CROSSING OVER AND NUCLEAR PASSING IN *NEUROSPORA CRASSA*¹

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BY tetrad analysis in *Neurospora* 2-, 3-, and 4-strand double exchanges may be distinguished, and on a basis of random exchange are expected to occur in a ratio of 1:2:1. A significant departure from this ratio is indicative of chromatid interference. Previous investigations have indicated an excess of 2-strand double exchanges, or negative chromatid interference, across the centromere of the sex chromosome in *Neurospora* (LINDEGREN and LINDEGREN 1942).

Critical evaluation of chromatid interference between two immediately adjacent transcentromere regions is technically difficult, because apparent 2-strand double exchanges between these two regions may result from certain ascospore misarrangements. Even an infrequent occurrence of misarrangements might produce an apparent excess of 2-strand double exchanges across the centromere, because such double exchanges are themselves relatively rare events. LINDEGREN and LINDEGREN (1942) considered ascospore misarrangements an unlikely explanation of their non-random results.

The present paper reports data consistent with random exchange across the centromere of the *Neurospora* sex chromosome, as well as the occurrence of nuclear passing at the second meiotic division, detected by means of an independent centromere marker. Biochemical markers were used to increase the accuracy of scoring genotypes. A preliminary report has been published (HOWE 1954). STADLER (1956) has recently found additional evidence of random strand relationships during multiple crossing over in *Neurospora* and also reports meiotic nuclear passing. Less conclusive crossing over studies with genetically analyzed tetrads in other forms, some indicating randomness, some nonrandomness, have been reviewed by WHITEHOUSE (1942), PAPAZIAN (1952), and PERKINS (1955).

Cytological analyses of chromatid interference based upon relative frequencies of compensating and noncompensating chiasmata, or using structural heterozygotes, have suggested both nonrandomness (DARLINGTON and DARK 1932; HEARNE and HUSKINS 1935; DARLINGTON 1936; FRANKEL 1937; UPCOTT 1937; HUSKINS and NEWCOMBE 1941) and randomness (DARK 1936; GILES 1944). Genetic recombination values exceeding fifty percent, interpretable as an excess of 4-strand exchanges (positive chromatid interference), have been reported (CLAUSEN 1926; WELLENSIEK 1929; FISHER and MATHER 1936; WRIGHT 1947). Most of the *Drosophila* workers have concluded that 2-, 3-, and 4-strand double exchanges occur in random frequencies (ANDERSON 1925; BRIDGES and ANDERSON 1925; REDFIELD, 1930; MORGAN 1933; EMERSON and BEADLE 1933; BEADLE and EMERSON 1935; STURTEVANT and

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BEADLE 1936; WEINSTEIN 1936; WELSHONS 1955), but a significant departure from random expectations was reported by BONNIER and NORDENSKIÖLD (1937, see WELSHONS 1955 for critique).

MATERIALS AND METHODS

Three mutants of *Neurospora crassa* originally produced by BEADLE and TATUM (1945) were used. The mutant *ad-5*(71104) has a partial requirement for adenine (MITCHELL and HOULAHAN 1946), but reading of growth tests at 48 hours obviated scoring difficulty; *rib-1*(516021) has a temperature-sensitive riboflavin requirement (MITCHELL and HOULAHAN 1946a); *vis*(3717) is a morphological mutant with no previously published description. Early growth (12–24 hours) on solid sucrose medium produces a circular patch with a regular margin easily distinguished from the irregularly spreading young hyphae characteristic of the wild allele. Fully developed growth on solid medium produces a limited mycelial patch with reduced aerial hyphae and no bright orange conidia. In liquid medium *vis*(3717) usually grows as a loose pellet at the bottom of the culture tube, producing neither pigment nor mycelial mat. The author is indebted to DR. R. W. BARRATT and to MRS. MARY B. MITCHELL for these mutant strains, and to DR. NORMAN H. GILES for wild types 73a-10a and 74A-4B.

