The frequency in *Neurospora* tetrads of multiple exchanges within short intervals*†

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This paper concerns the problem, whether meiotic exchanges that the geneticist has conventionally called single are really simple, or whether they may actually consist of clusters of exchanges within short regions of effective pairing. Evidence on this point is provided by tetrad data from short regions of the genetic map. Methods suitable for analysing such data have been developed (Papazian, 1952) and have been applied effectively in several cases (e.g. Strickland, 1958*b*, with *Aspergillus*; Ebersold & Levine, 1959, with *Chlamydomonas*). This problem has been discussed from various points of view by Pritchard (1955, 1960), Weinstein (1957, 1958) Papazian (1960) and Shult & Lindegren (1959).

Numerous data are now available from *Neurospora* which contribute information on the question of multiple exchanges within intervals. The present study brings these together and analyses them.

MATERIALS AND METHODS

Tetrad data from short gene-marked intervals within individual chromosome arms of *Neurospora crassa* have been collected in Table 1. Intervals showing more than 15% recombination have been excluded, as have intervals within which a centromere is located medially. Recombination values in column 2 are in each case computed from the data on the same line. In multiply-marked crosses, where data from several short intervals have come from the same tetrads, each interval is considered individually. In these cases the total number of interval-tetrads exceeds the actual number of tetrads analysed. No data are used redundantly.

Tetrads are classified into three segregation types with respect to linked markers. From a cross AB × ab, PD = parental ditype (AB + AB + ab + ab), T = tetratype (AB + Ab + aB + ab), and NPD = non-parental ditype (aB + aB + Ab + Ab). (PD's are expected from non-exchanges 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.)

Column 6, which gives the expectation with no interference, is based on the equation $\text{NPD} = \frac{1}{8}\text{T}^2(1 + \frac{3}{2}\text{T})$ (Papazian, 1952, as modified by Strickland, 1958*b*). For values of T not exceeding 0·6, this formula is a very close approximation of the

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Table 1. *Crossing over within short intervals in* Neurospora crassa

Interval	% recom-bination	Observed tetrad nos.			Expected NPD nos.		Source of data
		PD	T	NPD	No inter-ference	Cluster model	
hist-2–nic-2	8·4	538	108	0	2·8	13·8	Giles et al. (1957)
ad-3A–ad-3B	0·5	640	6	0	0·007	0·8	,,
ylo–ad-1	1·8	826	30	0	0·1	3·8	Case et al. (1958)
pan-2–tryp-2	7·8	1439	261	2	6·2	33·4	,,
hist-1–inos	5·8	7792	1025	2	17·5	131·1	Strickland (1961)
inos–bis	5·7	7815	1001	3	16·6	128·0	,,
bis–pab-2	10·2	7026	1793	0	59·5	229·3	,,
sex–gap*	9·3	149	25	4	0·5	3·2	Lindegren (1936)
sex–gap†	5·9	327	44	0	0·8	5·6	Lindegren et al. (1942)
sex–gap‡§	3·7	277	18	2	0·1	2·3	,,
cr–pa	8·1	1320	254	1	6·4	32·5	,,
sex–ad-5	4·4	1093	106	0	1·3	13·6	Howe (1956)
sex–ad-5§	9·7	651	157	0	4·9	20·1	Bole-Gowda et al. (1962)
ad-5–arg-3	3·3	2749	195	1	1·8	24·9	,,
arg-3–hist-2	3·3	2748	197	0	1·8	25·2	,,
hist-2–nic-2	7·9	2482	463	0	11·2	59·2	,,
nic-2–cr	3·9	2716	229	0	2·5	29·3	,,
sex–hist-2	9·5	81	19	0	0·6	2·4	Perkins (1962)
hist-2–cr	7·5	85	15	0	0·3	1·9	,,
cr–thi-1	10·6	994	268	0	9·4	34·3	,,
nit-1–aur	11·3	979	282	1	10·5	36·1	,,
aur–nic-1	8·2	1056	205	1	5·2	26·2	,,
col-4–pan-1	13·0	946	316	7	13·5	40·4	Maling (1959)
pan-1–pyr-2	10·1	1423	350	4	11·2	44·8	,,
nit-1–aur	10·7	62	17	0	0·6	2·2	Perkins, unpub.
leu-3–sex	9·1	45	10	0	0·3	1·3	Barratt & Ogata (1954)
sex–phen	2·2	87	4	0	0·02	0·5	,,
rib-2–pdx-1	1·2	39	1	0	0·003	0·1	Garnjobst et al. (1956)
rib-2–pyr-3	2·1	23	1	0	0·006	0·1	,,
chol-1–pyr-3	10·9	19	3	1	0·06	0·4	,,
ylo–ad-1	4·5	20	2	0	0·03	0·3	,,
ylo–rib-1	1·5	32	1	0	0·004	0·1	,,
un–cys-2	4·2	43	4	0	0·05	0·5	Stadler (1956)
cys-2–ylo	2·1	69	3	0	0·02	0·4	,,
cys-1–ylo	3·5	40	3	0	0·03	0·4	,,
ylo–ad-1	3·0	250	16	0	0·1	2·0	,,
ad-1–rib-1	0·3	145	1	0	0·001	0·1	,,
arg-5–pe	8·8	79	17	0	0·5	2·2	Gross et al. (1960)
arg-5–arom-1	13·6	84	29	1	1·3	3·7	,,
pe–arom-1	3·3	71	5	0	0·05	0·6	,,
me-1–pyr-1	5·0	18	2	0	0·03	0·3	N. Murray (1960)
me-2–col-1	7·6	50	9	0	0·2	1·2	,,

