CROSSING-OVER AND INTERFERENCE IN A MULTIPLY MARKED CHROMOSOME ARM OF NEUROSPORA[1]

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THE experiments reported here employ Neurospora tetrads for a type of investigation earlier carried out with multiply marked attached-X half-tetrads of Drosophila by EMERSON and BEADLE (1933), BEADLE and EMERSON (1935), BONNIER and NORDENSKIÖLD (1937) and WELSHONS (1955), within the theoretical framework developed by such workers as E. G. ANDERSON, C. B. BRIDGES, and A. WEINSTEIN (see WEINSTEIN 1936, 1958). Combinations of six or more markers have been used that enable the detection and characterization of nearly all exchanges occurring within a segment 74 units long in one chromosome arm. The results provide information on the main features of normal meiotic crossing-over and demonstrate a close resemblance between crossing-over in Neurospora and in Drosophila.

The previous major studies of crossing-over with Neurospora tetrads have employed as many as four linked markers distributed over a shorter portion of the genome, either in regions spanning the centromere (LINDEGREN and LINDEGREN 1942; HOWE 1956; STADLER 1956) or within arms (STRICKLAND 1961). These have agreed in showing that crossing-over normally occurs reciprocally between chromatids at a 4-strand stage, that 4-strand double exchanges are rare within short intervals, and that all four chromatids are involved in successive exchanges. They have been inconsistent with regard to chromatid interference, which is apparently manifested in some experiments but not in others (STRICKLAND 1961; EMERSON 1962 review).

Inasmuch as double crossing-over in Neurospora is rare within intervals as long as 20 units (except across a centromere), extensive data on multiple exchanges in short adjacent intervals have been difficult to obtain. STRICKLAND, for example, had to analyze over 10,000 tetrads from his four-point crosses in order to obtain 140 double exchanges between intervals totalling 20 units. One way in which efficiency can be increased is by using intervals across the centromere, where chiasma interference does not obtain (HOWE 1956; STADLER 1956). If this is done, and the centromere itself is used as a marker by collecting asci as ordered tetrads, errors may arise from such causes as spindle overlap. These errors can be controlled to some extent by employing markers close to unlinked centromeres, as shown by HOWE and by STADLER. Additional information can be obtained, how-

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ever, if a closely linked gene marks the centromere of the same linkage group with the other markers. Use of this approach in another study has enabled 238 double exchanges to be obtained among 2945 tetrads, within a segment 28 units long (BOLE-GOWDA, PERKINS and STRICKLAND 1962).

Within arms, the yield of information on multiple exchanges can be increased by using a larger number of markers, distributed over a larger portion of the chromosome. This design was adopted for the present study, where six markers were distributed over 74 units. In this situation, approximately one half of all tetrads were of rank 2 or higher, and nearly 700 pairs of usable contiguous exchanges were obtained from a total of 1262 tetrads.

The present paper includes the data abstracted in two preliminary reports (PERKINS 1956, 1958) and unpublished data of the author cited by EMERSON (1962), as well as more recent data.

MATERIALS AND METHODS

Table 1 describes six experiments that were carried out between 1955 and 1961. All of them involved the same six markers in the right arm of linkage group I. In addition, one experiment also employed mating type as a marker in the left arm, and *hist-2* at the centromere (from which it is separated by only 0.1 unit, according to GILES, DE SERRES and BARBOUR 1957). Three sets of parents were used in the six experiments. It was felt that useful information might result from the minor differences in parentage, design, and environment, that would not be obtained from the same number of tetrads originating from a single experiment

TABLE 1

Description of experiments

Experiment no.	Protoperithecial parent* / Fertilizing parent	Completely analyzable regular tetrads	Environment after fertilization
1.	$\frac{A\ +\ +\ +\ +\ +\ +}{a\ cr\ thi\text{-}1\ nit\text{-}1\ aur\ nic\text{-}1\ os}$	248 (83% of total isolated)	25°
2.	$\frac{A\ +\ +\ +\ +\ +\ +}{a\ cr\ thi\text{-}1\ nit\text{-}1\ aur\ nic\text{-}1\ os}$	48 (86%)	29°
3.	$\frac{A\ +\ +\ +\ +\ +\ +\ +\ +}{a\ hist\text{-}2\ cr\ thi\text{-}1\ nit\text{-}1\ aur\ nic\text{-}1\ os\ so}$	100 (87%)	25°
4.	$\frac{a\ +\ +\ +\ +\ +\ +\ \underline{cot}\ \underline{ylo}}{A\ cr\ thi\text{-}1\ nit\text{-}1\ aur\ nic\text{-}1\ os\ \underline{cot}\ +}$	341 (94%)	Shielded from vibration and mechanical shock 25°
5.	$\frac{a\ +\ +\ +\ +\ +\ +\ \underline{cot}\ \underline{ylo}}{A\ cr\ thi\text{-}1\ nit\text{-}1\ aur\ nic\text{-}1\ os\ \underline{cot}\ +}$	260 (93%)	Subjected to continuous vibration 25°
6.	$\frac{a\ +\ +\ +\ +\ +\ +\ \underline{cot}\ \underline{ylo}}{A\ cr\ thi\text{-}1\ nit\text{-}1\ aur\ nic\text{-}1\ os\ \underline{cot}\ +}$	265 (90%)	15° (135 tetrads) 19° (130 tetrads)

* The centromere is approximately at *hist-2*.

involving no variations. At the same time, the design was kept basically the same so as to enable data to be compared and combined.

Experiments 1 and 2 involved the same two parents. Experiments 4, 5, and 6 shared a different set of identical parents. In considering the effects of external variables on recombination, comparisons within each of these groups can thus be made independently of genotypic differences, but such comparisons between groups, or with Experiment 3, are not valid because of the effects of intrinsic genotypic differences on crossing-over.

The following markers were present in one or more experiments: *A*, *a*: mating type; *hist-2* : histidine-2 (Y152M14, WEBBER and CASE 1960); *cr* : crisp (LINDEGREN allele); *thi-1* : thiamine-1 (56501); *nit-1* : nitrate-1 (34547, see PERKINS 1959 for scoring); *aur* : aurescent (34508); *nic-1* : nicotinic-1 (3416); *os* : osmotic (B135, EMERSON and EMERSON 1958; PERKINS 1959); *so* : soft (B230, PERKINS 1959); *cot* : colonial temperature-sensitive (C102, linkage group IV; MITCHELL and MITCHELL 1954); *ylo* : yellow (Y30539y, linkage group VI). Additional information on most of these genes can be found in the 1954 compilation of BARRATT, NEWMEYER, PERKINS and GARNJOBST.

Wild type strain 74A, or derived strains, were used in crosses for stockbuilding, and in the experimental crosses. Markers were introduced from other backgrounds without extensive inbreeding, however, and multiply marked stocks were not inbred systematically to 74A before use in the experiments reported here.

Mating type was used as a marker only in Experiment 3. soft and yellow were scored in some isolates, but were not used as markers. (*ylo* was useful in identifying pairs of sister spores.)

The number of segregating markers was such that four visibly different types could usually be recognized among the eight cultures originating from an individual ascus, and each sister pair of segregants could readily be identified as such. Both members of each sister pair were completely scored except for half of the asci in Experiment 1, and a minority of asci in Experiments 4 and 5. In Experiment 3, both sisters were scored completely, except that mating type was determined only for one member of each pair. (This was also true of *hist* or *os* in a few asci.) In the remaining cases of incomplete scoring, one culture of each pair was scored completely, while its sister was usually scored only for the visible traits *cr*, *aur*, and usually *nit* (by indicator color) and *os* (by morphology or exudate). However, all germinants were scored for all markers in critical asci or in asci where some irregularity was evident or suspected.

In Experiment 1, an appreciable number of ascospores germinated prior to heat shock. These were accepted as legitimate if they contributed to making up the complement of a tetrad in which segregation was perfectly regular.

