Conventional and unconventional analysis of an inversion in *Neurospora*

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SUMMARY

In(IL; IR)OY348 is a pericentric inversion of linkage group I in *N. crassa*, with a breakpoint between *fr* and *un-5* in the left arm and a breakpoint between *ad-9* and *nit-1* in the right arm. Approximate breakpoint location was found by tabulating crossovers between the rearrangement and markers in normal chromosome sequence. Inversion structure was verified by marked *In OY348* × *In OY348* crosses. Precise mapping of breakpoints was by duplication coverage. Inversions like *OY348* do not produce progeny with segmental chromosome duplications when crossed to normal sequence, but duplications were produced by crossing it to *In(IL; IR)OY323* (Barry & Leslie, 1982), another standard pericentric inversion, and to *T(I → VI)NM103* (Turner, 1977), a translocation to a tip. Each of these rearrangements has a breakpoint within the inverted region of *In OY348*. Two duplications from *In OY348* × *In OY323* were converted to normal chromosome sequence by double mitotic recombination. Besides expediting mapping, the technique of intercrossing rearrangements increasingly enables us to make segmental duplications exactly tailored for studying specific included genes.

1. INTRODUCTION

By 'conventional mapping' we mean the ordering of points (gene loci or rearrangement breakpoints) on a linkage group by tabulating crossover and non-crossover classes. *In(IL; IR)OY348* is a pericentric inversion in *Neurospora*. Crosses to marked stocks with normal chromosome sequence showed that it has a breakpoint in the central region of each arm of linkage group I. After markers were recombined with the inversion, the structure was verified and approximate location of breakpoints was found by making marked crosses homozygous for the inversion. Markers very close to breakpoints cannot be mapped conveniently in this way because of the excessive number of progeny that must be screened in order to obtain recombinants.

Some rearrangement types produce segmental chromosome duplications when

crossed to normal-sequence testers. Breakpoints can be mapped precisely by finding out which markers are included in the duplications. A recessive marker that is included in heterozygous condition will be referred to as 'covered'. Inversions like *O Y348* do not make duplications when crossed to normal sequence but will do so when crossed to a second rearrangement that has one breakpoint within the inverted region. (Such rearrangements will be called 'overlapping'.) These duplications can be used exactly like those from Rearrangement × Normal. This is what we mean by 'unconventional analysis' of *In O Y348*. Overlapping re-arrangements have, of course, been widely used to produce segmental aneuploids, including duplications, in higher organisms; see, for example, Sturtevant & Beadle (1936), Lindsley *et al.* (1972), Rhoades & Dempsey (1956), Birchler (1980) and other reports cited by Perkins & Barry (1977). The present study illustrates the use of overlapping rearrangements in a new context and employs not only inversions but also a combination that involves inversion and translocation.

One classic pericentric inversion other than *O Y348* has been reported in Neurospora (Barry & Leslie, 1982). Three additional inversions are known, but instead of having both breakpoints within the functional region of the chromosome, they have one of the breakpoints at a tip, beyond the essential genetic material (Newmeyer & Taylor, 1967; Turner *et al.* 1969).

2. MATERIALS AND METHODS

In(IL; IR)O Y348 originated as a prototrophic survivor of a filtration enrichment experiment (Yoder, 1979). In this experiment ultraviolet irradiation killed 70 % to 90 % of (multinucleate) conidia from *al-2 a* (FGSC 3448), a stock that had been inbred to Oak Ridge wild type. The original *O Y348* stock also carried a temperature-sensitive mutant of unknown nature that was closely linked to the inversion. A recombinant free of the mutation was obtained, and inversion stocks used for precise mapping were all derived from that recombinant.

The highly fertile, non-conidiating fluffy strains fl^P *A* and fl^P *a* (Fungal Genetics Stock Center nos. 3249 and 3250) were used as normal-sequence standard testers for scoring chromosome structure and mating type (*mt*, *A* or *a*). In symbolizing rearrangements, T = translocation, In = inversion, and the numbers in parentheses are the linkage groups involved. Standard chromosome sequence is designated Normal (*N*).

Technical methods: See Perkins & Barry (1977) for background and techniques of analysing rearrangements in *Neurospora*. Chromosome structure (normal, rearrangement or duplication) was scored by crossing to normal-sequence *fl* testers on slants of synthetic cross medium (Westergaard & Mitchell, 1947) in 75 mm tubes. All tests were scored ten days after fertilization for percent black ascospores. Normal strains crossed to the testers produce at least 90 % viable (black) ascospores. Inversion *O Y348* strains produce about 70 % black; of the defective ascospores, half are white, and half are dark brown. When microscope illumination is from above, many of the inviable brown ascospores appear black.

(This technical problem leads to a large number of 'non-germinants' among ascospores isolated from Normal × *In O Y348* crosses.)

Progeny with segmental chromosome duplications were produced in crosses to other rearrangements. Like the majority of duplication-carrying strains in *Neurospora*, they were Barren. Barren strains make abundant perithecia with the testers, but most of these perithecia do not mature or produce ascospores. Barrens were rechecked at about 13 and 16 days; in some tubes individual fertile perithecia were evident in the later checks, but all tests that scored as Barren were clearly different from fertile tests. Besides being screened for Barrens, tests of progeny from crosses to the other rearrangements were screened for the presence of exceptional Normals. No attempt was made to distinguish between the two parental rearrangement types.

