TETRAD ANALYSIS OF SHORT CHROMOSOME REGIONS OF NEUROSPORA CRASSA[1]

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THE information concerning chromatid and chiasma interference which is now available from tetrads of a number of organisms shows many conflicting results and variable situations. An excess of 2-strand double exchanges has been found in *Neurospora crassa* (LINDEGREN and LINDEGREN 1942) and in yeast (DESBOROUGH, SHULT, YOSHIDA and LINDEGREN 1960). MALING (1959) found a deficiency of 3-strand double exchanges in *N. crassa* after treatment with 5-bromouracil, but other treatments produced no evidence of chromatid interference. HAWTHORNE and MORTIMER (1960) obtained a deficiency of 3-strand double exchanges in yeast. The same type of deficiency was also found for one pair of intervals in *Chlamydomonas reinhardi*, but no chromatid interference was found in two other pairs of intervals of the same cross (EBERSOLD and LEVINE 1959). No chromatid interference has been found in *N. crassa* (HOWE 1956; PERKINS 1956; STADLER 1956), in *Aspergillus nidulans* (STRICKLAND 1958) or in Sphaerocarpus (KNAPP 1960). In general, no chromatid interference has been found in Drosophila, but there was an almost significant excess of 2-strand double exchanges when only short intervals were considered (for review see WELSHONS 1955).

Chiasma interference cannot be determined from random strands without prior knowledge of the presence or absence of chromatid interference. Assuming chromatid interference to be absent, chiasma interference has been shown to be positive within the chromosome arms of Drosophila (ANDERSON and RHOADES 1931; MORGAN, BRIDGES and SHULTZ 1935) and absent across the centromeres. From tetrad data chiasma interference has been shown to be positive within the chromosome arms of Neurospora (PERKINS 1955, 1958 and unpublished; STADLER 1956; HOWE 1956), Chlamydomonas (EBERSOLD and LEVINE 1959) and Sphaerocarpus (KNAPP and MÖLLER 1955), but absent in *Aspergillus nidulans* (STRICKLAND 1958) and in yeast (SHULT and LINDEGREN 1959; DESBOROUGH, SHULT, YOSHIDA and LINDEGREN 1960). On the contrary, positive chiasma interference is reported in yeast by HAWTHORNE and MORTIMER (1960).

The extent to which chromatid and chiasma interference can be distinguished and separated has been discussed recently by SHULT and LINDEGREN (1959), while some errors of experimental design which may obscure chromatid inter-

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ference have been suggested by STRICKLAND (1958). In designing the present experiments, these features were taken into account in an effort to resolve previous conflicting results.

MATERIALS AND METHODS

In much of the work on chromatid and chiasma interference, intervals have been used that were too long to detect all multiple exchanges within the region under investigation. WHITEHOUSE (1956) has pointed out that undetected multiple exchanges within intervals may obscure interference. Therefore, the first problem in the present study was to select a chromosome region in *N. crassa* where known loci were spaced so that a minimum of undetected multiple exchanges occurred. On the other hand, the loci had to be sufficiently far apart to allow recovery of a reasonable number of double exchanges. Based on the analysis of 760 tetrads with various interval lengths, intervals of 5–10 units were found to be suitable. A region of linkage group V far out on the right arm appeared to carry several suitable closely spaced markers. The loci selected as markers in these experiments were *hist-1*, histidine-1 (C84); *inos*, inositol (37401); *bis* biscuit (B6) and *pab-2*, p-aminobenzoic acid-2 (H193). The linkage relations of these loci have been summarized recently (see STRICKLAND, PERKINS and VEATCH 1959). To facilitate recognition of spore pairs, two other independently segregating loci included in the cross were *aur*, aurescent (34508), in linkage group I and *ylo*, yellow (Y30539y), in linkage group VI. An unlocated gene determining slow growth also segregated in all the crosses. All the stocks containing the markers were backcrossed several times to derivatives of St. Lawrence wild-type strains prior to their incorporation into multiply marked strains. The alleles were incorporated into four strains in the following arrangements:

hist-1, bis; ylo; aur a (320)	*hist-1, bis; ylo; a* (324)
inos, pab-2; slo; A (927)	*inos, pab-2; slo; aur A* (72)

Numbers 320 and 324 were obtained from a single cross and 72 was obtained by backcrossing 927 to an inbred aurescent strain. Thus all four parents have fairly closely related genetic backgrounds. Progeny from all four possible crosses of these four strains were analyzed and the ascospores from individual asci were collected by a "spore shooting" technique (STRICKLAND 1960). This technique gives unordered asci. Each of the eight ascospores from each individual ascus to be analyzed was germinated separately on an agar slant. In order to detect all exchanges, it is unnecessary to determine the genotypes of all eight cultures derived from each ascus. If the pairs were recognizable by inspection of the germinated cultures, only two cultures need be tested; if the pairs were not recognizable, only three need be tested. The method is to test one culture from each of the two pairs containing biscuit or one culture from each of the two pairs containing biscuit$^+$. All double exchanges (and higher multiple exchanges) can be recognized by the fact that both cultures will result from single crossovers (or combinations of single and multiple crossovers) or one of the cultures will result from a double crossover (or multiple crossover) (Chart 1). Two exceptions to this