In all, 1378 asci were dissected and classified, 38 (ascus numbers 701–738) from the cross *A ad-5* +; *rib-1* × *a* + *vis*; + (table 1) and 1340 (ascus numbers 739–2078) from the cross *A ad-5 vis*; *rib-1* × wild type 73a-10a (table 2). Tables 1 and 2 include only the 1199 asci in which at least one member of three or more different ascospore-pairs germinated and upon which tetrad analysis was based. When repre-

TABLE 1

Classification of 38 asci from the cross A ad +; r × a + v; + in which at least one member of 3 or 4 ascospore-pairs germinated. Ascospore-pair arrangements are not necessarily ordered. Rib-1 (region IV) is not on the sex chromosome. A, a—mating type alleles, ad—adenine-5, v—visible; r—riboflavin-1

Crossover classes	Regions	Ascospore-pair genotypes				Number of asci
Non	(IV)	<i>A ad + r</i>	<i>A ad + r</i>	<i>a + v +</i>	<i>a + v +</i>	11
		<i>A ad + +</i>	<i>A ad + +</i>	<i>a + v r</i>	<i>a + v r</i>	15
		<i>A ad + r</i>	<i>A ad + +</i>	<i>a + v r</i>	<i>a + v +</i>	1
Single	I	<i>A ad + r</i>	<i>a ad + r</i>	<i>A + v +</i>	<i>a + v +</i>	4
	I	<i>A ad + +</i>	<i>a ad + +</i>	<i>A + v r</i>	<i>a + v r</i>	3
Single	II	<i>A ad v r</i>	<i>a + v r</i>	<i>A ad + +</i>	<i>a + + +</i>	1
Single	III	<i>A ad v +</i>	<i>A ad + +</i>	<i>a + v r</i>	<i>a + + r</i>	1
	III	<i>A ad v r</i>	<i>A ad + r</i>	<i>a + v +</i>	<i>a + + +</i>	1
3-strand double	I-II	<i>A ad v r</i>	<i>a + v r</i>	<i>a ad + +</i>	<i>A + + +</i>	1
Total						38

TABLE 2

Classification of 1161 asci from the cross $A\ ad\ v;\ r \times a\ +\ +$; + in which at least one member of 3 or 4 ascospore-pairs germinated. Ascospore-pair arrangements are not necessarily ordered. *Rib-1* (region IV) is not on the sex chromosome

Crossover classes	Regions	Ascospore-pair genotypes				Number of asci
Non	(IV)	$A\ ad\ v\ r$	$A\ ad\ v\ r$	$a\ +\ +\ +$	$a\ +\ +\ +$	407
		$A\ ad\ v\ +$	$A\ ad\ v\ +$	$a\ +\ +\ r$	$a\ +\ +\ r$	461
		$A\ ad\ v\ r$	$A\ ad\ v\ +$	$a\ +\ +\ r$	$a\ +\ +\ +$	20
Single	I	$A\ ad\ v\ r$	$a\ ad\ v\ r$	$A\ +\ +\ +$	$a\ +\ +\ +$	39
	I	$A\ ad\ v\ +$	$a\ ad\ v\ +$	$A\ +\ +\ r$	$a\ +\ +\ r$	43
	I (& IV)	$A\ ad\ v\ r$	$a\ ad\ v\ +$	$A\ +\ +\ r$	$a\ +\ +\ +$	2
	I (& IV)	$A\ ad\ v\ r$	$a\ ad\ v\ +$	$A\ +\ +\ +$	$a\ +\ +\ r$	1
Single	II	$A\ ad\ v\ r$	$a\ +\ v\ r$	$A\ ad\ +\ +$	$a\ +\ +\ +$	20
	II	$A\ ad\ +\ r$	$a\ +\ +\ r$	$A\ ad\ v\ +$	$a\ +\ v\ +$	23
Single	III	$A\ ad\ v\ r$	$A\ ad\ +\ r$	$a\ +\ v\ +$	$a\ +\ +\ +$	66
	III	$A\ ad\ v\ +$	$A\ ad\ +\ +$	$a\ +\ v\ r$	$a\ +\ +\ r$	59
	III (& IV)	$A\ ad\ v\ r$	$A\ ad\ +\ +$	$a\ +\ +\ r$	$a\ +\ v\ +$	1
2-strand double	I-III	$A\ ad\ v\ r$	$a\ ad\ +\ r$	$A\ +\ v\ +$	$a\ +\ +\ +$	2
	I-III	$A\ ad\ v\ +$	$a\ ad\ +\ +$	$A\ +\ v\ r$	$a\ +\ +\ r$	1
	II-III	$A\ ad\ v\ r$	$a\ +\ +\ r$	$A\ ad\ v\ +$	$a\ +\ +\ +$	2
3-strand double	I-III	$A\ ad\ v\ +$	$a\ ad\ +\ +$	$a\ +\ v\ r$	$A\ +\ +\ r$	2
	I-III	$a\ ad\ v\ r$	$A\ ad\ +\ r$	$A\ +\ v\ +$	$a\ +\ +\ +$	2
	I-III	$a\ ad\ v\ +$	$A\ ad\ +\ +$	$A\ +\ v\ r$	$a\ +\ +\ r$	1
	I-III	$A\ ad\ v\ r$	$a\ ad\ +\ r$	$a\ +\ v\ +$	$A\ +\ +\ +$	3
	II-III	$A\ ad\ v\ r$	$a\ +\ +\ r$	$A\ ad\ +\ +$	$a\ +\ v\ +$	2
4-strand double	I-II	$a\ ad\ v\ r$	$a\ +\ v\ r$	$A\ ad\ +\ +$	$A\ +\ +\ +$	1
	I-III	$a\ ad\ v\ +$	$A\ ad\ +\ +$	$a\ +\ v\ r$	$A\ +\ +\ r$	1
	II-III	$A\ ad\ +\ r$	$a\ +\ v\ r$	$A\ ad\ +\ +$	$a\ +\ v\ +$	2
Total						1161

