EVIDENCE FOR MEIOTIC RECOMBINATION IN ASCOBOLUS INVOLVING ONLY ONE MEMBER OF A TETRAD[1]

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THE models for genetic recombination which were designed by WHITEHOUSE (1963) and HOLLIDAY (1964) differ in some details, but both include a stage at which two chromatids of the tetrad carry corresponding segments of hybrid DNA (a region in which the two complementary strands come from different chromatids). The models were designed to account for both reciprocal and nonreciprocal recombination in crosses between allelic mutants. (Reciprocal recombination is defined here as intragenic recombination in which all marked sites in the involved gene segregate 2:2; if one or more sites shows 3:1 segregation, it is called nonreciprocal recombination.) The models' predictions about relative frequencies of these two results, among those tetrads which produced nonmutant recombinants, depended on the distance separating the mutant sites in comparison with the length of a hybrid DNA segment. The maximum proportion of nonreciprocal events should have been observed when the two mutant sites were close together. If they were close enough so that one or both usually fell in the hybrid DNA segment when recombination occurred between them, the models predicted similar frequencies of reciprocal and nonreciprocal events (see Figure 1). However, a proportion of nonreciprocal recombinants much higher than this prediction has been observed in tetrad analyses in Neurospora (STADLER and TOWE 1963), yeast (FOGEL and HURST 1967), and Ascobolus (KRUSZEWSKA and GAJEWSKI 1967).

The analysis of intragenic recombination at the his_1 locus in yeast by FOGEL and HURST (1967) yielded a second observation which was difficult to explain by the hybrid DNA models. The products of reciprocal and nonreciprocal recombination differed in their outside markers (Table 1). Virtually all the reciprocal recombination products carried the same nonparental combination of markers, while the nonreciprocal recombination products might have any of the four possible marker combinations. The models offer no basis for any difference in markers accompanying reciprocal as opposed to nonreciprocal recombination between sites which are close together (as the his_1 mutants must be, in view of the high proportion of nonreciprocal recombination). WHITEHOUSE (1967) has attempted to account for this kind of observation by "secondary crossing over"—an event which would result from a restriction of the length of the hybrid DNA region by certain segregating sites.

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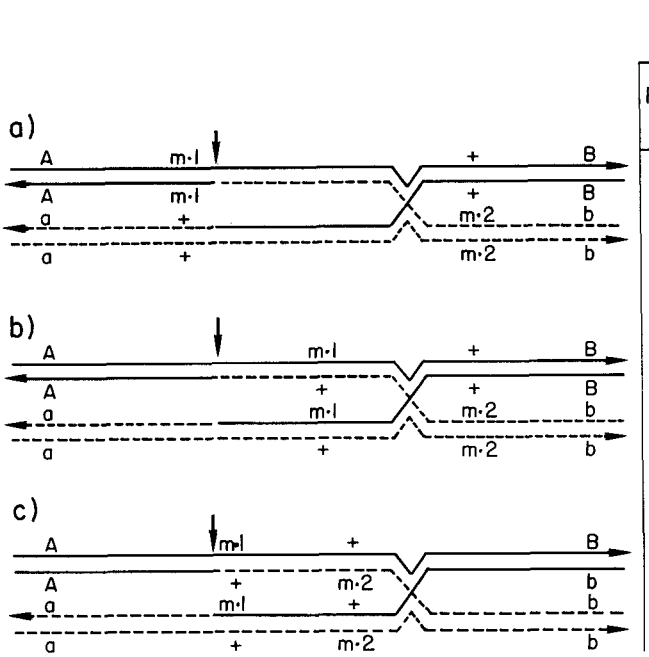

Proportion of Recombinant Tetrads with		
Reciprocal Recombination	Non-reciprocal Recombination	Two Wild-Type Chromatids
1	0	0
1/2	1/2	0
2/7	4/7	1/7

FIGURE 1.—Predictions of the HOLLIDAY model regarding the relative frequencies of different classes of tetrads producing recombinants (those having at least one $m+$ chromatid). These classes (reciprocal recombination, nonreciprocal recombination, two wild-type chromatids) refer only to segregation at the m locus and not to the outside markers A and B.

It has been assumed that segregating sites within the hybrid DNA are always corrected and that this correction is equally likely to involve the removal of either component at the mismatched site.