rule occur when there are 3-strand double exchanges involving the two intervals *hist-1—inos, inos—bis*. With one type of 3-strand double exchange in these two intervals only a single crossover strand in the *inos—bis* interval will be found if the cultures from the two *bis*$^-$ spore pairs are tested. With the other type of 3-strand double exchange in these two intervals only a single crossover strand in the *inos—bis* interval will be found if the two *bis*$^+$ spore pairs are tested. Therefore, when an exchange in the *inos—bis* interval was detected from the presence of a single crossover strand, one culture from each of the other two spore pairs was tested in order to insure that an exchange in the *hist-1—inos* interval would not be missed. When a double or multiple exchange of any type was detected, all the spores of that ascus were tested and scored completely for all the segregating loci, including sex.

All crosses were carried out at 25° on suitably supplemented synthetic crossing medium (WESTERGAARD and MITCHELL 1947). The ascospores were isolated to specifically supplemented minimal medium to avoid histidine inhibition by complete medium (LEIN, MITCHELL and HOULAHAN 1948; HAAS, MITCHELL, AMES and MITCHELL 1952).

RESULTS

The data from the four possible crosses are given in Tables 1 to 4. These tables include the incompletely classifiable tetrads. None of these incompletely classifiable samples showed any signs of being selected with respect to either allele ratios or tetrad classes.

(a) Chiasma interference (Table 5): If the strand relations of double and multiple exchanges could be predicted it would be more efficient to study chiasma interference by the use of ascospores isolated at random than by tetrad analysis. However, the information is also available from tetrads. By definition, with no chiasma interference, exchanges occurring in any one interval should be independent of those occurring in any other interval.

In all cases the observed number of double exchanges is significantly less than the expected number (Table 5). Therefore, positive chiasma interference exists; the coefficients of coincidence between exchanges in tetrads range from 0.14 to 0.45.

(b) Chromatid interference (Table 6): Because the intervals selected were sufficiently short to minimize undetected double exchanges, the number of multiple exchanges within the entire chromosome region studied is meager despite the large number of asci analyzed. Therefore, in order to have significant numbers of multiples some pooling of the data from the various crosses is necessary.

Cross 320 × 927 (Table 6, section a): Only 17 double exchanges were obtained in 1802 completely analyzable tetrads. The ratio obtained was nine 2-strand:one 3-strand:seven 4-strand double exchanges. If the expected ratio is 1:2:1, the probability of obtaining these values is 0.001. There was almost a complete absence of 3-strand double exchanges. Two other tetrads were double exchanges

CHART 1

Genotypes which will be recovered from asci having various types of double exchanges

Types of double exchange				Genotypes				Type of strand
hist-1	+	*bis*	+	*hist-1*	+	*bis*	+	Parental
hist-1	+	*bis*	+	*hist-1*	*inos*	*bis*	+	Double
+	*inos*	+	*pab-2*	+	+	+	*pab-2*	Double
+	*inos*	+	*pab-2*	+	*inos*	+	*pab-2*	Parental
hist-1	+	*bis*	+	*hist-1*	*inos*	*bis*	+	Double
hist-1	+	*bis*	+	+	+	*bis*	+	Single
+	*inos*	+	*pab-2*	*hist-1*	+	+	*pab-2*	Single
+	*inos*	+	*pab-2*	+	*inos*	+	*pab-2*	Parental*
hist-1	+	*bis*	+	*hist-1*	+	*bis*	+	Parental†
hist-1	+	*bis*	+	+	*inos*	*bis*	+	Single
+	*inos*	+	*pab-2*	*hist-1*	*inos*	+	*pab-2*	Single
+	*inos*	+	*pab-2*	+	+	+	*pab-2*	Double
hist-1	+	*bis*	+	+	+	*bis*	+	Single
hist-1	+	*bis*	+	+	*inos*	*bis*	+	Single
+	*inos*	+	*pab-2*	*hist-1*	+	+	*pab-2*	Single
+	*inos*	+	*pab-2*	*hist-1*	*inos*	+	*pab-2*	Single
hist-1	+	*bis*	+	*hist-1*	+	*bis*	+	Parental
hist-1	+	*bis*	+	+	+	*bis*	*pab-2*	Double
+	*inos*	+	*pab-2*	*hist-1*	*inos*	+	+	Double
+	*inos*	+	*pab-2*	+	*inos*	+	*pab-2*	Parental
hist-1	+	*bis*	+	*hist-1*	+	*bis*	+	Parental
hist-1	+	*bis*	+	+	+	*bis*	*pab-2*	Double
+	*inos*	+	*pab-2*	*hist-1*	*inos*	+	*pab-2*	Single
+	*inos*	+	*pab-2*	+	*inos*	+	+	Single
hist-1	+	*bis*	+	*hist-1*	+	*bis*	*pab-2*	Single
hist-1	+	*bis*	+	+	+	*bis*	+	Single
+	*inos*	+	*pab-2*	*hist-1*	*inos*	+	+	Double
+	*inos*	+	*pab-2*	+	*inos*	+	*pab-2*	Parental
hist-1	+	*bis*	+	*hist-1*	+	*bis*	*pab-2*	Single
hist-1	+	*bis*	+	+	+	*bis*	+	Single
+	*inos*	+	*pab-2*	+	*inos*	+	+	Single
+	*inos*	+	*pab-2*	*hist-1*	*inos*	+	*pab-2*	Single
hist-1	+	*bis*	+	*hist-1*	+	*bis*	+	Parental
hist-1	+	*bis*	+	+	*inos*	*bis*	*pab-2*	Double
+	*inos*	+	*pab-2*	*hist-1*	+	+	+	Double
+	*inos*	+	*pab-2*	+	*inos*	+	*pab-2*	Parental
hist-1	+	*bis*	+	*hist-1*	+	*bis*	*pab-2*	Single
hist-1	+	*bis*	+	+	*inos*	*bis*	+	Single
+	*inos*	+	*pab-2*	*hist-1*	+	+	+	Double
+	*inos*	+	*pab-2*	+	*inos*	+	*pab-2*	Parental

