CROSSING-OVER AND INTERFERENCE IN THE CENTROMERE REGION OF LINKAGE GROUP I OF NEUROSPORA[1]

B. N. BOLE-GOWDA,[2] D. D. PERKINS, AND W. N. STRICKLAND[3]

Department of Biological Sciences, Stanford University, Stanford, California

Received May 21, 1962

TWO previous studies have provided extensive tetrad data on crossing-over in the centromere region of linkage group I. LINDEGREN and LINDEGREN (1942) found an excess of multiple exchanges across the centromere. These multiple exchanges included a proportion of 2-strand pairs in excess of the 0.25 expected if all four chromatids participated in exchanges at random. Such a deviation might be attributed in part to centromere misassortment during meiosis, but a significant excess of 2-strand pairs of exchanges was still found in intervals that were not subject to this objection because they did not involve using the centromere as a marker. The experiments of HOWE (1956) employed not only linked group-I markers but also another independent gene close to the centromere of linkage group VI. This enabled him to detect nuclear passing. There appeared to be an excess of 2-strand double exchanges in HOWE's data when nuclear passing was not taken into account, but chromatid interference disappeared when the spurious multiple exchanges caused by nuclear passing were reclassified as non-exchanges. Similar observations were reported by STADLER (1956b) for regions in linkage group VI. There was no chiasma interference across the centromere in STADLER's experiments, and HOWE's data, though limited on this point, were in agreement.

In analyzing chromatid interference it has seemed preferable to use close markers so that undetected multiple exchanges would be kept to a minimum. STRICKLAND (1961) used four markers within an arm of linkage group V that met this requirement. Double exchanges between the short intervals were very infrequent, however, because chiasma interference was strongly positive. The present experiments were designed to take advantage of the absence of chiasma interference across a centromere. Five genes were used that were spaced from three to ten units apart in opposite arms of linkage group I, and a sixth gene was present 23 units away. Three of the intervals were located in each arm, and the centromere itself was marked with a closely linked gene, thus avoiding depend-

[1] Supported by a research grant (E1462) from the National Institute of Allergy and Infectious Diseases, Public Health Service.

[2] Supported by a Government of India Scholarship. Present address: Department of Zoology, Manasa Gangotri, University of Mysore, Mysore 2, India.

[3] Present address: Department of Biological Sciences, Dartmouth College, Hanover, New Hampshire.

ence on ordered asci for information regarding crossing-over in regions adjoining the centromere.

The crosses were carried out and the data were collected by the first author. All three authors participated in planning the experiments and analyzing the results. This paper includes the unpublished data of the first author cited by EMERSON (1962), as well as more recent data.

MATERIALS AND METHODS

The loci selected as markers in these experiments were *a*: mating type, *ad-5*: adenine-5 (Y152M40), *arg-3*: arginine-3 (30300), *hist-2*: histidine-2 (Y152M14), *nic-2*: nicotinic-2 (43002), *cr*: crisp (B123) and *aur*: aurescent (34508). (For information on these loci, see PERKINS 1959.) The ordered tetrad data of GILES, DE SERRES and BARBOUR (1957) place *hist-2* 0.1 map unit right of the centromere. To facilitate recognition of spore pairs, two other independently segregating genes were included in the crosses, *ylo*: yellow (Y30539y) in linkage group VI and *pt*: phenylalanine-tyrosine (NS1) (STRICKLAND 1961) in linkage group IV. The markers were incorporated into four strains, (1) *a ad-5 hist-2 cr*, (2) *a ad-5 hist-2 aur; ylo; pt*, (3) *A arg-3 nic-2 cr*, and (4) *A arg-3 nic-2 aur; ylo; pt*. Crosses were made between strains (1) and (4) (Cross A), and between strains (2) and (3) (Cross B).

