the segment carrying R^+. These derivatives acted as fertilizing parents for the perithecia that produced ascospores.

(Two control crosses confirmed that the production of nonround spores in the same cross with round spores was not due simply to the combination of two R^+ and one *R* in the two parents (which might alter the dominance relationship). In the controls, the *NM103* duplication was homozygous R^+/R^+ *A*, and the other parents were *T(NM103) R a* and Normal *R a*. Duplication transfers were allowed to grow in tubes of crossing meduim for ten days before being fertilized with the *R* parents. Two weeks later, spores were reasonably abundant on the walls of the tubes; these and spores that oozed out of the perithecia were examined several times over a period of four weeks. In both crosses only round spores were produced.)

It turned out that progeny tests of R/R^+ duplications verified the origin of round spores and nonround spores from fertilization by nuclei that had lost the R^+ or *R* allele, respectively, and along with it the entire segment of the duplication carrying it. Ascospores from 12 crosses involving eight different R/R^+ *NM103* duplications were isolated and germinated, and the cultures obtained were separated according to the shape of the spores from which they came. In

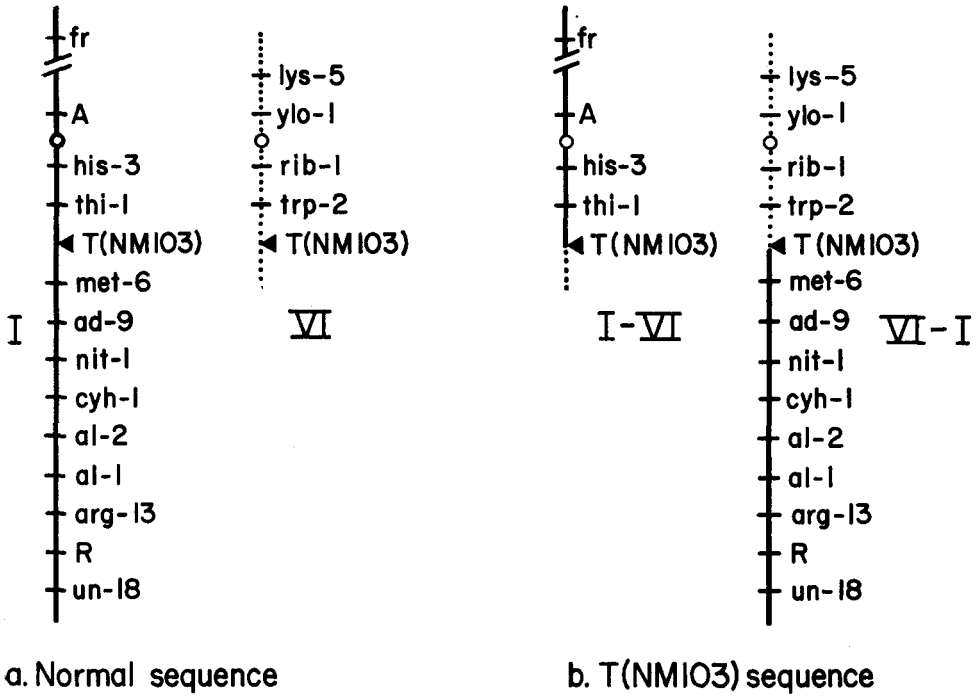

FIGURE 1.—a. Normal order on linkage groups I and VI for genes used in this study. b. Gene order in *T(NM103)*. By analogy with translocations studied cytologically in other organisms, it is assumed that *T(NM103)* originated from an exchange between a segment of IR and a dispensable tip of VI, as shown in this figure. In Normal × *T(NM103)* crosses, disjunction of the Normal I and *T(NM103)* VI-I centromeres to the same pole produces duplication of a segment of IR, as shown in Figure 2a, for example. If a tip of VI is attached to the Translocation I-VI chromosome, it must be missing from meiotic products which carry the duplication. Since progeny carrying such a duplication are viable, the tip, if it exists, must not contain essential material. It is omitted from the pairing diagrams in Figure 2.

