INTRAGENIC RECOMBINATION AT THE *mtr* LOCUS OF NEUROSPORA WITH SEGREGATION AT AN UNSELECTED SITE[1]

DAVID R. STADLER AND BEVERLY KARIYA

Department of Genetics, University of Washington, Seattle

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WHITEHOUSE (1963) and HOLLIDAY (1964) have proposed molecular models for recombination which are designed to account for the observed complexities of genetic experiments. These models include the following steps: 1) single-strand breaks in two homologous chromatids; 2) escape of the free ends of each broken strand followed by pairing (by hydrogen bonding) with the complementary unbroken strand of the other chromatid; (A segregating site falling within this region of "hybrid DNA" will result in an improper pairing which may be corrected by the subsequent steps.) 3) excision of a segment of one strand including the improperly paired site; 4) new synthesis to replace the excised segment with a sequence complementary to the unexcised strand.

Such a series of events would have a number of measurable parameters: frequency of formation of hybrid DNA; length of hybrid DNA; length of the excised region; relative likelihood of correction to wild type, correction to mutant or no correction.

To date we have made little progress in measuring these parameters, because most experiments have given such limited information about intragenic events. Typically a cross is made between strains carrying different mutant alleles in the same gene, and prototrophic recombinants are selected and counted. This frequency does not measure any single parameter in the hybrid DNA models or other complex models, but instead represents the product of the coincident frequencies for the required series of events. To measure the separate events, we need more information than that provided by the frequency of this single, selected product. Many studies have included unselected outside markers which have yielded information about the number of exchanges. However, to measure the parameters of intragenic recombination directly, we must have *unselected* information about the frequency of events at sites within the gene.

An old and hallowed assumption of genetics is that segregating markers only *reveal* recombination events; that is, these events would take place whether or not the markers were present. This assumption has been challenged by investigators of bacterial transformation (RAVIN and IYER 1962; EPHRUSSI-TAYLOR 1966). Recent studies of nonreciprocal recombination in tetrads of Ascobolus (KRUSZEWSKA and GAJEWSKI 1967; ROSSIGNOL 1969) provide strong evidence that segregating markers alter the frequency or the nature of the recombination

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event. Such "marker effects" cannot be measured by observations limited to frequencies of selected recombinants in two-point crosses. Again we need unselected information about intragenic events. Ideally, in this case, we should like to measure the frequency of the same event both in the presence and absence of segregation at a nearby site.

This study, dealing with recombination at the *mtr* locus of Neurospora, was designed to yield unselected information about intragenic events. We have employed a conditional (temperature) mutant to provide a gratuitous site of segregation in crosses in which we could select recombinants for other segregating sites in the same gene.

MATERIALS AND METHODS

Mutation at the *mtr* locus results in resistance to the inhibitory amino acid analogs 4-methyltrytophan (4MT) and *p*-fluorophenylalanine (FPA). The mutants have severely reduced rates of uptake for these substances as well as for several amino acids including the aromatics (LESTER 1966; STADLER 1966). The *mtr* locus may represent the structural gene for a permease for the uptake of these substances.

A two-way selection system has been developed to detect rare events (mutation and recombination) at the *mtr* locus (STADLER 1967). The mutant phenotype is selected on media supplemented with 4MT or FPA. Revertants or *mtr*+ recombinants are selected by employing an auxotroph with a requirement for an amino acid taken up by the *mtr* system. The use of a tryptophan auxotroph for this purpose presented some technical difficulties, but these have been overcome in the present work by using a histidine-requiring mutant.

Wild-type Neurospora can take up histidine through either of two systems: the *mtr* system and a second uptake system which has a high affinity for arginine (and lysine). Therefore, a histidine-requiring *mtr* strain can grow on minimal medium supplemented with histidine, but it cannot grow if arginine is added to that medium. This becomes the selective medium for reversion of *mtr*. The same medium also selects *hist*+ revertants, but these are easily recognized by growth tests.

All strains used in these studies were derived from two basic strains: *hist-2 col-4 a* (abbreviated *hca*) and *his-2 pdx-1 A* (*hpA*). Both strains carried the same allele at the *hist-2* (histidine-requiring; linkage group I) locus, so *all* progeny from crosses between them required histidine. *pdx-1* (pyridoxine-requiring; linkage group IV) and *col-4* (colonial morphology; linkage group IV) served as outside markers for the *mtr* locus; they gave 3% recombination in crosses between these strains. The *mtr* locus is between them, about one map unit from *col-4*.

To select *mtr* mutants, conidia of *hca* or *hpA* were treated with ultraviolet light (UV) and plated on minimal sorbose medium supplemented with 50 mg/l L-histidine, 5 mg/l pyridoxine, and 70 mg/l of filter-sterilized 4MT (this medium will be called HP4MT). Colonies appearing after two days at 33°C or 37°C were isolated on slants of minimal medium with the same supplements. Conidial suspensions from these slant cultures were checked (by drop tests on sorbose media) for resistance to 4MT and FPA. Resistance to 4MT may sometimes represent mutations at other loci; but, in our experience, nearly all isolates which were resistant to both inhibitors resulted from mutation at *mtr*.

Conidia from the slant cultures were restreaked on HP4MT, and a single-cell colony from this re-isolation was generally found to be genetically pure *mtr*. The *mtr* mutants are recessive, so only those cells which were pure *mtr* gave colonies in the restreaking.

The two-way selection system provided us with a simple spot test for genetic purity of mutants or recombinants. The purity of an *mtr* strain was established when a drop of a conidial suspension gave no growth on a medium called HPArg (10 mg/l L-histidine, 5 mg/l pyridoxine and 60 mg/l L-arginine), which was selective for *mtr*+. Purity of *mtr*+ strains was shown by the absence of growth on HP4MT.

Resistant strains isolated from *hca* cells were crossed to *hpA*. Linkage of the resistance mutation to *col-4* was established as follows: Approximately 200 ascospores were germinated on a plate of HP4MT to select *mtr*, and a similar number were plated on HPArg, the selective medium for *mtr*+. Overnight incubation at 33°C yielded colonies about 2 mm in diameter; the dense *col* colonies were easily distinguished from the more diffuse *col*+ colonies. About 99% of the colonies on HP4MT were *col*, while a similar majority on HPArg were *col*+. This established the close linkage of the resistance mutation to the *col* mutant which accompanied it in the cross parent. Resistant mutants isolated from *hpA* were crossed to *hca*, and the same test was performed yielding the opposite result: 99% *col*+ on HP4MT, 99% *col* on HPArg.

The location of *mtr* between *pdx-1* and *col-4* had been established earlier (STADLER 1966) by analysis of selected recombinants from crosses of the same type as described above. The same method was used in the present work, the approximate position of each new *mtr* mutation being determined by plating spores from these crosses on sorbose medium supplemented only with histidine. This plating selected *pdx*+ ascospores, and the *col*+ recombinants among them were visually selected and isolated in slants to test for *mtr*. The results revealed that all the mutants were located in a restricted region about three-quarters of the way from *pdx-1* to *col-4*.

The *mtr* mutants isolated in *hca* were assigned two-digit numbers, and the mutants in *hpA* were given three-digit numbers. About 30 *mtr* mutants were isolated, tested and located genetically. Eight of these were selected for the studies of recombination at *mtr*. These included three *hca* mutants (*mtr 10, 14* and *30*) and five mutants of *hpA* (*mtr 112, 117, 119, 120,* and *121*). Selection was based on fertility of interallelic crosses. Many *mtr* mutants were rejected because only a very small fraction of the ascospores from their crosses to other *mtr* strains would germinate. Even among the selected strains, crosses gave a wide range of germination frequencies (see Tables 1 and 2).

All crosses were made in slants of synthetic crossing medium supplemented with 250 mg/l L-histidine. The *hca* parent (whether it carried *mtr* or not) served as protoperithecial parent and was incubated five days at 25°C before fertilization with conidia of the *hpA* parent. The crosses were incubated at least three weeks after fertilization before analysis.

To prepare ascospores for germination, the ripe perithecia were squashed and teased with forceps in water. This mixture was shaken vigorously and filtered through gauze. The resulting suspension was added to liquid 1% agar at 60°C and incubated at this temperature for 25 min to activate the ascospores before pipetting onto plates of appropriately supplemented sorbose medium. To select recombinants in a cross between two *mtr* parents, the ascospores were plated on HPArg and incubated 1–2 days at 33°C or 3 days at 18°C before counting and isolating the selected recombinants. Microscopic counts of sample areas from these plates were used to determine total numbers of ascospores and percentage of germination. In the *mtr* × *mtr* crosses it was not unusual to have as many as 50,000 spores on each plate.