Crosses were carried out at 25° in plates containing suitably supplemented agar synthetic crossing medium (WESTERGAARD and MITCHELL 1947). Ascospores from individual asci were collected as groups of eight after they had been projected from the perithecia to an opposing agar surface, as described by STRICKLAND (1960). Each of the eight ascospores from each individual ascus was germinated separately on an agar slant containing specifically supplemented minimal medium, which avoids the inhibition of histidine-requiring strains typical of complete medium (HAAS, MITCHELL, AMES and MITCHELL 1952). All germinated cultures were scored completely for all group I markers except mating type, the determination of which was limited to those asci having one or more exchanges between *ad-5* and *cr*.

The following terminology has been used in classifying tetrads: From a cross $Ab \times aB$, PD = parental ditype ($Ab + Ab + aB + aB$), T = tetratype, ($Ab + AB + ab + aB$), and NPD = nonparental ditype ($AB + AB + ab + ab$).

RESULTS

Tetrad data from the two crosses are given in full in Table 1. Numbers in the main body of the table are for completely analyzable, regular tetrads, i.e., those with either three or four products represented. The data in subsequent tables can all be derived from those in Table 1. Completely analyzable regular tetrads make up over 90 percent of all that were isolated. All four products were recovered in 482 (88%) of the 547 completely analyzable multiple exchange tetrads. Tetrads which could not be used because of poor germination or because of some irregularity are enumerated at the end of Table 1.

TABLE 1

Unordered tetrads from the two crosses. Exchanges occurred in regions where letters are present to indicate the strand relations. The strands involved in the proximal exchange are arbitrarily designated bc. A dash indicates no exchange. The number of completely analyzable tetrads is given for each cross

a	*ad-5*	+	*hist-2*	+	*cr**	+*	strand a
a	*ad-5*	+	*hist-2*	+	*cr**	+*	strand b
A	+	*arg-3*	+	*nic-2*	+	*aur*	strand c
A	+	*arg-3*	+	*nic-2*	+	*aur*	strand d

Strands involved in exchanges in regions						Number of tetrads observed		
a–ad	*ad–arg*	*arg–hist*	*hist–nic*	*nic–cr*	*cr–aur*	Cross A	Cross B	Pooled
Not scored	—	—	—	—	—	423	581	1004
—	bc	—	—	—	—	35	43	78
—	—	bc	—	—	—	28	52	80
—	—	—	bc	—	—	80	143	223
—	—	—	—	bc	—	49	57	106
Not scored	—	—	—	—	bc	345	562	907
bc	bc	—	—	—	—	0	2	2
bc	ac	—	—	—	—	1	1	2
bc	bd	—	—	—	—	0	3	3
bc	—	bc	—	—	—	2	1	3
bc	—	ac	—	—	—	0	4	4
bc	—	bd	—	—	—	1	0	1
bc	—	—	bc	—	—	7	10	17
bc	—	—	ac	—	—	5	8	13
bc	—	—	bd	—	—	9	9	18
bc	—	—	ad	—	—	4	4	8
bc	—	—	—	bc	—	4	0	4
bc	—	—	—	ac	—	3	4	7
bc	—	—	—	bd	—	4	4	8
bc	—	—	—	ad	—	5	4	9
—	bc	ac	—	—	—	1	0	1
—	bc	—	bc	—	—	1	6	7
—	bc	—	ac	—	—	2	0	2
—	bc	—	bd	—	—	2	1	3
—	bc	—	ad	—	—	2	1	3
—	bc	—	—	bc	—	2	2	4
—	bc	—	—	ac	—	0	1	1
—	bc	—	—	bd	—	1	3	4
—	bc	—	—	ad	—	1	2	3
—	bc	—	—	—	bc	4	4	8
—	bc	—	—	—	ac	5	10	15
—	bc	—	—	—	bd	1	17	18
—	bc	—	—	—	ad	9	8	17
—	—	bc	bc	—	—	3	1	4
—	—	bc	bd	—	—	1	2	3
—	—	bc	ad	—	—	3	0	3
—	—	bc	—	bc	—	3	1	4
—	—	bc	—	ac	—	1	1	2
—	—	bc	—	—	bc	10	12	22
—	—	bc	—	—	ac	5	14	19
—	—	bc	—	—	bd	6	1	7
—	—	bc	—	—	ad	5	10	15
—	—	—	bc	bd	—	1	0	1
—	—	—	bc	ad	—	1	0	1
—	—	—	bc	—	bc	8	19	27
—	—	—	bc	—	ac	12	16	28
—	—	—	bc	—	bd	6	23	29
—	—	—	bc	—	ad	6	13	19