All markers shown have been crossed to *T(NM103)* and tested for coverage (heterozygosity) in duplications. Most have also been located with respect to interchange point by conventional recombination mapping. Map distances in Neurospora vary enormously for the same markers in different crosses. In Normal × *T(NM103)* crosses, *met* has never recombined with Translocation, and *ad* has shown less than 1% recombination with either *met* or Translocation. Crosses in this study and published mapping data for N × N crosses give distances on the order of 10 units in the regions *thi-met*, *met-ad*, and *ad-nit*. The smallest estimates of distance for the interchange point on linkage group I are: centromere to interchange point, 15 units; and interchange point to right tip, 60 units. In Table 1, crosses 5, 6, and 7, the distance from *trp* to *ad* or *trp* to the interchange point on VI is on the order of 10 units.

nine crosses both round and nonround ascospores were used. A total of 1329 cultures were tested for all markers carried by their duplication parent, and all were scored for duplication morphology. Except for 90 of them, they were also crossed to Normal testers and scored for fertility and percent of black ascospores. Cultures obtained from round spores segregated for *R* and for the alleles of the other markers coupled to *R* in the duplication. Cultures obtained from the non-

round spores were R^+ and segregated for the alleles coupled to R^+ in the duplication. Depending on which segment of the duplication carried *R*, in crosses to Normal testers, progeny with one spore shape gave only Normal tests, and progeny with the other spore shape gave Normal, Translocation, and barren tests, just like progeny from crosses of *T(NM103)* × Normal. In the next section, the analysis of one duplication is explained in detail and is shown in Figure 2 and Table 3.

Exceptional progeny were found in two selective crosses discussed below, in the section concerning somatic recombination, and shown in Table 4 and Figure 4. In the other ten crosses only three progeny were found that did not have the phenotype predicted by their spore shape. These three cultures all came from nonround spores but had none of the alleles that were coupled to R^+ in the respective parental duplications. They had the alleles and structure expected of cultures coming from round spores. It is very likely that they represent the expected rare instances of incomplete penetrance of *R*.

Intensive analysis of a marked R/R$^+$ *duplication.* Conidial platings of *Dp(NM103)10–1* gave conidial cultures of the structures shown in Figures 2b and 2c, showing that the original duplication was as shown in Figure 2a. Crosses 22 and 23 of Table 3 were made to obtain a complete analysis of the behavior of the duplication in crosses. In each cross the results are given separately for cultures from round and nonround spores. Using each of these four sets of results, it is possible to reconstruct what the genotype and structure of the parents must have been in each of the four cases. It turns out that cross diagrams produced in this way are just like Figures 2f-2i, which were drawn by pairing the nonduplication parents (Figures 2d and 2e) with the conidial isolates shown in Figures 2b and 2c. Progeny starting from nonround spores in both crosses are consistent with the class b parent genotype in Figure 2b, which has lost the entire translocated segment at or near the interchange point on Group VI. Progeny starting from round spores from both crosses are consistent with the class c parent genotype in Figure 2c, which has lost the entire duplicated segment from normal sequence, starting at or near the interchange point on Group I.

Markers from the nonduplication parents are seen to segregate normally. In cross 23. to the *T(NM103)* parent, *nit-1* occurs in roughly half the fertile progeny from both round and nonround spores. In cross 22, to the normal sequence parent, *met-6* occurs in the same pattern. These results show that deletion of the translocated segment began proximal to the *met*$^+$ allele and that deletion of the normal segment began proximal to *nit*$^+$. Had the deletion of the translocated segment begun distal to *met*$^+$, progeny receiving linkage group VI from the duplication parent and I from the Normal parent in cross 22 (nonround spores), would have been heterozygous *met*$^+$/*met*. Therefore that fourth of the progeny would be expected to be *met*$^+$ *ad*$^+$ in phenotype, and only one fourth of the progeny would be expected to be *met*. In fact, only one *met*$^+$ *ad*$^+$ was obtained, as expected from meiotic crossing over. Similarly, in cross 23 (round spores), had the deletion of the normal segment begun distal to *nit*$^+$, one fourth of the progeny would be exected to be *nit*$^+$/*nit* in genotype and *cyh*^{s} *nit*$^+$