Hybrid DNA extends from the point of the original single-strand breaks (vertical arrow) to the "half-chiasma" (point of exchange of single strands). It is assumed that the half-chiasma is always resolved by breakage and rejoining. This event may involve the two strands which contain the original single-strand breaks, or it may involve the other two strands. These two alternatives are assumed to be equally probable.

The predictions are shown for three situations:

a) both segregating sites lie outside the hybrid DNA;
b) one segregating site is within the hybrid DNA segment;
c) both segregating sites lie within the hybrid DNA.

The authors of unitary models for recombination are hard-pressed to account for the observed distribution of outside markers among progeny selected for intragenic recombination. There is a significant correlation of marker recombination with the intragenic event, but it is far from absolute. Roughly 50% of the intragenic recombinants show no evidence of marker recombination. To deal with this partial correlation, WHITEHOUSE (1963) proposed that while the formation of hybrid DNA *always* resulted in a reciprocal exchange of chromatids (and markers), there was a high probability of a second hybrid region adjacent to the first, and this would reverse the chromatid exchange. HOLLIDAY (1964) proposed that the formation of a hybrid DNA region set up a *potential* chromatid

TABLE 1

Markers accompanying intragenic recombination in yeast
Comparison of reciprocal and nonreciprocal recombinants (from FOGEL and HURST 1967)

Cross: $\frac{A \quad m\text{-}1 \quad + \quad B}{a \quad + \quad m\text{-}2 \quad b}$

Analysis of m^+ recombinants	$A\ m^+\ B$	$a\ m^+\ b$	$a\ m^+\ B$	$A\ m^+\ b$	Totals
Reciprocal recombinants	4	0	97	0	101
Nonreciprocal at *m-1*	474	5	265	103	847
Nonreciprocal at *m-2*	0	49	77	7	133
Totals	478	54	439	110	1081

m-1 and *m-2* represent two mutant alleles at the his_1 locus. Outside marker *A* is thr_3 (2.4 map units to the left of *his*), and *B* is ar_6 (ten map units to the right of *his*).

exchange, but that chance determined whether the subsequent DNA breaks would complete the exchange or negate it.

The suggestion of a separate mechanism for nonreciprocal recombination dates from its original descriptions (MITCHELL 1955; ROMAN 1956). These authors noted, however, that even the nonreciprocal event appeared to be correlated with marker exchange, and this has been confirmed by tetrad analyses (STADLER and TOWE 1963; FOGEL and HURST 1967). The observed excess of nonreciprocal over reciprocal recombination has led WHITEHOUSE (1967) and PASZEWSKI (1970) to suggest that recombination may sometimes occur in tetrads containing only a single hybrid chromatid. The present report presents a specific hypothesis of this type. We propose that while recombination is sometimes initiated by two (non-sister) chromatids becoming "recombinogenic," other recombination tetrads have only a single chromatid in this condition. It is proposed that the two-chromatid event is accompanied by reciprocal exchange of outside markers, while the one-chromatid event is not. Several of the predictions of this hypothesis are tested by observations of recombination among a set of allelic ascospore color mutants in Ascobolus.

MATERIALS AND METHODS

Minimal, complete, and crossing media were prepared according to the directions of YU-SUN (1964). The specific methods of isolating mutants, making crosses, and analyzing progeny were described in the preceding paper (STADLER, TOWE and ROSSIGNOL 1970).

HYPOTHESIS

The hypothesis will be presented as a specific modification of the hybrid DNA model of HOLLIDAY (1964); it will be generalized later.

We propose that hybrid DNA frequently forms in only one chromatid, while the second involved chromatid (the one which donated a strand to the hybrid) fails to receive a strand and fills the lesion by new synthesis (Figure 2). This repaired chromatid is genetically identical to its previous state, and any recombination events in the one hybrid chromatid are necessarily nonreciprocal. In other tetrads, two hybrid chromatids are formed, as described by HOLLIDAY.