sentatives of fewer than three different ascospore-pairs in an ascus germinate, not all crossover types have an equal probability of being inferred, and hence 178 asci were omitted to avoid possible bias from germination deficiency. The remaining ascus, a 4-strand double exchange in a single region, was omitted because the corresponding 2- and 3-strand double exchanges could not have been detected. Table 3 shows the linkage relations of *sex*, *ad-5*, and *vis* on the sex chromosome (linkage group I) and of *rib-1* in linkage group VI. For convenience, the region between *rib-1* and its centromere is designated as region IV.

Crosses were made by simultaneous inoculations of parental strains onto fresh slants of supplemented WESTERGAARD-MITCHELL (1947) medium, followed by incubation in the dark at 25°C. Only asci containing eight uniformly dark, seemingly mature ascospores were dissected, but every ascus dissected was classified to the

TABLE 3

Classification of 1199 asci summarized from tables 1 and 2. Expected numbers computed on assumption of no interference

Crossover classes	Regions	Number of asci		Crossovers per region			
		Observed	Expected	I	II	III	IV
Non		915	918.2				21
Single	I	92	89.1	92			3
	II	44	41.6		44		
	III	128	127.3			128	1
Double	I & II	2	4.1	2	2		
	I & III	12	12.3	12		12	
	II & III	6	5.8		6	6	
Triple	I & II & III	0	0.6				
Total		1199	1199	106	52	146	25
C.o. freq.				.088	.043	.122	.021

<i>sex</i>	<i>ad-5</i>	<i>cent.</i>	<i>vis</i>	<i>cent</i>	<i>rib-1</i>
· 4.4	· 2.2	· 6.1	·	· 1.0	·
I	II	III		IV	

maximum that ascospore germination would permit. All dissections were ordered. Ascospore isolates were heat-shocked immediately after dissection and then held at least two weeks to permit scoring of slow germinating types. Growth tests were run in duplicate on one member of each ascospore-pair, but on all members of asci having detectable ascospore misarrangements.

Equational division of centromeres at meiosis I, passing of the two center nuclei around each other at meiosis II, or dissecting errors could all produce ascospore misarrangements interpretable as 2-strand double exchanges between regions II and III, which bound the sex chromosome centromere. Thus an excess of apparent 2-strand double exchanges between two adjacent transcentromere regions may be a consequence of ascospore misarrangements rather than of actual negative chromatid interference. However, the strands involved in double exchanges between pairs of regions not adjacent to the centromere may be inferred from marker gene recombinations independently of ascospore order (LINDEGREN and LINDEGREN 1942).