* These segregants are stated to have shown low fertility (Lindegren et al. (1942), p. 2).

† 1937 data. Numbers as given by Lindegren et al. (1942), p. 3. Not all asci scored for sex.

‡ 1940 data.

§ Sex scored only in asci with one or more exchanges in other regions.

Table 1—*continued*

Interval	% recom- bination	Observed tetrad nos.			Expected NPD nos.		Source of data
		PD	T	NPD	No inter- ference	Cluster model	
me-2–hist-4	8·3	20	4	0	0·1	0·5	N. Murray (1960)
tryp-4–me-2	8·8	34	5	1	0·09	0·6	,,
me-2–pan-1	3·8	37	3	0	0·03	0·4	,,
tryp-4–pan-1	11·1	42	12	0	0·4	1·5	,,
cot–le-1	11·7	73	20	1	0·7	2·6	J. Murray (1959)
pyr-1–pdx-1	0·6	85	1	0	0·002	0·1	Mitchell *et al.* (1954)
pdx-1–col-4	2·3	127	6	0	0·04	0·8	,,
col-4-pyr-3	3·2	44	3	0	0·03	0·4	,,
pyr-3–cot	12·9	209	73	0	3·3	9·3	Mitchell (1960)
mac–al-2	3·6	13	1	0	0·01	0·1	Dubes (1953)
lys-3–aur	4·8	28	3	0	0·04	0·4	Barratt *et al.* (1954) compilation of data from many authors
un–sex	10·0	13	1	1	0·009	0·1	,,
arg-3–sex	3·8	48	4	0	0·04	0·5	,,
aur–nit-1	5·0	9	1	0	0·01	0·1	,,
ad-3–me-6	7·4	52	9	0	0·2	1·2	,,
al-2–arg-6	0·5	97	1	0	0·001	0·1	,,
leu-3–sex	8·2	56	11	0	0·3	1·4	,,
ad-5–sex	7·5	17	3	0	0·07	0·4	,,
tryp-3–fl	1·5	33	1	0	0·004	0·1	,,
ac-1–arom-1	12·5	15	5	0	0·2	0·6	,,
ac-1–fl	7·2	65	11	0	0·2	1·4	,,
arom-1–pe	7·5	17	3	0	0·07	0·4	,,
pe–tu	8·2	248	49	0	1·3	6·3	,,
ser-1–sc	4·2	22	2	0	0·02	0·3	,,
ser-1–prol-1	4·3	32	3	0	0·04	0·4	,,
ser-1–leu-1	6·0	37	5	0	0·09	0·6	,,
ser-1–tryp-1	14·2	76	30	0	1·5	3·8	,,
sc–prol-1	5·0	9	1	0	0·01	0·1	,,
sc–leu-1	4·5	40	4	0	0·05	0·5	,,
thi-2–thi-4	14·7	13	3	1	0·08	0·4	,,
thi-2–leu-1	9·7	25	6	0	0·2	0·8	,,
thi-2–prol-1	12·9	23	8	0	0·4	1·0	,,
tryp-1–prol-1	10·0	16	4	0	0·1	0·5	,,
ad-4–ad-2	5·7	40	3	1	0·03	0·4	,,
pyr-1–pyr-3	5·8	311	41	0	0·7	5·2	,,
pyr-1–tryp-2	1·1	129	3	0	0·01	0·4	,,
tryp-4–chol-1	8·1	26	5	0	0·1	0·6	,,
pan-1–chol-1	1·4	71	2	0	0·007	0·3	,,
col-1–chol-1	7·4	23	4	0	0·1	0·5	,,
pdx-1–pyr-3	1·3	38	1	0	0·003	0·1	,,
pdx-1–pdx-2	1·1	44	1	0	0·003	0·1	,,
pdx-1–col-4	14·3	16	4	1	0·1	0·5	,,
pab-1–pab-2	7·4	63	11	0	0·2	1·4	,,
pab-1–pab-2	12·0	111	35	0	1·4	4·5	,,