CHART 1—Continued

Genotypes which will be recovered from asci having various types of double exchanges

Types of double exchange				Genotypes				Type of strand
hist-1	+	*bis*	+	*hist-1*	+	*bis*	+	Parental
hist-1	+	*bis*	+	+	*inos*	*bis*	*pab-2*	Double
+	*inos*	+	*pab-2*	*hist-1*	+	+	*pab-2*	Single
+	*inos*	+	*pab-2*	+	*inos*	+	+	Single
hist-1	+	*bis*	+	*hist-1*	+	*bis*	*pab-2*	Single
hist-1	+	*bis*	+	+	*inos*	*bis*	+	Single
+	*inos*	+	*pab-2*	*hist-1*	+	+	*pab-2*	Single
+	*inos*	+	*pab-2*	+	*inos*	+	+	Single

* When a single exchange in the *inos—bis* interval is found by testing the *bis*+ spore pairs, then all genotypes must be tested.
† When a single exchange in the *inos—bis* interval is found by testing the *bis* spore pairs, then all genotypes must be tested.

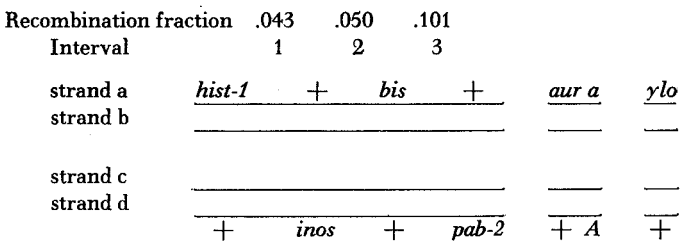
in the *inos—bis*, *bis—pab-2* intervals but they were both associated with a 3:1 ratio of *inos:inos*+. As a consequence they could have been either 3-strand or 4-strand double exchanges (see Asci numbers 3 and 4, Table 7). Even if both these double exchanges were 3-strand doubles, the deviation from 1:2:1 would still be significant.

Crosses 324 × 72; 324 × 927 and 320 × 72 (Table 6, sections b, c and d): Only 33 double exchanges were obtained in 1968 completely analyzable tetrads from 324 × 72. Similarly, only 45 double exchanges were obtained in 2239 completely analyzable tetrads from 324 × 927, and again only 40 double exchanges were obtained in 2810 completely analyzable tetrads from 320 × 72. There were generally more 2-strand double exchanges than 4-strand double exchanges in the adjacent intervals of all three crosses. This was not the case, however, for the nonadjacent pair of intervals. The pooled data from the adjacent intervals (Table 6, section e) gave 22 2-strand:41 3-strand:10 4-strand double exchanges. The probability that these results agree with a ratio of 1:2:1 is between 0.10 and 0.05. However, significantly more 2-strand than 4-strand double exchanges ($0.05>P>0.02$) were obtained. On the other hand, the pooled data from the nonadjacent intervals gave 11 2-strand:22 3-strand:12 4-strand double exchanges, clearly a good agreement with the hypothesis of random strand exchange. One of the 4-strand double exchange tetrads from the cross 324 × 72 gave an irregular segregation of 3 *ylo*+:1 *ylo* (see Ascus 12 of Table 7).

(c) Abnormal tetrads: The eight spores within any one ascus from a cross between two marked haploid strains of *N. crassa* usually show segregation in a 1:1 allele ratio with respect to each marker. Occasionally an exceptional ascus is found where one or more alleles segregate in a ratio other than 1:1. Ninety-eight such abnormal asci were recovered from the 10,269 collected from the four crosses. Since usually only two or three cultures arising from the eight spores of each ascus were tested for nutritional requirements, total numbers of irregularly segregating asci were observed only for the visible markers biscuit, yellow and

TABLE 1

Analysis of cross 320 × 927



Strands involved in apparent exchanges			Total no. of tetrads
Interval 1	Interval 2	Interval 3	
None	None	None	1120
b, c	None	None	148
None	b, c	None	168
None	None	b, c	349
b, c	b, c	None	1†
b, c	a, c	None	1†
b, c	a, d	None	1†
b, c	None	b, c	3†
b, c	None	a, d	2†
None	b, c	b, c	5†
None	b, c	a, d	4†
			1802
Incompletely analyzable tetrads*			
None	None	None	94
b, c	None	None	15
None	b, c	None	11
None	None	b, c	16
			136
No germination			9
Abnormal			14
		Total	1961

* Incompletely analyzable tetrads are those in which only one or two genotypes were recovered. In this cross there were 115 with two genotypes and 21 with one genotype.