An independent centromere marker was employed to detect asci resulting from meiotic nuclear passing or dissection errors, thereby making it possible to distinguish between false and legitimate 2-strand double exchanges in regions II and III. The independent centromere marker, *rib-1*, segregates at the first division in about 98 percent of asci. Passing, however, would convert a *rib-1* first division segregation into an apparent asymmetrical second division segregation, and similarly for *ad-5* and *vis* on the sex chromosome. Therefore, an ascus showing asymmetrical second division segregation arrangements of *ad-5*, *vis*, and *rib-1* would almost certainly

have been produced by meiotic nuclear passing or dissection error rather than by crossing over. An ascus produced by legitimate 2-strand double exchange in regions II and III would in all likelihood show first division segregation for *rib-1*, if independence of crossing over between tetrads is assumed.

Ascospore misarrangements resulting from centromere misassortment were not detectable as such, but failure to find negative chromatid interference makes it unlikely that appreciable misassortment of the sex chromosome centromere occurred. Dissecting errors could produce any ascospore misarrangements, but adjacent ascospores would most often be interchanged, and the correct order was usually inferable if enough ascospores germinated.

EXPERIMENTAL RESULTS

Chromosome interference

Table 3 summarizes the data from tables 1 and 2 on observed and expected crossovers in the three regions of the sex chromosome. The numbers of double crossovers by regions are too small for any definite conclusions, but these data best agree with an interpretation of no chromosome interference across the centromere (regions I and III and regions II and III), and positive interference in the left arm (regions I and II). No triple crossovers were found.

Chromatid interference

The ratio of 2-, 3-, and 4-strand double exchanges initially classified was 9:11:4. One of these 2-strand double crossover asci, 712, apparently involved regions II and III, directly across the centromere, and also showed apparent asymmetrical second division segregation of *rib-1*, the independent centromere marker (table 6). Ascus 712 almost certainly resulted from meiotic nuclear passing and is reclassified as a noncrossover ascus in the tables. The alternative interpretation requires a double crossover on the sex chromosome and a simultaneous crossover in the one map unit region between *rib-1* and its centromere. Similarly, Asci 1074, 1586, and 2022 are interpreted as nuclear passing or as a dissection error resulting in interchange of spores 3 and 6. This correction changes the double crossover ratio from 9:11:4 to 5:11:4 (table 4). This ratio does not differ significantly from the 1:2:1 expectation with random exchange between chromatids. Ordered ascospore arrangements of all double crossover asci are given in table 5.

TABLE 4

Observed and expected numbers of 2-, 3-, and 4-strand double crossovers among 1199 asci. Expected numbers computed on the assumption of no chromatid interference

Crossover class double	Regions			Totals	
	I-II	I-III	II-III	Observed	Expected
2-strand	0	3	2	5	5.0
3-strand	1	8	2	11	10.0
4-strand	1	1	2	4	5.0

TABLE 5

Ascospore arrangements in all double crossover asci. Ascospore number 1 is at the distal end of the ascus. Ascospores in parenthesis did not germinate but were inferable. Asci 701-738 are from the cross A ad +; r X a + v; +. Asci 739-2078 are from the cross A ad v; r X a + +; +