Table 1—*continued*

Interval	% recom-bination	Observed tetrad nos.			Expected NPD nos.		Source of data
		PD	T	NPD	No inter-ference	Cluster model	
asp–inos	8·9	23	5	0	0·1	0·6	Barratt *et al.* (1954) compilation of data from many authors
pab-1–inos	2·2	43	2	0	0·01	0·3	,,
iv-1–iv-2	3·4	56	2	1	0·009	0·3	,,
iv-1–inos	14·6	46	19	0	1·0	2·4	,,
rib-1–cys-1	1·2	39	1	0	0·003	0·1	,,
ad-1–un	13·1	14	5	0	0·2	0·6	,,
Total (58,068 interval-tetrads)				37	201·6||	1014·6	

|| Obtained by adding the numbers expected for individual intervals.

exact equation $2NPD = 1 - T - (1 - \frac{3}{2}T)^{2/3}$. These formulae for non-parental di-types as a function of tetratype frequency are based on the following assumptions: (1) Exchanges are distributed at random within and between tetrads (i.e. chiasma interference is absent). (2) Exchanges occur between chromatids at the 4-strand stage, and multiple exchanges involve non-sister chromatids at random (i.e. chromatid interference is absent).

In col. 7, expectations are computed using a specific 'cluster' model along the lines suggested by Pritchard (1955) and Pontecorvo (1958), and elaborated by Pritchard (1960). Column 7 values are based on the following assumptions: (1) Meiotic exchanges occur only within short regions of effective pairing. (2) Exchanges occur at random within each such region (i.e. there is no chiasma interference within a pairing region). (3) Interference may obtain between the regions of effective pairing. (4) Exchanges occur between chromatids at the 4-strand stage, and multiple exchanges involve non-sister chromatids at random (i.e. chromatid interference is absent).

For purposes of computation, we have made two further specific assumptions. (*a*) Following Pritchard, we have assumed the mean number of crossovers per effective pairing region to be 0·6 (i.e. 1·2 exchanges per tetrad). Column 7 is there-fore based on a Poisson distribution having a mean of 1·2, with tetrads of rank 2 and greater contributing NPD's in the proportion expected with no chromatid interfer-ence. (*b*) Coincidence of two effective pairing regions within the same marked region is assumed to be zero for the short intervals considered in Table 1. This assumption minimizes the expected number of NPD's. On the basis of these assumptions, $NPD = 0·1279T$ for the cluster model.

The net effect of such a cluster model is to give the appearance of negative interference (high coincidence) even though exchanges occur at random within

each effectively paired segment. This result does not depend on the specific assumptions or values adopted above for this particular model. Any cluster hypothesis (or other type of heterogeneity) will result in a coincidence greater than that expected if exchanges are random, and would thus be expected to produce more NPD's (representing double exchanges) relative to T's (which include the singles).

RESULTS

Data from 58,068 interval-tetrads of *Neurospora crassa* are analysed in Table 1. It is apparent that 4-strand double exchanges in *Neurospora* are extremely infrequent in the short intervals considered. Not only do the observed NPD numbers fall far short of the values predicted from a cluster hypothesis (col. 7); NPD's are also definitely less frequent than would be predicted in the complete absence of chiasma and chromatid interference (col. 6).