3. RESULTS

(i) *Initial observations*

In a screening of mutagenized cultures, isolate *O Y348* produced 30 % defective (white and brown) ascospores in crosses to testers with normal chromosome sequence. For several crosses of Normal × *O Y348* (the original strain and descendants carrying the rearrangement), individual groups of eight ascospores, representing unordered tetrads, were collected and classified according to their content of black : defective spores (method of Perkins, 1974). The following example is typical:

$$8:0 \quad 43\% \qquad 6:2 \quad 5\% \qquad 4:4 \quad 43\% \qquad 2:6 \quad 3\% \qquad 0:8 \quad 6\%.$$

Most of the asci are about equally divided between two major classes: those with eight viable, black ascospores and those with four black : four defective.

Among randomly collected progeny of Normal × *O Y348* crosses, markers that are usually very far apart on the left and right arms of linkage group I are closely linked to each other and to the rearrangement. Markers on all the other linkage groups show independent segregation from the rearrangement. These results suggested a long inversion with both breakpoints interstitial, so *O Y348* was tentatively classified as an inversion with breakpoints in linkage group I left and I right, symbolized as *In(IL; IR)O Y348*.

(ii) *Conventional mapping of the inversion*

Inversion *O Y348* was crossed to a series of normal-sequence stocks with markers in linkage group I. Table 1 gives the results of a typical cross. Fig. 1 shows the location of markers in normal sequence. Crossover classes are listed as they would be for a Normal × Normal cross to illustrate the apparent crossover suppression. Both chromosome arms are involved in the rearrangement. Region 1 lies in the left arm, and region 2 lies mostly in the right arm. In Normal × Normal crosses, *fr* to *mt* (region 1) usually shows 30 % to 50 % recombination; *mt* to *al* (region 2) is about the same. In the *In O Y348* × Normal cross, region 1 shows only 9 %

and region 2 only 15 % recombination. In both regions single crossovers are dramatically reduced compared to usual map distances and compared to the number of double crossovers – an important point because it distinguishes crossover suppression due to a rearrangement from crossover suppression due to genes that regulate recombination.

Table 1. *Progeny obtained from a cross of Inversion* (In) *OY348* a × *Normal sequence* (N) fr al-1^y R A

	fr	(1)	mt	(2)	al	(3)	R	No. of progeny	Structure
						Markers and regions			
Parental	+		a		+		+	33	*In*
	−		A		−		−	25	*N*
Singles region 1	−		a		+		+	1	*In*
	+		A		−		−	1	*N*
Singles region 2	+		a		−		−	2	*In*
	−		A		+		+	2	*N*
Singles region 3	+		a		+		−	18	*In*
	−		A		−		+	5	*N*
Doubles 1, 2	+		A		+		+	2	*In*
	−		a		−		−	2	*N*
Doubles 2, 3	+		a		−		+	3	*In*
	−		A		+		−	3	*N*
Doubles 1, 3	+		A		−		+	1	*In**
	−		a		+		−	1	*In*
Triple 1, 2, 3	−		a		−		+	1	*N*

Total   100

Markers are identified in Fig. 1. Total recombination: region 1, 9 %; region 2, 15 %; region 3, 32 %.

* This isolate was either a quadruple (two crossovers inside the loop and two around *al*) or a double that produced defective ascospores for some reason unrelated to *OY348*.

Fig. 2 shows complete pairing of the two parental chromosomes. Markers are shown in their correct relationship to the breakpoints based on further information from later crosses. Single crossovers inside the loop in either region 1 or region 2 make complementary duplication–deficiency products, both of which are inviable.

The only recombinants between *mt* and the inversion are four double crossovers in regions 1 and 2, one triple (also involving 1 and 2), and possibly a quadruple. The fact that recombinants of *mt* are found only in the multiple-crossover classes shows clearly that the locus is between the inversion breakpoints (inside the loop of Fig. 2).

Conversely, *R* is clearly outside the inversion because there are too many recombinants between *R* and the inversion to be accounted for by double crossovers. Region 3 (*al* to *R*) is 32 map units. This is within the range of values found in Normal × Normal crosses.

This cross does not provide conclusive information about the location of the inversion breakpoints relative to *al* and *fr*. For example, the class listed in Table 1 as Singles region 1 can be interpreted in two ways. As it turned out, *fr* is just to the left of the breakpoint, and these are really single crossovers in a short region between the breakpoint and *fr*. But this same genotype could have been produced
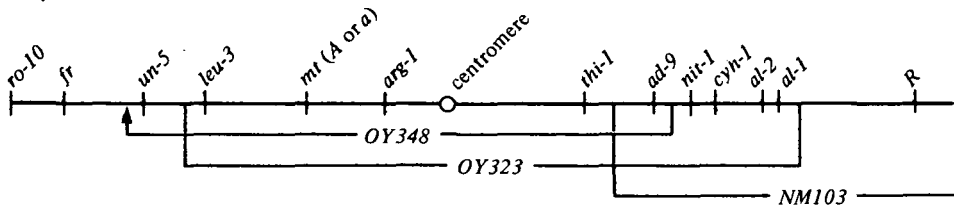