† Among the 17 completely analyzable multiple exchanges there were 16 with four genotypes and one with three genotypes.

aurescent. Abnormal ratios at the three nutritional loci were only recovered because the irregular segregation caused the semblance of a double exchange in that ascus. As a consequence the whole ascus was tested, and the putative double exchange was found to be due to an irregular segregation rather than a double exchange.

The 98 tetrads showing irregular segregations could have arisen either by genuine irregular segregation or by contamination. Exceptional asci were ascribed

TABLE 2

Analysis of cross 324 × 72

Recombination fraction		.072	.053	.090			
Interval		1	2	3			
strand a	*hist-1*	+	*bis*	+	+ *a*	*ylo*	(324)
strand b							
strand c							
strand d							(72)
	+	*inos*	+	*pab-2*	*aur A*	+	

Strands involved in apparent exchanges			Total no. of tetrads	
Interval 1	Interval 2	Interval 3		
None	None	None	1161	
b, c	None	None	262	
None	b, c	None	185	
None	None	b, c	327	
b, c	b, c	None	1	
b, c	a, c	None	1	
b, c	None	b, c	2	
b, c	None	a, c	3	
b, c	None	b, d	4	
b, c	None	a, d	3	
None	b, c	b, c	4	†
None	b, c	a, c	6	
None	b, c	b, d	3	
None	b, c	a, d	2	
b, c	a, c	a, c	1	
b, c	b, c	a, d	1	
b, c and a, d	None	None	1	
None	b, c and a, d	None	1	
				1968
Incompletely classifiable tetrads*				
None	None	None	250	
b, c	None	None	41	
None	b, c	None	27	
None	None	b, c	77	
None	b, c	a, c	1	
				396
No germination				33
Abnormal				26
			Total	2423

* Incompletely analyzable tetrads are those in which only one or two genotypes were recovered. In this cross there were 319 with two genotypes and 77 with one genotype.

† Among the 33 completely analyzable multiple exchanges there were 25 with four genotypes and eight with three genotypes. The latter group contained four 2-strand double exchanges with only one recombinant genotype recovered.

TABLE 3

Analysis of cross 324 × 927

Recombination fraction		.065		.059	.107		
Interval		1		2	3		
strand a	*hist-1*	+	*bis*	+	+ *a*	*ylo*	(324)
strand b							
strand c							
strand d							(927)
	+	*inos*	+	*pab-2*	+ *A*	+	

Strands involved in apparent exchanges			Total no. of tetrads	
Interval 1	Interval 2	Interval 3		
None	None	None	1254	
b, c	None	None	260	
None	b, c	None	239	
None	None	b, c	439	
b, c	b, c	None	1	
b, c	a, c	None	3	
b, c	b, d	None	1	
b, c	a, d	None	2	
b, c	None	b, c	7	
b, c	None	a, c	4	
b, c	None	b, d	4	
b, c	None	a, d	5	†
None	b, c	b, c	6	
None	b, c	a, c	6	
None	b, c	b, d	3	
None	b, c	a, d	1	
b, c	b, c	b, c	1	
b, c and a, d	None	None	1	
None	b, c and a, d	b, c	2	
				2239
Incompletely analyzable tetrads*				
None	None	None	220	
b, c	None	None	32	
None	b, c	None	39	
None	None	b, c	57	
b, c	None	b, c	1	
				349
No germination				10
Abnormal				35
			Total	2633

* Incompletely analyzable tetrads are those in which only one or two genotypes were recovered. In this cross there were 304 with two genotypes and 48 with one genotype.

† Among the 46 completely analyzable multiple exchanges there were 33 with four genotypes and 13 with three genotypes. The latter group contained two 2-strand double exchanges with only one recombinant genotype recovered.

TABLE 4

Analysis of cross 320 × 72

Recombination fraction		.054		.063		.106	
Interval		1		2		3	
strand a	*hist-1*	+	*bis*	+	*aur a*	*ylo*	(320)
strand b							
strand c							
strand d							(72)
	+	*inos*	+	*pab-2*	*aur A*	+	

Strands involved in apparent exchanges			Total no. of tetrads	
Interval 1	Interval 2	Interval 3		
None	None	None	1597	
b, c	None	None	282	
None	b, c	None	329	
None	None	b, c	564	
b, c	b, c	None	2	
b, c	a, c	None	2	
b, c	b, d	None	2	
b, c	None	b, c	2	
b, c	None	a, c	3	
b, c	None	b, d	4	
b, c	None	a, d	4	†
None	b, c	b, c	4	
None	b, c	a, c	4	
None	b, c	b, d	6	
None	b, c	a, d	3	
b, c	a, c	a, d	1	
b, c	a, d	b, d	1	
				2810
Incompletely analyzable tetrads*				
None	None	None	254	
b, c	None	None	34	
None	b, c	None	38	
None	None	b, c	77	
b, c	b, d	None	1	
b, c	None	b, c	1	
None	b, c	b, d or a, d	1	
				406
No germination				13
Abnormal				23
			Total	3252

* Incompletely analyzable tetrads are those in which only one or two genotypes were recovered. In this cross there were 360 with two genotypes and 46 with one genotype.