In the absence of chromatid interference, the observed deficiency of NPD's would require that chiasma interference be positive ($C < 1$). But in the presence of chromatid interference, a deficiency of NPD's could also occur, provided that neighbouring exchanges involved the same two strands preferentially. Four-strand double exchanges could thus be precluded by chromatid interference even though chiasma interference was negative ($C > 1$), so that exchanges occurred as clusters. Choice between these two alternatives is not possible by the methods employed here, or without a clearer understanding of chromatid interference than we now possess. The results in Table 1 do, however, serve the purpose of limiting speculations regarding the nature of meiotic crossing over, and of focusing attention on the interrelation between problems of chiasma and chromatid interference. We can conclude that unless there is chromatid interference, exchanges in *Neurospora* cannot occur as clusters, and chiasma interference must be strongly positive within short regions, just as is known to be the case between regions.

Data from longer intervals than those considered in Table 1 lead to similar conclusions regarding the relative infrequency of 4-strand double exchanges in *Neurospora crassa*. The same is true of most of the other organisms for which reliable pertinent tetrad data are available. Data from thirteen other organisms are given in Table 2. In many of these cases, information from short intervals is absent or limited; data have therefore been included from long intervals, provided that tetratype frequencies do not exceed 60%, or recombination frequencies 40%. In addition to the thirteen species that have contributed data for Table 2, tetrad data for linked genes are stated to have been obtained for *Coprinus* (Day & Anderson, 1961) and for *Schizosaccharomyces* (Leupold, 1958), but these have not yet been published. Extensive unpublished data must also have been obtained for *Sphaerocarpus* (Knapp & Möller, 1955). To our knowledge, this is the extent of existing data that lend themselves to analysis by the methods employed here. Older reports by Moewus on *Chlamydomonas*, and by Wettstein on the moss *Funaria*, have been rejected from our tabulation for reasons put forward by Gowans (1960) and by Knapp (1960).

Table 2. *Crossing over within intervals, in organisms other than Neurospora crassa*

Organism and interval	% recombination	Observed tetrad nos.			Expected NPD nos.		Source of data
		PD	T	NPD	No interference	Cluster model	
Venturia inaequalis							
br–pa	0·9	53	1	0	0·002	0·1	Boone *et al.* (1956)
br–wh-2	1·2	159	4	0	0·1	0·5	,,
p-8–p-9	12·4	130	43	0	1·8	5·5	Williams *et al.* (1957)
p-8–p-12	25·0	16	16	0	1·8	2·0	,,
							,,
Total (422 interval-tetrads)				0	5·5	8·1	
Glomerella cingulata							
try–th	13·8	29	11	0	0·5	1·4	Wheeler (1956)
th–A	14·7	36	15	0	0·8	1·9	,,
try–A	28·6	78	78	8	7·9	10·0	,,
or–arg	8·5	39	8	0	0·2	1·0	,,
arg–leu	28·9	17	20	1	2·4	2·6	,,
cy–ni	19·1	63	39	0	2·9	5·0	,,
Total (442 interval-tetrads)				9	14·8	21·9	
Sordaria fimicola							
st-64–st-52	14·2	43	17	0	0·9	2·2	El-Ani *et al.* (1961)
st-52–st-9	16·4	43	21	0	1·3	2·7	,,
st-9–st-60	13·5	57	21	0	1·0	2·7	,,
st-60–sp	15·4	36	16	0	0·9	2·0	,,
r–sp	3·6	130	10	0	0·1	1·3	,,
sp–mat	9·1	45	10	0	0·3	1·3	,,
mi–mat	0·6	253	3	0	0·004	0·4	,,
mat–g	0·4	1057	9	0	0·01	1·2	,,
g–cor	3·4	531	39	0	0·4	5·0	,,
cor–st-22	20·5	60	39	1	3·0	5·0	,,
Total (2441 interval-tetrads)				1	7·9	23·7	
Sordaria macrospora							
A-3–r_1	29·2	577	752	18	96·4	96·2	Heslot (1958)
r_1–A-1	29·2	149	189	6	23·7	24·2	,,
A-1–j_2	22·6	208	152	7	12·8	19·4	,,
A-19–j_1	25·5	53	49	2	4·9	6·3	,,
A-2–vo_2	12·5	36	12	0	0·5	1·5	,,
r_2–v_1	33·8	50	72	8	9·1	9·2	,,
Total (2340 interval-tetrads)				41	147·4	156·8	,,