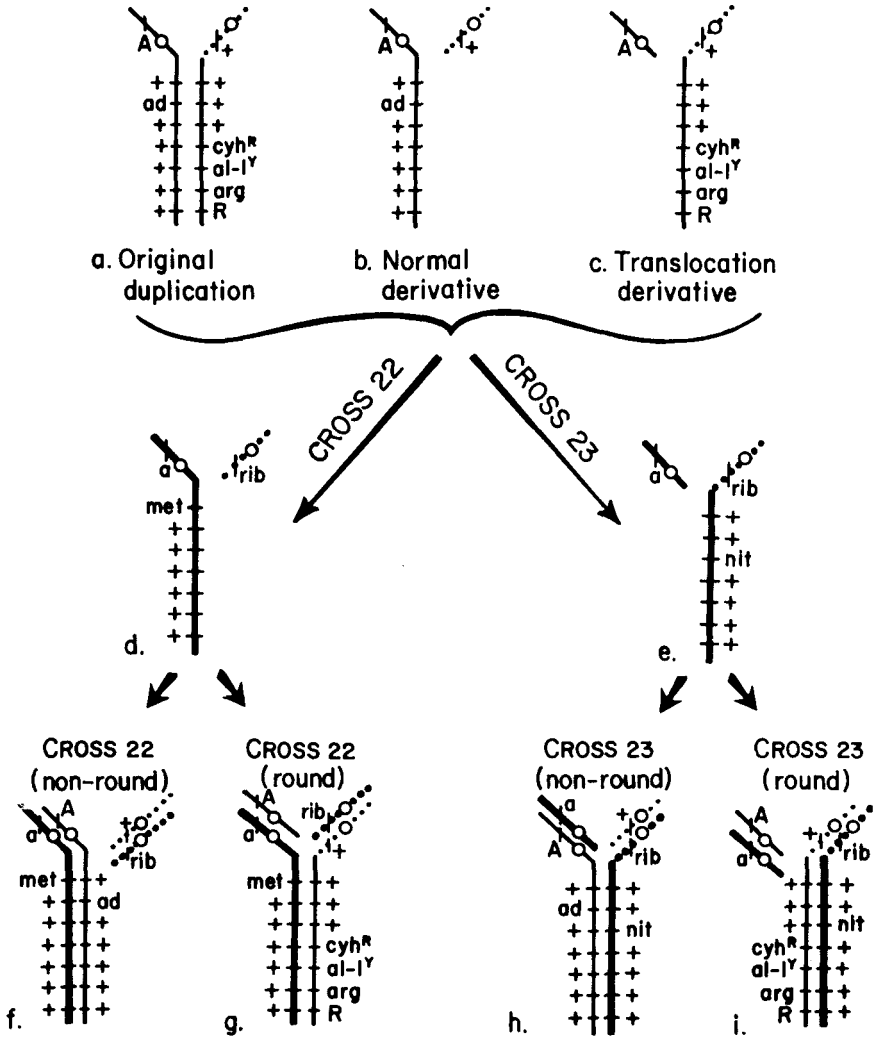

FIGURE 2.—Analysis of *Dp(NM103)10-1* (see Table 3). Linkage group I, solid line; linkage group VI, dotted line. a. Original duplication structure inferred from progeny and from conidial isolates shown in b and c. b. Conidial isolate reduced to normal structure by loss of segment in *T(NM103)* sequence. c. Conidial isolate reduced to *T(NM103)* structure by loss of segment in normal sequence. d. Normal sequence parent in cross 22. e. *T(NM103)* parent in cross 23. f.–i. Theoretical pairing diagrams for the isolates shown in b and c crossed to the parents shown in d and e. Spore shape expected from each pairing is indicated. Crosses 22 and 23 were actually fertilized by an unresolved culture containing a mixture of a, b, and c.

The dispensable tip shown in Figure 1 is omitted from these pairing diagrams.

in phenotype. Only four *cyh*^{s} *nit*$^{+}$ were obtained, a reasonable expectation from crossing over. Duplication 9–1 (Table 2) was also crossed to the parent shown in Figure 2e. In this cross the nonround spores are analogous to the round spores of cross 23. Of 39 cultures from nonround spores, none were *cyh*^{s} *nit*$^{+}$.

