DOUBLE CROSSING OVER IN NEUROSPORA

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A CROSSOVER involves exchange of genetic material between only two of the four products of meiosis. We should like to know whether there are any special properties of these two strands of the tetrad which enable them to participate in crossing over. Such knowledge might help us understand the mechanics of the crossing over process and would perhaps shed some light on the physical nature of the chromosomes themselves. One approach to this problem is the study of strand relationships in double crossovers (two crossovers in the same tetrad). This type of study can only be made in a situation in which at least two of the products of meiosis can be recovered and analysed genetically. Analysis of attached-X females in Drosophila permits a comparison of the frequencies of 2-strand and 4-strand double crossovers (BEADLE and EMERSON 1935; BONNIER and NORDENSKIÖLD 1937; WELSHONS 1955).

Tetrad analysis in Neurospora permits a direct scoring of 2-strand, 3-strand, and 4-strand double crossovers. With random association of non-sister strands, we would expect these three types of double crossover to occur with a frequency ratio of 1:2:1. Any significant deviation from this ratio is considered to be a demonstration of "chromatid interference". LINDEGREN and LINDEGREN (1939, 1942) studied double crossing over in neighboring regions of the same chromosome arm of Neurospora, and they found no evidence of chromatid interference. However, the same authors (LINDEGREN and LINDEGREN 1937, 1942) reported that among double crossovers involving regions in opposite chromosome arms, the majority involved only two strands. Recent work has not confirmed this report of an excess of 2-strand double crossovers. HOWE (1956), in a study of the same linkage group as was used by LINDEGREN and LINDEGREN, found no evidence of chromatid interference across the centromere. The findings of the present report, involving double crossing over across the centromere of another linkage group of *Neurospora crassa*, are in complete agreement with those of HOWE.

METHODS

All crosses in this study were segregating for the ascospore mutant, *asco* (37402). Spores carrying this mutant gene remain colorless while the wild type spores turn black. Crossing over in the region between the *asco* locus and the centromere can be detected in the whole ascus by the patterns of 2nd division segregation for the ascospore character (STADLER 1956). Only these asci need be dissected and grown to test for other segregating mutants. This method of selection of crossover asci greatly reduces the labor involved in a study of chromatid interference. The experiments reported below involve the analysis of 8455 asci for double crossing over, but the time-consuming operations of dissection and growth tests were only performed on the 1622 asci with patterns of 2nd division segregation for *asco*.

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One disadvantage of the mutant *asco* is that it is nearly always lethal. Only a small fraction of those spores carrying it will ever mature and grow. This means that only the spores carrying the wild allele of *asco* can be grown and classified for other segregating markers. However, even this amount of information is sufficient to determine the strand relationships of double crossovers in certain situations.

Comparative frequencies of 2-strand and 4-strand double crossovers

In these experiments, *asco* was crossed to a strain carrying a linked mutant in the opposite chromosome arm. Asci with crossing over between the *asco* locus and the centromere (region I) were selected by their patterns of 2nd division segregation. The mature (*asco*$^+$) spore pairs from these asci were grown and tested for the presence of the other segregating mutant. If we assume that both of the marked regions are short enough to insure that there will not be more than a single crossover within a region, the following interpretation can be made (see fig. 1): Those asci with no crossover between the centromere and the linked mutant (region II) have a single recombination event, resulting in one mutant and one wild spore pair. A crossover in region II making a 4-strand relationship with the one in region I results in double recombination, and both spore pairs are wild. A 2-strand double crossover across the centromere results in no recombination of the marker genes, and both spore pairs are mutant.