One-chromatid events never result in crossing over for the outside markers. Two-chromatid events *always* do so (this differs from HOLLIDAY's postulate). Reciprocal recombination between

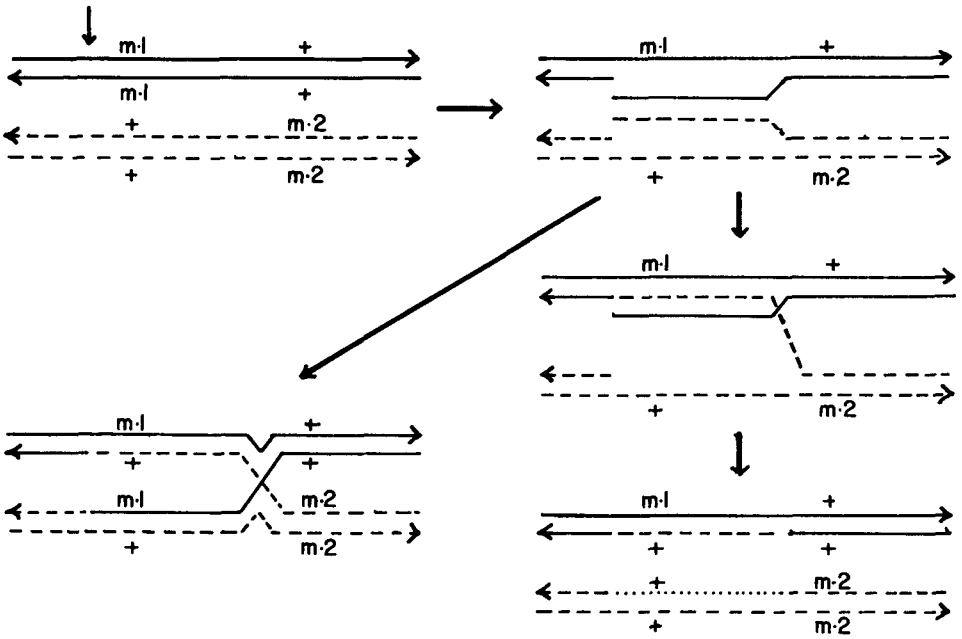

FIGURE 2.—The HOLLIDAY model for hybrid DNA formation with the one-chromatid modification. The first steps (single-strand breaks and unwinding), shown at the top, are unchanged. At lower left is the situation in which two chromatids carry hybrid DNA, as described by HOLLIDAY. The lower right figures show the formation of one hybrid chromatid with the donor chromatid restored to its previous form by new synthesis (dotted line).

alleles can only be generated by the latter type of event, and its products are always recombinant with regard to the markers. Nonreciprocal intragenic recombination can result from either one or two hybrid chromatids, so its products may carry either parental or recombinant markers.

One-chromatid events exercise no interference with crossing over in any of the four chromatids. This follows from the observation that in Neurospora (STADLER 1959) and in yeast (FOGEL and HURST 1967) intragenic recombinants with parental markers have normal recombination frequencies in neighboring regions, and from the demonstration in tetrads of yeast (FOGEL and HURST 1967) and Ascobolus (STADLER, TOWE and ROSSIGNOL 1970) that such recombinants are accompanied by sister strands with normal recombination even in the region immediately around the multiple allelic locus.

RESULTS

Mutation at the *w17* locus causes colorless ascospores. Over twenty mutant alleles at this locus have been studied in crosses to each other and to w^+ strains (STADLER, TOWE and ROSSINGOL 1970). Many of the crosses were segregating for the linked markers *colt* (temperature-colonial, 10 map units to the left of *w17*) and *fpr* (fluorophenylalanine-resistant, 3 map units to the right of *w17*).

The 5:3 and 3:5 segregations in crosses of *w17* × w^+ offer us a way of testing for the occurrence of one-chromatid events, on the basis of marker segregation. (Note: our segregation ratios state wild type first, mutant second; thus a 5:3

ascus has five wild type spores and three mutant spores.) These patterns occur in tetrads in which one chromatid remains heterozygous at the conclusion of meiosis. Assuming the event began with two hybrid chromatids as envisaged by HOLLIDAY (1964), one of them would have become homozygous by repair. The repair could have occurred in either chromatid, and it could have produced either allele. The allele in the repaired chromatid would be as likely to carry the markers which came into the cross with the other allele as those which were its own. This would lead to the prediction of equal frequencies of Type 1 and Type 2 asci (see Figure 3). On the other hand, a recombination event which began with one hybrid chromatid could not generate a Type 2 ascus.

The results of the analysis of 100 asci of these types (5:3 and 3:5) are shown in Table 2, and the great disparity between Type 1 and Type 2 clearly argues against the unfailing occurrence of two hybrid chromatids. The almost-complete absence of Type 2 may seem to make it doubtful that two-chromatid events had occurred at all. However, our hypothesis predicts the absence of Type 2 because it is a parental ditype for the markers, and we have stated that two-chromatid events must always result in a reciprocal recombination for the markers. Thus 5:3 and 3:5 asci resulting from two-chromatid events should be tetratypes in which the segregating spore pair has nonparental markers (Type 3 of Figure 4).