TABLE 1—Continued

Strands involved in exchanges in regions						Number of tetrads observed		
a–ad	*ad–arg*	*arg–hist*	*hist–nic*	*nic–cr*	*cr–aur*	Cross A	Cross B	Pooled
—	—	—	—	bc	bc	10	8	18
—	—	—	—	bc	ac	5	5	10
—	—	—	—	bc	bd	5	4	9
—	—	—	—	bc	ad	2	4	6
Not scored	—	—	—	—	bc, ad	14	20	34
bc	bc	—	bc	—	—	0	1	1
bc	bc	—	bd	—	—	0	1	1
bc	bc	—	ad	—	—	0	1	1
bc	ad	—	—	bd	—	0	1	1
bc	ad	—	—	ac	—	0	1	1
bc	bc	—	—	—	ac	0	1	1
bc	ad	—	—	—	ac	1	1	2
bc	ad	—	—	—	bd	0	1	1
bc	—	bc	—	—	bd	0	1	1
bc	—	bd	—	—	ad	0	2	2
bc	—	ad	—	—	ad	0	1	1
bc	—	—	bd	bc	—	1	0	1
bc	—	—	bc	—	bc	1	0	1
bc	—	—	bc	—	ac	0	1	1
bc	—	—	bc	—	bd	0	2	2
bc	—	—	ac	—	ad	1	1	2
bc	—	—	ac	—	bc	1	0	1
bc	—	—	bd	—	bd	2	1	3
bc	—	—	bd	—	ad	1	1	2
bc	—	—	bd	—	bc	1	0	1
bc	—	—	bd	—	ac	3	0	3
bc	—	—	ad	—	ad	1	1	2
bc	—	—	ad	—	ac	1	0	1
bc	—	—	ad	—	bd	0	3	3
bc	—	—	ad	—	bc	0	1	1
bc	—	—	—	bc	bc	1	0	1
bc	—	—	—	bc	ac	2	1	3
bc	—	—	—	bc	bd	1	0	1
bc	—	—	—	bd	bd	2	2	4
bc	—	—	—	bd	ad	1	2	3
bc	—	—	—	bd	bc	2	0	2
bc	—	—	—	bd	ac	1	0	1
bc	—	—	—	ad	bd	1	1	2
—	bc, ad	—	—	—	indeterminate	1	0	1
—	bc	—	bc	—	ad	0	2	2
—	bc	—	bd	—	ac	1	0	1
—	bc	—	ad	—	ad	1	0	1
—	bc	—	ad	—	ac	0	1	1
—	bc	—	ad	—	bd	1	1	2
—	bc	—	ad	—	bc	0	1	1
—	bc	—	—	bc	ac	0	1	1
—	bc	—	—	bc	ad	0	1	1
—	indeterminate	—	—	—	bc, ad	1	2	3
—	—	bc	bc	—	ac	0	1	1
—	—	bc	bc	—	bd	0	1	1
—	—	bc	ac	—	ad	1	0	1
—	—	bc	ac	—	bc	1	1	2
—	—	bc	ac	—	bd	0	2	2
—	—	bc	bd	—	ad	0	1	1
—	—	bc	ad	—	bc	1	2	3
—	—	bc	—	bc	bc	1	0	1
—	—	bc	—	ac	ac	0	1	1
—	—	bc	—	bd	bd	0	2	2
—	—	bc	—	ad	ac	0	1	1
—	—	bc	—	ac	bd	0	1	1