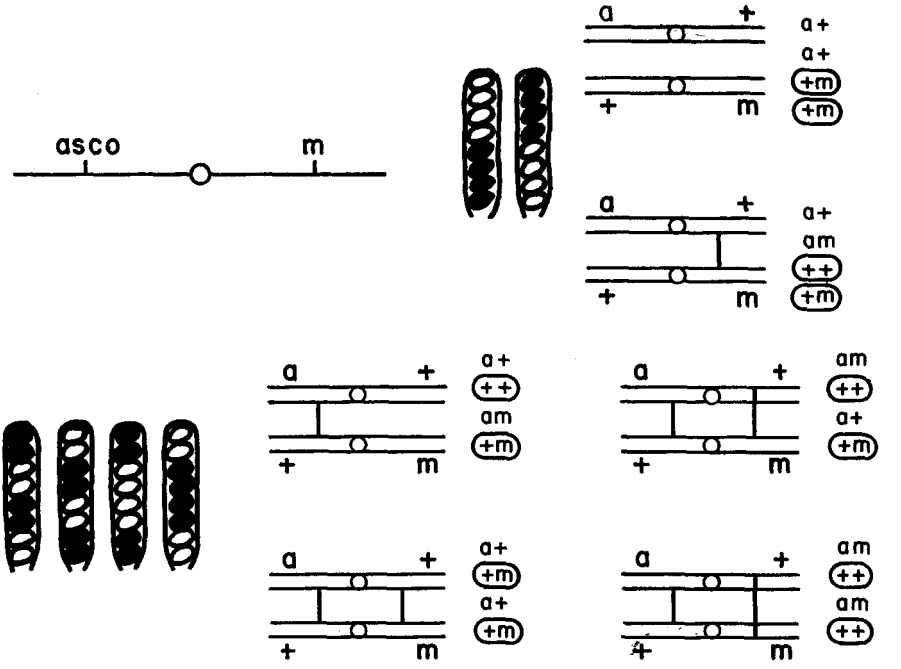

FIGURE 1.—Spore patterns and ascus types in a cross of *asco* to a linked mutant in the opposite chromosome arm. Ovals are drawn around the genotypes of those spore pairs which carry the wild allele of *asco* and can be grown and tested for the other segregating mutant. Above: asci with no crossing over between the *asco* locus and the centromere. Below: asci with crossing over between *asco* and the centromere.

TABLE 1

Counts of 2-strand and 4-strand double crossovers

Cross number	Mutant crossed to *asco*	Asci with 2nd division segregation for *asco*		
		Single recombination	Non-recombination (2-strand double crossover)	Double recombination (4-strand double crossover)
1	*rib-1*	395	1	1
2	"	13	0	0
3	"	2	0	0
4	"	19	1	1
5	"	19	0	0
6	"	14	0	0
7	"	12	0	1
8	"	242	1	2
9	"	10	1	1
10	"	8	0	0
11	"	10	0	1
12	"	7	0	1
13	"	33	0	0
14	"	65	3	1
15	"	17	0	0
16	"	3	1	0
17	"	602	5	3
Total	"	1471	13	12
18	*tryp-2*	105	12	9

A 3-strand double crossover yields one mutant and one wild spore pair and is thus indistinguishable from the single crossover ascus.

The results of these experiments are shown in table 1. It can be seen that there is no significant deviation from the 1:1 ratio of 2-strand and 4-strand double crossovers expected in the absence of chromatid interference. In cross # 1 there were three more asci with patterns of 2nd division segregation for *asco* and with both spore pairs mutant at the *rib-1* (51602) locus. These were not listed in the table with the 2-strand double crossovers, because they were shown by a method similar to that of HOWE (1956) to have resulted almost certainly from meiotic nuclear passing rather than crossing over. Segregating markers located near the centromeres of two other linkage groups (5801 in group III and mating type in group I) showed coincident patterns of 2nd division segregation with *asco* and *rib-1* in these asci. A less precise test for meiotic nuclear passing was made in all other crosses to *rib-1* (# 2 through 17), involving a marked locus in only one other linkage group. This was the mating type locus, which is about 6 units from the centromere. The test revealed that two of the twelve asci classified as 2-strand double crossovers in these crosses may have resulted instead from meiotic nuclear passing. In the cross to *tryp-2* (75001), no check was made for such aberrant meiotic movements. Crossing over was so frequent in region II of this cross that the results could only have been obscured by meiotic nuclear passing occurring much more often than has been observed in other crosses.