TABLE 3

Detailed analysis of an R/R+ *duplication of the genotype shown in Figure 2a, by crosses with testers of normal and of translocation sequence, shown in Figure 2d and Figure 2e*

Cross No. and ascospore shape	Sequence of tester parent	No. of progeny	Phenotype: mt A	mt a	met +	met —	ad +	ad —	nit +	nit —	cyh S	cyh R	al +	al —	R +	R —	rib +	rib —	Structure N	Structure T	Inferred zygote genotype
Nonduplications																					
22, nonround	Normal	57	26	31	27	30	28	29			57	0	57	0	57	0	13	14*	56	1†	Fig. 2f
22, round	(Fig. 2d)	40	18	22	25	15	40	0			17	23	16	24	22	18	24	16	19	21	Fig. 2g
23, nonround	Translocation	47	22	25			23	24	24	23	46	1‡	46	1	47	0	27	20	25	22	Fig. 2h
23, round	(Fig. 2e)	65	36	29			65	0	27	28	30	35	33	32	30	35	42	23	0	65	Fig. 2i
Duplications																					
22, nonround	Normal	0																			
22, round		17	5	12	17	0	17	0			17	0	12	5	12	4*	8	0*			Fig. 2g
23, nonround	Translocation	10	9	1			9	1	9	1	10	0	10	0	10	0	2	8			Fig. 2h
23, round		0																			

* Not all isolates were scored.

† This isolate (22–41) appears to result from a somatic recombination in the duplication culture before fertilization. It is *ad a*, and *NM103* structure (verified in crossses to *T(NM103)* testers).

‡ This isolate, which was also *al-1*^{y}, probably came from a spore that was or should have been round. In simple $R \times R+$ crosses rare nonround spores are produced.

Linkage group VI and the part of I not covered by the duplication are also marked. Allele ratios for markers in these regions are similar in all four parts of the experiment; that is, the mating type alleles occur in roughly a 50:50 ratio among fertile progeny and show linkage in duplication progeny as explained in MATERIALS AND METHODS, and the *rib-1* alleles are comparable except that *rib-1* is selected against, so that the *rib-1*+ allele carried by the duplication is found in more than half the euploid progeny. The mating-type alleles have segregated normally in all crosses that involved *NM103* duplications, and numerous crosses have been marked in VI and have shown normal segregation of VI markers.

Duplications do not go through meiosis intact: Duplications from at least one terminal rearrangement [*In(IL→IR)NM176*] can produce progeny with the parental chromosome duplication intact (TURNER *et al.* 1969; PERKINS, personal communication). That is, both segments of the duplication can enter and complete meiosis together. However, crosses 22 and 23 show that duplication nuclei derived from *T(NM103)* do not go through meiosis intact. There are two lines of evidence for this. (1) If the original duplication went through meiosis, some of the duplication progeny would be identical to it. None are found in cross 23, and the two found in cross 22 are expected from meiotic crossing over. The duplication progeny actually obtained (Table 3) are exactly the types expected from a cross of Translocation × Normal (Figures 2g and 2h). (2) If the original duplication went through meiosis, some of the Normal or Translocation progeny would have a combination of alleles from both segments of the duplication. This is not found.

Cross 23 results provide no evidence that duplications are transmitted intact. Duplication 10-1, the *A* parent in cross 23, is *rib*+; test crosses of it produce both round and nonround ascospores; and transfers from it to cycloheximide medium grow after a lag. All duplication progeny in cross 23 were aged for a week and then transferred to cycloheximide medium with perfectly negative results. No test crosses produced any round ascospores. One of the two *rib*+ duplications listed in Table 3 differs from the parent in mating type. The other *rib*+ duplication is *A* (but the scoring for duplication structure and for *rib*+ were both uncertain because the isolate grew poorly).

Cross 22 results also provide no evidence that duplications are transmitted intact. In cross 22, two of the 17 duplications had the same phenotype as the parent. However, from Figure 2g it can be seen that a crossover between *mt* and the interchange point gives a product differing from the parent duplication only in being heterozygous for *met*, which does not affect the phenotype.

Some of the other crosses of *R*/*R*+ duplications described previously were also sufficiently marked for this kind of analysis, and again results are consistent with the failure of intact duplications to go through meiosis.