Crosses were carried out on agar synthetic crossing medium (WESTERGAARD and MITCHELL 1947). Except for the early parts of Experiment 1, the crossing medium was supplemented with thiamine (25 μg/ml), nicotinamide (40 μg/ml), and in Experiment 3, L-histidine (0.1 mg/ml). Supplementation followed the unexpected observation that *nic-1* segregants germinated poorly when the crossing medium contained no nicotinamide, even though the supposedly recessive

nutritional deficiency was introduced by the fertilizing parent, whereas the protoperithecial parent contained the *nic*$^+$ allele and had no requirement. Protoperithecial parents were fertilized six days after inoculation. All crosses were maintained at 25° under identical conditions until two days after fertilization, when plates were placed under new conditions in those experiments that involved environmental differences. Experiment 5 cross plates were placed in contact with a metal housing that transmitted vibrations from a continually operating fan motor. Experiment 4 plates were wrapped in silicon sponge and placed on a vibration-free shelf at the same temperature.

At first, asci were removed from the perithecia and separated manually. After the first two thirds of Experiment 1, however, tetrads were collected exclusively as unordered groups of eight ascospores, which are projected spontaneously from the perithecia to an opposing agar surface (STRICKLAND 1960). Ascus shooting begins at about 12 days after fertilization, and continues for several days. Conidia or vegetative fragments were killed by transferring asci briefly to an agar surface spread lightly with a solution of sodium hypochlorite (2.5 percent active ingredients). Each of the eight ascospores from each individual ascus was isolated to an agar slant containing appropriately supplemented media.

Tubed ascospores were stored at 25° or 30° for at least six days in order to allow ripening and increase germination. After removal of any spontaneous germinants, ascospores were then activated by 30 to 45 minutes at 60°C in a water bath.

In Experiments 4 and 5, aging was at first attempted at 34°, with ascospores disposed as groups of eight on agar plates. Ascospores were then isolated to fresh tubes immediately before heat shock. This procedure was possible because *cot* restricts growth at 34° and assures that a rare preheat-shock germinant will not overgrow an entire plate. It was hoped that accelerated ripening of optimum germination would result. Germination proved to be low after storage at 34°, however, especially for *cr*$^-$ progeny. Apparently the high temperature interferes with the normal ripening process. The adverse effect on germination of storage at 34° was simultaneously pointed out to us by DR. M. J. MAYO (personal communication), on the basis of her experience with other crosses.

In all crosses, isolates that failed to germinate after the first heat shock were rehydrated and subjected to an additional heat shock. A substantial number of additional germinants were obtained in this way, even after a second or third repetition. These consisted almost exclusively of *cr*$^-$ types.

Terminology: Tetrads can be classified into three segregation types with respect to two markers. From a cross $AB \times ab$, PD = parental ditype ($AB + AB + ab + ab$), T = tetratype ($AB + Ab + aB + ab$), and NPD = nonparental ditype ($aB + aB + Ab + Ab$). For linked genes, PD's are expected from nonexchanges and 2-strand doubles, T's from single exchanges and 3-strand doubles, and NPD's from 4-strand double exchanges, but not from any simpler type of exchange.

The terms *exchange* and *crossover* have been used in a precise sense to designate different entities. *Exchange* refers strictly to a crossing-over event in a tetrad, and exchange frequencies are expressed in terms of the population of tetrads.

Crossover refers to a point in a chromatid where it has participated in an exchange, and crossover frequencies are expressed in terms of the population of single strands. Exchange frequencies (being expressed per tetrad) are therefore twice as great as the corresponding crossover frequencies and conventional map distances (being expressed per strand). *Recombination* frequencies refer to markers in strands.

Two exchanges have been termed *contiguous* if another exchange does not intervene; they need not be located in adjoining intervals to be called contiguous.

RESULTS

Tetrad data from the six experiments are given in full in Table 2, for the five intervals between *cr* and *os*. Numbers in the main body of the table are for com-

TABLE 2

Unordered tetrads from the six experiments. Genotypes are given to the left. 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. On the right, the number of completely analyzable tetrads of each genotype is given for each experiment; this number includes tetrads from which only three members were recovered. The number of three-membered tetrads is also italicized in parentheses.

+	+	+	+	+	+	strand a
+	+	+	+	+	+	strand b
cr	*thi-1*	*nit-1*	*aur*	*nic-1*	*os*	strand c
cr	*thi-1*	*nit-1*	*aur*	*nic-1*	*os*	strand d

Strands involved in exchanges					Numbers of tetrads observed						
Regions					Experiments						
cr–thi	*thi–nit*	*nit–aur*	*aur–nic*	*nic–os*	1	2	3	4	5	6	Pooled
Nonexchanges:											
—	—	—	—	—	**13**(*7*)	**0**	**6**(*4*)	**33**(*10*)	**21**(*5*)	**30**(*2*)	**103**(*28*)
Single exchanges:											
bc	—	—	—	—	10	1	4(*1*)	23(*4*)	10(*3*)	17(*1*)	65(*9*)
—	bc	—	—	—	48(*10*)	4	8(*2*)	54(*9*)	48(*8*)	39(*2*)	201(*31*)
—	—	bc	—	—	43(*12*)	6(*1*)	17(5)	21(*3*)	5	16(*3*)	108(*24*)
—	—	—	bc	—	17(*4*)	5(*1*)	8(*4*)	8(*2*)	5(*1*)	9(*1*)	52(*13*)
—	—	—	—	bc	12(*4*)	2(*1*)	8(5)	43(*9*)	29(*8*)	32(*1*)	126(*28*)
Total singles:					**130**(*30*)	**18**(3)	**45**(*17*)	**149**(*27*)	**97**(*20*)	**113**(*8*)	**552**(*105*)
Double exchanges:											
bc	bc	—	—	—	2	0	0	1	2	1	6
bc	ac	—	—	—	0	0	0	2	0	0	2
bc	bd	—	—	—	1	1	1	3(*1*)	0	0	6(*1*)
bc	ad	—	—	—	2(*1*)	0	0	2	1(*1*)	0	5(*2*)
bc	—	bc	—	—	2	0	0	0	2	3	7
bc	—	ac	—	—	1	0	3	2(*1*)	0	0	6(*1*)
bc	—	bd	—	—	2	0	0	0	1	2	5
bc	—	ad	—	—	4	0	1(*1*)	0	0	1	6(*1*)
bc	—	—	bc	—	4(*1*)	1	3	1	2	0	11(*1*)
bc	—	—	ac	—	4(*1*)	2	0	0	2(*1*)	1	9(*2*)
bc	—	—	bd	—	0	2	2(*2*)	0	1	1	6(*2*)
bc	—	—	ad	—	2	0	0	1	1	1	5
bc	—	—	—	bc	2	1	1	7(*3*)	3	4	18(*3*)