	Ascus number	Ordered ascospore genotypes							
		1	2	3	4	5	6	7	8
Two-strand	I-III	a ad + r	a ad + r	A ad v r	A ad v r	A + v +	A + v +	a + + +	a + + +
	I-III	a ad + r	a ad + r	A ad v r	A ad v r	A + v +	A + v +	a + + +	a + + +
	I-III	A ad v +	A ad v +	a ad + +	a ad + +	A + v r	A + v r	a + + +	a + + +
	II-III	a + + + r	a + + + r	A ad v r	A ad v r	A ad v +	A ad v +	a + + +	a + + +
	II-III	(a + + + r)	a + + + r	A ad v r	A ad v r	a + + +	a + + +	A ad v +	A ad v +
Three-strand	I-II	a + v r	a + v r	A ad v r	A ad v r	a ad + +	a ad + +	A + + +	A + + +
	I-III	(a + v r)	a + v r	(A + + r)	A + + r	(a ad + +)	(a ad + +)	A ad v +	A ad v +
	I-III	A ad + r	A ad + r	a ad v r	a ad v r	a + + +	a + + +	A + v +	A + v +
	I-III	a + v +	a + v +	A + + +	A + + +	a ad + r	a ad + r	A ad v r	A ad v r
	I-III	A ad v +	A ad v +	a ad + +	a ad + +	a + v r	a + v r	A + + r	A + + r
	I-III	a + + r	a + + r	A + v r	A + v r	a ad v +	a ad v +	A ad + +	A ad + +
	I-III	a ad + r	a ad + r	(A ad v r)	(A ad v r)	a + v +	a + v +	A + + +	A + + +
	I-III	A + v +	A + v +	a + + +	a + + +	A ad + r	A ad + r	a ad v r	a ad v r
	I-III	A + + +	A + + +	a + v +	a + v +	A ad v r	A ad v r	(a ad + r)	(a ad + r)
	II-III	a + v +	a + v +	A ad + +	A ad + +	a + + r	a + + r	A ad v r	A ad v r
	II-III	a + v +	a + v +	A ad + +	A ad + +	A ad v r	A ad v r	a + + r	a + + r
	II-III	1564							
Four-strand	I-II	A + + +	A + + +	A ad + +	A ad + +	a + v r	a + v r	a ad v r	a ad v r
	I-III	a ad v +	(a ad v +)	A ad + +	A ad + +	A ad + +	A ad + +	a + v r	a + v r
	II-III	813	A ad + r	a + v r	a + v r	A ad + +	A ad + +	a + v +	a + v +
	II-III	1420	a + v +	A ad + +	A ad + +	a + v r	a + v r	A ad + r	A ad + r

* Also listed in Table 7.

Ascospore misarrangements

Two asci showed evidence of having been produced by meiotic nuclear passing rather than by crossing over (table 6). Ascus 712 showed apparent double crossovers in regions II and III, as well as apparent asymmetrical second division segregation of *rib-1*. Passing in ascus 2075 was detected by exceptional behavior of a *vis* modifier (see below). *Rib-1* also showed apparent asymmetrical second division segregation in ascus 2075, an indication of the reliability of the independent centromere marker technique. Asci 1074, 1586, and 2022 involve positional interchange of spores 3 and 6. This may be due to incomplete meiotic nuclear passing, meiotic and mitotic nuclear passing, or dissection errors. Similar explanations can account for 1001, 1913, and 2020 which show interchange of spores 4 and 6.

Fifty-five asci showed evidence of having resulted either from dissecting errors or from (third division) nuclear passing (table 7). These two causes are indistinguishable in any given ascus. Comparison of the frequency of misarrangements between

TABLE 6

Summary of asci showing evidence of nuclear passing or dissection errors. Asci 712, 1074, 1586, and 2022 might be regarded as 2-strand double exchanges except for post reduction of the riboflavin centromere marker. Ascospores in parentheses did not germinate but were inferable. Ascus 712 is from the cross A ad +; r × a + v; +; the others are from A ad v; r × a + +; +

Ascus number	Ordered ascospore genotypes							
	1	2	3	4	5	6	7	8
712	A ad + +	A ad + +	a + v r	a + v r	A ad + +	A ad + +	a + v r	a + v r
2075	a + + +	a + + +	(A ad + r)	A ad + r	a + v +	a + v +	(A ad v r)	A ad v r
1074	A ad v r	A ad v r	a + + +	A ad v r	a + + +	A ad v r	a + + +	a + + +
1586	a + + +	a + + +	A ad v r	a + + +	A ad v r	a + + +	A ad v r	A ad v r
2022	a + + r	a + + r	A ad v +	(a + + r)	A ad v +	(a + + r)	A ad v +	A ad v +
1001	a + + r	a + + +	A ad v r	A ad v r	A ad v r	a + + +	A ad v r	A ad v r
1913	A ad v r	A ad v r	A ad v r	a + + +	a + + +	A ad v r	a + + +	a + + +
2020	A ad v r	A ad v r	A ad v r	a + + +	a + + +	(A ad v r)	a + + +	a + + +

TABLE 7

Summary of asci showing evidence of dissecting errors or third division nuclear passing among 1199 analyzed asci

Type of error. Transposition of ascospores	Number of asci	Number of asci per crossover class						
		NCO	NCO (IV)	SI	SII	SIII	SIII (IV)	DI-III
2 & 3	10		3	3	1	3		
4 & 5	32	24	2		2	3		1
3 & 6	3	3						
4 & 6	3	3						
6 & 7	3					2	1	
2 & 3; 4 & 5	2					2		
2 & 3; 6 & 7	1			1				
4 & 5; 6 & 7	1				1			
Total	55	30	5	4	4	10	1	1

ascospores two and three and between ascospores six and seven shows that misarrangements were about three times as frequent at the distal end of the ascus as at the proximal end, perhaps because of a greater tightness at the attached proximal end.