Table 2—*continued*

Organism and interval	% recom-bination	Observed tetrad nos.			Expected NPD nos.		Source of data
		PD	T	NPD	No inter-ference	Cluster model	
Podospora anserina							
s–i	27·2	122	137	3	16·0	17·5	Monnot (1953)
Neurospora sitophila							
pink–sex	19·0	31	19	0	1·4	2·4	Whitehouse (1956)
pink–sex	19·8	69	40	2	2·8	5·1	Whitehouse (1948)
sex–b	15·2	32	14	0	0·8	1·8	,,
c–w	21·4	20	15	0	1·3	1·9	Fincham (1952)
w–al	11·4	27	8	0	0·3	1·0	,,
al–g	25·7	34	33	1	3·5	4·2	,,
Total (345 interval-tetrads)				3	10·1	16·5	
Aspergillus nidulans							
ribo–an	16·5	181	79	4	4·3	10·1	Strickland (1958*b*)
an–ad-14	5·5	237	25	2	0·3	3·2	,,
ad-14–pro-1	25·4	143	108	13	8·9	13·8	,,
pro-1–paba-1	7·1	1067	154	10	2·9	19·7	,,
paba-1–y	12·8	929	288	14	11·4	36·8	,,
y–bi	4·6	1122	105	4	1·3	13·4	,,
Total (4485 interval-tetrads)				47	29·0	97·1	
Saccharomyces cerevisiae							
IN–PY	23·8	24	16	2	1·2	2·0	Lindegren (1949)
AD–IN	30·2	22	23	3	2·4	2·9	,,
PY–TH	26·0	52	44	4	4·0	5·6	,,
AN–HI	25·2	68	63	2	6·4	8·1	Lindegren *et al.* (1951)
CA–CH	2·9	242	15	0	0·1	1·9	Desborough *et al.* (1959)
CH–HI	32·8	154	232	16	31·2	29·7	,,
HI–IS	15·9	387	166	6	8·9	21·2	,,
IS–AN	12·8	278	96	0	4·3	12·3	,,
CH–NI	18·2	14	8	0	0·6	1·0	,,
NI–IS	27·3	10	12	0	1·5	1·5	,,
TZ–AG	10·9	50	14	0	0·5	1·8	Desborough *et al.* (1960)
AG–IS	5·2	122	12	1	0·2	1·5	,,
IS–NI	10·3	97	23	1	0·7	2·9	,,
GA–AC-1	22·7	74	50	4*	3·9	6·4	,,
(Lindegren, Desborough *et al.*: 2407 interval-tetrads)				39	65·9	98·8	
his–try	19·5	25	16	0	1·2	2·0	Leupold *et al.* (1954)
me-2–p-2	9·7	50	12	0	0·4	1·5	Hawthorne *et al.* (1960)
α–thr-4	16·2	25	12	0	0·7	1·5	,,

* Markers may be in opposite arms, with centromere medial.