TABLE 2—Continued

Strands involved in exchanges					Numbers of tetrads observed						
Regions					Experiments						
cr–thi	*thi–nit*	*nit–aur*	*aur–nic*	*nic–os*	1	2	3	4	5	6	Pooled
bc	—	—	—	ac	2(*2*)	0	0	8(*2*)	5	10	25(*4*)
bc	—	—	—	bd	0	0	0	8(*3*)	6(*2*)	8(*1*)	22(*6*)
bc	—	—	—	ad	0	0	2(*1*)	4	4(*2*)	5	15(*3*)
—	bc, ad	—	—	—	1	0	0	1	0	3	5
—	bc	bc	—	—	0	1	0	0	1	1	3
—	bc	ac	—	—	3(*1*)	1	0	0	6	3(*1*)	13(*2*)
—	bc	bd	—	—	3	1	3(*2*)	3	1	1	12(*2*)
—	bc	ad	—	—	5(*1*)	1	0	0	0	2	8(*1*)
—	bc	—	bc	—	7(*2*)	1	1(*1*)	3	2	1	15(*3*)
—	bc	—	ac	—	1	2(*1*)	1(*1*)	3	0	2(*1*)	9(*3*)
—	bc	—	bd	—	6(*2*)	0	0	0	1(*1*)	0	7(*3*)
—	bc	—	ad	—	4(*1*)	0	0	2	1	2	9(*1*)
—	bc	—	—	bc	5	1	1	15(*3*)	19(*4*)	7	48(*7*)
—	bc	—	—	ac	4(*2*)	2	0	19(*4*)	19(*5*)	7(*1*)	51(*12*)
—	bc	—	—	bd	1	0	1(*1*)	24(*3*)	14(*2*)	8(*1*)	48(*7*)
—	bc	—	—	ad	6(*1*)	0	0	16(*3*)	9(*3*)	10(*1*)	41(*8*)
—	—	bc, ad	—	—	1	0	0	0	0	0	1
—	—	bc	bc	—	1	0	0	0	0	0	1
—	—	bc	ac	—	0	1	0	1	0	1	3
—	—	bc	bd	—	1(*1*)	0	2(*1*)	2(*1*)	0	1	6(*3*)
—	—	bc	ad	—	2(*1*)	1	2	0	0	0	5(*1*)
—	—	bc	—	bc	2	2(*1*)	3	4(*1*)	1	3	15(*2*)
—	—	bc	—	ac	2	0	6(*3*)	2(*2*)	3(*1*)	0	13(*6*)
—	—	bc	—	bd	1	0	1	1	1(*1*)	1	5(*1*)
—	—	bc	—	ad	1	0	2	5(*1*)	3(*1*)	3	14(*2*)
—	—	—	bc	bc	1	0	0	1	1	3	6
—	—	—	bc	ac	0	0	0	0	1(*1*)	1	2(*1*)
—	—	—	bc	bd	0	0	1	2	0	6(*1*)	9(*1*)
—	—	—	bc	ad	1(*1*)	0	0	2	1	1	5(*1*)
Total doubles:					**87**(*18*)	**21**(*2*)	**37**(*13*)	**145**(*28*)	**114**(*25*)	**104**(*7*)	**508**(*93*)
Triple exchanges:											
indet.	bc, ad	—	—	—	0	0	0	1	0	0	1
bc	bc	bc	—	—	0	1	0	0	0	0	1
bc	ac	ac	—	—	0	0	0	0	1	0	1
bc	bc	—	bc	—	0	0	0	0	0	1(*1*)	1(*1*)
bc	bc	—	ac	—	0	0	1	0	0	0	1
bc	bc	—	bd	—	1	0	0	0	0	0	1
bc	ac	—	ac	—	0	0	0	0	1	0	1
bc	ac	—	bd	—	1	0	0	0	0	0	1
bc	bd	—	ad	—	0	1	1	0	0	0	2
bc	ad	—	ad	—	0	0	0	0	1	0	1
bc	bc	—	—	ac	0	0	0	0	1	0	1
bc	ac	—	—	ac	0	0	0	0	1(*1*)	0	1(*1*)
bc	ac	—	—	bc	0	0	0	1(*1*)	0	0	1(*1*)
bc	ac	—	—	ad	0	0	0	0	1	1	2
bc	bd	—	—	bc	0	0	0	0	0	2	2
bc	bd	—	—	ad	0	0	0	0	1	0	1
bc	bd	—	—	ac	0	0	0	0	1	0	1
bc	ad	—	—	ad	0	0	1	0	2	0	3
bc	ad	—	—	ac	0	0	0	0	0	1	1
bc	ad	—	—	bd	0	0	1	0	0	0	1
bc	—	bc	bd	—	0	1	0	0	0	0	1
bc	—	ac	bd	—	0	1	0	0	0	0	1
bc	—	ac	ad	—	0	0	0	0	1	0	1
bc	—	bd	bc	—	0	1	1(*1*)	0	0	0	2(*1*)
bc	—	ac	—	ac	0	0	1	0	0	0	1
bc	—	ac	—	ad	1(*1*)	0	0	0	0	0	1(*1*)

TABLE 2—Continued

Strands involved in exchanges					Numbers of tetrads observed						
Regions					Experiments						
cr–thi	*thi–nit*	*nit–aur*	*aur–nic*	*nic–os*	1	2	3	4	5	6	Pooled
bc	—	bd	—	bd	0	0	0	0	1	1(*1*)	2(*1*)
bc	—	bd	—	ad	0	0	1	1	0	0	2
bc	—	ad	—	ad	0	0	1	0	0	0	1
bc	—	ad	—	ac	0	0	0	0	1(*1*)	1	2(*1*)
bc	—	ad	—	bc	0	0	0	0	1	0	1
bc	—	—	bc	ac	1	0	0	0	0	0	1
bc	—	—	bc	bd	0	0	0	0	1	0	1
bc	—	—	ac	bd	0	0	0	1	0	0	1
bc	—	—	bd	bd	0	0	1	0	0	0	1
bc	—	—	bd	bc	0	0	0	0	1	0	1
bc	—	—	bd	ad	0	0	0	0	0	1(*1*)	1(*1*)
—	bc, ad	indet.	—	—	1(*1*)	0	0	0	1	0	2(*1*)
—	bc, ad	—	indet.	—	2	0	0	0	0	0	2
—	bc, ad	—	—	indet.	0	0	0	2(*1*)	1(*1*)	2	5(*2*)
—	bc	bc	bc	—	0	0	0	0	0	1	1
—	bc	bc	ad	—	0	0	0	1	0	0	1
—	bc	bd	bd	—	0	0	1	0	0	0	1
—	bc	ad	ad	—	1	0	0	0	0	0	1
—	bc	ad	ac	—	2(*1*)	0	0	0	0	0	2(*1*)
—	bc	ad	bc	—	0	1	0	0	0	0	1
—	bc	bc	—	bc	0	0	1	0	0	1	2
—	bc	bc	—	ac	0	0	0	0	1(*1*)	0	1(*1*)
—	bc	ac	—	ac	0	0	0	1	1	0	2
—	bc	ac	—	ad	0	0	0	0	0	1	1
—	bc	ac	—	bc	0	0	0	0	0	1	1
—	bc	bd	—	bd	0	0	0	1	1	0	2
—	bc	bd	—	ad	0	0	0	1	0	2	3
—	bc	ad	—	ad	0	0	0	2	1	1	4
—	bc	ad	—	ac	1	0	0	0	0	0	1
—	bc	ad	—	bc	0	0	0	0	0	1	1
—	indet.	—	bc, ad	—	0	1	0	0	0	0	1
—	bc	—	bc	ac	0	0	0	0	1	0	1
—	bc	—	bc	ad	1(*1*)	0	0	0	0	0	1(*1*)
—	bc	—	ac	ac	1(*1*)	1(*1*)	0	0	0	0	2(*2*)
—	bc	—	ac	ad	1	0	0	0	1	0	2
—	bc	—	bd	bd	1	0	0	0	1	0	2
—	bc	—	bd	bc	0	0	0	0	1(*1*)	0	1(*1*)
—	bc	—	ad	bc	0	1(*1*)	0	0	0	0	1(*1*)
—	—	bc	bc	ac	0	0	0	1	0	0	1
—	—	bc	ac	ad	1	0	0	0	0	0	1
Total triples:					**16**(*5*)	**9**(*2*)	**11**(*1*)	**13**(*2*)	**26**(*5*)	**18**(*3*)	**93**(*18*)
Quadruple exchanges:											
bc	ad	bc	ac	—	1(*1*)	0	0	0	0	0	1(*1*)
bc	ac	ac	—	ad	0	0	0	0	1	0	1
bc	—	ac	bc	ad	1	0	0	0	0	0	1
—	bc	bc	bc	bd	0	0	1	0	0	0	1
—	bc	bc	ad	ad	0	0	0	0	1	0	1
—	bc	ad	ac	bc	0	0	0	1	0	0	1
Total quadruples:					**2**(*1*)	**0**	**1**	**1**	**2**	**0**	**6**(*1*)
Total completely analyzable tetrads					**248**	**48**	**100**	**341**	**260**	**265**	**1262**
Tetrads with 4 products					187	41	65	274	205	245	1017
Tetrads with 3 products					61	7	35	67	55	20	245
Tetrads with 2 products					15	5	11	13	12	11	67
Tetrads with 1 product					22	1	2	0	0	0	25
Tetrads with 0 products					2	0	0	0	2	0	4
Irregular segregations					1	0	1	4	2	3	11
Mixups or contaminants					10	0	1	1	1	12	25
Scoring inadequate					0	2	0	3	4	3	12
Total isolated:					**298**	**56**	**115**	**362**	**281**	**294**	**1406**

pletely analyzable regular tetrads, i.e., those with either three or four products represented. The number of asci from which only three products were recovered is italicized in parentheses. (These are also included in the number which precedes them.) When the three-membered tetrads are omitted, and an analysis is carried out solely on tetrads with all four products, no significant difference is apparent from the results with completely analyzable tetrads. In the tables that follow, all completely analyzable tetrads have therefore been used, including the three-membered tetrads. Data from intervals left of crisp in Experiment 3 have not been included in Table 2 but are included where relevant in subsequent tables, as noted. With this exception, data in subsequent tables are all derivable from those in Table 2. Experiment 6 results obtained at the two temperatures below 20° were homogeneous, and were therefore pooled.