The preponderance of misarrangements between ascospores four and five (table 7) is at least partially due to the fact that four and five are the only two adjacent ascospores between which misarrangements can be detected in the most frequent ascus type, the noncrossovers. By assuming that the 32 interchanges between ascospores four and five represent about the average frequency of dissecting errors or third division passing, the total number of adjacent interchanges can be estimated, since practically all four-five interchanges are detectable.

If all seven possible adjacent ascospore interchanges had 32 misarrangements, the total number of asci with adjacent interchanges would be $32 \times 7 = 224$, or about 19 percent of the 1199 asci analyzed. Four of the seven possible adjacent interchanges are between sister ascospores and would not be detected in analysis. The correct order of detectable interchanges was usually inferable from the remaining members of the ascus.

To improve germination older asci were dissected, and this might have favored dissecting errors to some extent. Nevertheless, misarrangements did not increase appreciably with the period between crossing and dissecting, which averaged 35 days for all asci and 38 days for the 55 asci in table 7.

Modifiers of vis

Two additional markers were found in the course of this investigation, both modifiers of *vis* and linked to it. The first modifier, m_1 , produces a browning of the agar and, about two thirds of the time, a few pale pink conidia; the complementary

TABLE 8

Frequency of crossovers between vis and two vis modifiers, m_1 and m_2 , in all asci in which these modifiers were determinable

m_1		m_2	
Regions	Number of crossover asci	Regions	Number of crossover asci
a	96	c	108
b	50	d	197
a-b	1	c-d	20
Total asci	1200	Total Asci	1061

Map Distance, region a = $97/1200 \times 50 = 4.0$

Map Distance, region b = $51/1200 \times 50 = 2.1$

Map Distance, region d = $217/1061 \times 50 = 10.2$

<i>sex</i>	<i>ad-5</i>	<i>cent.</i>	m_1	<i>vis</i>	m_2
.	4.4	.	2.2	.	4.0
.	.	.	4.0	.	2.1
.	.	.	.	.	10.2
.	.	.	.	.	.
		a		b	d
		c			

crossover type produces a rapidly growing, fluffy mycelium which lacks conidia. The second modifier, m_2 , produces a slowly growing, button-like mycelium; the complementary crossover type cannot be recognized. Table 8 gives the crossover data for these two modifiers and shows their locations.

The centromere- m_1 and the m_1 - vis regions were not used as separate regions in the crossing over study, because m_1 decreased somewhat the accuracy of identifying progeny. The vis - m_2 region was not used, because the crossover complement of m_2 could not be recognized. The mutant m_1 was used to detect meiotic nuclear passing in one instance, however, in the following manner. This modifier always appeared coincidentally with crossing over in region III, locating it proximal to vis . In ascus 2075, however, m_1 appeared coincidentally with apparent crossovers in regions II and IV. The apparent second division segregation of $rib-1$ was asymmetrical, as would be expected with passing. Ascus 2075 was therefore reclassified as a single crossover in region III (table 6).

Selection among the progeny

In 1378 total asci dissected, ascospore germination was 85.4 percent (table 9). Highest ascospore germination percentages were obtained with asci dissected six to seven weeks subsequent to setting up crosses. Table 9 shows germination according to ascospore position. Although suggestive of somewhat lower germination at the proximal end, the differences are not statistically significant.