Table 2—*continued*

Organism and interval	% recombination	Observed tetrad nos.			Expected NPD nos.		Source of data
		PD	T	NPD	No interference	Cluster model	
Saccharomyces cerevisiae							
le-1–tr-5	10·6	226	61	0	2·1	7·7	Hawthorne *et al.* (1960)
p-1–ar-4	5·0	163	18	0	0·3	2·3	,,
ar-4–thr-1	11·3	213	62	0	2·3	7·9	,,
p-1–thr-1	15·2	119	52	0	2·9	6·7	,,
thr-1–CU-1	25·6	106	111	0	12·5	14·2	,,
thr-3–hi-1	0·8	64	1	0	0·002	0·1	,,
hi-1–is-1	14·5	49	20	0	1·0	2·6	,,
is-1–tr-2	9·4	56	13	0	0·4	1·7	,,
hi-1–tr-2	23·0	118	95	2	8·7	12·1	,,
le-1–ad-6	30·5	125	175	6	23·2	22·4	,,
(Hawthorne *et al.*: 1954 interval-tetrads)				8	54·5	80·7	
Total for *Saccharomyces* (4402 interval-tetrads)				47	121·6	181·5	
Ustilago maydis							
me-1–ad-1	30·6	49	74	1	10·5	9·5	Holliday (1961)
ad-1–leu-1	20·2	74	50	0	4·0	6·4	,,
Total (248 interval-tetrads)				1	14·5	15·9	
Schizophyllum commune							
A–s	19·7	21	11	1	0·7	1·4	Papazian (1951)
Chlamydomonas moewusii							
mt–a	37·2	9	15	2	2·0	1·9	Lewin (1953)
t–l	28·9	8	11	0	1·5	1·4	,,
(45 interval-tetrads)				2	3·5	3·3	
Chlamydomonas reinhardi							
arg-1–slo	34·4	54	30	16	1·9	3·8	Ebersold (1956)
arg-1–pgd(26°)	31·8	71	34	24	1·6	4·3	,,
arg-1–pgd(5°)	32·8	31	16	11	0·8	2·0	,,
na–lg	26·8	55	32	10	2·0	4·1	,,
(384 interval-tetrads)				61	6·2	14·3	
thi-1–ac-157b	13·8	200	64	5	2·6	8·2	Eversole (1956)
pab-1–nic-5	22·4	113	89	1	8·1	11·4	,,
arg-1–arg-2	6·5	372	43	6	0·6	5·5	,,
(893 interval-tetrads)				12	11·3	25·1	

Table 2—*continued*

Organism and interval	% recom-bination	Observed tetrad nos.			Expected NPD nos.		Source of data
		PD	T	NPD	No inter-ference	Cluster model	
Chlamydomonas reinhardi							
arg-1–arg-2	6·5	198	23	3	0·3	2·9	Eversole *et al.* (1956)
arg-1–arg-2	5·2	1542	179	0	2·7	22·9	Ebersold *et al.* (1959)
arg-2–pab-2	15·3	1365	595	3	32·8	76·1	,,
pab-2–thi-3	29·9	697	1019	5	142·4	130·3	,,
(Total, Ebersold *et al.*, 5404 interval-tetrads)				8	177·9	229·2	
Sphaerocarpus donnellii							
s–crispa	2·4	768	38	0	0·2	4·9	Knapp *et al.* (1955)
crispa–a	13·6	590	213	3	9·8	27·2	,,
Total (1612 interval-tetrads)				3	10·1	32·1	

The data from all but two of the organisms in Table 2 agree with the data from *Neurospora crassa* given in Table 1. NPD'S are typically in deficit compared to the number expected without any interference at all, and are far less than predicted from the cluster hypothesis.

The one outstanding exception is *Aspergillus nidulans*, which differs in having a consistent excess of NPD's, such as would be expected from a clustering of exchanges. These data are from the only extensive tetrad analysis that has been made in *Aspergillus* (Strickland, 1958*b*). Strickland noted the high frequency of NPD's, and subjected the data to a detailed analysis, cross by cross, using Papazian's formula.

Early (1956) results with *Chlamydomonas reinhardi* resemble *Aspergillus*, but more recent, and far more extensive, tetrad data for *Chlamydomonas* (Ebersold & Levine, 1959) fall conclusively into the *Neurospora* pattern, with a striking deficit of NPD's within intervals.

DISCUSSION

Except for the early results with *Chlamydomonas*, *Aspergillus nidulans* with its high frequency of NPD's stands apart from all the other organisms, in which interference consistently appears to be positive. This observation could mean that true negative interference obtains between close exchanges in meiosis in *Aspergillus*, so that crossovers occur as clusters, or it could mean that effective pairing is erratic or incomplete during meiosis in *Aspergillus*, in contrast to the other organisms for which information is available. Heterogeneity in crossing over, from this or other causes, would have the effect of raising coincidence values, perhaps to the level where interference appeared to be negative (Weinstein, 1918; Sturtevant, 1955). If heterogeneity is responsible for the unique behaviour of *Aspergillus*, it must be of

such a nature that it is not manifested in recombination values, which are extremely uniform and reproducible (Käfer, 1958).

The excess NPD's in *Aspergillus* might also have originated from positive chromatid interference, or from occasional crossing over at the 2-strand stage, but no independent evidence exists for either of these explanations.