Completely analyzable regular tetrads make up 90 percent of all the tetrads isolated either as intact asci or as groups of eight ascospores. Tetrads which could not be used because of poor germination or for other reasons are listed separately at the end of Table 2. Irregular tetrads that are attributable to gene conversion rather than to technical errors represent less than 1 percent of the total. Ten otherwise normal tetrads were found that each showed aberrant segregation for a single pair of alleles as follows: 2+:6− (*aur*, *os*), 6+:2− (*nic*), 5+:3− (*thi*, *nit*, *aur*, *nic*), 3+:5− (*os*), 2+:6− or 3+:5− (*cr*), 6+:2− or 5+:3− (*nic*). Among these irregular tetrads, seven had undergone reciprocal crossing-over between adjoining outside markers, one had not, and two involved terminal genes. Another tetrad showed 2+:6 − simultaneously for the three markers *nit-1*, *aur*, and *nic-1*. These tetrads were not studied further. The observed number of gene conversions is probably a minimum because some cases may have been missed where sister spores were not scored completely and where only three chromatids were recovered. Aberrant segregations involving more than one locus may have been classified as mixups or contaminants. In a number of cases, again well under one percent, some inadequacy or inconsistency of scoring was discovered in the records too late for rechecking, after cultures had already been discarded.

Frequency of exchanges: Numbers and frequencies of exchanges in the various experiments are given according to regions in Table 3. The tetrad exchange frequencies are translated into conventional maps in Figure 1. A comparison of Experiments 1 and 2 indicates that crossing-over is significantly more frequent at 29° than at 25°, but no such difference is apparent between Experiments 4 (25°) and 6 (<20°). Despite the differences in recombination attributed to environmental causes, the maps from experiments that involve the same parents are recognizably similar to one another. A significant decrease in the intervals between *nit* and *nic*, and an increase in the *nic—os* region, are characteristic of Experiments 4, 5, and 6, relative to Experiments 1 and 2.

Chiasma interference: Coincidence values between exchanges in immediately adjacent regions are derived in Table 4, and shown in Figure 1. Altogether, 199 double exchanges were observed in adjoining regions, whereas 374 would be expected in the absence of interference. Chiasma interference is clearly positive for

TABLE 3

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

	Experiments													
	1		2		3*		4		5		6		Pooled	
Regions	No.	Freq.	No.	Freq.	No.	Freq.	No.	Freq.	No.	Freq.	No.	Freq.	No.	Freq.
cr–thi	44	.177	13	.271	26	.260	66	.194	57	.219	62	.234	268	.212
thi–nit	117	.472	21	.438	23	.230	163	.478	148	.569	107	.404	579	.459
nit–aur	84	.339	19	.396	47	.470	50	.147	36	.138	48	.181	284	.225
aur–nic	66	.266	24	.500	26	.260	30	.088	28	.108	33	.124	207	.164
nic–os	49	.198	10	.208	34	.340	173	.507	142	.546	125	.472	533	.422
Total exchanges	360	1.45	87	1.81	156	1.56	482	1.41	411	1.58	375	1.42	1871	1.48
Total tetrads	248		48		100		341		260		265		1262	

* Additional values from Experiment 3 are *A–hist*: 18 (including 8 three-membered) and *hist–cr*: 15 (including 6 three-membered).

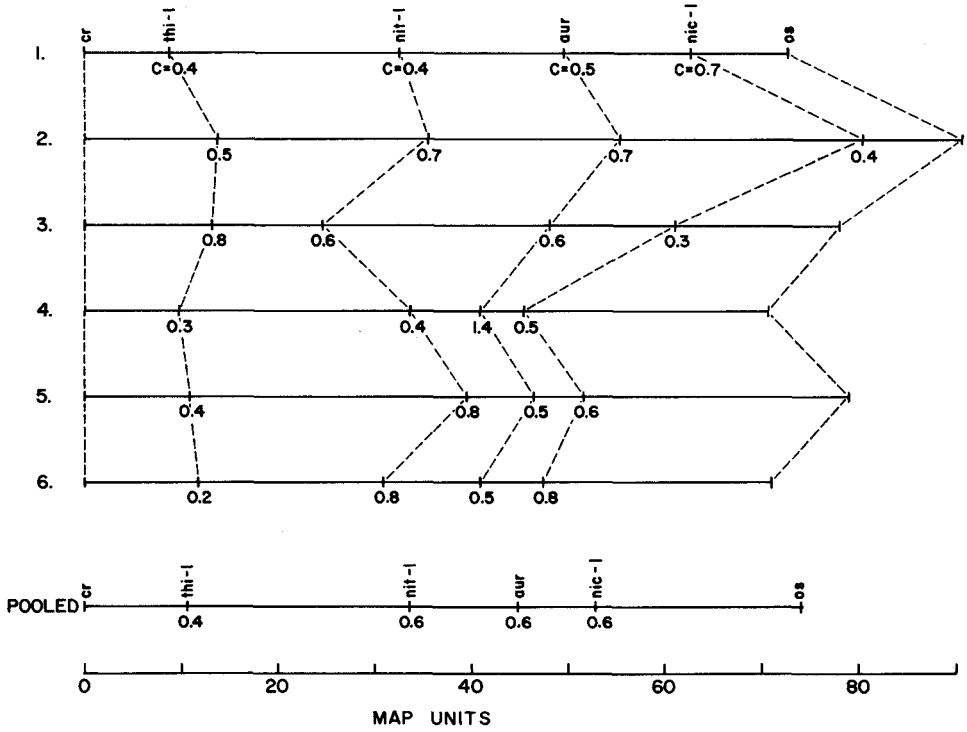

FIGURE 1.—Genetic maps summarizing recombination in the six experiments. Coincidence values shown under the gene symbols refer to exchanges in tetrads in the two intervals adjoining the locus. Experiments 1 and 2 shared one set of parents; 4, 5, and 6 shared a different set of parents. Recombination occurred at 25° except for Experiments 2 (29°) and 6 (<20°).

TABLE 4

Coincidence of exchanges in immediately adjacent regions. 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 regions are counted as though they were tetratypes

	Observed numbers/expected numbers of tetrads						
	Experiments						
Pairs of regions	1	2	3*	4	5	6	Pooled†
cr–thi, thi–nit	8/20.0	3/5.7	5/6.0	10/30.8	14/32.0	6/23.9	46/118.4
	C=0.4	C=0.5	C=0.8	C=0.3	C=0.4	C=0.3	C=0.4
thi–nit, nit–aur	17/37.8	6/8.3	6/10.8	10/23.3	16/20.2	15/18.5	70/118.9
	C=0.4	C=0.7	C=0.6	C=0.4	C=0.8	C=0.8	C=0.6
nit–aur, aur–nic	10/22.1	6/9.1	7/12.2	6/4.4	2/3.9	3/6.0	34/57.7
	C=0.5	C=0.7	C=0.6	C=1.4‡	C=0.5	C=0.5	C=0.6
aur–nic, nic–os	9/13.0	2/4.8	3/8.8	8/15.2	10/15.3	12/15.6	44/72.7
	C=0.7	C=0.4	C=0.3	C=0.5	C=0.7	C=0.8	C=0.6

* Additional values from Experiment 3 are: *hist–cr, cr–thi*: 0 obs./3.9 exp. (C=0), and *A–hist, hist–cr*: 5 obs./2.7 exp. (C=1.9, > 0.6 at 2.5% level, one-sided). The centromere is located near *hist-2*.
† Expected values are the sums from individual experiments.
‡ C > 0.5 at 2.5% level, one-sided.

all adjacent pairs of intervals except *a–hist* and *hist–cr*, which span the centromere. These coincidence values are for exchanges observed in tetrads, and are therefore not subject to the objection sometimes raised against coincidence values from crossovers in strands, that a low coincidence might reflect chromatid interference rather than chiasma interference.