The hypothesis states that one-chromatid events do not exert any interference. Therefore, about 26% of them should be tetratypes as a result of a separate crossover in the 13-map-unit interval between the markers; these should be di-

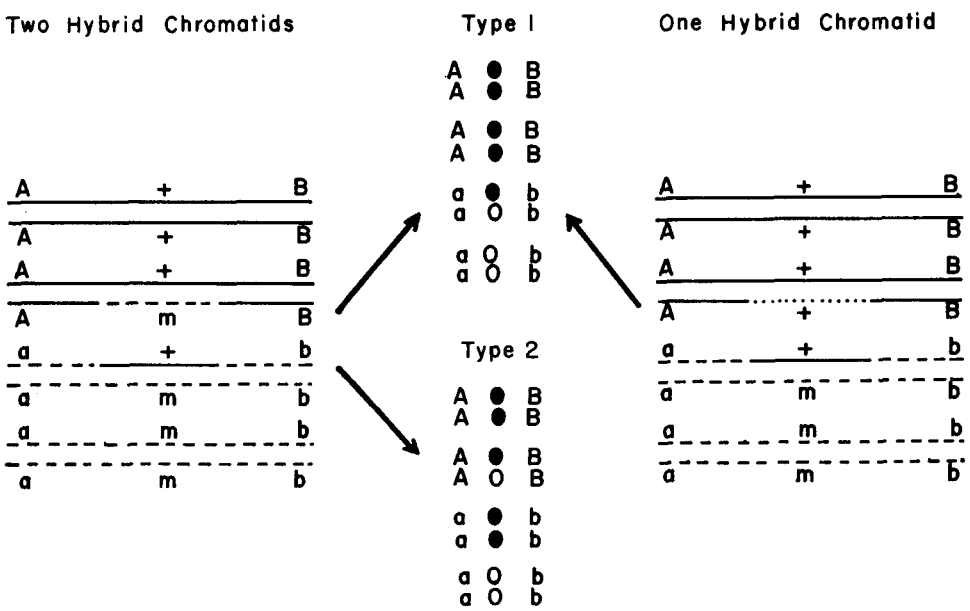

FIGURE 3.—Parental type marker distributions accompanying 5:3 segregation in the cross $w \times w^+$. As shown on the left, the HOLLIDAY model predicts equal likelihood of Type 1 and Type 2, while our hypothesis (on the right) predicts the absence of Type 2.

TABLE 2

Marker distributions accompanying 5:3 and 3:5 segregation in crosses of w17 × w+*

	Parental ditypes		Tetratypes	
	Type 1	Type 2	Type 3	Type 4
5:3	21	0	14	9
3:5	32	1	16	7
Totals	53	1	30	16

Type 1: Nonsegregating pairs are all parental types.
Type 2: One nonsegregating pair is double crossover type.
Type 3: Segregating pair has nonparental marker combination.
Type 4: Segregating pair has parental marker combination.

* Summed data from ten crosses which were all heterozygous for *w17* and for the flanking markers *colt* and *fpr*. The mutant alleles at *w17* were *a,b,c,k,l,n,q*, and *z*.

vided evenly between Type 3 and Type 4. The excess of Type 3 over Type 4 should represent the two-chromatid events.