In *Chlamydomonas*, deviant results are limited to data published in 1956. Levine & Ebersold (1958b) suggest misscoring as a possible explanation of Ebersold's 1956 results. Another possibility is that *pgd*, *slo* and *lg* may actually be unlinked to *arg-l*, and that the apparent linkage was due to preferential segregation, of which examples are known in *Chlamydomonas* (Gowans, 1960) and in yeast (see Shult & Lindegren, 1959; Hawthorne & Mortimer, 1960).

Similar deviations in *Chlamydomonas* were found by Eversole & Tatum (1956) who reported also that the frequency of NPD segregations for closely linked markers is strikingly increased by the chelating agent ethylene diamine tetra-acetic acid. Levine & Ebersold (1958a) later failed to confirm this effect in experiments carried out under similar but not identical conditions.

It may be that these high NPD ratios in *Chlamydomonas* are real, and that the inconsistencies reflect variability in some feature of the underlying mechanism, such as chromatid interference. Strickland (1961) has provided a well-documented case of variability of chromatid interference from cross to cross in *Neurospora*.

Negative interference and gene conversion

Evidence from random meiotic products in a number of organisms has established a high coincidence between intragenic recombination and the recombination of out-side markers. Both complementary classes of outside markers are found among a particular class of intragenic recombinants, however. So long as only random segregants are considered, these results can be visualized in terms of several alternatives: (1) a positive correlation between distinctly different non-reciprocal events, on the one hand, and reciprocal events, on the other, (2) localized high negative interference between like reciprocal events, or (3) a single meiotic process capable of producing both reciprocal and non-reciprocal intragenic recombinants, and characterized by high negative interference.

A distinction between reciprocal and non-reciprocal recombination is possible only with tetrads. The existing evidence from tetrad analysis indicates that the first alternative is tenable. Non-reciprocal exchanges do occur (albeit rarely) in *Neurospora* (Mitchell, 1956), *Aspergillus* (Strickland, 1958a), and other organisms. These account for a majority of intragenic recombinations in the most thoroughly studied cases (e.g. Case & Giles, 1958; Lissouba & Rizet, 1960). Furthermore, evidence from *Neurospora* tetrads has established the existence of a high coincidence between gene conversion and reciprocal recombination between markers on either side of the converted locus (see e.g. Case & Giles, 1958). Much more extensive evidence for the same correlation has recently been obtained from tetrads of *Sordaria fimicola* (Kitani, Olive & El-Ani, 1961, 1962).

In contrast, evidence from meiotic tetrads, including the data gathered in the

present paper, does not lend support to the second alternative (negative interference between reciprocal meiotic events) except in *Aspergillus*. For all the other organisms considered, the evidence presented in the present paper would seem to support an interpretation that purported cases of negative interference among random segregants are due to non-reciprocal recombination, because localized negative interference between reciprocal exchanges cannot be occurring unless multiple exchanges are restricted so that not all four chromatids are involved.

Some tetrad evidence exists that bears on the third alternative, and indicates that more than one mechanism is involved. Factors that increase reciprocal crossing over do not simultaneously affect non-reciprocal events (Stadler, 1959*b*). 6 : 2 segregations do not interfere with reciprocal crossing over in an interval one removed from the conversion, either in *Neurospora* (Stadler, 1959*a*) or *Sordaria* (Kitani, Olive & El-Ani, 1962). 5 : 3 segregations, on the other hand, apparently do show positive interference with such reciprocal exchanges (Kitani *et al.*, 1962).

SUMMARY

Tetrad data from short gene-marked intervals provide information on the frequency of multiple exchanges within intervals. Non-parental ditype and tetratype frequencies from 58,000 interval-tetrads of *Neurospora crassa* show that 4-strand double exchanges are far less frequent than would be expected in the absence of chiasma or chromatid interference. These results are in general agreement with meiotic tetrad data from other organisms, except *Aspergillus nidulans*. They preclude the occurrence of reciprocal meiotic exchanges as clusters unless multiples within each cluster are restricted so as not to involve all four chromatids. If this is not the case, and chromatid interference does not occur, then chiasma interference must be strongly positive within short regions. Known cases of apparent negative interference among random meiotic segregants are probably the result of non-reciprocal conversion of a middle marker, rather than of multiple reciprocal crossing over.

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