In contrast to adjoining regions, C = 1 for the nonadjacent regions *cr–thi, nic–os* (112 observed:114 expected, 42 to 74 units intervening), *cr–thi, aur–nic* (52:44, 34 to 53 units), and *thi–nit, nic–os* (239:243, 19 to 63 units). (These pooled observed numbers include both contiguous and noncontiguous exchanges, i.e., coincidence is inclusive. Expected numbers were obtained by summing the numbers expected in individual experiments.)

Distribution of ranks: If there were no interference, exchanges would be distributed at random among tetrads according to a Poisson distribution. In Figure 2 observed values are compared with the values expected at random, for exchanges falling in the entire region between *cr* and *os*. Observed pooled totals are shown as solid bars. Open bars show the proportions expected in the absence of interference. These were obtained by adding the values derived from a Poisson distribution for each experiment. The observed excess of single- and double-exchange tetrads, and the observed deficiency of nonexchange tetrads and tetrads of higher ranks, are manifestations of positive chiasma interference.

Tetratype frequencies: An excess of rank 1 tetrads, combined with a deficiency in the numbers of rank 0 and of high ranks, should be reflected in a high frequency of tetratype tetrads, exceeding that expected if there were no chiasma interference. This is a consequence of the fact that all single exchanges in a gene-marked interval result in tetratype segregations, whereas tetrads of other

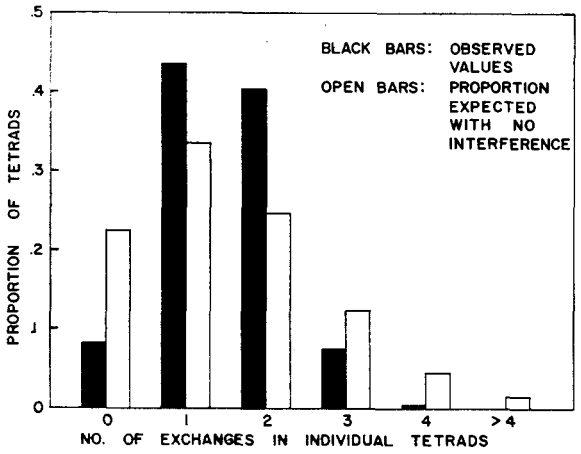

FIGURE 2.—Chiasma interference as manifested in the distribution of exchanges among tetrads. Based on 1262 tetrads from pooled data for intervals between *cr* and *os*. Expected values are the sums of expectations from individual experiments, which were derived individually for each experiment from a Poisson distribution.

ranks produce tetratypes in lower proportions. The expectation of high tetratype frequencies is borne out by values in Table 5, which are shown graphically in Figure 3. The observed tetratype frequencies are in each case plotted against map distances derived from data of the same experiment. The theoretical curves show the tetratype frequencies that are expected with complete interference (A), and with no interference at all (B). The latter is described by the equation $y = \frac{1}{3}(1-e^{-3x})$, based on a Poisson distribution of exchanges (RIZET and ENGELMANN 1949; PAPAZIAN 1951).

The observed frequency of tetratype segregations with respect to the proximal marker crisp rises as map distance increases, passes through a maximum, and declines (Figure 3). This relation does not have its basis in a polarized relation of chiasmata to the centromere, because the same type of curve is obtained if the distal gene *os* is substituted for crisp as the reference marker.

The fact that tetratype frequencies attain a value significantly in excess of 2/3 (which is the maximum expected in the absence of interference) not only indicates that interference between exchanges is positive. The excess over 2/3 also excludes the occurrence of a sister-chromatid exchange in such a way as to participate in chiasma interference (WEINSTEIN 1933).

Numerous cases are known from tetrads of various other organisms, where tetratype (or second-division segregation) frequencies exceed two thirds (PERKINS 1955). Another example in Neurospora has been provided by GILES, DE SERRES and BARBOUR (1957), who obtained 487 tetratypes:159 ditypes ($P < 0.001$) for the genes *nic-2* and *al-2*.

The relation between tetratype frequencies and map distance can be compared directly with homozygosis frequencies of genes at various distances from the

TABLE 5

Tetratype frequencies with respect to the proximal marker crisp (top half) and with respect to the distal marker osmotic (bottom half)

	Experiments													
	1		2		3		4		5		6		Pooled	
Genes	No.	Freq.	No.	Freq.	No.	Freq.	No.	Freq.	No.	Freq.	No.	Freq.	No.	Freq.
cr–thi	44	.177	13	.271	26	.260	66	.194	57	.219	62	.234	268	.212
cr–nit	139	.560	30	.625	41	.410	209	.613	180	.692	151	.570	750	.594
cr–aur	180	.726	37	.771	70	.700	242	.710	186	.715	163	.615	878	.696
cr–nic	182	.734	38	.792	73	.730	249	.730	187	.720	181	.683	910	.721
cr–os	177	.714	37	.771	75	.750	240	.704	178	.685	176	.664	883	.700
os–nic	49	.198	10	.208	34	.340	173	.507	142	.546	125	.472	533	.422
os–aur	100	.403	28	.583	56	.560	191	.560	156	.600	142	.536	673	.533
os–nit	157	.633	34	.708	69	.690	202	.592	165	.635	160	.604	787	.624
os–thi	186	.750	38	.792	77	.770	233	.683	179	.688	176	.664	889	.704
os–cr	177	.714	37	.771	75	.750	240	.704	178	.685	176	.664	883	.700

centromere in attached-X half-tetrads of *Drosophila melanogaster*. This has been done in Figure 3, using homozygosis values reported by BONNIER and NORDENSKIÖLD (1937). Their data were chosen because the X chromosome in their experiments was most nearly comparable in length to the *cr–os* portion of linkage group I in our experiments. (The homozygosis and crossover frequencies used for Figure 3 are both based on the 516 completely analyzed phenotypically wild daughters in their Table 2. The crossover value for the most proximal marker *f* is derived from its homozygosis frequency. Percent dominant homozygosis is expressed in terms of total daughters recovered in the experiment). It is apparent that crossing-over and interference are very similar in Neurospora and in Drosophila.

Multiple crossing-over within short intervals: The frequency with which two linked genes show nonparental ditype segregation provides a minimum estimate of the frequency of double crossing-over between them, because NPD's represent 4-strand double exchanges (or higher multiples). In the absence of both chiasma and chromatid interference, the frequency of 4-strand doubles (NPD's) is given by the equation $NPD = 1/8\, T^2(1 + \frac{3}{2}T)$ (PAPAZIAN 1952, modified by STRICKLAND 1958), where T is the tetratype frequency. Table 6 gives for each interval the observed numbers of tetrads of these types, and for comparison, the number of NPD tetrads expected in the absence of chiasma or chromatid interference. A total of 17 NPD's was observed among 6510 interval-tetrads, whereas 121 would be expected. This observation is consistent with positive chiasma interference within intervals. It is possible that such a deficiency of NPD's could also result if most double exchanges within intervals involved the same two strands (negative chromatid interference), but there is no independent evidence favoring such an explanation. It is reasonable to conclude that very few multiple exchanges have gone undetected in the experiments reported here.