Fig. 1. Normal sequence of markers on linkage group I (not to scale). Breakpoints found in this study are shown for *In(IL; IR)OY348*. Other chromosome rearrangements used were *T(I → VI)NM103* (Turner, 1977) and *In(IL; IR)OY323* (Barry & Leslie, 1982). *In OY348* and *In OY323* are pericentric inversions. *T NM103* has the part of IR distal to the breakpoint translocated to the end of linkage group VI. Markers used were *ad-9*, adenine (Y154M614); *al-1ʸ*, albino yellow (ALS4); *al-2*, albino (15300); *arg-1*, arginine (B369); *cyh-1* (KH52) (the wild type allele *cyh-1ˢ* is sensitive to cycloheximide, *cyh-1ᴿ*, is resistant; *cyh-1ᴿ* is recessive – see Turner, 1976); *fr*, frost (B110); *leu-3*, leucine (R156); *mt* (*A* or *a*), mating type; *nit-1*, nitrate non-utilizer (34547); *R*, Round spore (35408R); *ro-10*, ropy (AR7); *thi-1* (56501) thiamine; *un-5* (b39t) and *un-18* (T54M94) unknown lesions, heat-sensitive. Because of differences in genes controlling recombination, genetic distances in *Neurospora* are extremely variable in crosses of different parentage.
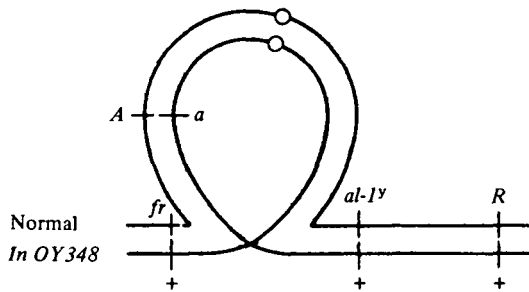

Fig. 2. Complete meiotic pairing of linkage group I in *In OY348* × Normal *fr al-1ʸ R A*. For convenience, only two of the four strands are shown.

if *fr* had been right of the breakpoint (inside the loop of Fig. 2). In this case the recombinants would arise from doubles around *fr* inside the loop (just as the *mt* recombinants are doubles around *mt*).

When markers (like *al-1* and *fr*) that are located close to breakpoints can be recombined into the inversion sequence, their position can be determined with certainty by making a marked isosequential cross. From further mapping crosses, we obtained *In OY348 ad-9 A* and *In OY348 fr al-2 a* (*al-2* is very close to *al-1*). The cross between them is shown in Table 2. Compared to normal sequence (Fig.

1) the order of *mt* and *ad-9* is reversed in relation to *fr* and *al-2*. Therefore, the first two markers must be within the inversion, and *fr* and *al-2* are outside. Besides resolving the location of breakpoints, the isosequential cross substantiates the simple two-break model shown in Fig. 1. A (complex) translocation is ruled out because the flanking markers are still linked to the markers that are within the inverted region.

Table 2. *Progeny obtained from a cross with the inversion homozygous:*
In OY348 ad-9 A × In OY348 fr al-2 a

					Progeny genotypes*					
						Single crossovers			Double crossovers		
	Parents			Parental	1	2	3	1, 2	2, 3	1, 3
+	*ad-9*	*A*	+	17	5	2	5	1	2	2
fr	+	*a*	*al-2*	17	2	3	8	2	3	2
				Total: 71						

* *fr⁺* progeny listed on top line. Normal order is *fr mt* (*A* or *a*) *ad-9 al-2*. Total recombination: region 1, 20%, region 2, 17%, region 3, 31%. This 68% contrasts with 24% between *fr* and *al-1* in Table 1, where the inversion was heterozygous.

(iii) *Genetic mapping by duplication coverage*

For markers close to breakpoints, it is often necessary to obtain and score hundreds of progeny in order to get recombinants with a rearrangement, and in some cases linkage is so close that getting a recombinant is a practical impossibility. In such cases, duplication coverage provides a practical alternative for the precise mapping of breakpoints. Chromosome duplications were obtained in two ways: by crossing *In OY348* to *T NM103*, a translocation to a tip, and by crossing to *In OY323*, another pericentric inversion in linkage group I.

(iv) *Mapping the right breakpoint of* In OY348 *with novel duplications produced by crossing Inversion × Translocation*

Description of Translocation NM103. *T NM103* has part of the right arm of linkage group I translocated to the tip of VI (Turner, 1977). In Fig. 3A the translocation is drawn so that the two segments of linkage group I are in the same sequence as in Fig. 1. All distances in Fig. 3 are greatly distorted for convenience. Figs. 3B and 3C show the translocation paired with Normal. Fig. 3B is the conventional way of showing a translocation; Fig. 3C shows exactly the same chromosomes, but a loop has been added to facilitate comparison with Fig. 2 and Fig. 3D. In the cross *T NM103* × Normal, when the two centromeres shown as solid circles go to the same pole, the meiotic products are duplicated for linkage group I distal to the *T NM103* breakpoint. Progeny carrying the duplication have very distinctive morphology, so they are easy to identify. For all the loci shown the wild type allele is completely dominant, so for markers distal to breakpoint all duplication progeny have the wild type phenotype, regardless of which parent carries the wild type allele. *T NM103* was first mapped (Turner, 1977) by crossing it to normal sequence marked with the recessive alleles of the loci in the vicinity of the breakpoint. Markers

proximal to the breakpoint were not 'covered' in duplications, so the duplication progeny expressed the recessive phenotype, which is illustrated here by *thi-1*.