Table 10 shows that neither of the two alleles at the sex , $ad-5$, or vis loci had a significant selective advantage. However, the wild type allele at the $rib-1$ locus survived more often than did the mutant allele, and the difference is highly significant. Because $rib-1$ was used only as an independent centromere marker, whereas crossing over was studied in the sex chromosome, this differential effect of $rib-1$ could not influence the crossing over analysis made. The absence of any significant effect of the sex chromosome markers on ascospore germination, together with the

TABLE 9

The relation between ascospore germination and position of the ascospore in all 1378 asci dissected. Position 1 is the most distal

Ascospore position	Number of ascospores		Percent germination
	Germinated	Non-germinated	
1	1176	202	85.3
2	1194	184	86.6
3	1194	184	86.6
4	1184	194	85.9
5	1182	196	85.8
6	1183	195	85.8
7	1156	222	83.9
8	1142	236	82.9
Total	9411	1613	85.4

$$\chi^2_7 = 13.7, P = 0.07.$$

TABLE 10

Effect of marker genes on ascospore germination in all 1378 asci dissected. The number of germinated and non-germinated, but inferable, ascospores is given for each pair of alleles. The chi-square is the interaction chi-square computed for each set of four

Alleles	Number of ascospores		χ^2_1	Prob.
	Germinated	Non-germinated, genotype inferable		
<i>A</i> <i>a</i>	4736 4675	403 436	1.62	> .05
<i>ad-5</i> <i>ad-5</i> ⁺	4728 4683	412 427	.40	> .05
<i>vis</i> <i>vis</i> ⁺	4727 4684	419 420	.02	> .05
<i>rib-1</i> <i>rib-1</i> ⁺	4632 4779	496 343	30.2	< .001

high overall germination rate, makes it extremely unlikely that germination deficiency could have appreciably biased the estimates of crossing over and interference.

Spindle orientation

Table 11 gives first meiotic division spindle orientations for all asci for which these could be inferred. There was no evidence for preferential segregation in either chromosome, nor for any interaction between the *sex* and *rib-1* tetrads. Second meiotic division spindle orientation data also indicate randomness, since the frequencies of symmetrical and asymmetrical arrangements are not significantly different (table 12).

TABLE 11

First meiotic division spindle orientation in the 1188 asci for which spindle orientation was inferable

Tetrads	Ordered ascospore-pair arrangements				Number of asci	Totals	χ^2_1	Prob.
	1.2	3.4	5.6	7.8				
<i>sex</i>	<i>A ad v r</i>	<i>A ad v r</i>	<i>a + + +</i>	<i>a + + +</i>	298	620	2.28	> .05
	<i>A ad v +</i>	<i>A ad v +</i>	<i>a + + r</i>	<i>a + + r</i>	322			
	<i>a + + +</i>	<i>a + + +</i>	<i>A ad v r</i>	<i>A ad v r</i>	266			
	<i>a + + r</i>	<i>a + + r</i>	<i>A ad v +</i>	<i>A ad v +</i>	302			
<i>rib-1</i>	<i>A ad v r</i>	<i>A ad v r</i>	<i>a + + +</i>	<i>a + + +</i>	298	600	.12	> .05
	<i>a + + r</i>	<i>a + + r</i>	<i>A ad v +</i>	<i>A ad v +</i>	302			
	<i>a + + +</i>	<i>a + + +</i>	<i>A ad v r</i>	<i>A ad v r</i>	266			
	<i>A ad v +</i>	<i>A ad v +</i>	<i>a + + r</i>	<i>a + + r</i>	322			
Tetrad In- teraction	<i>A ad v r</i>	<i>A ad v r</i>	<i>a + + +</i>	<i>a + + +</i>	298	564	3.03	> .05
	<i>a + + +</i>	<i>a + + +</i>	<i>A ad v r</i>	<i>A ad v r</i>	266			
	<i>A ad v +</i>	<i>A ad v +</i>	<i>a + + r</i>	<i>a + + r</i>	322			
	<i>a + + r</i>	<i>a + + r</i>	<i>A ad v +</i>	<i>A ad v +</i>	302			

TABLE 12
Second division segregations for four loci in all asci from tables 1 and 2

Segregation type	Locus				Total		χ^2_1	Prob.
	<i>sex</i>	<i>ad-5</i>	<i>vis</i>	<i>rib-1</i>	Obs.	Exp.		
Symmetrical	72	27	68	12	179	189	1.06	> .05
Asymmetrical	83	25	78	13	199	189		

DISCUSSION

The conclusion has been reached that crossing over was at random between chromatids along that portion of the sex chromosome analyzed. Meiotic nuclear passing seems to occur, but with very low frequency.