† Among the 38 completely analyzable multiple exchanges there were 30 with four genotypes and eight with three genotypes. The latter group contained one 2-strand double exchange with only one recombinant genotype recovered.

TABLE 5

Number of double exchanges observed and expected from different pairs of intervals in the four crosses 320 × 927, 324 × 72, 324 × 927, and 320 × 72

Cross	Pairs of intervals: *hist-1—inos* / *inos—bis*		*inos—bis* / *bis—pab-2*		*hist-1—inos* / *bis—pab-2*	
	Observed	Expected	Observed	Expected	Observed	Expected
320 × 927	3	15.7	9	36.7	5	31.6
324 × 72	4	29.5	17	37.6	14	50.0
324 × 927	7	34.2	18	56.4	21	61.5
320 × 72	8	38.2	19	75.2	15	64.3
Pooled	22	117.6	63	205.9	55	207.4
	C = 0.19		C = 0.31		C = 0.27	

to one or the other of these two possibilities on the following basis: If the separated allele ratio of an ascus could be rendered normal by the rejection of a single ascospore which possessed a genotype that occurred frequently in the progeny of the cross, then contamination would be more probable than irregular segregation. On the other hand, if normalization depended on the rejection of one (or more) ascospore(s) having a very rare genotype, then contamination would seem to be a less plausible explanation than irregular segregation. In this paper contamination is not considered plausible if the ascospore(s) to be rejected had genotypes occurring with a frequency of one in 100 or less in a large random sample of ascospores. On the basis of this rigorous criterion for establishing the "genuineness" of abnormal asci, 79 out of the 98 observed exceptions could have resulted from contamination. The remaining 19 are listed in Table 7, and their origin and significance are considered in the discussion.

(d) New mutant: A spontaneous mutant strain was found which requires phenylalanine plus tyrosine for growth at 34°C, but shows no requirement at 25°C (B.N. BOLE-GOWDA, unpublished). This strain is assigned the symbol *pt* (N.S. 1) (see Table 7, Ascus Number 11). *pt* strains secrete a substance into the medium which fluoresces blue (detectable with a Blak-Ray long wave ultraviolet lamp manufactured by Ultra-Violet Products, Inc., San Gabriel, Calif.). After several days the medium becomes dark brown. *pt* is located on linkage group IV (B.N. BOLE-GOWDA, unpublished) between *pdx-1* and *pyr-3* (BARRY 1960). It has not been tested for allelism with *pt* (S4342), which is in the same region (COLBURN 1958).

DISCUSSION

Chiasma interference: Positive interference was found between exchanges in tetrads. This finding is in agreement with all previous work on chiasma interference within chromosome arms in Neurospora.

Chromatid interference: The total data from the cross 320 × 927 showed a significant deficiency of 3-strand double exchanges. In the other three crosses the proportion of 2-strand, 3-strand and 4-strand double exchanges from the

TABLE 6

Strand relationships of double exchanges

	Adjacent intervals			Nonadjacent intervals	
(a) Cross 320 × 927	*hist-1—inos* *inos—bis*	*inos—bis* *bis—pab-2*	Pooled	*hist-1—inos* *bis—pab-2*	Pooled
2-strand	1	3	4	5	9
3-strand	1	0	1	0	1
4-strand	1	2	3	4	7
					P=0.001
(b) Cross 324 × 72	*hist-1—inos* *inos—bis*	*inos—bis* *bis—pab-2*	Pooled	*hist-1—inos* *bis—pab-2*	Pooled
2-strand	2	5	7	2	9
3-strand	2	9	11	7	18
4-strand	0	3	3	3	6
(c) Cross 324 × 927	*hist-1—inos* *inos—bis*	*inos—bis* *bis—pab-2*	Pooled	*hist-1—inos* *bis—pab-2*	Pooled
2-strand	2	7	9	7	16
3-strand	4	9	13	8	21
4-strand	2	1	3	5	8
		0.20>P>0.10			
(d) Cross 320 × 72	*hist-1—inos* *inos—bis*	*inos—bis* *bis—pab-2*	Pooled	*hist-1—inos* *bis—pab-2*	Pooled
2-strand	2	4	6	2	8
3-strand	5	12	17	7	24
4-strand	1	3	4	4	8
(e) Crosses (324 × 72); (324 × 927) and (320 × 72)	*hist-1—inos* *inos—bis*	*inos—bis* *bis—pab-2*	Pooled	*hist-1—inos* *bis—pab-2*	Pooled
2-strand	6	16	22	11	33
3-strand	11	30	41	22	63
4-strand	3	7	10	12	22
	N.S.	$\chi^2_{[2]} = 4.73$ 0.10>P>0.05	$\chi^2_{[2]} = 5.05$ 0.10>P>0.05	N.S.	N.S.