The *mtr*+ recombinants were grown in slants of minimal medium plus HPArg and scored visually for *col*. Conidial suspensions of these cultures were prepared for drop tests on plates of sorbose medium to score for *pdx* and to confirm their classification as *mtr*+. The latter involved both a positive (HPArg) and a negative (HP4MT) test. If the *mtr*+ phenotype of an ascospore had resulted from its being a pseudowild type (PWT; MITCHELL, PITTENGER and MITCHELL 1952) rather than a true recombinant, it would have produced homokaryotic *mtr* conidia and heterokaryotic *mtr*+ conidia; thus it would have grown on both HPArg and HP4MT. No such result was observed in any of the *mtr* × *mtr* crosses. (The few cultures which grew on both media were shown to result from mixed isolates—an *mtr*+ recombinant accompanied by an *mtr* strain; this was demonstrated by the successful isolation of a pure *mtr*+ strain from a single-cell colony on HPArg.) The occurrence of PWT's in an interallelic cross results from complementation between the parent mutants. Complementation does not seem to occur at the *mtr* locus. No evidence of it was seen in 113 pairwise combinations which were crossed to select recombinants, or in another 30 combinations tested vegetatively in forced heterokaryons.

In our attempt to map *mtr* by interallelic crosses, we have designated the recombination frequency as the number of *mtr*+ per million ascospores. We have chosen to use *total* spores rather than *germinated* spores as the denominator for these measurements. This is the more meaningful

figure, if, as we believe, the *mtr+* ascospores nearly always germinate, even in crosses with low germination frequency for the general population. As described below, there is strong evidence that the sites of the mutants studied here are confined to several clusters. Recombination frequencies for different crosses between the same two clusters were calculated per total spores and per germinated spores. The former method gave fairly homogeneous results (see Figures 1 and 2), while the latter gave huge fluctuations showing a consistent inverse proportionality to germination frequency.

After most of the crosses had been analyzed, DR. DONALD BOONE discovered that the *hpA-mtr119* stock was segregating for *two* genes which determined the *mtr* phenotype. One was *mtr119* at the *mtr* locus, and the other was unlinked to it. The presence of either gene resulted in the failure to grow on HPArg. Therefore the selection of recombinants in crosses to other *mtr* alleles was only 50% efficient. The mapping data were corrected by dividing the numbers of total ascospores by two. The *mtr119* allele was separated from the second gene in a cross-reisolate designated *mtr119*^{s}. Several of the two-point mapping crosses were repeated with this strain (Table 1), and the recombination frequencies were used in the map (Figure 1). No further study has been made of the unlinked mutant which determines an *mtr*-like phenotype. Strains carrying this mutant grow rather poorly on HP4MT or HPFPA, which probably explains why they were not recovered during the selection of *mtr* mutant strains.

Isolation of the temperature mutant: About 200 resistant mutants were isolated at 33°C or 37°C from plates of HP4MT which contained UV-treated conidia of *hpA*. These isolates were tested for *mtr* at both 18°C and 37°C. One strain was recovered which at 37°C showed the mtr phenotype (growth on HP4MT, no growth on HPArg), while at 18°C it showed intermediate growth on both test plates. All the other isolates were clearly *mtr* at both temperatures. The strain exhibiting the temperature effect was crossed to *hca*, and *pdx col+* progeny were isolated. The selected progeny proved to be of two types: one which was mtr at both temperatures and a second which was mtr at 37°C but *fully* mtr+ (no growth on HP4MT) at 18°C. This latter type was designated *mtr-t*. The original isolate appears to have been a heterokaryon between *mtr-t* and an absolute *mtr* mutant. However, all attempts to purify *mtr-t* vegetatively from the original isolate were unsuccessful, suggesting that the *mtr-t* nucleus contained a recessive lethal lesion in some other part of the genome.

Isolation of mtr *double mutants:* Conidia of *hpA–mtr-t* were treated with UV and plated in HP4MT at 18°C. Nine resistant colonies were isolated. All of these isolates were resistant to 4MT and FPA at both 18°C and 37°C, and crosses showed that the resistance was determined by the *mtr* locus. Crosses of these strains to other *mtr* mutants and to each other led to the conclusion that each of these stocks carried the mutant *mtr-t* as well as a secondary mutant at some other site in the *mtr* gene. These double mutants were designated *mtr-t–1*, *mtr-t–2*, etc.

Nonreciprocal segregation at mtr: Tetrad analyses of intragenic recombination (FOGEL and HURST 1967; KRUSZEWSKA and GAJEWSKI 1967) have shown that most of the recombinants result, not from a reciprocal exchange between two chromatids, but from a nonreciprocal (3:1) segregation at one of the mutant sites. Nonreciprocal segregation can be observed directly in tetrads of a one-point (mutant × wild type) cross (KITANI and OLIVE 1967, FOGEL and MORTIMER 1969), and it is found to occur at a low frequency comparable to that of interallelic recombination. Tetrads have not been analyzed in the present study, but an indirect method has been used to estimate the frequencies of nonreciprocal segregation of the various *mtr* alleles.

In a cross of the form *hca–mtr* × *hpA*, spores were plated on HArg to select *pdx+ mtr+* recombinants. Approximately 1% of the viable spores formed colonies on this selective medium, and of these about 1% were *col*. By this method, we obtained a measure of the frequency with which *mtr+* emerged from the cross with outside markers from the other parent. We propose that these markers are too close together (three map units) for any progeny to result from two separate recombination events between them. On this assumption, the selected progeny must result from 3:1 segregation at *mtr*. KITANI and OLIVE (1967) found that the majority of the 3:1 segregations they observed in tetrads of Sordaria were not accompanied by any recombination for the outside markers; therefore, one of the three wild-type products emerged with the outside markers from the mutant parent.

The frequency of 1:3 segregation was similarly estimated in crosses of the form *hca* × *hpA*–*mtr*, by plating ascospores on H4MT and selecting pdx^+ *mtr col* progeny.

RESULTS AND DISCUSSION

Mapping the mtr *locus:* One hundred and thirteen pairwise crosses between different *mtr* strains were analyzed for recombination. Ninety-six of these crosses were segregating for the outside markers *pdx* and *col*. The mtr^+ recombinants were counted and scored for the unselected markers. The results of the two-point crosses are in Table 1 and a map based on these results is shown in Figure 1.

Ascospores from three-point crosses (those involving the secondary mutants in double mutant combinations with the temperature mutant) were plated at 18°C to provide nonselective conditions for the temperature mutant site. The mtr^+ recombinants selected at 18°C were subsequently tested for the *mtr-t* phenotype at 37°C. Results of the three-point crosses are shown in Table 2; the numbers of selected recombinants carrying the temperature mutant allele are shown in parentheses. Figure 2 is a map representation of these results.

Seventeen pairwise combinations of the secondary mutant strains (crosses #39–55) were also analyzed for mtr^+ recombinants. These crosses were segregating at only two points in the *mtr* locus, because both parents carried the mutant lesion at the *mtr-t* site. A total of 216 mtr^+ recombinants were recovered from these crosses on HPArg at 18°C. Every one of them was found to carry *mtr-t* when scored at 37°C. This provided an important confirmation of our assumption that the secondary mutant stocks each carried two lesions in the *mtr* gene, one of which was *mtr-t*. Germination was very poor in these crosses, and they were not

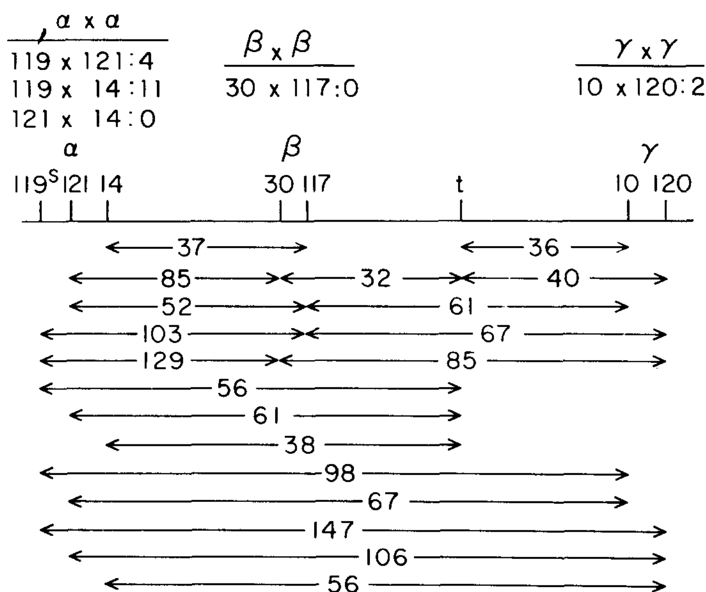

FIGURE 1.—Recombination map of the primary *mtr* mutants. The frequencies are numbers of mtr^+ progeny per million ascospores.