KITANI and OLIVE (1967) reported an analysis of this kind involving spore

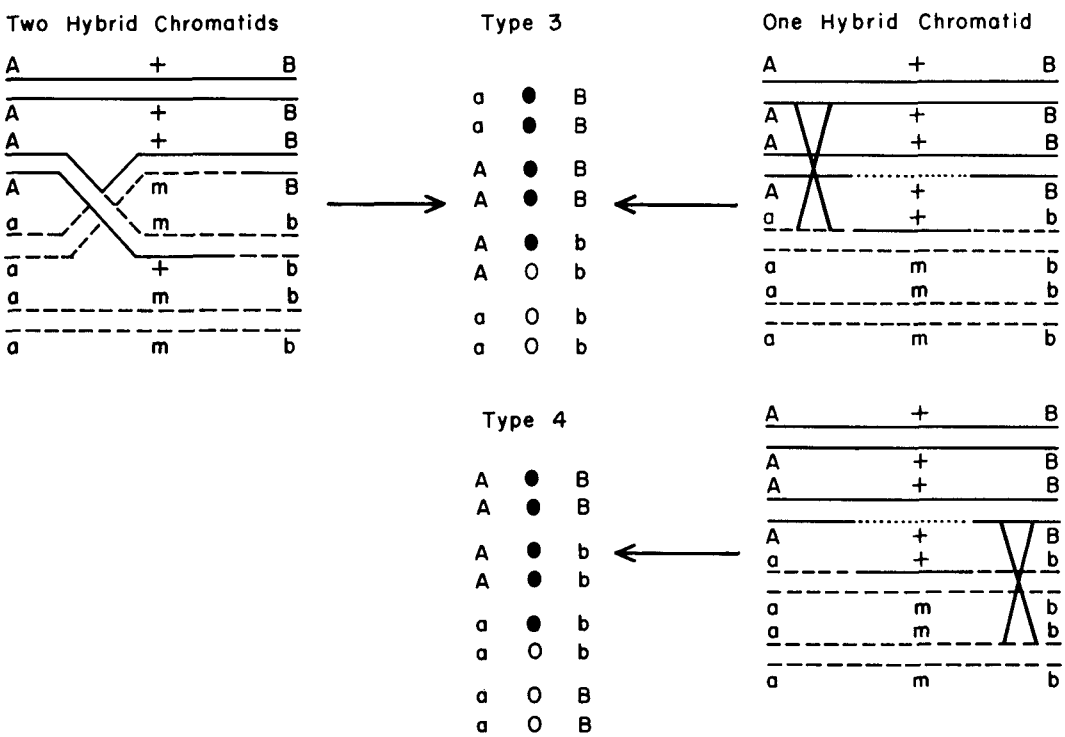

FIGURE 4.—Tetratype marker distributions accompanying 5:3 segregation in the cross $w \times w^+$ as explained by our hypothesis. Two-chromatid events result in marker exchange for the involved chromatids and can only produce Type 3. One-chromatid events accompanied by separate crossing over are equally likely to yield Type 3 or Type 4.

TABLE 3

Segregation of markers in tetrads with intragenic recombination at the w17 *locus**

Cross	Marker segregation in 2:6 asci							
	P-1		P-2		R-1		R-2	
	PD	T	PD	T	T	NPD	T	NPD
Ca+ × *+ cR*	27	1	0	0	19	0	6	0
+aR × *Cd+*	30	5	1	2	12	1	3	0
+a+ × *CdR*	0	0	0	1	0	1	0	0
Ca+ × *+eR*	10	8	0	1	10	1	4	0
Ca+ × *+fR*	27	3	0	0	14	1	1	0
Ca+ × *+iR*	0	0	0	1	2	0	0	0
Ca+ × *+jR*	1	0	0	0	0	0	0	0
Ca+ × *+kR*	14	2	4	1	2	0	1	0
Ca+ × *+lR*	1	0	1	0	0	0	0	0
+aR × *Cl+*	1	0	0	0	0	0	0	0
+a+ × *CoR*	11	1	4	0	9	1	2	0
	7	0	2	1	2	0	1	0
Ca+ × *+sR*	14	3	3	0	5	0	1	0
+b+ × *CdR*	11	7	0	0	20	1	3	0
+bR × *Cd+*	33	3	0	0	11	0	1	0
Cb+ × *+mR*	4	1	0	0	1	0	0	0
Totals	191	34	15	7	107	6	23	0

* From Table 5 of STADLER, TOWE and ROSSIGNOL (1970).

The cross parents are abbreviated by using capital letters to represent the mutant alleles of the markers: *C* represents *colt* and *R* represents *fpr*. The lower-case letters represent *w17* alleles. Column headed P-1 refers to those 2:6 asci in which the w^+ spore pair had the markers of the first parent, and P-2 refers to those with the markers of the second parent. The w^+ spores in an R-1 ascus had the left-hand marker from the first parent and the right-hand marker from the second parent; R-2 asci had the reverse combination. PD, T, and NPD refer to asci which were parental ditypes, tetratypes, and nonparental ditypes, respectively, with regard to the outside markers.

color mutants in Sordaria. Among 5:3 and 3:5 asci they found 162 which correspond to our Type 1 and 57 of Type 2.