P values indicate probability of 1:2:1 ratios.

adjacent pairs of intervals was different from that in the nonadjacent pair of intervals. In these adjacent pairs of intervals there was an excess of 2-strand double exchanges relative to the expected number of 4-strand doubles, indicating negative chromatid interference, while in the nonadjacent pair of intervals there was no evidence of chromatid interference.

Previous work on chromatid interference sometimes has indicated that it is present and sometimes that it is absent. LINDEGREN and LINDEGREN (1942) using intervals of 3.5 to 8.0 units in the centromere region of linkage group I of *N. crassa*

TABLE 7

Asci with segregation ratios other than 1:1

Cross and ascus number	Genotypes							Number of spores germinating
hist-1 bis; ylo; aur (320) *a*	*hist-1*	+	*bis*	+	+	$\frac{+}{aur}$	$\frac{A}{a}$	2
× *inos pab-2* (927) *A*	*hist-1*	+	*bis*	+	+	$\frac{aur}{a}$		2
Ascus 1	+	*inos*	+	*pab-2*	*ylo*	$\frac{aur}{a}$		2
	+	*inos*	+	*pab-2*	*ylo*	$\frac{aur}{a}$		2
hist-1 bis; ylo; aur (320) *a*	$\frac{hist-1}{hist-1}$	*inos*	+	*pab-2*	+	*aur*	*a*	2
× *inos pab-2* (927) *A*	$\frac{hist-1}{hist-1}$	+	*bis*	+	*ylo*	+	*A*	2
Ascus 2	$\frac{hist-1}{hist-1}$	+	*bis*	*pab-2*	+	*aur*	*a*	2
	$\frac{+}{+}$	*inos*	+	+	*ylo*	+	*A*	2
hist-1 bis; ylo; aur (320) *a*	+	$\frac{inos}{inos}$	+	+	*ylo*	+	*A*	1
× *inos pab-2* (927) *A*	*hist-1*	$\frac{inos}{inos}$	*bis*	*pab-2*	+	+	*A*	2
Ascus 3	*hist-1*	$\frac{+}{inos}$	+	*pab-2*	+	*aur*	*a*	2
	+	$\frac{inos}{inos}$	*bis*	+	*ylo*	*aur*	*a*	2
hist-1 bis; ylo; aur (320) *a*	+	$\frac{inos}{inos}$	+	+	*ylo*	*aur*	*a*	2
× *inos pab-2* (927) *A*	*hist-1*	$\frac{inos}{inos}$	*bis*	*pab-2*	+	*aur*	*A*	2
Ascus 4	*hist-1*	$\frac{+}{inos}$	+	*pab-2*	+	+	*A*	2
	+	$\frac{inos}{inos}$	*bis*	+	*ylo*	+	*a*	2
hist-1 bis; ylo (324) *a*	*hist-1*	+	+	*pab-2*	*ylo*	$\frac{+}{+}$	$\frac{A}{A}$	2
× *inos pab-2; aur* (72) *A*	+	*inos*	*bis*	+	+	$\frac{+}{+}$	$\frac{A}{A}$	2
Ascus 5	*hist-1*	+	*bis*	+	+	$\frac{+}{+}$	$\frac{A}{A}$	2
	+	*inos*	+	*pab-2*	*ylo*	$\frac{+}{+}$	$\frac{A}{A}$	2
hist-1 bis; ylo (324) *a*	*hist-1*	+	*bis*	+	+	$\frac{+}{+}$	$\frac{A}{A}$	2
× *inos pab-2; aur* (72) *A*	*hist-1*	+	*bis*	+	*ylo*	$\frac{+}{+}$	$\frac{A}{A}$	2
Ascus 6	+	*inos*	+	*pab-2*	+	$\frac{+}{+}$	$\frac{A}{A}$	2
	+	*inos*	+	*pab-2*	*ylo*	$\frac{+}{+}$	$\frac{A}{A}$	2
hist-1 bis; ylo (324) *a*	*hist-1*	+	*bis*	+	+	$\frac{+}{+}$	*a*	2
× *inos pab-2; aur* (72) *A*	+	*inos*	+	*pab-2*	*ylo*	$\frac{+}{+}$	*A*	2
Ascus 7	+	*inos*	+	*pab-2*	+	$\frac{+}{+}$	*a*	1
hist-1 bis; ylo (324) *a*	*hist-1*	+	*bis*	+	*ylo*	$\frac{aur}{aur}$	$\frac{A}{A}$	2
× *inos pab-2; aur* (72) *A*	+	*inos*	+	*pab-2*	+	$\frac{aur}{aur}$	$\frac{A}{A}$	2
Ascus 8	+	*inos*	+	*pab-2*	*ylo*	$\frac{aur}{aur}$	$\frac{A}{A}$	2
hist-1 bis; ylo (324) *a*	*hist-1*	+	*bis*	+	+	$\frac{aur}{aur}$	$\frac{A}{A}$	2
× *inos pab-2; aur* (72) *A*	*hist-1*	+	*bis*	+	*ylo*	$\frac{+}{+}$	$\frac{A}{A}$	2
Ascus 9	+	*inos*	+	*pab-2*	+	$\frac{aur}{aur}$	$\frac{A}{A}$	2
	+	*inos*	+	*pab-2*	*ylo*	$\frac{aur}{aur}$	$\frac{a}{a}$	1
hist-1 bis; ylo (324) *a*	*hist-1*	+	*bis*	+	*ylo*	$\frac{aur}{aur}$	$\frac{A}{A}$	2
× *inos pab-2; aur* (72) *A*	*hist-1*	+	*bis*	+	*ylo*	$\frac{+}{+}$	$\frac{a}{a}$	1
Ascus 10	+	*inos*	+	*pab-2*	+	$\frac{+}{+}$	$\frac{a}{a}$	2
	+	*inos*	+	*pab-2*	+	$\frac{+}{+}$	$\frac{a}{a}$	2