TABLE 1

Intragenic recombination at the mtr *locus in two-point crosses.*

Cross No.	Parents	Total spores × 10^{-3}	Percent germination	Total mtr^+	mtr^+ per 10^6	pdx^+ mtr^+ col	pdx mtr^+ col^+	pdx^+ mtr^+ col^+	pdx mtr^+ col
1	*10 × 112*	940	50	27	29	8	15	3	1
2	*10 × 117*	2150	30	132	61	35	69	15	13
3	*10 × 119*	860†	70	85	99	20	41	12	12
3a	*10 × 119*^{s}	693	85	68	98	14	35	12	7
4	*10 × 120*	1500	35	3	2	1	1	1	0
5	*10 × 121*	210	40	14	67	5	8	1	0
6	*10 × t*	1525	85	60	39	11	35	7	7
7	*14 × 112*	989	35	7	7	2	5	0	0
8	*14 × 117*	493	50	18	37	4	11	2	1
9	*14 × 119*	510†	85	9	18	5	4	0	0
9a	*14 × 119*^{s}	708	85	8	11	3	4	0	1
10	*14 × 120*	357	55	20	56	5	12	2	1
11	*14 × 121*	357	50	0	0	.	.	.	.
12	*14 × t*	954	90	36	38	15	11	4	6
13	*30 × 112*	730	30	30	41	11	7	10	2
14	*30 × 117*	417	30	0	0	.	.	.	.
15	*30 × 119*	250†	90	35	140	19	7	6	3
15a	*30 × 119*^{s}	722	90	93	129	16	50	18	9
16	*30 × 120*	330	50	28	85	9	14	4	1
17	*30 × 121*	742	35	63	85	9	27	22	5
18	*30 × t*	1898	85	60	32	12	27	14	7
19	*112 × 117*	479	7	4	8	1	2	0	1
20	*112 × 119*^{s}	726	75	26	36	5	17	1	3
21	*112 × 120*	606	8	39	64	15	16	7	1
22	*112 × 121*	1041	4	28	27	4	17	6	1
23	*112 × t*	2547	75	15	6	8	3	4	0
24	*117 × 120*	413	5	35	85	16	9	4	6
25	*119 × 112*	88†	55	1	11	0	0	0	1
26	*119 × 117*	145†	55	15	103	6	5	4	0
27	*119 × 120*	305†	70	22	72	10	6	4	2
27R	*119 × 120*	279†	60	28	100	12	11	2	3
28	*119 × t*	626†	80	35	56	18	10	3	4
29	*120 × 112*	432	15	17	39	7	4	6	0
30	*120 × 117*	448	12	22	49	3	7	10	2
31	*120 × 119*	571†	80	65	114	12	40	10	3
31R	*120 × 119*	467†	75	44	94	16	13	15	0
31a	*120 × 119*^{s}	584	85	86	147	16	42	18	10
32	*120 × 121*	410	12	27	66	5	17	4	1
33	*120 × t*	1026	85	41	40	13	17	6	5
34	*121 × 112*	458	5	16	35	5	6	5	0
35	*121 × 117*	619	6	34	52	12	9	11	2
36	*121 × 119*^{s}	1581	70	7	4	1	5	1	0
37	*121 × 120*	422	5	62	147	35	13	7	7
38	*121 × t*	1483	80	91	61	39	27	18	7

The numbers in the "Parents" column indicate which *mtr* alleles were involved in each cross. In every case the first parent was *hist-2 mtr col-4 a* and the second parent was *hist-2 pdx-1 mtr A*. In crosses 27R and 31R the protoperithecial parent was the second parent listed; in all other crosses it was the first parent.

† Total spores recorded here are half of the actual total because the presence of an unlinked resistant mutant prevented detection of half of the recombinants.

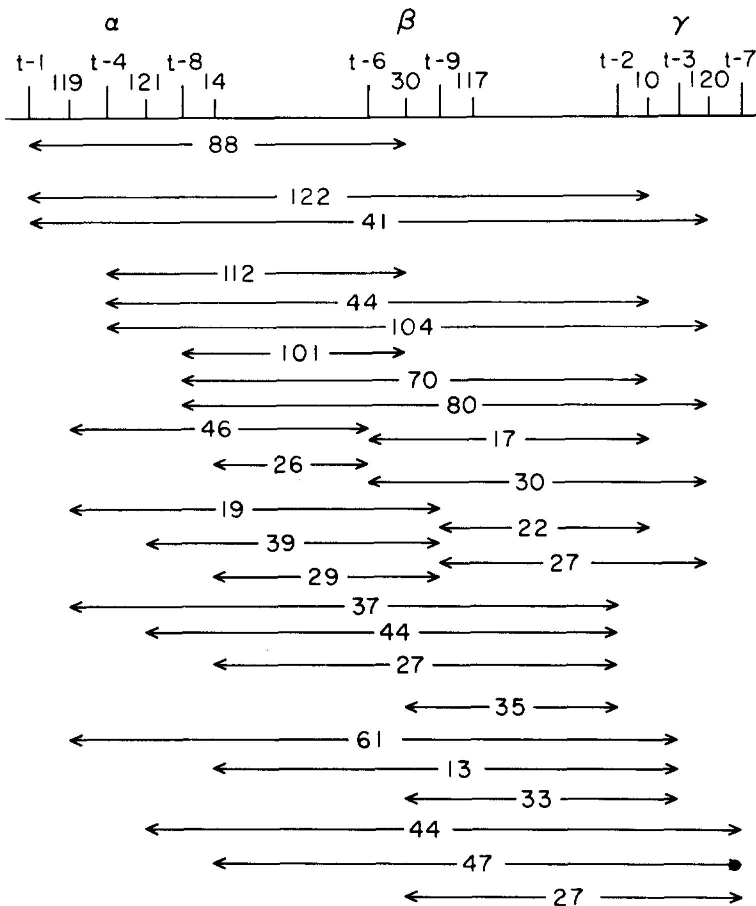

FIGURE 2.—Recombination map of the three clusters of primary and secondary *mtr* mutants.

segregating for the outside markers. Therefore, they have not been included in Table 1.

Two methods of mapping the mutant sites were attempted without success. The first method is based on the assumption that the frequency of recombinants is directly proportional to the distance along the gene between the mutant sites. The best evidence for success of this method is the construction of a one-dimensional map of mutant sites for which the frequencies are additive. Figures 1 and 2 show an order of sites for which the frequencies are not additive, and no other order would appear to diminish the departure from additivity.

TABLE 2

Intragenic recombination at the mtr *locus in three-point crosses*

Cross No.	Parents	Total spores × 10^{-3}	Percent germination	Total *mtr*+	*mtr*+ per 10^6	*pdx*+ *mtr*+ *col*	*pdx* *mtr*+ *col*+	*pdx*+ *mtr*+ *col*+	*pdx* *mtr*+ *col*
56	*10 × t-1*	579	70	71(44)	122	11(5)	43(28)	13(9)	4(2)
57	*10 × t-2*	346	70	5(0)	14	1(0)	3(0)	0	1(0)
58	*10 × t-3*	433	65	2(1)	5	1(1)	0	0	1(0)
59	*10 × t-4*	428	50	19(7)	44	4(2)	13(5)	2(0)	0
60	*10 × t-5*	398	12	0	0	.	.	.	.
61	*10 × t-6*	826	65	14(1)	17	4(0)	6(1)	3(0)	1(0)
62	*10 × t-7*	362	65	1(0)	3	0	1(0)	0	0
63	*10 × t-8*	374	45	26(9)	70	3(1)	11(3)	3(2)	9(3)
64	*10 × t-9*	784	85	17(3)	22	1(0)	8(2)	6(0)	2(1)
65	*14 × t-1*	833	25	8(7)	10	2(1)	5(5)	0	1(1)
66	*14 × t-2*	962	40	26(8)	27	11(1)	11(5)	1(1)	3(1)
67	*14 × t-3*	455	40	6(0)	13	2(0)	2(0)	0	2(0)
68	*14 × t-4*	873	30	0	0	.	.	.	.
69	*14 × t-5*	781	10	0	0	.	.	.	.
70	*14 × t-6*	303	50	8(1)	26	2(1)	4(0)	2(0)	0
71	*14 × t-7*	827	55	39(2)	47	17(1)	6(1)	5(0)	11(0)
72	*14 × t-8*	957	35	8(5)	8	3(1)	2(2)	2(2)	1(0)
73	*14 × t-9*	1056	80	31(5)	29	17(3)	7(0)	5(2)	2(0)
74	*30 × t-1*	936	35	82(54)	88	28(18)	36(22)	16(12)	2(2)
75	*30 × t-2*	967	40	34(17)	35	6(3)	11(6)	15(8)	2(0)
76	*30 × t-3*	307	30	10(4)	33	3(1)	2(1)	4(2)	1(0)
77	*30 × t-4*	846	40	95(68)	112	18(10)	44(34)	25(19)	8(5)
78	*30 × t-5*	1049	10	0	0	.	.	.	.
79	*30 × t-6*	652	50	2(1)	3	0	1(0)	1(1)	0
80	*30 × t-7*	637	55	17(12)	27	2(1)	9(7)	4(3)	2(1)
81	*30 × t-8*	584	40	59(44)	101	10(6)	33(27)	11(8)	5(3)
82	*30 × t-9*	1130	80	12(10)	11	3(2)	1(1)	4(4)	4(3)
83	*112 × t-1*	1015	7	15(14)	15	1(1)	9(8)	4(4)	1(1)
84	*112 × t-2*	1061	6	14(9)	13	6(3)	7(5)	0	1(1)
85	*112 × t-4*	667	6	15(15)	23	3(3)	6(6)	5(5)	1(1)
86	*112 × t-5*	794	8	0	0	.	.	.	.
87	*112 × t-7*	1026	15	16(6)	16	9(4)	4(1)	1(1)	2(0)
88	*112 × t-8*	770	9	9(5)	12	1(1)	7(3)	1(1)	0
89	*112 × t-9*	808	55	2(0)	2	0	0	2(0)	0
90	*119 × t-1*	386†	50	3(2)	8	3(2)	0	0	0
91	*119 × t-2*	438†	70	16(2)	37	5(0)	3(1)	4(0)	4(1)
92	*119 × t-3*	147†	65	9(0)	61	3(0)	1(0)	1(0)	4(0)
93	*119 × t-4*	71†	35	0	0	.	.	.	.
94	*119 × t-5*	154†	8	0	0	.	.	.	.
95	*119 × t-6*	175†	60	8(2)	46	2(1)	2(0)	4(1)	0
96	*119 × t-8*	145†	30	0	0	.	.	.	.
97	*119 × t-9*	158†	75	3(0)	19	2(0)	1(0)	0	0
98	*120 × t-1*	834	15	34(17)	41	9(0)	12(9)	10(8)	3(0)
99	*120 × t-2*	774	20	4(0)	5	3(0)	1(0)	0	0
100	*120 × t-3*	283	20	4(1)	14	0	3(1)	0	1(0)
101	*120 × t-4*	499	15	52(36)	104	9(6)	24(15)	14(13)	5(2)
102	*120 × t-5*	601	20	0	0	.	.	.	.