TABLE 1—Continued

Strands involved in exchanges in regions						Number of tetrads observed		
a–ad	*ad–arg*	*arg–hist*	*hist–nic*	*nic–cr*	*cr–aur*	Cross A	Cross B	Pooled
—	—	indeterminate	—	—	bc, ad	4	2	6
—	—	—	bc	ac	ac	1	0	1
—	—	—	bc	bd	ad	1	0	1
—	—	—	bc	ad	ac	1	0	1
—	—	—	indeterminate	—	bc, ad	1	1	2
bc	bc	—	bd	—	ac	1	0	1
bc	—	bd	—	—	indet. NPD	0	1	1
bc	—	—	bd	—	indet. NPD	0	2	2
—	bc	ad	—	ac	ad	1	0	1
Total completely analyzable tetrads						1187	1758	2945
Only two products recovered						98	182	280
Only one product recovered						2	1	3
Abnormal asci or technical errors						6	30	36
Total Isolated						1293	1971	3264

* Or opposite coupling phase in cross B.

Frequency of exchanges: Exchange frequencies and map distances are given in Table 2 for the intervals right of *ad-5*. Mating type is situated left of *ad-5*, with a minimum map distance of 7.8 units based on 157 exchanges in the 1000 tetrads in which it was scored in the present experiments. (All 1000 had undergone one or more exchanges in other regions.)

Exchanges between *cr* and *aur* total 46 percent, based on 0.43 tetratype and 0.02 nonparental ditype segregations. These values are much lower than the 0.70 T and 0.04 NPD frequencies reported for the same markers by PERKINS 1962b, and the 0.75 T and 0.05 NPD reported for the comparably placed markers *nic-2* and *al-2* by GILES, DE SERRES and BARBOUR 1957. Marked variations in crossing-over in a given region, in crosses of different parentage, are not uncommon in Neurospora (see for example STADLER 1956a). In the present study, values for all intervals in the two crosses A and B are statistically homogeneous, however. Exchange frequencies are homogeneous in the incompletely and the completely

TABLE 2

The frequency of exchanges in tetrads, according to regions. Nonparental ditypes are counted as two exchanges within an interval. Lengths are in conventional map units

Region	Cross A			Cross B			Pooled		
	No.	Freq.	Length	No.	Freq.	Length	No.	Freq.	Length
ad–arg	76	.0640	3.2	121	.0688	3.4	197	.0669	3.3
arg–hist	78	.0657	3.3	119	.0677	3.4	197	.0669	3.3
hist–nic	177	.1491	7.5	286	.1627	8.1	463	.1572	7.9
nic–cr	114	.0960	4.8	115	.0654	3.3	229	.0778	3.9
cr–aur	521	.4389	21.9	830	.4721	23.6	1351	.4587	22.9
Total exchanges	966	.8138	40.7	1471	.8367	41.8	2437	.8275	41.4
Total tetrads	1187			1758			2945		

analyzable tetrads for all regions except *cr–aur*, where crossing-over is somewhat lower in the 283 tetrads having only one or two surviving members.

Chiasma interference: Coincidence values between exchanges in adjoining regions are derived in Table 3 (top half). Altogether, 80 such exchanges were observed between pairs of intervals within the same arm, whereas 149 would be expected in the absence of interference. If the long interval *cr–aur* is omitted, the totals are eight observed against 49 expected.

Coincidence is approximately one for intervals across *hist-2* (Table 3, bottom half), as is expected for exchanges in opposite arms of a chromosome on the basis of past experience in other organisms and in Neurospora. The observed numbers of doubles on opposite sides of *hist-2* are somewhat less than would be expected if exchanges were independent, though not significantly so. This may reflect the fact that *hist-2* is not exactly at the centromere. The coincidence values for exchanges in opposite arms are inclusive, i.e. tetrads are included wherein one or more additional exchanges occurred in intervening intervals.