TABLE 2

Test for chromosome interference

Cross number	Mutant crossed to *asco*	2 & 4-strand double crossovers among asci with 2nd division segregation for *asco*	Recombination asci among those with 1st division segregation for *asco*	Crossover frequency in region II (map units)	
				With crossing over in region I	With no crossing over in region I
1	*rib-1*	2 in 397 asci	2 in 104 asci	0.50	0.96
8	*rib-1*	3 in 245 asci	3 in 100 asci	1.22	1.50
17	*rib-1*	8 in 610 asci	7 in 198 asci	1.31	1.77
18	*tryp-2*	21 in 126 asci	32 in 100 asci	16.7	16.0

In crosses 1, 8, 17 and 18, asci with no crossover in region I were analysed for crossing over in region II (table 2). These data enable us to test for the occurrence of chromosome interference (a correlation of crossing over in two neighboring regions). However, we must make an assumption as to the frequency of 3-strand double crossovers among the asci with crossing over in region I. In the calculations shown in table 2, it has been arbitrarily assumed that the observed 2-strand and 4-strand double crossovers represent one half of the total double crossovers: i.e., that there is no chromatid interference. The calculations indicate no chromosome interference.

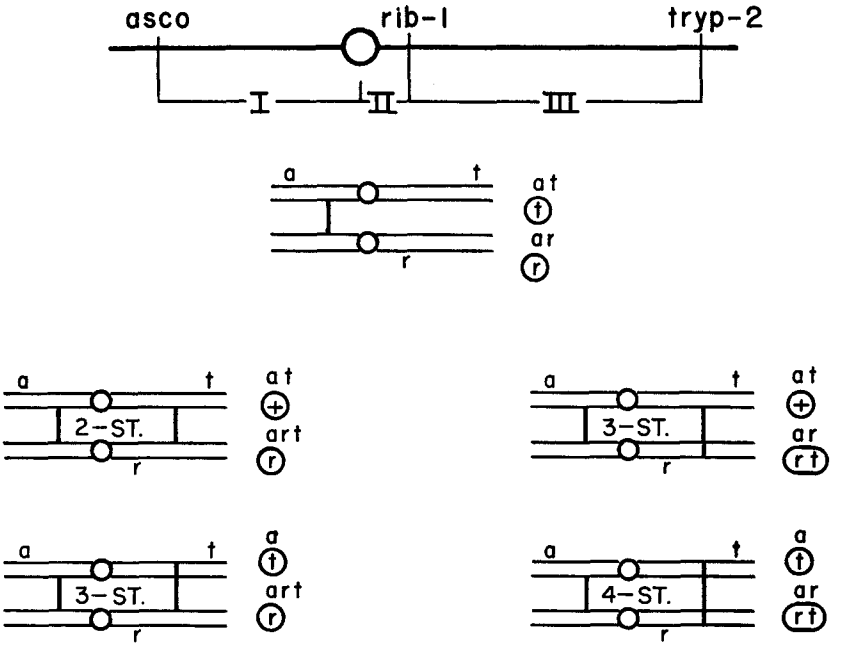

FIGURE 2.—Expected ascus types in the cross *ascotryp-2* × *rib-1* with crossing over between *asco* and the centromere (region I) and between *rib-1* and *tryp-2* (region III). Encircled genotypes are those of spore pairs carrying the wild allele of *asco* which can thus be tested for the other segregating mutants. Genotypes are abbreviated by listing only the mutants (and not the wild alleles) present.

Comparative frequencies of 2-strand, 3-strand and 4-strand double crossovers

The cross analysed in this experiment was segrating for *asco* and two linked mutants in the opposite chromosome arm: *rib-1* and *tryp-2*. The *rib-1* locus is very near the centromere. Crossing over was scored in three marked regions: *asco* to centromere (region I), centromere to *rib-1* (region II), and *rib-1* to *tryp-2* (region III). 2-strand and 4-strand double crossovers involving regions I and II could be analysed as in the previous experiments; these data are listed in table 1 as cross ✱17. Among double crossovers between regions I and III, the 2-strand and 4-strand types and one of the two 3-strand types are all detectably different from each other and from other crossing over events of the same or lower rank (see fig. 2).

Counts of segregation patterns of 5085 asci from the cross *asco tryp-2* × *rib-1* revealed a frequency of 2nd division segregation for *asco* of 20.8% (4026 1st division segregations, 523 alternating and 536 symmetrical 2nd division segregations). 781 asci with patterns of 2nd division segregation were dissected, and the two mature spore pairs of each ascus were transferred to slants of complete medium. There were 610 asci in which both spore pairs were viable. These were tested for riboflavin and tryptophane requirements. The results of the analysis of these asci are shown in table 3.