TABLE 2—Continued

Cross No.	Parents	Total spores × 10^{-3}	Percent germination	Total mtr^+	mtr^+ per 10^6	pdx^+ mtr^+ col	pdx mtr^+ col^+	pdx^+ mtr^+ col^+	pdx mtr^+ col
103	*120* × *t-6*	594	25	18(0)	30	1(0)	13(0)	0	4(0)
104	*120* × *t-7*	702	45	5(0)	7	3(0)	2(0)	0	0
105	*120* × *t-8*	628	20	50(30)	80	6(4)	32(19)	5(2)	7(5)
106	*120* × *t-9*	860	85	23(7)	27	10(3)	12(4)	0	1(0)
107	*121* × *t-1*	895	5	2(1)	2	1(0)	1(1)	0	0
108	*121* × *t-2*	569	10	26(4)	46	12(0)	10(4)	2(0)	2(0)
109	*121* × *t-4*	697	5	1(1)	1	0	1(1)	0	0
110	*121* × *t-5*	731	10	0	0	.	.	.	.
111	*121* × *t-7*	697	25	31(2)	44	11(0)	6(0)	9(0)	5(2)
112	*121* × *t-8*	763	10	9(0)	12	4(0)	1(0)	2(0)	2(0)
113	*121* × *t-9*	614	50	24(0)	39	10(0)	7(0)	5(0)	2(0)

The numbers in the "Parents" column indicate which *mtr* alleles were involved in each cross. In every case the first parent was *hist-2 mtr col-4 a* and the second parent was *hist-2 pdx-1 mtr A*. In every case the protoperithecial parent was the first one listed.

Numbers in parentheses are the *t* mutants among the selected recombinants.

† Total spores recorded here are half of actual total because presence of unlinked resistant mutant prevented detection of half of the recombinants.

The second method makes use of the unselected outside markers. If the selected event results from a single exchange between the mutant sites, it should always produce an mtr^+ recombinant with the same nonparental combination of outside markers. Recombinants with the other nonparental combination would require at least a triple exchange. On the assumption that single-exchange events are more frequent than triples, the more frequent marker combination has been assumed to reveal the order of the mutant sites. In most of the crosses reported here this method could not be used because *neither* nonparental marker combination was found frequently among the mtr^+ recombinants. Over 70% of them had parental marker combinations, and the two nonparental combinations were typically found in low and nearly equal frequencies.

KRUSZEWSKA and GAJEWSKI (1967) analyzed tetrads from two-point crosses involving seven allelic mutants in Ascobolus. They concluded that the frequency of recombinants was not a valid measure of distance separating the mutant sites. They placed the mutant sites in two clusters on the basis that crosses between two mutants in the same cluster always gave very low frequencies of recombination, while crosses between mutants in separate clusters *sometimes* gave much higher frequencies. They also crossed each mutant to wild type to determine its frequency of gene conversion (3+:1*m* segregation). In a cross between two mutants in separate clusters, the frequency of asci with wild-type recombinants was closely correlated with the sum of the conversion frequencies of the two mutants involved. These frequencies varied over a 50-fold range, even though they were all presumed to represent crosses between mutant sites about the same distance apart.

The cluster method of KRUSZEWSKA and GAJEWSKI has been used to map the *mtr* mutants. When all pairwise crosses of a set of mutants gave very low recombination frequencies, they were assumed to be in a cluster. On this basis there

appear to be three clusters which include seven of the eight primary mutants (Figure 1) and eight of the nine secondary mutants (Figure 2). The remaining primary mutant (*mtr-112*) and the temperature mutant itself appear to be outside these clusters. The one remaining secondary mutant (*mtr-t-5*) has not recombined with any other *mtr* mutant and may be a deletion.

The clusters have been designated α, β and γ, and they are shown in that order along a linear map in Figures 1 and 2. The order of clusters shown is perhaps the one most compatible with all the observations. The few crosses which do show a pronounced inequality in the two nonparental marker combinations suggest that α is to the left of the other clusters. There are four crosses (#15a, 17, 74, 77) which indicate that α is to the left of β, while one (#35) indicates the reverse. Five crosses (#31R, 56, 76, 98, 101) argue that α is to the left of γ, while one (#63) argues the reverse. However, the decision to place the mutant sites along a line is based mainly on custom and convenience. Certainly the recombination frequencies do not dictate that the *mtr* mutant sites must be located on a one-dimensional structure.

The position of the temperature-mutant site: We have attempted to locate the temperature mutant by determining its linkage to each of the clusters. Among the three-point crosses in which the two absolute mutants are in separate clusters, we may ask which of the selected wild-type sites carries the temperature mutant site with it into the recombinants. Figure 3 shows the summed results for the various cluster combinations. It may be seen that the unselected site stays with β (the allele which came into the cross with β^+ appears in most of the recombinants) in crosses with either α or γ. In the $\alpha \times \gamma$ recombinants the temperature mutant shows linkage to γ. These results are compatible with the order α-β-t-γ, shown in Figure 1, as well as with two other possible orders: α-γ-β-t and α-γ-t-β.

Figure 3 shows results for each of the three pairwise combinations of clusters with the two reciprocal alignments of the temperature mutant. We might expect

Cross	Alignment (upper / lower)	+	t	%t
α x t-β	α ⊕ + / ⊕ β t	66	8	11
β x t-α	⊕ β + / α ⊕ t	70	166	70
α x t-γ	α + ⊕ / ⊕ t γ	135	18	12
γ x t-α	⊕ + γ / α t ⊕	109	143	57
β x t-γ	β + ⊕ / ⊕ t γ	28	33	54
γ x t-β	⊕ + γ / β t ⊕	61	11	15

FIGURE 3.—Segregation at the unselected t site in selected recombinants. Summed results of crosses from Table 2. Circles indicate the selected mtr^+ sites.

the "%t" for two reciprocal sets to add up to 100%, but it is less than that for each of the three pairs of data. This disparity might be explained if spores carrying *mtr-t* were less viable than *mtr*$^+$, but platings of control crosses show that this is not the case. ROSSIGNOL (1969), studying gene conversion in Ascobolus, observed that the frequency of 3:1 segregation for a given mutant may be significantly different from the frequency of 1:3 segregation. The deficient recovery of the mutant temperature allele among the selected recombinants may indicate that 3:1 segregation is more frequent than 1:3 at this site. (This could account for the disparity only if gene conversion at this site were frequent; while it is rare in the general population, it may be the normal result when the segregating site finds itself in a recombination region.)