Positive chiasma interference within chromosome arms is manifested not only in low coincidence values, but also in the proportions of tetrads containing various

TABLE 3

Coincidence of exchanges in immediately adjacent regions (top portion) and inclusive coincidence in regions across hist-2, *which is close to the centromere (bottom)*

Regions	Cross A	Cross B	Pooled
Immediately adjacent:			
ad–arg, arg–hist	2/4.9	0/8.2	2/13.1
	C=0.4	C=0	C=0.2
arg–hist, hist–nic	10/11.6	11/19.4	21/31.0
	C=0.9	C=0.6	C=0.7
hist–nic, nic–cr	6/17.0	0/18.7	6/35.7
	C=0.4	C=0	C=0.2
nic–cr, cr–aur	38/48.1	34/52.5	72/100.6
	C=0.8	C=0.6	C=0.7
On opposite sides of *hist-2*:			
ad–arg, hist–nic	11/11.2	16/19.7	27/30.9
	C=1.0	C=0.8	C=0.9
ad–arg, nic–cr	5/7.2	12/7.9	17/15.1
	C=0.7	C=1.5	C=1.1
ad–arg, cr–aur	27/31.7	51/55.2	78/86.9
	C=0.9	C=0.9	C=0.9
arg–hist, hist–nic	10/11.6	11/19.4	21/31.0
	C=0.9	C=0.6	C=0.7
arg–hist, nic–cr	6/7.5	7/7.8	13/15.3
	C=0.8	C=0.9	C=0.8
arg–hist, cr–aur	35/32.9	57/54.3	92/87.2
	C=1.1	C=1.0	C=1.1
Total across *hist-2*:	94/102.1	154/164.3	248/266.4
	C=0.92	C=0.94	C=0.93

The observed number of tetrads having exchanges in each designated region is compared with the number expected in the absence of interference. Nonparental ditypes within intervals are treated here as though they were tetratypes.

numbers of exchanges. These are given in Table 4. Exchanges were not randomly distributed within chromosome arms, there being an excess of tetrads with one exchange within an arm, and a deficiency of all other tetrad ranks.

Positive chiasma interference within intervals would be expected to result in a deficit of nonparental ditype segregations for immediately adjoining markers. NPD's represent 4-strand double exchanges (or higher multiples), and thus provide a basis for estimating the frequency of double exchanges within intervals. In the absence of both chiasma and chromatid interference, the frequency of 4-strand doubles (NPD's) is given by the equation $NPD = 1/8\ T^2(1 + 3/2\ T)$ (PAPAZIAN 1952, as modified by STRICKLAND 1958), where T is the tetratype frequency. Table 5 gives for each interval the observed numbers of tetrads of these types, and the number of NPD tetrads that would be expected in the absence of interference. Only 49 NPD's were observed among the 15,533 interval-tetrads, whereas 132 would be expected. Most of these were contributed by the long region *cr–aur*. If this region is ignored, the NPD numbers become one observed and 22.2

TABLE 4

Distribution of exchanges among tetrads

	Left arm (*ad-5–hist-2*)				Right arm (*hist-2–aur*)			
	Cross A		Cross B		Cross A		Cross B	
Rank	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.
0	1036	1042.3	1518	1528.3	491	589.4	687	873.0
1	148	135.5	240	214.0	584	412.6	914	611.1
2	3	8.8	0	15.0	108	144.4	154	213.9
3	0	0.4	0	0.7	4	33.7	3	49.9
>3	0	0.0	0	0.0	0	6.8	0	10.1
Total tetrads	1187		1758		1187		1758	
Total exchanges	154		240		812		1231	
Exchanges per tetrad	0.13		0.14		0.68		0.70	

Right and left arms are considered separately. The observed number of tetrads having a designated number of exchanges in the arm is given for each experiment. The numbers expected in the absence of interference are based on a Poisson distribution.

TABLE 5

Crossing-over within individual intervals

Interval	Percent recombination	Observed tetrad nos.			NPD nos. expected with no interference
		PD	T	NPD	
a–ad	9.7	651	157	0	4.9
ad–arg	3.3	2749	195	1	1.8
arg–hist	3.3	2748	197	0	1.8
hist–nic	7.9	2482	463	0	11.2
nic–cr	3.9	2716	229	0	2.5
cr–aur	22.9	1642	1255	48	109.6
Total (15,533 interval-tetrads)				49	131.8

The observed number of nonparental ditypes is compared with the number of 4-strand double exchanges expected in each interval in the absence of chiasma and chromatid interference. Crosses pooled.