Markers in recombination tetrads: Previous recombination models have failed to predict the distribution of linked markers in tetrads with intragenic recombination. We shall test the predictive value of our hypothesis on recombination data from the *w17* locus of Ascobolus (Table 3). Although there are not sufficient data from any single cross, STADLER, TOWE and ROSSIGNOL (1970) reported marker distribution for 383 recombination asci from 15 crosses in which *w17a* or *w17b* was crossed to another *w17* allele. We shall consider these to be homogeneous crosses as *w17a* and *w17b* are both high-conversion alleles and map at one end of the locus, while all the other alleles have lower conversion frequencies and map toward the other end of the locus.

In order to predict the marker distribution, we must assume values for the relative frequencies of one-chromatid and two-chomatid events and the relative conversion frequencies for the two participating alleles. We must also make an assumption regarding the length of the hybrid DNA region relative to the distance separating the two segregating sites (Figure 1). We shall assume that the situation is always the one shown in Figure 1b—one segregating site in the

hybrid DNA, the other outside (see DISCUSSION for justification of this assumption). This situation leads to equal numbers of reciprocal and nonreciprocal recombinations arising from two-chromatid events. Thus the observed frequency of reciprocal recombination among recombination tetrads should represent one-half of the two-chromatid events and should enable us to estimate the relative frequencies of one-chromatid and two-chromatid events. STADLER, TOWE and ROSSIGNOL (1970) made backcross tests on 213 recombination asci from crosses of *w17a* and *w17b* to other *w17* alleles. They observed 150 cases of nonreciprocal segregation at *w17a, b* and 20 at the other segregating site (which we shall refer to as *w17ε*). There were 14 cases of reciprocal segregation and 29 asci in which the backcross analysis was incomplete. Eleven of the incomplete analyses were cases in which nonreciprocal segregation at *w17a* was ruled out by the backcross test, and the *w17ε* allele (*d, f,* or *o*) was one which had *never* shown nonreciprocal (6:2) segregation in crosses to other mutant alleles or to w^+. We have assumed that these 11 are very probably cases of reciprocal recombination and have included them for the calculation of the frequency of two-chromatid events. If there were 25 reciprocal recombinations and 170 nonreciprocal, the proportion of recombinations resulting from two-chromatid events was $(2 \times 25)/195$ or 0.26.

The same backcross analyses may be used to estimate the relative frequencies of nonreciprocal events at the two segregating sites: 150/170 or 0.88 at *w17a, b* and 20/170 or 0.12 at *w17ε*. Another measure of relative frequencies of nonreciprocal events can be derived from the frequencies of 6:2 segregations in crosses of the various *w17* alleles to w^+ (Table 4a). Average frequencies of 6:2 segrega-

TABLE 4a

Relative frequencies of nonreciprocal segregation

	Allele	6:2 asci per 1000*	Number of asci		
w17a,b:	*a*	3.8	287	2.85	
	b	4.9	96	1.23	
			383	4.08	per 1000 = weighted average
w17ε:	*c*	0.2	53	0.0277	
	d	0	146	0	
	e	0.9	34	0.0888	
	f	0	46	0	
	i	2.5	3	0.0196	
	j	3.3	1	0.0086	
	k	1.1	24	0.0690	
	l	2.4	3	0.0188	
	m	0	6	0	
	o	0	41	0	
	s	0.1	26	0.0068	
			383	0.2393	per 1000 = weighted average

* From Table 2 of STADLER, TOWE and ROSSIGNOL (1970).

TABLE 4b

Expected marker distribution among 2:6 asci from interallelic crosses at w17
Assumptions: 74% one-chromatid events; 26% two-chromatid events; 88% nonreciprocal segregation at *w17a,b*, 12% at *w17ε*.

	Markers with w^+	Ascus type	Expected	Observed	Expected on alternate assumption*
One-chromatid events:					
Nonreciprocal					
segregation at *w17a,b*:			383		
$0.74 \times 0.88 \times 0.74 = 0.482$	P1	PD	$\times 0.482 = 185$	191	197
$\times 0.13 = 0.085$	P1	T	$\times 0.085 = 33$	34	34
$\times 0.03 = 0.020$	R1	T	$\times 0.289 = 111$	113†	109
$\times 0.10 = 0.065$	R2	T	$\times 0.068 = 26$	23	27
Nonreciprocal					
segregation at *w17ε*:					
$0.74 \times 0.12 \times 0.74 = 0.065$	P2	PD	$\times 0.065 = 25$	15	13
$\times 0.13 = 0.012$	P2	T	$\times 0.012 = 4$	7	2
$\times 0.10 = 0.009$	R1	T			
$\times 0.03 = 0.003$	R2	T			
Two-chromotid exents:					
0.260	R1	T			