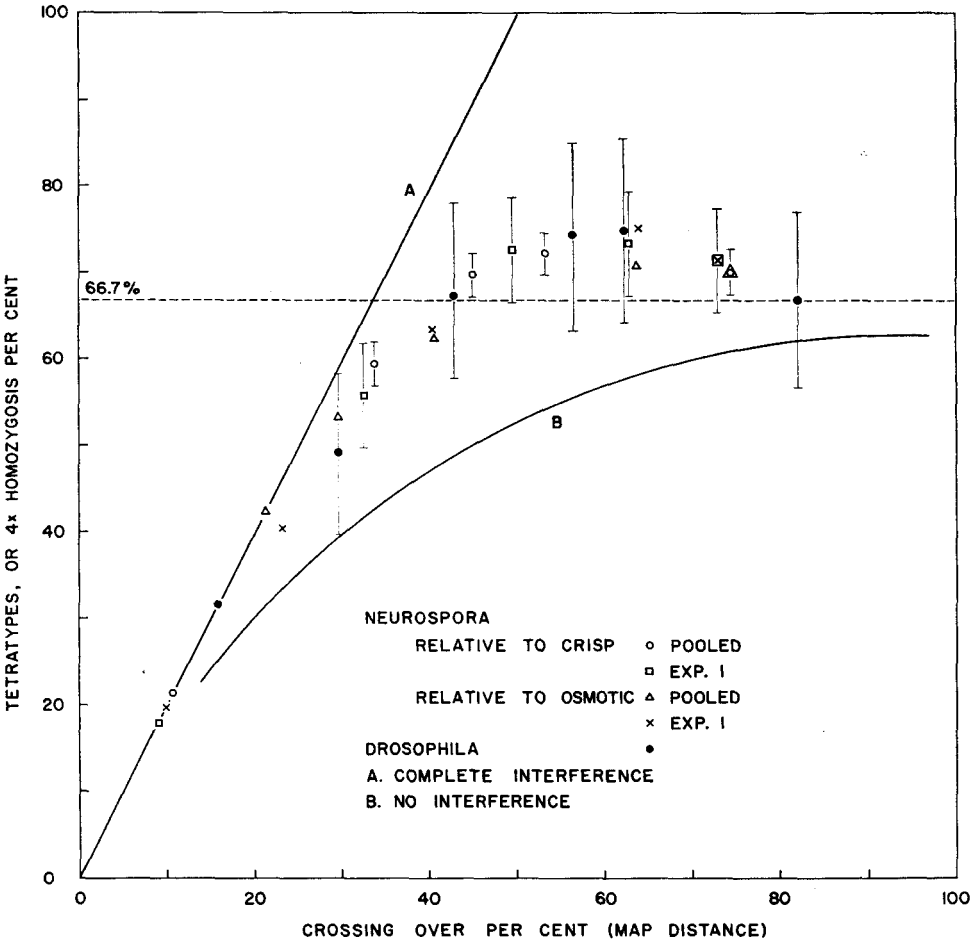

FIGURE 3.— Interference as manifested in the frequency of tetratype segregations relative to the terminal markers *cr* or *os*, and in the frequency of homozygosis in Drosophila (BONNIER and NORDENSKIÖLD 1937). Ninety-five percent confidence limits are indicated for critical points. Crossover values are in each case from the same tetrads that provided the information on tetratype or homozygosis frequency.

The marked deficiency in 4-strand double exchanges in our Neurospora data is consistent with extensive other data from the same organism, and with comparable data from a number of other organisms, among which only *Aspergillus nidulans* is apparently an exception (see PERKINS 1962).

The estimation of multiple exchanges within long intervals: Observed NPD frequencies from two-point data can be used to estimate the total number of undetected double exchanges within an interval, and to compute an interval length that compensates for the undetected exchanges by the formula: *map length* $= 50(T + 6\,NPD)$ (PERKINS 1949). The formula assumes no chromatid

TABLE 6

Crossing-over within individual intervals. 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. All experiments pooled

Interval	Percent recombination	Observed tetrad nos.			NPD nos. expected with no interference
		PD	T	NPD	
A–hist	9.0	82	18	0	0.5
hist–cr	7.5	85	15	0	0.3
cr–thi	10.6	994	268	0	9.4
thi–nit	23.0	698	549	15	49.3
nit–aur	11.3	979	282	1	10.5
aur–nic	8.2	1056	205	1	5.2
nic–os	21.1	729	533	0	46.0
Total	(6510 interval-tetrads)			17	121.2

interference, and is based on double exchanges, so that it does not correct fully for the contribution of triples and quadruples.

The data in Table 7 provide an opportunity to test this formula using NPD numbers that are relatively large, from experiments where chromatid interference and map distances are known empirically. When the correction is applied to increasingly long intervals (column 7), map lengths calculated using only the two outside markers, and ignoring intermediate genes, closely approximate the actual map values obtained by adding short intervals (column 2) except for the 74-unit interval *cr–os*, for which the computed value is somewhat too low. This discrepancy can be attributed to a slight deficit of 4-strand doubles and to the occurrence of triple and quadruple exchanges.

Applying the correction formula to the shortest component intervals in the present experiments would increase the *thi–nit* length only from 23.0 to 25.3 units, and the total *cr–os* distance from 74.2 to 76.8 units.

Strand recombination frequencies and nonparental ditype tetrad frequencies at various map distances: The intensity of interference determines both the frequency of chromatid recombination and of NPD segregation for markers at various distances apart. Recombination frequencies and NPD frequencies are given in Table 7 for markers increasingly far from the proximal gene crisp. The proportion of NPD tetrads rises slowly compared to the recombination fraction. Figure 4 shows that the recombination/map distance relationship in Neurospora is comparable to that in maize and in Drosophila.

For detecting linkage, the proportion of PD to NPD tetrads is used rather than the proportion of NPD's among total tetrads. The fraction NPD/Total ditypes is therefore given in Table 7 and Figure 4 for each gene with reference to *cr*. Interference is such that linkage between the distant markers *cr* and *os* could be detected with about 120 tetrads (providing 25 PD's and 12 NPD's), or with 360 random segregants (providing 199 parentals and 161 recombinants). Spore for spore, random isolates are thus somewhat more informative than tetrads for the purpose (PERKINS 1953).

TABLE 7

The frequencies of recombination in strands, and of nonparental ditype segregations in tetrads, for increasingly distant markers. The 601 tetrads from Experiments 4 and 5 were used as an example

Markers	Map distance by adding component intervals	Recombination among strands (percent)	Tetrad nos. PD:NPD:T	NPD's/total tetrads (percent)	NPD's/total ditypes (percent)	Computed map distance*
cr–thi	10.2	10.2	478: 0:123	0	0	10.2
cr–nit	36.1	34.2	201:11:389	1.8	5.2	37.9
cr–aur	43.3	38.3	157:16:428	2.7	9.2	43.6
cr–nic	48.1	39.6	145:20:436	3.3	12.1	46.3
cr–os	74.3	44.8	123:60:418	10.0	32.8	64.7

* From *map distance* $=50(T + 6\,NPD)$, using tetrad frequencies.