LINDEGREN and LINDEGREN (1942) analyzed four regions of the *Neurospora* sex chromosome delimited by the *sex* locus and three morphological markers, as follows: region I, *sex-gap*, 4 units; region II, *gap*-centromere, 3.5 units; region III, centromere-*crisp*, 3.5 units; region IV, *crisp-pale*, 8 units. Departures from random exchange between chromatids were found.

In the present investigation only about 0.2-0.5 percent of the asci analyzed showed evidence of meiotic nuclear passing. Alternatively, these may be due to dissection errors or postmeiotic transpositions. MCCLINTOCK's (1945) analysis of arrangements of visibly different ascospores in 869 asci revealed seven arrangements (0.8 percent) that could be attributed to passing. Table 2 (LINDEGREN and LINDEGREN 1942) gives ordered arrangements of triple and quadruple crossovers found. Four of the nine triple crossovers, 3243, 2968, 3150, and 3249, become single crossovers on an assumption of passing, and all four involve exchanges directly across the centromere, with asymmetrical *gap* and *crisp* arrangements. On the basis of this reinterpretation, about 0.3 percent passing occurred in the LINDEGRENS' study, and the frequency might have been somewhat higher, if all occurrence of passing had been discernible among their double crossovers.

The frequency of passing may tend to vary in different stocks, but evidence so far accumulated indicates that frequencies somewhat under one percent are to be expected. In studies depending upon ascospore order, two independent centromere markers instead of one would be more precise in validating critical asci, but would require more time and labor in classifying progeny.

The ratio of 2-, 3-, and 4-strand double exchanges reported by LINDEGREN and LINDEGREN (1942) was 28:13:15, a significant departure from random expectation ($P < .01$). The LINDEGRENS' double crossover ratio for their first three regions, 13:4:3, also differed significantly from expectation ($P < .05$), and from the writer's double crossover ratio, 5:11:4, for three similarly placed regions ($P < .05$). The finding of evidence for nuclear passage, undetected except by a centromere marker, suggests that at least some of the LINDEGRENS' excess of 2-strand doubles might be attributed to this cause. In fact, the original classification ratio of 9:11:4 before correction for nuclear passage or errors of dissection does not differ significantly from LINDEGREN's 13:4:3.

PERKINS (1955) showed that evidence for chromatid interference is greatly reduced in the total data if retabulation is made on the assumption that all the LINDEGRENS' 2-strand double crossovers in regions II and III, about one percent of asci, resulted from various misarrangements. The LINDEGRENS' 15:7:6 double crossover ratio for regions I and IV only, however, shows evidence of negative chromatid interference not readily attributable to misarrangements, since these two regions are not adjacent to the centromere.

LINDEGREN and LINDEGREN (1942) reported an excess of triple and quadruple exchanges. Their total multiple exchanges were not in excess, however, because the most frequent multiple type, the doubles, were fewer than expected (total multiples observed:total multiples expected = $70:80.06 = 0.87$). Moreover, reinterpretation of the ordered arrangements of their nine triple crossovers, as discussed above, indicates that four of the triples probably resulted from passing and should be reclassified as single crossovers. The expected number of triples was 4.8. Their quadruple exchanges, however, are in marked excess even if reinterpreted on the passing hypothesis, but not if assumptions of nonadjacent rearrangements, as by centromere misassortment, are made (see PERKINS 1955).

SUMMARY

Crossing over was studied in the sex chromosome of *Neurospora crassa* by means of genetically analyzed tetrads. Data were obtained from 1199 asci having at least one member of three different ascospore-pairs germinating. Three regions on the sex chromosome were considered, regions I and II to the left of the centromere and region III to the right.

The ratio of 2-, 3-, and 4-strand double crossovers found was 5:11:4 and does not differ significantly from the 1:2:1 chance expectation. Thus there is no suggestion in our data for chromatid interference, or of any interference across the centromere.

An independent centromere marker technique was employed for detecting meiotic nuclear passing or errors in dissection. Such misarrangements were of rare occurrence, having been found in but 0.2–0.5 percent of the asci analyzed. The effects of meiotic nuclear passing and other misarrangements upon conclusions drawn from chromatid interference data are considered.

Differences between the present findings and those of LINDEGREN and LINDEGREN (1942) are discussed.

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