TABLE 6

Chromatid interference. The number of tetrads containing 2-, 3-, and 4-strand pairs of contiguous exchanges is given for each set of intervals, and totals are derived for various segments of the data

Region	Cross A			Cross B			Pooled			2-str / 2-str + 4-str	P for devn. from 1:1 for 2-str:4-str
	2-str	3-str	4-str	2-str	3-str	4-str	2-str	3-str	4-str		
a–ad, ad–arg	1	1	1	6	4	4	7	5	5		
ad–arg, arg–hist	0	1	1	0	0	0	0	1	1		
arg–hist, hist–nic	3	3	4	3	6	2	6	9	6		
hist–nic, nic–cr	0	4	2	0	0	0	0	4	2		
Total for short adjacent intervals	4	9	8	9	10	6	13	19	14	0.48	. . .
nic–cr, cr–aur	15	20	3	13	15	6	28	35	9	0.76	0.003
Total for all adjacent intervals	19	29	11	22	25	12	41	54	23	0.64	0.03
a–ad, arg–hist	2	1	0	2	7	1	4	8	1		
ad–arg, hist–nic	1	6	4	9	2	5	10	8	9		
arg–hist, nic–cr	4	2	0	1	5	1	5	7	1		
hist–nic, cr–aur	13	26	12*(1)	21	53	21*(3)	34	79	33		
Total where one region intervenes	20	35	16	33	67	28	53	102	44	0.55	. . .
a–ad, hist–nic	8	24	6	13	22	9	21	46	15		
ad–arg, nic–cr	2	1	1	4	6	2	6	7	3		
arg–hist, cr–aur	10	11	5*(4)	13	18	10*(3)	23	29	15		
Total where two regions intervene	20	36	12	30	46	21	50	82	33	0.60	(0.08)
a–ad, nic–cr	8	13	6	1	12	5	9	25	11		
ad–arg, cr–aur	4	7	9*(2)	4	30	8*(2)	8	37	17		
Total where three regions intervene	12	20	15	5	42	13	17	62	28	0.38	(0.1)
Grand total including all regions	71	120	54	90	180	74	161	300	128	0.56	(0.06)
Total, omitting pairs in immediately adjacent intervals	52	91	43	68	155	62	120	246	105	0.53	. . .
Total within arms	31	53	19	42	79	32	73	132	51	0.59	(0.06)
Total across centromere	40	67	35	48	101	42	88	168	77	0.53	. . .
Total omitting *cr–aur*	29	56	25	39	64	29	68	120	54	0.56	. . .

* Plus the indicated number of pairs of indeterminate type, owing to a 4-strand double within one of the intervals.

expected. These observations are consistent with positive chiasma interference within intervals. Negative chromatid interference would provide a possible alternative explanation, but there is no independent evidence to support it. It therefore seems probable that very few multiple exchanges have gone undetected within the short intervals in these crosses. The marked deficiency in 4-strand double

exchanges in these crosses agrees with extensive other data from Neurospora and a variety of other organisms (see PERKINS 1962a).

Chromatid interference: Pairs of contiguous exchanges are enumerated according to type in Table 6, for comparison with the 1:2:1 ratio expected for 2-strand:3-strand:4-strand types if nonsister chromatids are involved at random in successive exchanges. (Two exchanges are termed contiguous if another exchange does not intervene. Exchanges need not be located in adjoining regions to be called contiguous.) A total of 589 such pairs were recovered in which the strand relations could be ascertained. (An additional 15 pairs were indeterminate as to type because they also involved multiple crossing-over within a single interval.) 2-strand and 4-strand pairs taken together are nearly equal in number to the 3-strand pairs. However, 2-strand pairs of exchanges are somewhat in excess over 4-strand pairs among the totals in a ratio of 56:44. The excess does not attain statistical significance in the totals ($P = 0.06$), but among pairs of individual intervals a striking deviation in the same direction occurs in the case of the right-arm intervals *nic-cr* and *cr-aur*, with 28 2-strand and nine 4-strand types ($P < 0.01$). The two types of 3-strand pairs were nearly equal: 143 involved strands *bc* + *ac* or *bd* + *ad*, and 157 involved *bc* + *bd* or *ac* + *ad*.