Even in crosses with few markers, if a zygote were trisomic for the *R* locus (*R*/*R*+ × *R*+), a product carrying the original duplication should be detectable. For the crosses in Table 3, if spores from such a zygote were round, then duplications from them would be found among the round spores in cross 23. No duplications were found. If spores from such a zygote were nonround, then duplications from them would be found among the nonround spores in cross 22. Again, none was found. Except for the special situation of cross 27, Table 4, in all crosses of this study when a spore shape was tested that was not expected to produce duplications, none was found.

Allowing for possible rare exceptions, then, the duplication genome does not go through meiosis intact, and the shape of the ascospore reveals which allele of an *R*/*R*+ duplication took part in meiosis. It can also be seen that for any given spore only the reduced (class b or class c)

nucleus contributes alleles to the meiotic products. For example, in cross 22 (Table 3), the progeny from nonround ascospores are all $R+$ as predicted from spore shape, and they are also cyh^S and $al+$, the alleles linked to $R+$. The progeny from round ascospores all have the $ad+$ allele which was coupled to R in the duplication. All the foregoing is true of cross 23 except that one culture which was listed as coming from a nonround spore had the alleles that were coupled to R. As explained previously, this is probably an instance of incomplete penetrance of R. The exceptional culture is $ad+$ cyh^R $al\text{-}1^y$ $R+$ just like many of the cultures from round spores.

Fertile perithecia result from fertilization by conidia that already contain reduced euploid nuclei. Perithecia derived from fertilization by *NM103* R/R^+ duplications generally contain either all round or all nonround ascospores. While crosses were being examined for other purposes, many perithecia were opened and observed unsystematically, and it was noted that they contained either one shape or the other, not a mixture. Four crosses that were shooting both round and nonround spores were observed more extensively, and a total of 250 perithecia were opened, of which 245 contained only one shape. The five mixed perithecia were only poorly fertile, probably representing instances where breakdown occurred after fertilization. The other 245 perithecia were probably fertilized by conidia that carried only one allele of R. JOHNSON (1975) observed that when heterokaryons of R and R^+ are used as protoperithecial (female) parents, a significant fraction of individual perithecia contain some asci with all round spores and some asci with all nonround. This means that each ascus is autonomous for the expression of R. Therefore the fact that such mixed perithecia are rare in crosses of *NM103* duplications indicates that fertilization by conidia carrying both R and R^+ is rare.

Factors shown not to affect reduction of duplications. The following factors were varied without significant effect on the frequency of loss: (1) origin of the Normal linkage group I; (2) genetic background of the *NM103* translocation stock, other than in the immediate vicinity of the interchange points; (3) presence of markers and their coupling phase (normal or translocation sequence), including *R*, *cyh-1*, *ad-9*, *al-1*, and *arg-13*; (4) environmental factors—temperature and media. The effect of cycloheximide in the medium is not known quantitatively; however, there does not appear to be a qualitative effect. Many stocks that have shown loss of the cyh^S allele on cycloheximide medium have also shown it on medium without cycloheximide, and the same loss of linked alleles and reduction to euploidy is found.

Somatic recombination: Previously the study of somatic recombination in Neurospora has relied upon disomics. Duplications provide a method which is more reproducible and stable. In the present study, evidence of somatic crossing over has been obtained directly in one case and indirectly by analysis of progeny in three crosses.

The directly obtained recombinant came from duplication 11–3 (Table 2) shown in Figure 3a. From a conidial plating, seven *al-2* cultures were sampled and tested. All of them had the cyh^R and $arg+$ markers still linked to *al-2*, and six of them had *T(NM103)* sequence, Figure 3c. The seventh culture (Figure 3d) had normal sequence, evidently resulting from crossing over between the interchange point and the first marker, *cyh*.

Examples of progeny tests that imply somatic crossing over are shown in Table 4. Crosses in

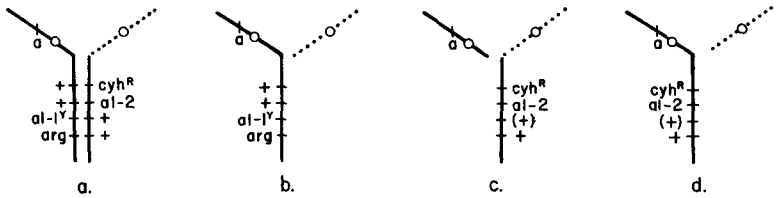

FIGURE 3.—A somatic recombinant obtained from conidial plating. Linkage group I, solid line; linkage group VI, dotted line. a. *Dp(NM103)11–3*. Genotype was inferred from progeny tests of original duplication culture, which was wild type in color. b. Product reduced to Normal by loss of *T(NM103)* segment. Inferred from progeny but not found in plating. c. Conidial isolates No. 1–6, which had a genotype consistent with simple loss of the Normal segment. d. Conidial isolate No. 7, which shows somatic recombination between interchange point and *cyh* before or during loss of the segment which was originally in normal sequence. c and d were tested directly, and their progeny from crosses to Normal were obtained and tested.