TABLE 7—Continued

Asci with segregation ratios other than 1:1

Cross and ascus number	Genotypes							Number of spores germinating
hist-1 bis; ylo (324) *a*	*hist-1*	+	*bis*	+	+	*aur*	*A*+	2
× *inos pab-2; aur* (72) *A*	+	*inos*	+	*pab-2*	+	*aur*	*A pt*	2
Ascus 11	+	*inos*	+	*pab-2*	*ylo*	+	*a pt*	2
hist-1 bis; ylo (324) *a*	*hist-1*	+	+	*pab-2*	+	+	*a*	2
× *inos pab-2; aur* (72) *A*	+	*inos*	*bis*	+	+	+	*a*	1
Ascus 12	*hist-1*	*inos*	+	*pab-2*	*ylo*	*aur*	*A*	2
	+	+	*bis*	+	+	*aur*	*A*	1
hist-1 bis; ylo (324) *a*	*hist-1*	+	*bis*	+	+	+	*A*	1
× *inos pab-2; aur* (72) *A*	*hist-1*	+	*bis*	+	*ylo*	+	*a*	1
Ascus 13	+	*inos*	+	*pab-2*	+	+	*a*	2
	+	*inos*	+	*pab-2*	*ylo*	+	*A*	2
hist-1 bis; ylo (324) *a*	*hist-1*	+	*bis*	+	*ylo*	*aur*	*A*	2
× *inos pab-2; aur* (72) *A*	*hist-1*	+	*bis*	+	+	*aur*	*A*	2
Ascus 14	+	*inos*	+	*pab-2*	*ylo*	*aur*	*A*	2
	+	*inos*	+	*pab-2*	+	+	*a*	2
hist-1 bis; ylo (324) *a*	*hist-1*	+	+	*pab-2*	+	+	*a*	2
× *inos pab-2; aur* (72) *A*	*hist-1*	*inos*	+	*pab-2*	+	*aur*	*a*	2
Ascus 15	+	+	*bis*	+	*ylo*	*aur*	*A*	2
	+	*inos*	+	+	*ylo*	+	*A*	2
hist-1 bis; ylo (324) *a*	*hist-1*	+	*bis*	+	*ylo*	*A*		2
× *inos pab-2* (927) *A*	*hist-1*	+	*bis*	*pab-2*	+	*a*		2
Ascus 16	+	*inos*	+	+	+	*a*		1
	+	*inos*	+	*pab-2*	*ylo*	*A*		2
	+	+	+	+	+	*a*		1*
hist-1 bis; ylo (324) *a*	*hist-1*	+	*bis*	+	*ylo*	*a*		2
× *inos pab-2* (927) *A*	*hist-1*	+	*bis*	+	*ylo*	*a*		1
Ascus 17	+	*inos*	+	*pab-2*	+	*A*		2
	+	*inos*	*bis*	*pab-2*	+	*A*		2
hist-1 bis; ylo (324) *a*	*hist-1*	+	+	*pab-2*	+	*A*		2
× *inos pab-2* (927) *A*	+	*inos*	*bis*	+	*ylo*	*a*		2
Ascus 18	+	*inos*	*bis*	+	*ylo*	*a*		2
	hist-1	+	*bis*	*pab-2*	+	*A*		2
hist-1 bis; ylo; aur (320) *a*	*hist-1*	+	*bis*	+	*ylo*	*A*		2
× *inos pab-2; aur* (72) *A*	*hist-1*	+	*bis*	*pab-2*	*ylo*	*a*		2
Ascus 19	+	*inos*	+	+	+	*a*		2
	+	*inos*	+	+	+	*A*		1

* Outcrossed—no segregation.

found an excess of 2-strand doubles. This interference was found in a pair of intervals in which the centromere was not used as a marker. MALING (1959) found a deficiency of 3-strand double exchanges in a cross of *N. crassa* which had been treated with 5-bromouracil. HOWE (1956) and STADLER (1956) did not find chromatid interference in *N. crassa*. PERKINS (1956) using intervals of ten percent to 23 percent recombination did not find any significant deviation from a 1:2:1 ratio although the ratio of 2-strand to 4-strand double exchanges was 20:10 ($0.10>P>0.05$). An excess of 2-strand double exchanges has also been found in yeast (DESBOROUGH, *et al.* 1960). In contrast, a deficiency of 3-strand double exchanges has been found in yeast (HAWTHORNE and MORTIMER 1960) and in Chlamydomonas (EBERSOLD and LEVINE 1959). Finally, chromatid interference has not been found in Aspergillus (STRICKLAND 1958) or in Sphaerocarpus (KNAPP 1960).