* Alternate assumption: 94% nonreciprocal segregation at *w17a,b*, 6% at *w17ε*.
† Six of the 113 asci with R1 recombinants were nonparental ditypes; none was predicted on the stated assumptions, but the weakness of chiasma interference in Ascobolus (STADLER, TOWE and ROSSIGNOL 1970) makes them not unexpected.

tion have been weighted according to how many of the 383 asci of Table 4a involved any given allele. The weighted average for *w17a, b* is 4.08 per thousand and for *w17ε* 0.24 per thousand, leading to the assumption that 94% of the nonreciprocal events occur at *w17a, b* and 6% at *w17ε*. (It should be pointed out that our use of nonreciprocal segregation frequencies from mutant × wild type crosses involves the assumption that the same event produces the nonreciprocal recombinants in mutant × mutant crosses.)

Table 4b shows the predictions of our hypothesis for the frequencies of different marker combinations accompanying intragenic recombination. Note that one-chromatid events exert no interference, so 26% of them are accompanied by a separate crossover in the 13-map-unit region between the markers. Note that all two-chromatid events give the same marker distribution, regardless of whether the recombination is reciprocal or nonreciprocal. Both estimates of relative frequencies of nonreciprocal segregation have been tested, and both generate a very good fit to the observed distribution.

DISCUSSION

General form of the hypothesis: Our hypothesis has been presented as a variation of the HOLLIDAY (1964) model for recombination mediated by the formation of hybrid DNA. However, recombination may be considered to be a two-

step process of which our hypothesis deals only with the first step: the event by which chromatids become "recombinogenic" and the relationship of the exchange of outside markers to this event. The hypothesis is not concerned with the second step: excision-repair in hybrid DNA or some other specific mechanism for the production of a recombinant from a recombinogenic chromatid.

We propose that intragenic recombination sometimes results from an event which involves only a single chromatid, and this event is in no way related to reciprocal exchange between chromatids. We propose that intragenic events involving two chromatids are always accompanied by reciprocal exchange between those chromatids. An involved chromatid could be the site of hybrid DNA formation as visualized by HOLLIDAY. It could as well be a chromatid which incorporates a segment of genetic information from its homologue by the sequence of events envisaged by BOON and ZINDER (1969): neighboring breaks in both DNA strands of one chromatid and one strand of another set up a "replicating fork" of DNA synthesis which is terminated by a second set of breaks followed by rejoining; this model does not involve any recognition of mis-matched DNA strands by an excision-repair system.

Relative frequencies of one-chomatid and two-chromatid events: We have estimated that 74% of the recombination in crosses between alleles at the *w17* locus results from one-chromatid events and 26% from two-chromatid events. This proportion does not necessarily correspond with the absolute ratio of the two kinds of events. That would be true only if the chance of yielding a recombinant were the same for both kinds of events. Certainly it would not be true if the two segregating sites were very far apart, because a one-chromatid event can produce a recombinant only when it occurs *at* a segregating site, while a two-chromatid event can produce a recombinant if it falls anywhere between the segregating sites. Even when the sites are so closely linked that one of them is always included in the region of the event (as we have assumed in the *w17* crosses), the relative efficiency of the two events in producing recombinants depends on the specific nature of the process. It may even be subject to variations according to the specific configurations at the segregating sites. For the present analysis we have been able to avoid this complication by assuming that *on the average* two-chromatid events as pictured in Figure 1b should yield reciprocal and nonreciprocal recombinants with equal frequencies.

In the analysis of the 5:3 and 3:5 segregations from crosses of $w \times w^+$ it was pointed out that the two classes of tetratypes (3 and 4) should be produced in equal frequencies by one-chromatid events while two-chromatid events should yield only Type 3. Therefore, the difference (Type 3 minus Type 4) is a measure of frequency of two-chromatid events among these asci. The result is 14%, as opposed to 26% two-chromatid events among recombinants produced in $w \times w$ crosses. There is not necessarily any conflict between these findings; the relative efficiencies of the two kinds of events in producing 5:3 and 3:5 segregations, like their efficiencies in recombinant production, are open to a wide spectrum of possibilities.