Recessive markers carried by *T NM103* would not be expressed by duplication progeny whether proximal or distal to breakpoint. Coupling phase is critical in duplication mapping.
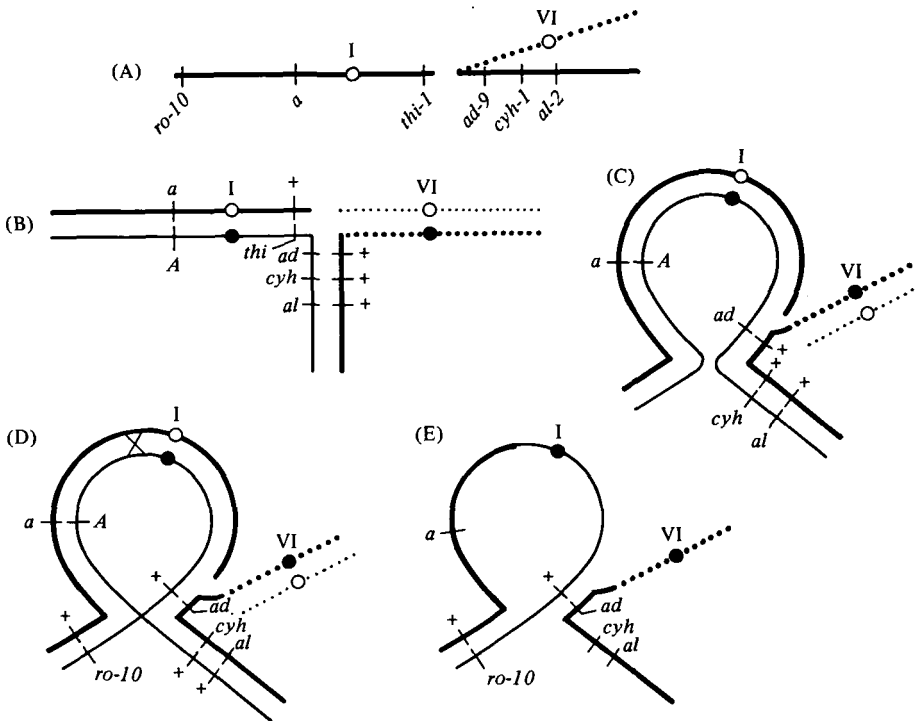

Fig. 3. Production of segmental chromosome duplications by $T(I \rightarrow VI)NM103$ (represented by heavy solid and dotted lines) in crosses to Normal and to $In(IL; IR)OY348$. Linkage group I (represented by solid lines) and linkage group VI (represented by dotted lines) are shown. (A) Structure of *T NM103* with linkage group I loci shown in same order as in Fig. 1. (B) Meiotic pairing of *T NM103 a* × Normal *thi-1 ad-9 cyh-1 al-2 A*, using classical translocation figure. Only two of the four strands are shown. (C) Same pairing as B, but drawn with a loop for comparison with D. Duplications are produced when strands with centromeres shown as solid circles go to the same pole. (D) Meiotic pairing of *T NM103 ad-9 cyh-1 al-2 a* × *In OY348 ro-10 A*, with *In OY348* oriented as in Fig. 2. Only two strands shown. (E) A duplication produced by crossing over at the top of the inversion loop. The segment containing *ad-9* and the segment containing *ro-10* are duplicated.

Fig. 3D shows pairing of the rearrangement chromosomes in the cross *T NM103 ad-9 al-2 cyh-1 a* × *In OY348ro-10 A*. When the two strands with centromeres shown as solid circles go to the same pole, the resulting progeny have the same segment duplicated as in standard *T NM103* duplications. Five of the 84 progeny had the same morphology as duplications from *T NM103* × Normal (evidently the changed sequence of linkage group I has no effect on the morphology).

All five were al^+ as expected. (ro-10 cannot be scored in the presence of duplication morphology, and the other markers were not scored.)

A novel type of duplication is predicted when there is a crossover in the loop. Fig. 3E shows an example derived from crossing over to the right of mt. (A crossover to the left of mt differs only in having A mating type.) Linkage group I is duplicated distal to the left breakpoint of $In\,OY348$ and for the region between the breakpoint of $NM103$ and the right breakpoint of $In\,OY348$. In this study only the second of these overlapping regions was utilized for mapping by duplication coverage (because there was no available stock of $T\,NM103$ with markers near the left breakpoint of $In\,OY348$).

The expected genotype of progeny carrying novel duplications is ad/ad^+ $al\,ro/ro^+$. Eight progeny had the phenotype ad^+ $al\,ro^+$ and were Barren (seven of them were mating type a). Besides being al, they also differed from standard $NM103$ duplications in not having the duplication morphology. There were no progeny of the reciprocal class, $ad\,al^+\,ro$, which corroborated that these Barren progeny were not simple euploid recombinants.