The size of a converted region: The great majority of interallelic recombinants result from gene conversion (3:1 segregation) at one of the segregating sites rather than from reciprocal recombination; this has been demonstrated by tetrad analysis in Neurospora (STADLER and TOWE 1963), in yeast (FOGEL and HURST 1967) and in Ascobolus (KRUSZEWSKA and GAJEWSKI 1967). We should like to know whether this miscopying event involves a point, a continuous segment or an interrupted segment. We have attempted to answer this question by observing the segregation of the unselected site in those recombinants resulting from gene conversion at one of the selected sites. The occurrence of gene conversion cannot be directly demonstrated without tetrad analysis, but it has been shown in the Neurospora and yeast studies mentioned above that the outside markers signal the conversion event. Specifically, it was found that the recombinants which carried a parental combination of markers nearly always resulted from conversion to wild type at the site which was mutant in that parent. For example, in the cross *pdx*$^+$ *mtr-β col* × *pdx mtr-α col*$^+$, the *pdx*$^+$ *mtr*$^+$ *col* progeny are believed to result from gene conversion at the β site.

Figure 4 illustrates three possible models for conversion and their predictions for a nearby unselected site. The first is a point conversion model and predicts that the neighboring site will always remain unconverted. The second model represents conversion of a continuous segment and predicts up to 100% co-

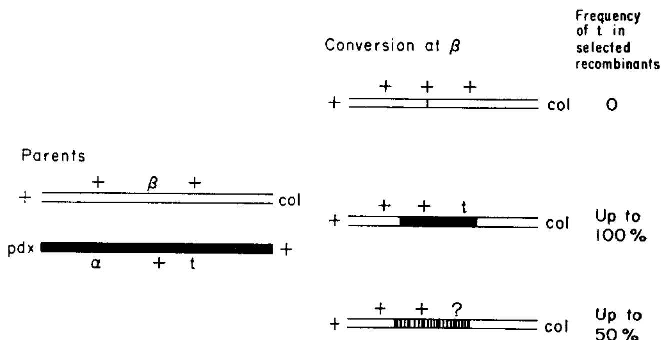

FIGURE 4.—Three models for the genetic constitution of a product of gene conversion (non-reciprocal segregation). See Text.

conversion for the neighboring site. The third model shows conversion of an interrupted segment (corresponding segments of two homologous chromosomes engage in a recombination process whereby each point in that segment independently converts to one or the other of the parent types); this model predicts up to 50% co-conversion of the neighboring site. The results shown in Table 3 support the second model (conversion of a continuous segment). Conversion at β results in co-conversion at the temperature mutant site in 72% of cases. (Again, as in Figure 3, we see nonidentical results for reciprocal sets of data which may be a consequence of 3+:1*m* conversion being more frequent than 1+:3*m* at the temperature mutant site.) Conversion of a continuous segment was also indicated in the recent study by FOGEL and MORTIMER (1969) of tetrads from crosses

TABLE 3

Gene conversion at the mtr *locus; inclusion of the unselected* t *site in the converted region*

Parents	Cross No.	Included	Excluded
α conversion:			
pdx+ α + + *col* / *pdx* + β *t col*+	70, 73, 95, 97, 113	5 *pdx*+ *mtr-t col*	28 *pdx*+ *mtr*+ *col*
pdx+ + β + *col* / *pdx* α + *t col*+	74, 77, 81	30 *pdx mtr*+ *col*+	83 *pdx mtr-t col*+
pdx+ α + + *col* / *pdx* + *t* γ *col*+	66, 67, 71, 91, 92, 108, 111	2 *pdx*+ *mtr-t col*	59 *pdx*+ *mtr*+ *col*
pdx+ + + γ *col* / *pdx* α *t* + *col*+	56, 59, 63, 98, 101, 105	56 *pdx mtr*+ *col*+	79 *pdx mtr-t col*+
β conversion:			
pdx+ + β + *col* / *pdx* α + *t col*+	74, 77, 81	34 *pdx*+ *mtr-t col*	22 *pdx*+ *mtr*+ *col*
pdx+ α + + *col* / *pdx* + β *t col*+	70, 73, 95, 97, 113	21 *pdx mtr*+ *col*+	0 *pdx mtr-t col*+
pdx+ β + + *col* / *pdx* + *t* γ *col*+	75, 76, 80	5 *pdx*+ *mtr-t col*	6 *pdx*+ *mtr*+ *col*
pdx+ + + γ *col* / *pdx* β *t* + *col*+	61, 64, 103, 106	32 *pdx mrt*+ *col*+	7 *pdx mtr-t col*+
γ conversion:			
pdx+ + + γ *col* / *pdx* α t + *col*+	56, 59, 63, 98, 101, 105	18 *pdx*+ *mtr-t col*	24 *pdx*+ *mtr*+ *col*
pdx+ α + + *col* / *pdx* + *t* γ *col*+	66, 67, 71, 91, 92, 108, 111	28 *pdx mtr*+ *col*+	11 *pdx mtr-t col*+
pdx+ + + γ *col* / *pdx* β *t* + *col*+	61, 64, 103, 106	3 *pdx*+ *mtr-t col*	13 *pdx*+ *mtr*+ *col*
pdx+ β + + *col* / *pdx* + *t* γ *col*+	75, 76, 80	8 *pdx mtr*+ *col*+	14 *pdx mtr-t col*+

between allelic mutants in yeast; they found that two sites in the *arg-4* locus were co-converted with a frequency of 75%.

Something may be said about the size of a converted region with respect to the size of the marked region in the *mtr* gene. The converted region generally appears to be longer than the distance from the temperature mutant site to β, about as long as the distance to γ (48% co-conversion) and shorter than the distance to α (27% co-conversion). However, this analysis may lead to an underestimate of the length of the converted region. In scoring co-conversion of the unselected site we are automatically excluding those conversions which extended as far as the second selected site. The frequency of detected co-conversion may be severely limited in those cases in which the unselected site and the second selected site lie in the same direction from the site of the selected conversion. We will return to this question later with an approach that does not suffer from this same limitation (Table 5). That experiment will indicate that conversion in any of the three clusters results in co-conversion at *t* most of the time.

Multiple gene conversion events: When interallelic recombinants were first selected in crosses with outside markers (MITCHELL 1955), the recovery of all four marker combinations among the recombinants meant that the simplest event which could account for one of the nonparental classes was a triple exchange. The other nonparental class could be produced by a single exchange, while the parental classes required double exchange. With the demonstration of gene conversion, it became clear that this event by itself could produce what had been called a double exchange. The "triple-exchange" class could be accounted for by gene conversion accompanied by a crossover. Thus, it appeared that all the multiple exchanges could be explained by the frequent involvement of gene conversion in the production of intragenic recombinants.

The presence of a third segregating site at the *mtr* locus permits us to detect events more complex than a "triple exchange." We would like to know whether two separate conversion events ever occur in the same gene. The hybrid DNA models of HOLLIDAY (1968) and WHITEHOUSE (1963) account for conversion by excision and replacement of a segment of one strand which includes the mismatched site. There is nothing in these models which forbids two such events in separate segments of the same hybrid DNA region. However, the recent study of FOGEL and MORTIMER (1969) of unselected tetrads in yeast crosses segregating at two sites in the same gene revealed many cases of single conversion at either site and of co-conversion of both sites; conspicuously absent was the class representing conversion to one parent type at the first site and conversion in the opposite direction at the other. They found no evidence of multiple conversion events even though the procedure should have revealed them had they occurred.

We can detect conversion of two separate segments in a cross which is segregating at three sites within the same gene. In the $\alpha \times \beta$ crosses which are also segregating at *t*, we look at the recombinants with the marker combination representing conversion at α and ask whether it is ever accompanied by conversion at the *t* site. If so, there must be two separate, converted segments because the intervening β site is necessarily not converted. The pertinent data are in the

first two rows of Table 3, and we see that 35 of the 146 α convertants reveal a second converted segment.

As mentioned earlier, there is a possibility that the order of sites is α-γ-t-β. If so, co-conversion of α and t in the cross with β could result from a single converted segment. However, the α conversions in the crosses to γ would provide the test for multiple conversion segments. Table 3 shows that the critical classes are even more frequent here than in the $\alpha \times \beta$ crosses.

The large number of double conversions revealed in this analysis represents a *minimal* estimate of the frequency of such events; we could only identify those cases in which separate segments including the outside sites were converted in the same direction, and in which the middle site was not included in either converted segment. In the earlier discussion of the size of a converted region, a model for the conversion of an interrupted segment was rejected because co-conversion of neighboring sites was seen to occur with frequencies well above fifty percent. The double conversions described here represent a special class of conversion of an interrupted segment. However, this does not conflict with the earlier result, which only argued against a model with interruptions at each successive point in the segment; both results are compatible with interruptions spaced at larger intervals.

FOGEL and MORTIMER (1969) observed no multiple conversion events, but it is important to note that the type they were able to detect differed from ours in two ways: 1) it required conversion in *opposite* directions in the two segments; 2) it required coincident conversions in two members of the tetrad.