A similar excess of 2-strand over 4-strand pairs is also apparent in the totals reported from this laboratory for Neurospora by MALING (1959, 29:24:19), STRICKLAND (1961, 42:64:29) and PERKINS (1962b, 161:300:128). When pooled with the present data, the totals are 423:759:329 for 2-, 3-, and 4-strand types. The excess of 2's over 4's is highly significant ($P < 0.001$). An analysis of the data of PERKINS (1962b) indicates that the excess is not due to such causes as misscoring or gene conversion of a marker. In the present study, and in those of MALING and PERKINS, excess 2-strand pairs are contributed by intervals that are rather long or far apart, at least as much as by short regions that are close together.

SUMMARY

2945 completely analyzable tetrads from seven-point crosses have been scored for markers whose map relations in linkage group I are: *a* 10 *ad-5* 3 *arg-3* 3 *hist-2* 8 *nic-2* 4 *cr* 23 *aur*. Chiasma interference is positive within arms. Coincidence values for exchanges in adjoining regions are less than one, and the proportion of tetrads having single exchanges in individual arms is in excess over the expected proportion. There is no or little interference between exchanges across the centromere, which is located near *hist-2*. 4-strand double exchanges within individual intervals were significantly less frequent than expected in the absence of chiasma or chromatid interference. This observation is consistent with strongly positive chiasma interference, and precludes negative interference unless clustered multiples occur in such a way as not to involve all four chromatids. 589 contiguous pairs of exchanges were examined for deviations from the 1:2:1 ratio expected for 2-, 3- and 4-strand types in the absence of chromatid interference. 2- and 4-strand pairs taken together were nearly equal to 3-strand pairs, but 2-strand were somewhat in excess over 4-strand pairs (161:128, $P = 0.06$). In the two right-most intervals, the ratio was 28 2-strand:nine 4-strand ($P < 0.01$).

LITERATURE CITED

EMERSON, S., 1962 Meiotic recombination in fungi with special reference to tetrad analysis. *Symposium on Methodology in Basic Genetics.* Edited by W. J. BURDETTE. Holden-Day, Inc., San Francisco, California.

GILES, N. H., F. J. DESERRES, JR., and E. BARBOUR, 1957 Studies with purple adenine mutants in *Neurospora crassa.* II. Tetrad analyses from a cross of an *ad-3A* mutant with an *ad-3B* mutant. Genetics **42:** 688–697.

HAAS, F., M. B. MITCHELL, B. N. AMES, and H. K. MITCHELL, 1952 A series of histidineless mutants of *Neurospora crassa.* Genetics **37:** 217–226.

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

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

MALING, B. D., 1959 The effect of environmental factors on crossing over in *Neurospora crassa.* Ph.D. thesis, Stanford Univ. (Abstracted in Diss. Abstr. **20:** 1135 (1959)).

PAPAZIAN, H. P., 1952 The analysis of tetrad data. Genetics **37:** 175–188.

PERKINS, D. D., 1959 New markers and multiple point linkage data in Neurospora. Genetics **44:** 1185–1208.

1962a The frequency in Neurospora tetrads of multiple exchanges within short intervals. Genet. Res. **3:** 315–327.

1962b Crossing-over and interference in a multiply marked chromosome arm of Neurospora. Genetics **47:** 1253–1274.

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

1956b Double crossing over in Neurospora. Genetics **41:** 623–630.

STRICKLAND, W. N., 1958 An analysis of interference in *Aspergillus nidulans.* Proc. Roy Soc. B **149:** 82–101.

1960 A rapid method for obtaining unordered Neurospora tetrads. J. Gen. Microbiol. **22:** 583–588.

1961 Tetrad analysis of short chromosome regions of *Neurospora crassa.* Genetics **46:** 1125–1141.

WESTERGAARD, M., and H. K. MITCHELL, 1947 Neurospora. V. A synthetic medium favoring sexual reproduction. Am. J. Botany **34:** 573–577.