There were also three fertile, normal-sequence $ad^+\,al\,ro^+\,a$ progeny. They could have been derived by meiotic recombination, but another reasonable explanation is duplication breakdown. This is common in *Neurospora* (Perkins, Newmeyer & Turner, 1972); in fact, an example of duplication breakdown is given in section 3 (vi).

With respect to the $In\,OY348$ breakpoint, this cross confirmed the locations of ad-9 and al-2 and located cyh-1. The duplications cover ad-9 (are ad/ad^+), but the recessive alleles cyh-1^R and al-2 are both expressed in the novel duplications – they are not covered, so the breakpoint is between ad-9 and cyh-1.

To complete right-breakpoint mapping we crossed $T\,NM103\,nit$-$1 \times In\,OY348$. Forty-three progeny were obtained, and those with standard $T\,NM103$ duplication morphology were discarded. Among the others, ten were Barren duplications, and these were all recessive nit phenotype. Therefore, nit is not covered, and the breakpoint lies between ad-3 and nit-1.

In both crosses of $In\,OY348 \times T\,NM103$ some aneuploid progeny of unexpected types were observed; these are attributed to non-disjunction and are probably irrelevant to the analysis. They had the characteristics associated with carrying both A and a: discoloration of the agar and inhibited, abnormal growth (Newmeyer & Taylor, 1967). The presence of both mating types was verified in three of seven cultures analysed. There were 13 inhibited progeny out of 84 in the first cross and four of 81 in the second. From the information already known for both rearrangements, segmental duplication is not a reasonable explanation. They were probably disomics resulting from non-disjunction involving the I–VI quadrivalent shown in Fig. 3D. The incidence in these crosses is unusually high, but disomics are common in crosses involving unlike chromosome structures.

(v) *Mapping the left breakpoint with duplications produced by crossing*
Inversion × Inversion

Inversion $OY348$ and Inversion $OY323$ (mapped by Barry & Leslie, 1982) were found to be overlapping – they produce viable duplication progeny when inter-
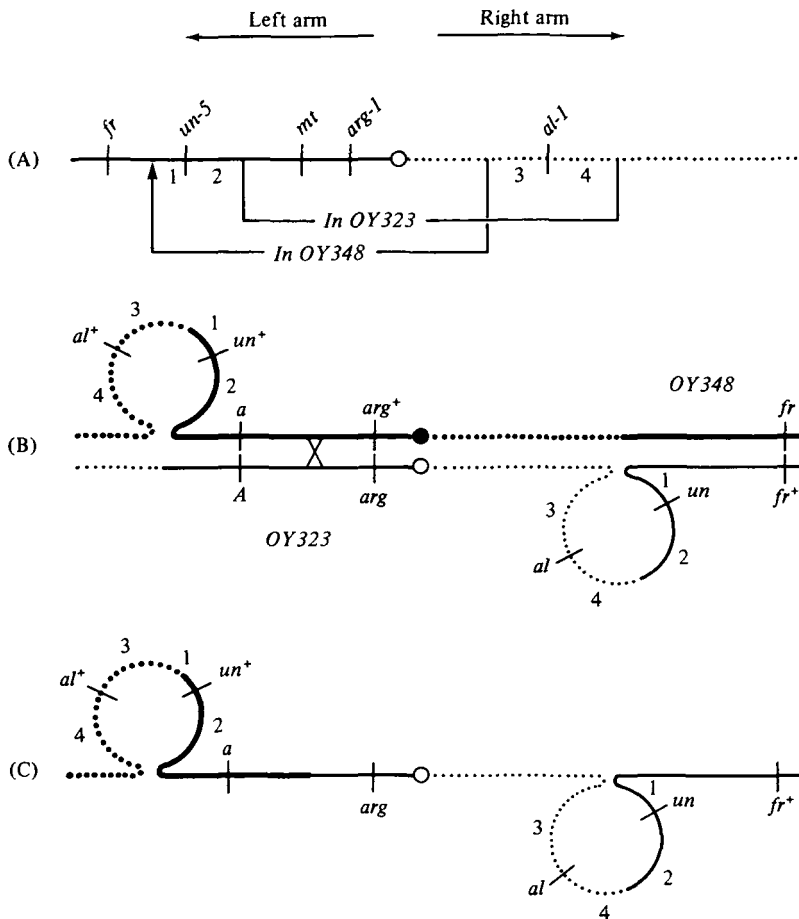
Fig. 4. Production of segmental chromosome duplications by crossing *In O Y348 fr a* × *In O Y323 al-1 un-5 arg-1 A* (adapted from a diagram of Perkins & Barry, 1977). (A) Normal sequence – includes left breakpoint of *In O Y348* mapped by this cross (arrow). (B) Meiotic pairing of the inversions. For convenience only two of the four strands are shown. Numbers will be used in connection with presentation of Fig. 5. (C) Product of a crossover between *mt* and *arg-1*, but similar duplications would result from crossovers anywhere in the common inverted region (the central paired segment). In (A), (B), and (C) solid lines represent material originally located in the left arm, and dotted lines represent material originally located in the right arm. In (B) and (C), segments from *In O Y348* are represented by darker dots and lines. Not to scale.

crossed. Fig. 4 relates to the cross *In O Y348 fr a* × *In O Y323 arg-1 al-1 un-5 A*. Fig. 4 A shows the normal sequence of the markers and the breakpoints of the two inversions (the *In O Y348* left breakpoint found by this cross is marked with an arrow). Among the 52 progeny obtained, five were Barren in crosses to normal-sequence testers. All five had *al+ un+ fr+* phenotype, and all were *mt a*. Three were *arg+* and two were *arg*. Fig. 4 C shows the structure of these

duplications, and Fig. 4B shows the meiotic pairing that gave rise to them by a crossover in the central paired region, the common inverted region.