Gene conversion frequencies of different mtr *mutants:* LISSOUBA *et al.* (1962) studied two-point crosses involving a series of allelic spore color mutants in Ascobolus. In a given cross, recombination *always* resulted from conversion of the *same* mutant parent. According to their recombination map of these mutants, conversion showed a consistent polarity. For any given pair, the conversion always involved the mutant to the right. They concluded that a converted segment could begin anywhere within this region (called a "polaron") and would always extend to the right end. This interpretation leads to the prediction that gene conversion frequency should be minimal for mutants at the left end of the polaron and show a constant rise for sites farther and farther to the right.

RIZET and ROSSIGNOL (1966) also found polarity in the frequencies of conversion of spore color mutants at another locus in Ascobolus. In this study, conversion was minimal for mutants at both ends of the region and higher for mutants toward the middle.

KITANI and OLIVE (1967) found that crosses of wild type to four allelic spore color mutants in Sordaria all gave similar total frequencies of "aberrant segregation" (including 6:2, 5:3, 3:5, 2:6 and certain kinds of 4:4), but for a single class, such as 6:2, there was considerable fluctuation between the different mutants.

Recent studies of this type in Ascobolus (KRUSZEWSKA and GAJEWSKI 1967; ROSSIGNOL 1969) have shown no simple polarity of conversion frequencies. In

TABLE 4a

Estimates of the frequency of conversion to mtr+ *of different* mtr *alleles in the cross* hca–mtr × hpA

mtr allele	Total spores $\times 10^{-3}$	Percent germination	"Convertants" (*pdx*+ *mtr*+ *col*)	Convertants per 10^6 spores
14 (α)	520	70	17	33
119 (α)	121†	40	4	32
121 (α)	269	50	6	22
30 (β)	500	65	48	96
117 (β)	165	60	14	85
10 (γ)	370	70	37	100
120 (γ)	405	60	29	72
112	534	50	25	47

† Total spores recorded here are half of the actual total because the presence of an unlinked resistant mutant prevented detection of half of the convertants.

one case a mutant with a very high frequency of conversion mapped at or very near the same site as one showing no conversion.

We have attempted to estimate the relative frequencies of conversion to wild type for the primary *mtr* mutants in the cross *hca-mtr* × *hpA* by the method described earlier. Specifically, we have measured the frequency with which the *mtr*+ allele is recovered with both markers from the other parent. We have assumed that this could only happen by gene conversion at *mtr*. The results are shown in Table 4a. There is a suggestion of polarity in that the α mutants show low frequencies, while the frequencies for β and γ are somewhat higher.

Frequencies of conversion from wild type to mutant have been estimated for each of the primary mutants crossed to *mtr*+, by a method parallel to that employed above. In this case the cross is *hca* × *hpA–mtr*. Selection is made for *mtr* in the presence of the markers which came into the cross with its wild type allele (*pdx*+ *col*). The results are shown in Table 4b. Most of the frequencies

TABLE 4b

Estimates of the frequency of conversion of mtr+ *to mtr for different alleles in the cross* hca × hpA–mtr

mtr allele	Total spores $\times 10^{-3}$	Percent germination	"Convertants" (*pdx*+ *mtr* *col*)	Convertants per 10^6 spores
14 (α)	59	75	1	17
121 (α)	151	40	2	13
30 (β)	25	60	1	40
117 (β)	408	35	2	5
10 (γ)	34	80	7	206
120 (γ)	236	60	10	39
112	251	30	5	20

are very low, and there does not appear to be any simple relationship between frequency and position of the mutant site.

The influence of a nearby segregating site on recombination: KRUSZEWSKA and GAJEWSKI (1967), in the study mentioned earlier of seven allelic spore mutants of Ascobolus, measured the frequency of conversion to wild type (3:1 segregation) for each mutant in a cross to wild type. They found that the frequency of wild-type recombinants in a cross between two mutants in separate clusters was very close to the sum of the conversion frequencies of the two mutants. However, a cross between mutants in the same cluster produced wild-type progeny with a frequency much lower than the sum of the conversion frequencies. There are two ways by which the proximity of the segregating sites might lower the rate at which convertants are recovered as wild-type recombinants: 1) it might prevent the occurrence of the conversion event; 2) it might prevent the *detection* of the event. The first alternative represents the suggestion of HOLLIDAY (1964) that heterozygous sites could inhibit genetic pairing. However, the same author (HOLLIDAY 1968) later pointed out that the similarity of recombination to the dark repair of pyrimidine dimers suggested an obvious basis for the second alternative: if the second site were close enough to be included in the same converted segment with the first, the event would not yield a wild-type recombinant.

We have tested these hypotheses by using the temperature mutant as the second site. This mutant does not prevent the detection of conversion of the other segregating mutant. Thus, it should permit us to look directly at the effect of nearby heterozygosity on the frequency of gene conversion.

Frequencies of conversion to mtr^+ were estimated for each of the primary mutants, just as they were in the experiment reported in Table 4a, with the exception that the *hpA* parent carried *mtr-t* instead of mtr^+. Frequencies were compared to those of the previous experiment, and the selected progeny were

TABLE 5

Estimates of the frequency of conversion for different mtr *alleles in the cross to the temperature mutant* (hca–mtr × hpA–mtr-t)

mtr allele	Total *spores* $\times 10^{-3}$	Percent germination	Convertants pdx^+ mtr^+ *col*	Convertants pdx^+ *mtr-t* *col*	Convertants per 10^6 spores	Convertants per 10^6 spores in the cross to mtr^+ (from Table 4a)
14 (α)	175	65	1	14	86	33
119 (α)	94†	50	3	8	117	32
121 (α)	211	45	3	12	71	22
30 (β)	170	45	1	17	106	96
117 (β)	144	55	0	23	160	85
10 (γ)	101	70	0	13	129	100
120 (γ)	145	70	1	13	97	72
112	96	55	0	10	104	47

† Total spores recorded here are half of the actual total because the presence of an unlinked resistant mutant prevented detection of half of the convertants.

tested for *mtr-t* to determine what fraction showed conversion at both sites. The results are shown in Table 5, and they clearly favor the second hypothesis. The presence of a nearby segregating site does not lower the frequency of conversion; in fact, the frequencies are somewhat higher. Furthermore, the great majority of selected convertants show co-conversion at the *mtr-t* site; thus, they would have gone undetected if the analysis had been designed to select wild-type recombinants.

In crosses to mtr^+ (Table 4a) the α mutants gave low conversion rates while those in the β and γ clusters gave high rates. In crosses to *mtr-t*, mutants in all three clusters give similarly high rates. Perhaps the presence of at least one segregating site in the β–t–γ region is required to stimulate the maximum incidence of conversion events.

Table 6 gives the data on the effect of segregation at *t* on intragenic recombination between mutants in separate clusters. Frequencies in two-point crosses (no segregation at *t*) are compared to those in three-point crosses (segregation

TABLE 6

The effect of segregation at t *on the frequency of recombination between* mtr *mutants in separate clusters*

$\alpha \times \beta$ Cross No.	Parents	mtr^+ per 10^6	$\beta \times \gamma$ Cross No.	Parents	mtr^+ per 10^6	$\alpha \times \gamma$ Cross No.	Parents	mtr^+ per 10^6
Two-point crosses								
8	*14 × 117*	37	2	*10 × 117*	61	3	*10 × 119*	99
15	*30 × 119*	140	16	*30 × 120*	85	3a	*10 ×* 119^s	98
15a	*30 ×* 119^s	129	24	*117 × 120*	85	5	*10 × 121*	67
17	*30 × 121*	85	30	*120 × 117*	49	10	*14 × 120*	56
26	*119 × 117*	103				27	*119 × 120*	72
35	*121 × 117*	52				27R	*119 × 120*	100
						31	*120 × 119*	114
						31R	*120 × 119*	94
						31a	*120 ×* 119^s	147
						32	*120 × 121*	66
						37	*121 × 120*	147
Three-point crosses								
70	*14 × t-6*	25	61	*10 × t-6*	17	56	*10 × t-1*	122
73	*14 × t-9*	29	64	*10 × t-9*	22	59	*10 × t-4*	44
74	*30 × t-1*	88	75	*30 × t-2*	35	63	*10 × t-8*	70
77	*30 × t-4*	112	76	*30 × t-3*	33	66	*14 × t-2*	27
81	*30 × t-8*	101	80	*30 × t-7*	27	67	*14 × t-3*	13
95	*119 × t-6*	46	103	*120 × t-6*	30	71	*14 × t-7*	47
97	*119 × t-9*	19	106	*120 × t-9*	27	91	*119 × t-2*	37
113	*121 × t-9*	39				92	*119 × t-3*	61
						98	*120 × t-1*	41
						101	*120 × t-4*	104
						105	*120 × t-8*	80
						108	*121 × t-2*	46
						111	*121 × t-7*	44

at t). There is no clear trend in the $\alpha \times \gamma$ crosses, and there is large variation in the frequencies in each class. The same is true of $\alpha \times \beta$. However, crosses between $\beta \times \gamma$ (the clusters believed to embrace the t region) give high frequencies in the two-point crosses, while frequencies in the three-point crosses are consistently low.