The experiments reported here, taken together with previous results by other workers, suggest that chromatid interference is variable in its manifestations. This variability can be explained by the two-phase model of exchange which has been thoroughly discussed by SCHWARTZ (1953, 1954). This two-phase model of exchange may be extended by postulating variation in the frequency of sister-strand exchanges between adjacent new-strand exchanges. In the present study there is an excess of 2-strand double exchanges in the two adjacent pairs of intervals from the three crosses 324 × 927, 324 × 72 and 320 × 72 but there is randomness when the two nonadjacent intervals are considered. Perhaps in this case the frequency of sister-strand exchanges is sufficiently low so that a deviation from randomness shows when the nonsister-strand exchanges are 10–15 units apart. On the other hand, when the nonsister-strand exchanges are 15–20 units apart it is not low enough to show a deviation. In the case of the cross 320 × 927 where there is a deficiency of 3-strand double exchanges, the sister-strand exchanges may be infrequent but they may be symmetrically placed when they do occur.

Irregular segregations: WINKLER (1930) first reported that alleles do not always segregate in a 1:1 ratio. LINDEGREN (1953) revived interest in the phenomenon with his work on irregular segregations in yeast; MITCHELL (1955) first reported irregular segregations in Neurospora tetrads. The data on irregular segregations have been reviewed by PONTECORVO (1958). More recently such studies as those of CASE and GILES (1958), and STADLER (1959) have focused attention on the correlation of crossing over with gene conversion. PAPAZIAN and LINDEGREN (1960) describe the phenomenon as follows: ". . . alleles, in the heterozygous state, mutate to or are replaced by other alleles at a rate much higher than the spontaneous mutation rate." This change is not random as mutations appear to be, but the new allele is the same as one of the parental alleles (ROMAN 1956).

All of the irregular segregations to be discussed are listed in Table 7. Four of them (numbers 1, 9, 10 and 14) gave 3:1 ratios for both *aur* and sex: two (numbers 5 and 6) gave 4:0 for both *aur* and sex; and one incomplete tetrad (number 8) gave either 4:0 or 3:1 ratios for both *aur* and sex. Ascus number 7 is at least 3:1 with respect to *aur* but the segregation of sex cannot be determined. Since these two markers are far apart on opposite arms of the same chromosomes, prob-

ably these ratios were caused by irregular chromosomal segregations before and during meiosis. There may be something peculiar about the segregation of linkage group I in the cross 324 × 72 since seven of these eight asci involving *aur* and sex came from this cross.

The frequencies of single irregular segregations at the three visible loci were extremely low. *aur* was segregating in only two of the crosses (320 × 927, and 324 × 72) so only 3814 completely analyzable tetrads with respect to this marker were observed. Only one 4:0 ratio of *aur*$^+$*:aur* (Ascus 13) was recovered. The two markers *ylo* and *bis* were segregating in all four crosses and 8921 completely analyzable tetrads with respect to these markers were observed. Again, only one tetrad showed a 3:1 segregation of *ylo*$^+$*:ylo* (number 12). Three tetrads were abnormal with respect to *bis* (numbers 15, 17 and 18). Numbers 17 and 18 gave ratios of 3:1 *bis:bis*$^+$ while number 15 gave a ratio of 3:1 *bis*$^+$*:bis*. In one of these asci (number 17) the irregular segregation was not associated with an exchange, but in the other two (numbers 15 and 18) the irregular segregation was accompanied by two other exchanges. These irregular 3:1 segregations at the *bis* loci were most probably the result of gene conversion. The number of conversions is insufficient to give a statistically significant correlation of exchanges and conversion, as was suggested by STADLER (1959).

As a consequence of a conversion occurring in an ascus which also contained a reciprocal exchange, one 3:1 ratio of *hist-1:hist*$^+$ (number 2), two 3:1 ratios of *inos:inos*$^+$ (numbers 3 and 4) and one 3:1 ratio of *pab-2*$^+$*:pab-2* (number 19) were recovered. Thus among the eight asci which gave 3:1 ratios with respect to a single locus, there were three in which conversion was from the auxotroph to the prototroph and five in which conversion was in the opposite direction.

One ascus (number 16) had a ratio of 5:3 of *inos*$^+$*:inos*. This type of ratio has been reported in *Sordaria fimicola* and is discussed at length by OLIVE (1959).

SUMMARY

10,269 asci were analyzed from four crosses involving closely related stocks which contained four markers on linkage group V of *Neurospora crassa*.

Chiasma interference was uniformly positive in all regions of all four crosses.

Chromatid interference was present in all four crosses. In one cross there was a statistically significant deficiency of 3-strand double exchanges. In the other three there was a statistically significant excess of 2-strand double exchanges from adjacent intervals but no evidence of chromatid interference from nonadjacent intervals. An attempt has been made to explain these results in terms of a two-phase model of exchange.

Eighteen tetrads with irregular segregations and one tetrad with a new spontaneous mutation are listed and discussed.

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