Pairing is also shown for the ends but not for the segment between the two left breakpoints and the segment between the two right breakpoints.

Before the inversions were crossed, it was already known that the right breakpoints flanked *al-1*, with the breakpoint of *In O Y348* on the left. The production of duplication progeny showed that the two inversions are overlapping, so they must have the same relationship for their left breakpoints – that is with *In O Y348* on the left. (If they had not made duplications, we would have inferred that *In O Y348* was completely included within *In O Y323* with the left breakpoint proximal to *un-5*.)

If the breakpoint of *In O Y348* had been between *un-5* and the *In O Y323* breakpoint, the *un⁺* allele of *In O Y348* would have been in the same segment as *fr* and would not have been included in the duplication progeny. Since these progeny did score as *un⁺*, the breakpoint must be distal to *un-5*.

(vi) *Derivatives with normal chromosome sequence produced by mitotic recombination*

It is not unusual for *Neurospora* duplications to break down mitotically by the loss of one or the other duplicated segment (see Section 4). Interstitial duplications, such as those from insertional translocations or from overlapping inversions, would require a precise interstitial deletion in order to restore a euploid parental sequence. Duplications from *In O Y348 × In O Y323* might have regenerated one of the parental (inversion) sequences by a two-break deletion of one of the loops in Fig. 4C. A fertile test cross of a culture where this had happened would be scored as an inversion, and its origin by deletion from a duplication progeny would be overlooked.

Another type of segmental loss evidently happened during the growth of two *un⁺ al⁺* progeny from *In O Y348 fr a × In O Y323 al-1 arg-1 un-5 A* (Fig. 4). Isolate *T700-4 arg a* was one of the five progeny scored as Barren ten days after fertilization, but when it was rechecked a few fertile perithecia were found. Ninety-five percent of the ascospores were black, indicating that *T700-4*, unlike either of its parents, had normal chromosome sequence. Isolate *T700-25 a* was fully fertile and it, too, shot 95 % black ascospores.

We collected and germinated ascospores from both of these test crosses and scored the resulting cultures. In both cases about half of the progeny were *un* even though the parent cultures had been phenotypically *un⁺*. Therefore, *T700-4* and *T700-25* must have carried *un* as well as the expressed *un⁺* allele.

Table 3 lists the progeny of *T700-4 arg-1 a × Normal A*. In order to explain these results we must assume that the genotype of the original *T700-4* culture was *al/al⁺ un/un⁺ arg a*. Only a few *al-1* progeny were obtained; a likely explanation is that at different times during the growth of the culture, separate events produced at least two different fertile derivatives. The final result was a mixed culture that

contained two derived subcultures, *al-1 un-5 arg-1 a* and *al-1⁺ un-5 arg-1 a*, as well as the original duplication. Fig. 5 traces the probable origin of these two subcultures. We propose that they arose by recombination rather than by chromosome breakage and loss.

It is now necessary to consider pairing of the regions that were shown as unpaired loops in Fig. 4B. Each loop contains two segments – the segment between the left

Table 3. *Progeny obtained from cross of Normal* A × *Duplication* (OY348-OY323) al-1/al-1⁺ un-5/un-5⁺ arg-1 T700-4 a

	un-5	mt	arg-1	al-1	No. observed
Parental genotypes					
Normal parent	+	A	+	+	.
Inferred derivatives	−	a	−	+	.
from *Dp* breakdown	−	a	−	−	.
Progeny classes	+	A	+	+	19
	−	a	−	+	16
	−	a	−	−	4
	−	A	+	+	4
	+	a	−	+	3
	−	a	+	+	2
	+	A	−	+	3
	−	A	−	+	1
	+	a	−	−	1
	+	A	−	−	1
	+	A	+	−	2
	−	A	+	−	1

Total progeny 57

All isolates had normal chromosome sequence (produced 95 % black ascospores in test crosses to Normal). Evidently the fertilizing parent was a mixed culture with at least two fertile components (*al* progeny must have had the *al* component as parent; *al⁺* classes include progeny from fertilizations by both components).

breakpoint of *OY348* and the left breakpoint of *OY323*, and the segment between the two right breakpoints. Although the two loops have identical genetic content, the gene *sequence* is different. This has been shown by numbering the regions on either side of *al* and *un*. In one segment the order is 2 1 3 4, and in the other, 1 2 4 3. Homologous pairing thus requires contortion into a double loop, as represented by the two small loops of Fig. 5. Fig. 5A shows the same meiotic pairing as Fig. 4B. Single crossovers in the large loop are, of course, the crossovers in the central paired segment of Fig. 4B.