If segregation at t lowers the production of $\beta^+ \gamma^+$ recombinants, the previous finding that such segregation does not diminish 3:1 frequency at either β or γ would appear to be contradicted. However, both observations might be explained if the effect of an added segregating site were to extend the length of a converted region. This would prevent the production of a prototroph in those cases in which the converted segment extended as far as the second absolute mutant site, but it would not prevent the occurrence of 3:1 segregation. In terms of the hybrid DNA models, we might imagine that the excision system would "see" two heterozygous sites if they were close together and excise a segment long enough to include both; but when heterozygous sites were too far apart, the excised segment would end between them.

The factors determining recombination frequency: Genetic recombination maps are based on the assumption that the frequency of recombinants in a cross between two mutants bears a simple relationship to the distance between the mutant sites. However, this frequency may have a more complicated relationship to distance. According to formal genetic models like the switch model of FREESE (1957) or the fixed-pairing-region models of MURRAY (1961) and of STAHL (1961), the recombination frequency is the product of the frequencies of two events: the frequency of special pairing for recombination in the marked region multiplied by the likelihood of the required combination of exchange events when such pairing occurs. (In the hybrid DNA models the first event would be the formation of hybrid DNA in the marked region, and the second would be the required combination of excision-repairs.)

The data of Table 5 permit us to estimate the frequencies of these two events separately. If conversion is evidence that the first event has occurred, we may ask how often the second event (recombination) accompanies it. The answer is: very seldom. Less than ten percent of the recombination events, revealed to us by conversion, have culminated in the production of an *mtr*$^+$ recombinant; in all the others the converted segment has included both segregating sites. In this respect, the full array of *mtr* mutants corresponds to a single cluster of the ascospore color mutants of Ascobolus (KRUSZEWSKA and GAJEWSKI 1967).

The influence of homology in the marked region on recombination: The preceding section dealt with the effects of heterozygosity at t on recombination events within the *mtr* gene. We shall now consider the effects of nonhomology at other sites in the marked region (between *pdx-1* and *col-4*). There have been several studies in Neurospora indicating that the degree of parental homology affects the frequency of recombination. STADLER and TOWE (1962) and CAMERON, HSU and PERKINS (1966) observed increased recombination with inbreeding (increased homology). DESERRES (1958) observed the reverse correlation (although there were important differences in the experimental design), and NAKAMURA (1966) found that inbreeding caused increased recombination in some crosses and de-

creased recombination in others. All these observations dealt with intergenic recombination. STADLER (1959) found that increased parental homology had no effect on the frequency of intragenic recombination at the *cys-2* locus although it caused a three-fold increase in recombination between outside markers. STADLER and TOWE (1968) found that inbreeding resulted in an increase in prototrophic recombinants in a cross of two closely-linked but separate genes (*cys-1* and *cys-2*). However, the increase was confined to the marker class representing reciprocal recombination; the frequency of "gene conversion" classes was not markedly changed. These studies revealed no effect of inbreeding on intragenic recombination, but the inbreeding procedure systematically maintained any nonhomology in the genetically marked region. In the present study we wished to look directly at the effect of homology within the marked region on intragenic recombination at *mtr*.

The crosses of Tables 1 and 2 can be divided into two classes. Class 1 refers to all crosses in which the parents were vegetative derivatives of *hca* and *hpA*. These crosses should have been identical in genetic constitution except at the mutant sites in *mtr*; Class 1 represents the *minimum* homology in the *pdx–col* region. Class 2 refers to those crosses in which the *hca* parent carried an *mtr* mutant which was originally isolated in *hpA* and had been inserted into the marked region of the *hca* parent, presumably by gene conversion. Whatever unknown heterology was present in the *pdx–col* region in the Class 1 crosses is maintained in Class 2, except for the converted segment; this segment must be homologous at all points except the *mtr* mutant sites (see Figure 5). The length of the converted segment is unknown. It could be about as long as the *mtr* gene, which is one percent of the distance from *pdx* to *col* (in recombination frequencies).

Table 7 compares the average recombination frequencies for Class 1 and Class 2 crosses for the different pairwise combinations of clusters at *mtr*. It is not clear whether the crosses segregating at *t* (three-point crosses) should be included in these calculations, since that factor itself caused lowered recombination frequencies in some crosses. The averages are shown both including and excluding the three-point crosses. Both methods of calculation are alike in showing no pronounced or consistent differences in recombination frequencies between Class 1 and Class 2.

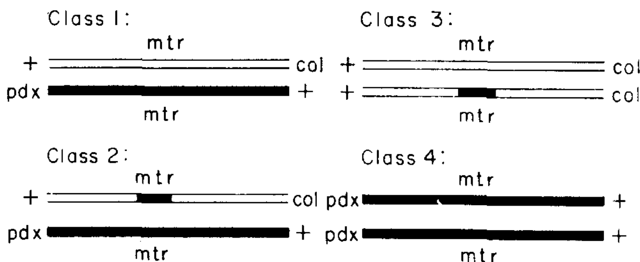

FIGURE 5.—The degree of homology within the marked region in the various classes of crosses between *mtr* alleles. See Text.

TABLE 7

Effect of genetic homology in the marked region on intragenic recombination frequency

	Average recombinant frequencies		
	$\alpha \times \beta$	$\alpha \times \gamma$	$\beta \times \gamma$
Class 1	all crosses: 747/9=83	all crosses: 643/10=64	all crosses: 280/7=40
	2-point crosses: 391/4=98	2-point crosses: 320/4=80	2-point crosses: 146/2=73
Class 2	all crosses: 259/5=52	all crosses: 812/11=74	all crosses: 191/4=48
	2-point crosses: 155/2=77	2-point crosses: 399/4=100	2-point crosses: 134/2=67

Comparison of frequencies in crosses of same mutants with different degrees of homology

Parents	Recombinant frequency Class 1	Class 3	Parents	Recombinant frequency Class 2	Class 4
10 × 119	99	193	*119 × 117*	103	134
10 × 121	67	69	*120 × 117*	49	91
14 × 120	56	40	*120 × 119*	114	239
30 × 120	85	44	*121 × 117*	52	224
30 × 121	85	128	*121 × 120*	147	228

Two more classes of crosses were made in which the homology was increased. For Class 3, the *hca–mtr* parents from Class 2 crosses were isolated in the opposite mating type and crossed to the *hca–mtr* parents from Cross 1 crosses. This resulted in complete homology in the *pdx–col* region except for the converted segment at *mtr* (see Figure 5). In Table 7 the five Class 3 crosses are compared to the corresponding Class 1 crosses. There are considerable fluctuations but no consistent direction of change in frequency.

The parents for the Class 4 crosses were derived by reisolating *hpA–mtr* strains from Class 1 in the opposite mating type and crossing to other *hpA–mtr* strains. This resulted in complete homology throughout the marked region (see Figure 5). The five crosses of Class 4 were compared to the Class 2 crosses involving the same pairs of mutants (Table 7). All pairs showed a pronounced increase in recombination in the Class 4 crosses.

We conclude that heterology in the *pdx–col* region of the original parents limited intragenic recombination at *mtr*. It is curious that this limitation was removed only by complete homology for the whole region; it was not removed either by homology for the small segment at *mtr* or by homology for all of the region excluding that segment. However, the small number of crosses makes this a tentative conclusion.

Lindegren gene conversion: The term "gene conversion" has been used in earlier sections of this report to refer to any departure from a 2:2 segregation at a heterozygous locus. LINDEGREN (1953) used the term in reference to a proposed

mechanism for the nonreciprocal segregation: "the conversion of a dominant gene into a recessive allele in the heterozygote." He stated that the converted gene might not be identical to either allele in the parent heterozygote. This *mutational* quality of LINDEGREN's gene conversion hypothesis lent itself to experimental testing. Several investigators have examined the products of nonreciprocal segregation or of intragenic recombination to see whether they carried genetic information different from that carried by either parent. Specifically, mutant products of nonreciprocal (1:3) segregation have been compared to their mutant parents, and prototrophs resulting from intragenic recombination (3:1) have been compared to their wild-type ancestors.

The *mtr* mutants originally isolated in the *hpA* strain were crossed to *hca*, and ascospores were plated in H4MT to select the genotype *hist* pdx^+ *mtr col.* These spores, in which the *mtr* mutant was inserted between the markers from the other parent, were assumed to represent 1:3 segregation at *mtr*; they were also used as the *hca–mtr* parents in subsequent mapping crosses to *hpA–mtr* strains (Class 2 crosses of Table 7). Control crosses were made in which each of these *hca–mtr* strains was crossed to the *hpA–mtr* strain carrying the same *mtr* allele. One of the first of such crosses was *hca–mtr116* × *hpA–mtr116*, and this cross unexpectedly produced mtr^+ progeny (Table 8). This led to the suspicion that the *hca–mtr* parent might not be *mtr116*, but a new mutant allele produced in the preceding cross by Lindegren gene conversion. A number of experiments were designed to detect Lindegren gene conversion at the *mtr* locus, because various selective plating techniques made this locus especially suitable for the purpose.