In the absence of *al-2*+, *al-1*^{y} cannot be scored.

this table involve *Dp(NM103)12–1* (Table 2). Platings yielded only class b and class c cultures (Figure 4), which must have been derived from the structure shown in Figure 4a. No somatic recombinants were found among the 59 conidial isolates tested. In all three crosses of Table 4, progeny from round spores were of the types expected if the fertilizing parent had the genotype shown in Figure 4b (note that it is *ad*+). In cross 25, with an *ad*+ tester, the nonround spores are as expected if the fertilizing parent had the genotype shown in Figure 4c. In control experiments under the same (standard) conditions, structurally homozygous *ad-9* × *ad-9* crosses do not produce any viable ascospores. The nonround spores of crosses 26 and 27 were expected to come from crosses homozygous for *ad*. The infertility of such crosses acted as a highly selective system which revealed the presence of somatic recombinants between *ad*+ and *R*+ much more efficiently than conidial plating and testing could do. Both crosses had progeny that must have come from a parent like Figure 4d. In addition, cross 27 had 17 progeny that must have resulted from fertilization by a different somatic recombinant, as shown in Figure 4e.

TABLE 4

Progeny showing somatic recombination in crosses to Dp(NM103) *shown in Figure 4*

Cross No.	Genotype of euploid tester	Source of progeny	No. tested*	Structure†	Exceptional progeny
25	N *al-1*^{aur} *A*	round spores	94	N	none
		nonround spores	94	N, T, Dp	none
26	N *ad al-1*^{aur} *A*	round spores	83	N	none
		nonround spores	80	N	many
27	T *ad cyh*^{R} *al-2 A*	round spores	90	N, T, Dp	none
		nonround spores	88	N, T, Dp	many

The original duplication has the genotype shown in Figure 4a. Progeny starting from round spores in all three crosses are consistent with a fertilizing parent of the genotype shown in Figure 4b (*R* is coupled to *ad*+). In cross 25 (to an *ad*+ tester) progeny starting from nonround spores are as expected from a fertilizing parent of the genotype shown in Figure 4c. In crosses 26 and 27 to the *ad* testers, nonround spore progeny (except for those that were like the euploid tester parent and therefore not diagnostic) could not have come from fertilization by the parent shown in Figure 4c (which is *ad*). Instead they show selection for somatic recombinants in which *ad*+ is coupled to *R*+.

* Scored for all markers and structure.

† Scored by fertility and percent black ascospores in crosses to normal sequence testers. See MATERIALS AND METHODS.

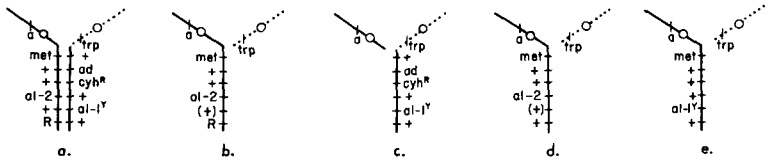

FIGURE 4.—Analysis of *Dp(NM103)12-1*, showing products of somatic recombination. Linkage group I, solid line; linkage group VI, dotted line. a. Original duplication structure. b. Conidial isolate reduced to normal structure. c. Conidial isolate reduced to *T(NM103)* structure. d. and e. Products of somatic recombination accompanied or followed by reduction. Inferred from progeny, only. See Table 5.

In the absence of *al-2+*, *al-1^v* cannot be scored.

A single isolate from cross 22 (22-41, Table 3, see †) provides another example of inferring somatic recombination from progeny analysis.