Fig. 5B shows the presumed original structure of *T700-4*, with the duplicated regions paired. If there is a crossover in each loop, the resulting products are an acentric circle and a reconstituted normal linkage group I (Fig. 5C). A crossover in segment 3 would give an *al-1⁺* derivative, and a crossover in segment 4 would give an *al-1* derivative. The accompanying crossover in segment 2 would give an
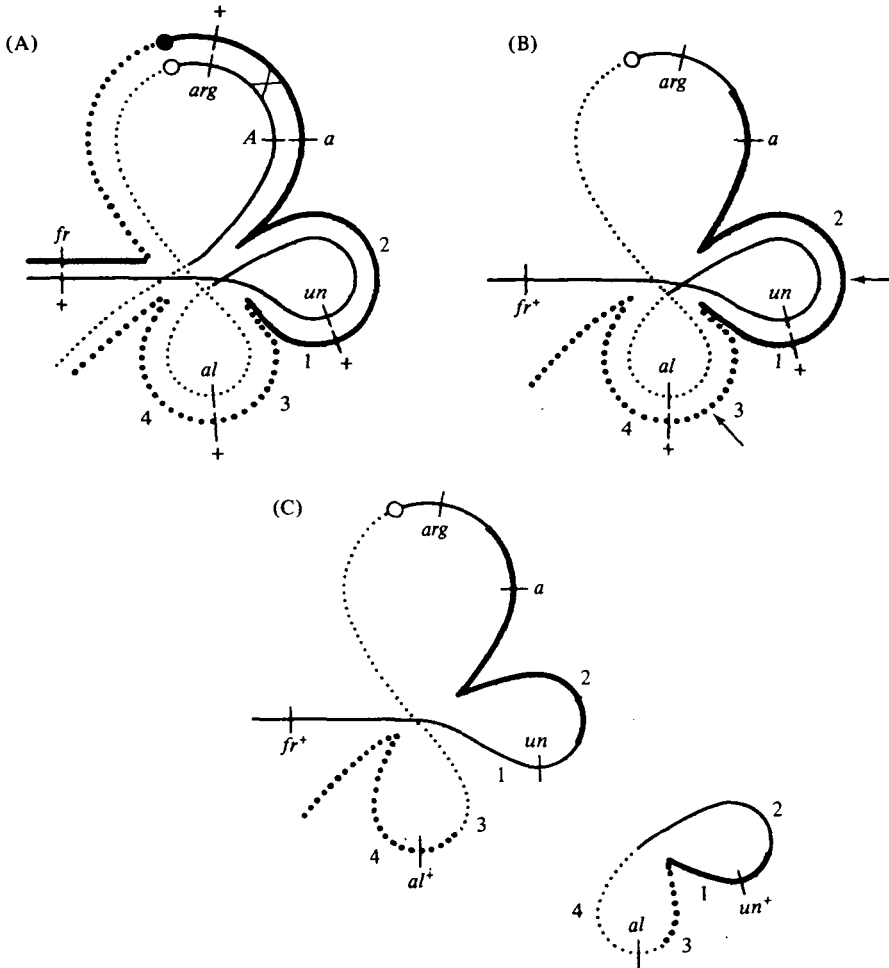
Fig. 5. (Same cross as Fig. 4.) Normal chromosome sequence reconstituted by mitotic crossing over in a duplication. Segments from *In O Y348* are represented by dark dots and lines, *In O Y323* by light dots and lines. Solid lines represent material located in the left arm of normal sequence, dotted lines, material in right arm. (A) *In O Y348 fr a × In O Y323 al-1 un-5 arg-1 A*. Same meiotic pairing as 4B but completely paired. Only two of the four strands are shown. (B) Same duplication as 4C but with mitotic pairing of duplicated regions. (C) Products of somatic recombination. One crossover in each small loop (arrows) gives an acentric circle and a normal-sequence chromosome.

un-5 derivative. An *un-5*+ derivative is unlikely because segment 1, the segment between *un-5* and the left breakpoint of *O Y348*, yielded no crossovers in conventional mapping crosses.

The progeny test of *T700-25* was much simpler than that of *T700-4*, since all progeny were *arg*+ and *al*+ like the parent, and only *un* was segregating in the test

cross to Normal (32 un:38 un^+). Presumably a fertile derivative similar to the al^+ derivative of *T700-4* arose early in the history of the culture. This would explain the early and full fertility of *T700-25*. Theoretically a normal-sequence meiotic product can be derived by a two-strand triple recombinant involving one crossover in each of the three loops. If *un-5* had not been present in the cross (or if the breakdown product had carried the un^+ allele), *T700-25* would have been indistinguishable from such a meiotic product.

4. DISCUSSION

Several major rearrangement types in *Neurospora* have previously been reported in detail: for example, terminal pericentric inversions (Newmeyer & Taylor, 1967; Turner, Taylor, Perkins & Newmeyer, 1969), insertional translocations (Perkins, 1972), terminal translocations (Turner, 1977; Perkins, Raju & Barry, 1980); see Perkins & Barry (1977) for review. The present study and that of Barry & Leslie (1982) now provide similar analyses for typical interstitial pericentric inversions. These *Neurospora* rearrangements resemble corresponding rearrangements in higher organisms in all major aspects of their genetic and cytological behaviour. The principles of higher eukaryote cytogenetics can thus be extended to the fungi, with their tiny genomes and small DNA content.