"Selfed" crosses of putative 1:3 segregants from five other *mtr* mutants were analyzed (Table 8). In every case they failed to produce any mtr^+ recombinants.

Sixteen mtr^+ recombinants from crosses between different mutant alleles were examined to determine whether they were the products of gene conversion (Table 9). They were crossed to wild type (the ancestral mtr^+ allele which was present before the *mtr* mutants were isolated), and large numbers of progeny were plated on HP4MT to detect rare *mtr* recombinants. The results were negative (the two *mtr* colonies recovered from platings of over six million ascospores were not considered significant). The same testcross was performed on a putative product of conversion to mtr^+ from the experiment reported in Table 4. It appeared to be identical to the mtr^+ parent by this test (Table 9).

An experiment was designed to permit direct selection of *mutant* gene conversion products. This involved the use of an *mtr* mutant parent of unique phenotype—one which failed to grow on a selective medium which supported the growth of strains carrying the fully mutant alleles. The unique mutant, *mtr-t*, was crossed to mtr^+, and the progeny were plated on HP4MT and on HPFPA at 18°C. There were no resistant spores in a population of over a million.

The instability of *mtr116* appeared to be unique. Nine more cross-reisolates of this mutant were crossed back to the parent (*hpA–mtr116*), and mtr^+ "recombinants" were produced in every case (Table 8). The only cross in which it failed to give mtr^+ progeny was to the putative deletion mutant *mtr-t-5*. The

TABLE 8

Selection of mtr+ *progeny from "selfed" crosses of* mtr *mutants*

Cross	Total spores × 10^{-3}	Percent germination	*mtr*+
hca–mtr116(CR1) × *hpA–mtr116*	160	8	7*
hca–mtr116(CR2) × *hpA–mtr116*	588	6	34
ha–mtr116 (CR3) × *hpA–mtr116*	840	6	6
hpa–mtr116(CR4) × *hpA–mtr116*	571	26	33
hpa–mtr116(CR5) × *hpA–mtr116*	497	5	17
hpa–mtr116(CR6) × *hpA–mtr116*	513	11	9
hpa–mtr116(CR7) × *hpA–mtr116*	90	7	5
hpa–mtr116(CR8) × *hpA–mtr116*	70	4	1
ha–mtr116 (CR9) × *hpA–mtr116*	650	10	91
ha–mtr116(CR10) × *hpA–mtr116*	142	5	2
hca–mtr112 × *hpA–mtr112*	1674	5	0
hca–mtr119 × *hpA–mtr119*	3352	75	0
hca–mtr120 × *hpA–mtr120*	739	40	0
hca–mtr121 × *hpA–mtr121*	848	4	0
hca–mtr × *hpA–mtr-t*	294	85	0
Crosses between cross-reisolates of *mtr116*			
hpa–mtr116 *hpA–mtr116*			
CR11 × CR13	371	10	3
CR12 × CR17	100	10	0
CR14 × CR17	192	20	0
CR15 × CR19	210	4	0
CR16 × CR19	428	9	0
CR18 × CR23	454	10	127
CR20 × CR21	480	15	82
CR22 × CR24	371	25	0
CR26 × CR28	287	14	0
CR27 × CR37	176	4	0

* 4 *pdx mtr+ col+*, 1 *pdx+ mtr+ col*, 1 *pdx+ mtr+ col+*, 1 *pdx mtr+ col*.

instability which was shown by *mtr116* in meiosis was not found in vegetative cells. A selective plating experiment involving 3 × 10^7 viable conidia of this strain gave only two *mtr*+ colonies.

Up to this point the results with *mtr116* resembled those of certain yeast mutants studied by MAGNI (1963). He concluded that meiotic instability was characteristic of reading frame mutants: in selfed crosses a low frequency of nonmutant progeny were produced as a result of unequal exchange which added or deleted a single nucleotide. However, further studies of *mtr116* indicated a different explanation. Mrs. AGNES TOWE pointed out that the original vegetative isolate of *hpA–mtr116* might have been a heterokaryon between two newly-induced *mtr* mutants. Possibly there were recessive lethal damages elsewhere in both genomes, insuring the perpetuation of the heterokaryon in vegetative transfer. Such a strain would have been phenotypically indistinguishable from a homokaryotic *hpA–mtr* strain. Cross-reisolates of *mtr116* would have been homokaryons representing

TABLE 9

Recombination test for identity of mtr+ *alleles*

Cross	Parents of tested *mtr*+ strain	Total spores $\times 10^{-3}$	Percent germination	*mtr* progeny
Control:				
hca × *hpA*	....	2097	75	0
(sum of four analyses)				
mtr+ recombinants				
crossed to *hca* or *hpA*:				
(sum of analyses	*hca–mtr119*	1013	60	1
of six *mtr*+)	× *hpA–mtr120*		(average)	
(sum of analyses	*hca–mtr120*	2486	70	1
of six *mtr*+)	× *hpA–mtr119*		(average)	
(sum of analyses	*hca–mtr112*	2705	75	0
of four *mtr*+)	× *hpA–mtr121*		(average)	
mtr+ "convertant"	*hca–mtr10*	932	85	0
crossed to *hca*:	× *hpA*			

only one of the two mutant alleles; therefore, a backcross to the original parent strain would have included two crosses, of which one would have produced *mtr*+ recombinants.

This hypothesis was tested by crosses between different pairs of cross-reisolates of *mtr116*. Some should carry the same mutant allele in both parents and give no recombinants. Among ten such crosses (Table 8), only three gave recombinants, supporting the two-mutant explanation of *mtr116*. (Such an explanation cannot apply to the yeast mutants which show meiotic instability; uninucleate haploid cells would not support the survival of such a heterokaryon.)

Thus, we have found no evidence of meiotic mutability at the *mtr* locus. Earlier tests in other systems also gave negative results. Roman (1956) examined the mutant members of 1:3 asci from crosses heterozygous for an adenine mutant in yeast. He consistently found all three to be identical to the parent mutant in terms of recombination. Fogel and Mortimer (1968) performed a very sensitive test for identity on the 1:3 asci from a yeast cross segregating for a suppressible arginine mutant; all the mutant spores were suppressible, indicating that the same nonsense codon was present in all of them.

Selected prototrophic recombinants from crosses between alleles have been examined for differences from true wild type. Giles (1958) measured the adenylosuccinase activity of recombinants from a Neurospora cross between two mutants of the gene controlling this enzyme and found them all identical to wild type. A similar test by Zimmermann (1968) on the threonine dehydratase activity of recombinants between allelic isoleucine mutants of yeast also showed no differences from wild type.

The present study provides the first extensive demonstration that prototrophs resulting from intragenic recombination are indistinguishable from wild type by a recombinational test. We have also performed an experiment which would directly select products of the hypothetical process: meiotic mutation at a hetero-

zygous locus. The consistently negative result of all these experiments argues strongly against the general occurrence of this process. This result is especially interesting in connection with the recent proposal of WITKIN (1968) that recombination in bacteria is mutagenic. She suggests a sequence of events much like those which have been envisaged for the hybrid DNA region in meiotic recombination: breakdown along one strand followed by repair synthesis. She proposes that breakdown is arrested by the enzymatic modification of a base, and that this may change the pairing properties of the base and result in a subsequent replication error.

MAGNI (1963) proposed that unequal exchange in meiosis results in additions and deletions of nucleotides. This hypothesis leads to the prediction of *forward* mutation in a cross between parents carrying identical wild-type alleles; the *mtr* system provides a sensitive test of this prediction, and the result is negative (Table 9). Furthermore, BRINK *et al.* (1969) have classed another allele (*mtr26*) as a reading frame mutant on the basis of studies of induced reversion, and it has failed to give any mtr^{+} progeny in extensive analyses of selfed crosses.

SUMMARY

A two-way selection system has permitted the recovery of both mutant and nonmutant products of recombination at the *mtr* locus of Neurospora. A conditional mutant at this locus has been used to provide an unselected site of segregation among progeny selected for recombination within the same gene. These five-point crosses (two outside markers plus three sites within *mtr*) have permitted us to draw several conclusions about the role of gene conversion (nonreciprocal segregation) in intragenic recombination:

1) conversion involves a continuous segment of genetic material;
2) a recombination event frequently involves the conversion of two separate segments within the same gene;
3) recombination between mutant sites in the *mtr* gene only occurs in a small fraction of those meioses which involve gene conversion at this locus.

Extensive analyses of mutant and nonmutant products of recombination at the *mtr* locus have revealed no evidence of new genetic sequences (different from those provided by the two parents). It appears that meiotic recombination at this locus is not a mutagenic event.

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