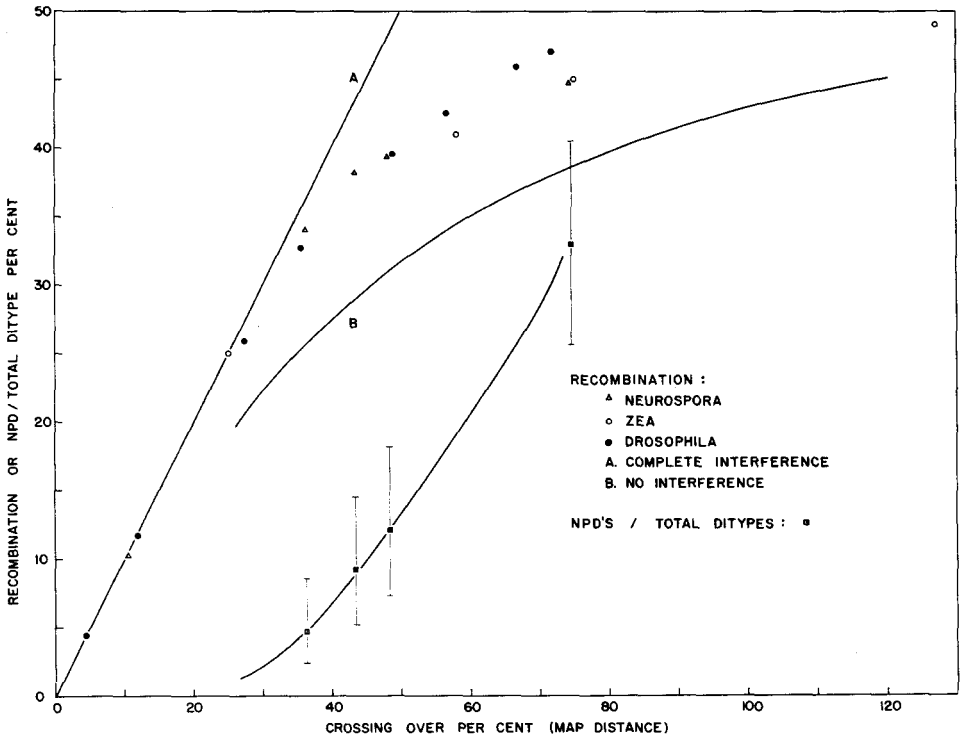

FIGURE 4.—Interference as manifested in the relation between recombination and crossing-over, and in the proportion of nonparental ditype tetrads at various map distances. Sources: Experiments 4 and 5 (Neurospora). EMERSON and RHOADES 1933 (Zea), MORGAN, BRIDGES and SCHULTZ 1935 (Drosophila). With no interference, recombination $= 1/2(1-e^{-2x})$ (HALDANE 1919).

Interference in triple and quadruple exchanges: With triple and higher exchanges, the question can be examined whether interference between two regions (say 2 and 3) is altered by the occurrence of a third exchange (in region 1) to

the left or to the right of the others. This can be tested by comparing the ratio *2, 3 doubles:2 singles* with the ratio *1, 2, 3 triples:1, 2 doubles*. Such a comparison was made using all triple exchange tetrads and contiguous sets of three exchanges within quadruples. With region 1 proximal, the ratios based on pooled numbers are .200 and .192. When region 1 is distal to the others, the ratios are .142 and .136. In neither case does the presence of the third exchange result in a significant difference in the ratios. This is consistent with a "unilateral" interference such as might be expected if exchanges occurred in a temporal sequence rather than all at the same time (BOOST and LUDWIG 1940).

Chromatid interference

Pairs of contiguous exchanges are classified according to type in Tables 8–11, 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. Both the raw totals (Tables 9 and 10) and the totals derived in various ways in Table 11 agree in showing a significant excess of 2-strand over 4-strand pairs of exchanges. indicating that the same two chromatids are more likely than not to be involved in successive exchanges. The overall ratio of 2-strand to 4-strand pairs is 5.6:4.4. The excess of two's over four's in the total is statistically significant, but only at the five percent level. On the other hand, the number of 3-strand pairs is not significantly different from the number of 2-strand plus 4-strand pairs, either in the raw or the derived totals.

Among the individual experiments (Table 9) a marked excess of 2-strand types was found in Experiments 3 and 5, and 2-strand doubles were slightly deficient in Experiment 1. These results are not statistically heterogeneous, however.

When pairs of exchanges are classified according to distance apart (Table 10), it is seen that 2-strand types are in excess except for those pairs of exchanges located in immediately adjacent intervals.

Among the 3-strand pairs of exchanges, two types can be distinguished: those in which the second exchange involves an additional chromatid from the wild-type parent (bc-ac or bd-ad pairs, in the terminology of Table 2), and those in which it involves an additional chromatid from the multiple mutant parent (bc-bd or ac-ad pairs). These two types are equal in the total data (189 *vs.* 182 pairs), as well as in individual experiments and in tetrads isolated at the same time.

Evaluation of the evidence for chromatid interference: Conclusions regarding chromatid interference in the present study depend upon 38 excess 2-strand pairs of exchanges among a total of 1262 tetrads. It is necessary to inquire whether any alternative explanation may account for this small number of critical asci (three percent of the total).

A simple change in any single medial marker arising from some cause other than double crossing-over, could most easily be mistaken for a 2-strand double crossover, and might result in a spurious excess of apparent 2-strand types. Such a single-gene substitution might be recorded illegitimately as a result of misscoring or contamination, or it might be recorded legitimately as a result of gene

TABLE 8

Chromatid interference. The number of tetrads containing 2-, 3-, and 4-strand pairs of contigous exchanges is given for each set of intervals. This number includes tetrads from which only three members were recovered; the number of three-membered tetrads is also italicized in parentheses

	cr–thi, thi–nit			*cr–thi, nit–aur*			*cr–thi, aur–nic*			*cr–thi, nic–os*			*thi–nit, nit–aur*		
Experiment	2-str	3-str	4-str	2-str	3-str	4-str	2-str	3-str	4-str	2-str	3-str	4-str	2-str	3-str	4-str
1	3	2	3 (*2*)	2	5 (*1*)	4	5 (*1*)	4 (*1*)	2	2	2 (*2*)	0	0	6 (*1*)	10*(1) (*3*)*(1)
2	1	2	0	1	2	0	1	4	0	1	0	0	2	2	2
3	1	2	2	0	6 (*1*)	2 (*1*)	3	3 (*2*)	0	1	0	2 (*1*)	2	4 (*2*)	0
4	1	6 (*2*)	2*(1)	0	3 (*1*)	0	1	1	1	7 (*3*)	16 (*5*)	4	1	6	3
5	3	7 (*1*)	4 (*1*)	2	3	2 (*1*)	3	4 (*1*)	1	3	11 (*2*)	4 (*2*)	5 (*1*)	9	1*(1)
6	2 (*1*)	3	1	3	3 (*1*)	2	0	3 (*1*)	1	4	18 (*1*)	5	3	8 (*1*)	4
Totals	11 (*1*)	22 (*3*)	12 (*3*)	8	22 (*4*)	10 (*2*)	13 (*1*)	19 (*5*)	5	18 (*3*)	47 (*10*)	15 (*3*)	13 (*1*)	35 (*4*)	20 (*3*)
	thi–nit, aur–nic			*thi–nit, nic–os*			*nit–aur, aur–nic*			*nit–aur, nic–os*			*aur–nic, nic–os*		
Experiment	2-str	3-str	4-str	2-str	3-str	4-str	2-str	3-str	4-str	2-str	3-str	4-str	2-str	3-str	4-str
1	8 (*3*)	11 (*3*)	5*(2) (*1*)	5	5 (*2*)	6 (*1*)	2	6 (*3*)	2 (*1*)	2	5 (*1*)	1	3 (*1*)	3	3 (*2*)
2	1	4 (*2*)	1*(1) (*1*)	1	2	0	0	3	3	2 (*1*)	0	0	1 (*1*)	0	1 (*1*)
3	1 (*1*)	3 (*1*)	0	2	2 (*1*)	0	2	3 (*2*)	2	6	8 (*3*)	2	1	2	0
4	3	3	2	15 (*3*)	44 (*8*)	16*(2) (*3*)*(1)	1	4 (*1*)	1	8 (*1*)	5 (*2*)	5 (*1*)	1	4	3
5	5	4 (*2*)	1	22 (*5*)	36 (*7*)	10*(1) (*3*)*(1)	0	1	1	5	7 (*4*)	4 (*1*)	3	6 (*2*)	1
6	2 (*1*)	2 (*1*)	2	7	19 (*2*)	10*(2) (*1*)	1	2	0	6 (*1*)	6	4	3	8 (*2*)	1
Totals	20 (*5*)	27 (*9*)	11 (*2*)	52 (*8*)	108 (*20*)	42 (*8*)	6	19 (*6*)	9 (*1*)	29 (*3*)	31 (*10*)	16 (*2*)	12 (*2*)	23 (*4*)	9 (*3*)
	A–hist, hist–cr			*A–hist, cr–thi*			*A–hist, thi–nit*			*A–hist, nit–aur*			*A–hist, aur–nic*		
Experiment	2-str	3-str	4-str	2-str	3-str	4-str	2-str	3-str	4-str	2-str	3-str	4-str	2-str	3-str	4-str
3	1 (*1*)	2	2 (*1*)	1	0	1	0	2 (*1*)	0	3 (*1*)	2 (*1*)	0	1 (*1*)	0	0
	A–hist, nic–os			*hist–cr, thi–nit*†			*hist–cr, nit–aur*			*hist–cr, aur–nic*			*hist–cr, nic–os*		
Experiment	2-str	3-str	4-str	2-str	3-str	4-str	2-str	3-str	4-str	2-str	3-str	4-str	2-str	3-str	4-str
3	0	1 (*1*)	0	1	2	0	1	5 (*3*)	1	1	1	0	0	3 (*3*)	0

* Plus the indicated number of pairs of indeterminate type, owing to a 4-strand double within one of the intervals.
† There were no double exchanges in *hist–cr, cr–thi*.

conversion, of mutation, or of single crossing-over in an unsuspected translocation heterozygote (see EMERSON 1962).