The various *Neurospora* rearrangements have been used experimentally in many ways: for example, to map genes relative to breakpoints by a right–left test, to map chromosome tips and centromeres, to determine the effect of aneuploidy on viability and phenotype, and to provide segmental aneuploids for studies of dosage, dominance, and gene amplification. For examples in *Neurospora*, and references, see Perkins & Barry (1977); also Metzenberg & Chia (1979) and Rodland & Russell (1982).

The haploid vegetative phase of the fungal life cycle enables certain genetic analyses that are difficult or impractical in most diploid organisms to be carried out with facility in *Neurospora*. For example, segmental duplications result in partial trisomics in a diploid organism, but they are seen as partial disomics against a haploid background in *Neurospora*. *Neurospora* and some of its relatives have the further advantage that viable meiotic products result in black ascospores, while inviable ascospores remain unpigmented. Therefore, any rearrangement that generates deficiencies signals its presence by producing easily seen white ascospores in characteristic frequencies and patterns. Aberrant chromosome structure can thus be inferred by quick visual scanning of ascospores from routine test crosses. Using this tool, we have been able to recognize and analyse rearrangements of considerable complexity, such as double translocations having four breaks in three chromosomes. It has also enabled us to detect and confirm rare types of recombinants; such recombinants can be predicted from rearrangement topology, but their detection would be extremely difficult without the assistance of ascospore patterns. The present study provides an example – the normal-sequence derivatives that arose from multiple crossing over when the two overlapping inversions

were intercrossed (Fig. 5). This part of the study also illustrates the use of barrenness as a signal for partial disomy.

The production of segmental duplications by intercrossing rearrangements, which is presented here as a way of mapping new inversions, has a more significant role. Obtaining effective diploidy for a specific portion of the genome is often vital for understanding gene action. In *Neurospora*, diploids and disomics cannot be used for this purpose because they break down to haploidy too rapidly (cf. Smith, 1974). Duplications, however, are often stable enough to be used as partial diploids, and they can be produced by crossing insertional and terminal rearrangements to normal sequence. Many such rearrangements are now available, and much of the *Neurospora* genome is represented among the duplications that can be produced in this way. Some regions are not yet covered, however. By selecting appropriate pairs of overlapping inversions or of overlapping reciprocal translocations, and by intercrossing them, we are able to extend significantly the proportion of the genome that can be included in made-to-order duplications.

(a) *Reliability of inversion mapping.* Some rearrangements prove to be more complex than they first appear. (See, for example, translocations S1229 and S1325 (Perkins & Barry, 1977), originally thought to be inversions.) Other rearrangements may appear more complex than they really are because recombination or non-disjunction may generate aneuploids which complicate the analysis. It is therefore prudent to use alternate methods and redundant crosses in assigning structures to a new rearrangement type. This was done in the present study, both for the conventional mapping and for the rearrangement intercrosses. Fifteen conventional crosses were made, for mapping and to obtain marked stocks, though only two are reported in detail. The second of these, the isosequential Inversion × Inversion cross (Table 2), helps rule out possible alternative structures. By including previously mapped loci, *ad-9* and *al-1*, in the cross of Fig. 3B, we greatly increased our confidence in using the novel Barrens when they turned out to be phenotypically ad^+ al as predicted. In the cross of Fig. 5, the breakdown of *T700-4* directly confirmed the assumption that the *al-1* and *un-5* recessive alleles were carried in the phenotypically al^+ un^+ duplication culture.

(b) *Recombinational origin of normal-sequence derivatives from the* In OY348 × In OY323 *intercross.* Duplications that break down so as to form fertile derivatives have previously been reported. The duplication derivatives that were studied in detail have all involved terminal segments that were translocated to chromosome tips. (See, for example, Perkins, Newmeyer & Turner, 1972; Turner, 1977; Newmeyer & Galeazzi, 1977.) Usually the entire duplicated segment is deleted precisely so as to restore the normal sequence (or sometimes so as to restore the rearrangement sequence). Newmeyer & Galeazzi (1977) have proposed a recombinational model to account for the precise deletion of terminal duplications, but deletion by breakage is an alternative explanation.

A coordinated cluster of events would be required to restore the normal chromosome sequence from duplication progeny derived from intercrossing over-lapping rearrangements, where neither parent itself was of normal sequence. In

the present study the complex interstitial duplications from *In O Y348 × In O Y323* gave rise to normal-sequence derivatives (Fig. 5). Similarly, certain combinations of overlapping translocations produce segmental duplications when they are intercrossed, and some of these have been observed to yield normal-sequence derivatives (Perkins & Barry, 1977; Perkins, unpublished). Such derivatives cannot be obtained by one- or two-break deletions. We believe that for both overlapping translocations and overlapping inversions, the best explanation for the normal-sequence derivatives is mitotic recombination. In the example given here in Fig. 5, two crossover events are required – one in each of the regions shown as small loops in the diagram.

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