TABLE 3

Analysis of 610 asci with 2nd division segregation for asco *from the cross* asco tryp-2 × rib-1

Number of asci	Genotypes of viable (*asco*$^+$) spore pairs	Simplest crossing over events to yield this type of ascus
452	*rib-1, tryp-2*	Single crossover in region I*
43	*rib-1*, +	2-strand double in regions I and III
54	*rib-1 tryp-2*, +	3-strand double in regions I and III
53	*rib-1 tryp-2, tryp-2*	4-strand double in regions I and III
4	*rib-1, rib-1*	2-strand double in regions I and II
3	*tryp-2, tryp-2*	4-strand double in regions I and II
1	*rib-1 tryp-2, rib-1*	triple crossover in regions I, II and III

* This class also includes the undetectable 3-strand double crossovers involving regions I and III.

TABLE 4

Analysis of 200 asci with 1st division segregation for asco *from the cross* asco tryp-2 × rib-1

Number of asci	Genotypes of viable (*asco*$^+$) spore pairs	Simplest crossing over events to yield this type of ascus
129	*rib-1, rib-1*	No crossover
62	*rib-1 tryp-2, rib-1*	single crossover in region III
4	*rib-1, tryp-2*	single in region II
2	*rib-1*, +	2-strand double in regions II and III
2	*tryp-2, tryp-2*	4-strand double *within* region I*
1	*rib-1 tryp-2, tryp-2*	3-strand double in regions II and III

Crossing over in region III in asci with no crossing over in region I: 65 exchanges in 198 asci—16.4 map units.

Crossing over in region III in asci with crossing over in region I (data from table 3): 201 exchanges in 610 asci—16.5 map units.

* Because these two asci had crossing over in region I, they were excluded from the above calculations testing for chromosome interference.

The observed ratio of 2-strand, detectable 3-strand, and 4-strand double crossovers of 43:54:53 does not depart significantly from the 1:1:1 ratio expected in the absence of chromatid interference.

A test for chromosome interference was also made on this cross. 200 asci with patterns of 1st division segregation for *asco* were grown and classified for *rib-1* and *tryp-2*. These data are shown in table 4. In comparing the frequencies of crossing over in region III with and without crossing over in region I, the assumption is made that the 150 double crossovers of the three detected types represent 75% of the total double crossovers between regions I and III (the one triple crossover ascus must be added to this total for the calculation). The calculation reveals that crossing over in region III is not correlated in any apparent way with that taking place in region I.

The frequencies of crossing over in regions I and III of this experiment are high enough to justify some concern over possible double crossovers *within* one of these regions. Such undetectable events might conceivably occur with a low frequency, so it is necessary to determine how they might obscure the results. By working out the expected ascus types for all combinations of double crossovers within one region coupled with single or noncrossovers in the other regions, it can be shown that the only such event which would bias the results in favor of one of the three types of detected double crossovers between regions I and III would be a single crossover in region I together with a 4-strand double crossover in region III. Regardless of the strand relationships of the crossover in region I with those in III, such an event would be erroneously scored as a detectable 3-strand double crossover between the two regions. 4-strand double crossovers within region III could not be detected in the analysis of the asci with 2nd division segregation for *asco*, but such an event would be detectable in an ascus with 1st division segregation for *asco*. It is reassuring to note that no such event occurred among the 200 asci in which it might have been detected.

The counts of double crossovers involving regions I and III are not affected by meiotic nuclear passing. Such an event would be scored as a 2-strand double crossover involving regions I and II. Five asci among the 610 analysed fell into this class (4 *rib-1*, *rib-1* and 1 *rib-1 tryp-2*, *rib-1*). Mating types were determined on these strains as a partial check for meiotic nuclear passing. In one of the five asci the mating type locus showed a coincident pattern of 2nd division segregation with all the other markers. This one ascus may have resulted from meiotic nuclear passing rather than crossing over.