Cases of gene conversion or mutation would more likely be mistaken for 2-strand than for 4-strand double exchanges. In four-membered tetrads, careful scoring should reveal their nonreciprocality and lead to their rejection. But conversions and mutations would be recorded as 2-strand double exchanges among a majority of tetrads wherein only three members had survived. Even where all four products survived, misscoring of only a single critical marker in one strand would suffice for conversions or mutations to be classified spuriously as 2-strand double exchanges. Conversion is known to occur at some loci with a frequency approaching one percent of tetrads, an incidence too high to be ignored.

On the other hand, 2-strand doubles would be the easiest type to overlook when exchanges occurred in adjacent intervals, because only a single medial marker would have been recombined. A spurious deficiency of 2-strand doubles would result if a portion of such 2-strand doubles were scored as noncrossover tetrads, which they resemble in all respects except for the recombined medial marker.

TABLE 9

Chromatid interference. The strand relations in pairs of contiguous exchanges according to experiments, in completely analyzable tetrads (intervals pooled)

Experiment	Number of pairs					2-str / (2-str + 4-str)	P for devn. from 1:1 for 2-str:4-str
	2-str	3-str	4-str	indeter-minate	4-str within†		
1	32	49	36	3	5	0.47	(0.8)
2	11	19	7	1	1	0.61	(0.5)
3*	28	51	14	0	0	0.67	<0.05
4	38	92	37	3	4	0.51	(1.0)
5	51	88	29	2	2	0.64	<0.05
6	31	72	30	2	5	0.51	(1.0)
Total	191	371	153	11	17	0.56	0.05

* Includes intervals *A–hist* and *hist–cr*.
† Not included in comparisons for evaluating chromatid interference

TABLE 10

Chromatid interference. The strand relations in pairs of contiguous exchanges according to distance between intervals, in completely analyzable tetrads (experiments pooled). Data for A–hist, *and* hist–cr *are included from Experiment 3*

No. of intervals intervening	Number of pairs			2-str / (2-str + 4-str)	P for devn. from 1:1 for 2-str:4-str
	2-str	3-str	4-str		
0	43	101	52	0.45	(0.4)
1	59	82	38	0.61	<0.05
2	66	134	48	0.58	(0.1)
3	22	50	15	0.59	(0.3)
4	1	3	0	. . .	. .
5	0	1	0	. . .	. .
Total	191	371	153	0.56	0.05

TABLE 11

Chromatid interference. Pairs of contiguous exchanges. Totals derived by excluding segments of the data that might bias the comparison of types by introducing errors

Mode of deriving total	Number of pairs			2-str / 2-str + 4-str	P for devn. from 1:1 for 2-str:4-str
	2-str	3-str	4-str		
Raw Total	191	371	153	0.56	0.05
Derived Totals:					
a. Excluding adjoining regions	148	270	101	0.59	<0.01
b. Excluding 3-membered tetrads	164	287	125	0.57	<0.05
c. Excluding Experiment 1	159	322	117	0.58	0.01
d. Excluding *thi–nit, nit–aur*	178	336	133	0.57	0.01
e. Excluding *cr–thi, thi–nit*	180	349	141	0.56	<0.05

It would be more difficult to overlook 4-strand doubles in adjoining regions, because not only would all four strands be involved, but markers both to left and to right of the two exchanges would be recombined with each other, as well as with the medial marker. However, misscoring of the medial marker in such a case would result in the pair being misclassified as a 4-strand double (NPD) within a single interval, rather than between two adjoining intervals. As such, it would not be included in the numbers for comparison in ascertaining chromatid interference. This error would result in a spuriously high ratio of 2-strand to 4-strand types.

As a result of considerations such as these, the evidence for chromatid interference has been reexamined in Table 11 by the device of excluding portions of the data that would be expected to reflect errors from the various causes discussed above. (a) Misclassifications due to misscoring, conversion, or mutation, would be most likely to involve pairs of exchanges in immediately adjacent regions. However, the deletion of the data from adjoining regions (Table 11a) increases the excess of 2-strand over 4-strand types, indicating that the excess was not due to these causes. (b) Misclassification due to scoring errors would be more likely to occur in tetrads having only three members surviving, and a majority of gene conversions would be indistinguishable from double reciprocal exchanges of the 2-strand type when a fourth member was not present. Deletion of the data from 3-membered tetrads does not decrease the excess of 2-strand types from what it was in the raw total, however. (c) It is expected that the earliest experiments would be least reliable owing to inexperience, and to the fact that both sister-spore cultures were usually not scored for all markers. Deletion of the data from Experiment 1 does not, however, decrease the evidence for chromatid interference. (d, e) Of all the markers, *nit-1* and *thi-1* might be suspected as being most susceptible to misscoring. The nitrate mutant is scored by indicator color change, and it is known that a spurious color can result if the agar medium

becomes dehydrated or if scoring is mistimed. *thi-1* eventually adapts and grows upon test media without thiamine, so that false positives might result if scoring were delayed sufficiently. Precautions against these errors were taken throughout the experiments. Nevertheless, the possibility of errors must be considered. Indeed, the intervals across *nit-1* are atypical of other intervals in having no 2-strand doubles represented in Experiment 1 (Table 9). Deletion of the data from exchanges immediately across *nit-1* and immediately across *thi-1* increases rather than decreases the excess of 2-strand types.

In none of these procedures has any evidence emerged to suggest that the ratio of 2-strand:4-strand pairs is too high. On the contrary, the fact that the ratio is lower among the doubles in immediately adjacent intervals might be interpreted as indicating that some 2-strand types have been overlooked, and that the ratio of two's to four's is too low.

SUMMARY

1. 1262 completely analyzable tetrads have been scored for six markers distributed over 74 units within the right arm of linkage group I.

2. The results confirm that individual meiotic exchanges normally occur reciprocally between nonsister chromatids, and that all four strands can be involved in multiple exchanges. Exceptions to reciprocality occurred in fewer than one percent of tetrads.

3. Chiasma interference is positive, as manifested by tetrad coincidence values of approximately 0.6 for exchanges in adjoining 15-unit intervals, by an observed excess over the expected proportion of single and double exchange tetrads, and by tetratype (T) segregations that achieved a maximum proportion exceeding 2/3 for genes spaced 60 units apart.

4. Four-strand double exchanges within individual regions were infrequent, as manifested in a rarity of nonparental ditype (NPD) segregations. This is consistent with strongly positive chiasma interference, and precludes negative interference in short regions unless clustered multiples occur in such a way as not to involve all four chromatids.

5. As a result of interference, NPD segregations constituted only 33 percent of all ditypes for genes 74 units apart, whereas recombinant strands for the same genes made up 43 percent of all products.

6. The formula $map\ distance = 50(T + 6\,NPD)$ provides a useful correction for undetected multiple exchanges within long intervals, using tetrad frequencies.

7. Among 716 contiguous pairs of exchanges, 2-strand types were in excess over 4-strand types in a ratio of 191:153 ($P = 0.05$), whereas 2-strand plus 4-strand together were approximately equal to the 3-strand pairs. The excess of 2-strand types arose at least as much from pairs far apart as from pairs close together.

8. Differences in the frequency and distribution of exchanges were found between crosses that differed in parentage. Exchanges were more numerous at a temperature above normal.

9. Meiotic recombination in Neurospora resembles crossing-over in Drosophila and maize in all its main features.

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