DISCUSSION

T(NM103) duplications undergo precise loss of one segment, either from normal linkage group I or from the translocated VI-I chromosome, thereby restoring, respectively, translocation or normal sequence. Because the frequency of loss is comparable for the two segments, the explanation cannot be some kind of defect at the interchange point of the rearrangement. No other rearrangements in Neurospora are known to produce duplications which generate T and N derivatives with equal frequency as *Dp(NM103)* does. The other terminal duplications known to be unstable make all Normal or many more Normal than Rearrangement products. A model that would explain both this behavior and the *NM(103)* results is proposed by NEWMEYER and GALEAZZI 1977.

The other terminal duplications have been studied in two ways: (1) by testing vegetative cultures that were originally inhibited and subsequently "escaped" and grew normally, and (2) by examining the ascospores or testing the progeny from test crosses of duplications which showed no initial inhibition.

(1) Duplications that are initially inhibited in vegetative phenotype can be obtained by making them heterozygous for vegetative (heterokaryon) incompatibility loci (examples: *In(IL→IR)H4250*, NEWMEYER and TAYLOR 1967; NEWMEYER and GALEAZZI 1977; *T(II→V)NM149*, *T(VII→IV)T54M50* PERKINS 1975, seven other duplications, MYLYK 1975). In the well analyzed cases, the escaping sectors are often not fertile, apparently because there is not precise elimination of the entire duplicated segment.

(2) Some duplications that do not have initially inhibited phenotypes and that have not been analyzed vegetatively are nevertheless known to be unstable because of the results of many test crosses. Both spore patterns (95% black spores in crosses to Normal testers) and progeny tests show that the duplications have been reduced to normal sequence before going through the cross. Duplications from *T(I→V)AR190* and *T(VI→IV)AR209*, among others, are reduced so readily that duplication cultures are as fertile as their Normal siblings from the same cross (PERKINS and BARRY 1977). At the other extreme, although duplications from *In(IL→R)NM176* appear to be perfectly stable under standard

conditions, test crosses may produce spores after a delay. Under conditions designed to prevent dehydration, test crosses began to produce ascospores as late as one to two weeks after spores would normally have been expected (NEWMEYER and GALEAZZI, unpublished). Evidently the instability in this case results from events within the perithecium. Again, breakdown predominantly involves the rearranged segment of the duplication.

Duplications from another ascomycete, *Aspergillus nidulans*, are also unstable and vary in the characteristics of the reduced products (ROPER 1973). (For a comparison of the breakdown products of Neurospora and Aspergillus duplications see DISCUSSION in NEWMEYER and GALEAZZI 1977.) Unlike Neurospora, Aspergillus has the advantage of uninucleate conidia with autonomous color markers. Plating is usually not necessary because the various products grow into separate sectors rather than becoming intermixed as in Neurospora. Colonial morphology of duplications is more extreme and recognizable than that of most Neurospora duplications. For these reasons, vegetative analysis has been much easier in Aspergillus than in Neurospora. However, in the case of *T(NM103)*, the presence of albino markers and the different colony morphology of duplications *vs.* euploids makes this Neurospora duplication nearly as easy to study as an Aspergillus duplication.

It would be of interest to examine the behavior of duplications obtained by crossing *T(NM103)* to various wild strains from nature. If quantitative and qualitative aspects of duplication instability are under genetic control, changes might be found either in the overall frequency of loss of duplicated segments, or in the frequency with which one or the other segment was lost preferentially. Change might be found in either direction. Duplications could become completely stabilized; for example, by the loss of some nuclease. At the other extreme, duplications could become more unstable, so that fresh (not aged) duplications were completely fertile. Increased fertility could be brought about either through facilitation of the existing pattern of breakdown or through a different process, such as a change allowing intact duplications to go through meiosis. An example of increased fertility was found by OREST MYLYK (personal communication) using duplications from another rearrangement, *In(IL→IR)NM176*. He found that when their normal sequence parent was any one of a number of new wild-collected isolates from Louisiana, duplications were approximately as fertile as their Normal siblings. It is hoped that the unique characteristics of *NM103* duplications will be useful in further studies of the genetic control of instability.

DAVID PERKINS provided the rearrangement and preliminary information on its structure. I am also grateful to him and to DOROTHY NEWMEYER for guidance and suggestions throughout the study.

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