Length of the recombination region with respect to the distance separating the

segregating sites: In order to make numerical predictions about markers accompanying intragenic recombination (Table 4), it was assumed that the two segregating sites were such a distance apart that the recombination region (hybrid DNA in the HOLLIDAY model) always included one or the other of them but not both—the situation shown in Figure 1b. The situation in Figure 1a (recombination region falling between segregating sites) was ruled out because of the great preponderance of nonreciprocal recombinations. An alternative possibility would be that the segregating sites were far enough apart for the recombination event to sometimes occur between them as in Figure 1a, but in that case the relative frequency of two-chromatid events would be even lower than 26%. Such an interpretation has not been adopted here because it leads to changes in the predictions of Table 4b which diminish their accuracy.

The situation in Figure 1c (recombination region including both segregating sites) was ruled out because it should produce an appreciable frequency of tetrads with *two* wild-type chromatids; 4:4 patterns should be one-sixth as frequent as 2:6. There were only two 4:4 asci in the total sample of over 100,000 asci which were examined from the fifteen crosses of Table 3; this sample included 548 recombination asci with 2:6 segregation.

It is notable that nearly all the 101 reciprocal recombinants reported by FOGEL and HURST (1967) carried the *same* nonparental recombination of markers (Table 1). This indicates that the mutant sites were too far apart to both be included in the recombination region as shown in Figure 1c, because reciprocal recombination in that situation would lead to similar numbers of m^+ recombinants with *both* nonparental marker combinations; it would also lead to significant numbers of tetrads in which *two* of the four meiotic products were nonmutant recombinants, and these were not observed. We conclude that the situation in Figure 1b best accounts for the results of their experiment. Note that this situation leads to the same markers with the m^+ product of reciprocal recombination regardless of which segregating site falls in the recombination region.

In order to produce recombinants in the situation shown in Figure 1c, it is required not only that both sites fall within the recombination region but also that two separate "correction" events occur within this region. In yeast, FOGEL and MORTIMER (1969) observed the absence of a class of tetrads which would have required two separate correction events in each of two chromatids. However, a random spore analysis in Neurospora (STADLER and KARIYA 1969) yielded significant numbers of a recombinant class which appeared to require two separate correction events in the *mtr* gene.

Tests and predictions of the hypothesis: According to the models of WHITEHOUSE (1963) and HOLLIDAY (1964), the marker exchange which accompanies intragenic recombination occurs at the same site and its frequency should be independent of the distance separating the markers. According to our hypothesis, the marker exchange which accompanies one-chromatid events is proportional to map distance. The proper test of these alternatives would involve a comparison of the segregation of differently spaced markers among recombinants between the same pair of alleles.

KITANI and OLIVE (1969) examined spore color patterns in asci of Sordaria. They were able to distinguish a large number of different aberrant segregation patterns in crosses segregating for two mutants at the same locus: an absolute mutant causing colorless spores and an intermediate (gray) color mutant. They separated aberrant patterns into a "substitution" group, which by our interpretation would be two-chromatid events, and a "restoration" group, which we would interpret as resulting from either one- or two-chromatid events. They observed that the majority of the substitution patterns had exchange of markers while the majority of the restoration patterns did not. The correlation is in the right direction to support our hypothesis. However, we would predict that *all* of the aberrant patterns which necessarily involve two chromatids should have marker exchange, and this was not found.

Our hypothesis states that one specific difference between one- and two-chromatid events is their interference with neighboring recombination. This feature of the hypothesis may not be testable in Ascobolus, because interference in this organism is weak, if present at all (STADLER, TOWE and ROSSIGNOL 1970). This has not impeded the analyses reported here, because our markers were close enough to make multiple exchange tetrads rare, and they have been ignored in the predictions of Table 4. However, there were a few (see footnote to Table 4b).

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SUMMARY

It is proposed that a large part of the intragenic recombination in meiosis results from an event which involves only one of the four chromatids. Another chromatid must donate genetic information to the recombinant, but this donor is not altered by the event. The one-chromatid event is completely unrelated to reciprocal recombination and to the exchange of outside markers. The balance of the intragenic recombination results from two-chromatid events. These may produce either reciprocal or nonreciprocal recombination and *always* result in the exchange of outside markers.—Crosses segregating for ascopore color mutants in Ascobolus produce rare asci with 5:3 and 3:5 segregation. The distribution of markers in these asci provides evidence in support of the occurrence of one-chromatid events.—Estimates of the relative frequencies of one-chromatid and two-chromatid events and of the frequencies of nonreciprocal segregation of the various mutant alleles were used to predict the marker distributions in asci with interallelic recombination. The predictions fit closely the observed distributions from crosses between alleles at the *w17* locus of Ascobolus.

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