Of the 781 asci with *asco* segregating in the 2nd division only those 610 in which

TABLE 5

Frequencies of genotypes among asco$^+$ *progeny of the cross* asco tryp-2 × rib-1

	rib-1	*tryp-2*	*rib-1 tryp-2*	+	Total
Cultures from the 610 viable 2nd division segregation asci	504	510	109	97	1220
Cultures from the 89 partially viable 2nd division segregation asci	46	33	6	4	89

Test for homogeneity: chi-square = 4.34 $P > 0.20$.

both spore pairs grew could be included in the experiment. It would be desirable to know whether the 171 asci which could not be included were a selected group in any sense. In 89 of these asci, one of the spore pairs was viable. These 89 cultures were classified for riboflavin and tryptophane requirements. In table 5 the frequencies of the four possible genotypes among these cultures are compared to the frequencies among the 1220 cultures of the asci counted in the experiment. There is no apparent selection against a particular genotype in the partially viable asci.

DISCUSSION

LINDEGREN and LINDEGREN (1937, 1942) reported strong negative chromatid interference in double crossovers involving both chromosome arms; 37 out of 64 involved only two strands. There has been considerable speculation as to whether some of the apparent 2-strand double crossovers observed by LINDEGREN and LINDEGREN might have resulted from meiotic nuclear passing (WHITEHOUSE 1942; PERKINS 1955; HOWE 1956). HOWE (1956) has devised a technique for detecting meiotic nuclear passing, thus ruling out this possible source of error. This method has also been used in the experiments reported here.

In designing an experiment to study double crossing over, some compromise must be made between precision and yield. HOWE's experiment involved short map regions and was thus precise in the sense that undetected double crossovers *within* regions were highly improbable. Because the regions were so short, he observed only a small number of double crossovers (20 in 1199 asci). The ratio of the 2, 3, and 4-strand types of 5:11:4* is certainly in good agreement with the 1:2:1 ratio expected with no chromatid interference. But because the numbers are small, the result is compatible with quite a wide range of ratios of the three types.

The present experiment has sacrificed some degree of precision in favor of yield of double crossovers. In the cross of *asco tryp-2* × *rib-1*, the 610 asci with 2nd division segregation for *asco* represented a total of 2928 asci and yielded 150 detectable double crossovers involving regions I and III. The relative frequencies of the 2, 3, and 4-strand types are again in good agreement with the expectation with no chromatid interference. In this case the numbers are large enough to put somewhat narrower limits on the statistical fluctuation of the ratio.

The present findings, like those reported by HOWE, indicate that crossing over events in one chromosome arm are independent of those going on in the other arm of the same tetrad. They tell us nothing about the relationship of crossovers in neighboring regions of the same chromosome arm. Studies in other forms have demonstrated that the interference relations between regions on opposite sides of the centromere are different from those between regions on the same side.

SUMMARY

Crossing over analyses involving 8455 asci have been made on marked regions on both sides of the centromere of linkage group VI of *Neurospora crassa*. Tests were

* Two of these involved regions in the same arm. The ratio for doubles across the centromere was ıctually 5:10:3.

made for chromatid and chromosome interference. The results indicate that crossing over events in one arm of a chromosome are independent of those in the other arm.

LITERATURE CITED

BEADLE, G. W., and S. EMERSON, 1935 Further studies of crossing over in attached-X chromosomes of *Drosophila melanogaster*. Genetics **20:** 192–206.

BONNIER, G., and M. NORDENSKIÖLD, 1937 Studies in *Drosophila melanogaster* with attached X:s. Hereditas **23:** 257–278.

HOWE, H. B., 1956 Crossing over and nuclear passing in *Neurospora crassa*. Genetics **41:** 610–622.

LINDEGREN, C. C., and G. LINDEGREN, 1937 Non-random crossing-over in Neurospora. J. Heredity **28:** 105–113.

1939 Non-random crossing-over in the second chromosome of *Neurospora crassa*. Genetics **24:** 1–7.

1942 Locally specific patterns of chromatid and chromosome interference in Neurospora. Genetics **27:** 1–24.

PERKINS, D. D., 1955 Tetrads and crossing over. J. Cellular Comp. Physiol. **45** (supplement 2): 119–149.

STADLER, D. R., 1956 A map of linkage group VI of *Neurospora crassa*. Genetics **41:** 528–543.

WELSHONS, W. J., 1955 A comparative study of crossing over in attached-X chromosomes of *Drosophila melanogaster*. Genetics **40:** 918–936.

WHITEHOUSE, H. L. K., 1942 Crossing-over in *Neurospora*. New Phytologist **41:** 23–62.