RECOMBINATION OF ALLELIC CYSTEINE MUTANTS IN NEUROSPORA[1]

D. R. STADLER AND AGNES M. TOWE

Department of Genetics, University of Washington, Seattle 5, Washington

Received May 20, 1963

UNTIL 1955 it was generally believed that genetic recombination between homologous chromosomes normally resulted from a precisely reciprocal exchange between two of the four strands of a meiotic tetrad, producing a tetrad with 2:2 segregation at every locus. However, in 1955, MITCHELL demonstrated that tetrads showing recombination between two closely-linked mutant sites frequently had nonreciprocal ratios (other than 2:2) at one of these sites. This suggested the possibility that recombination can be imprecise, yielding nonreciprocal products. MITCHELL (1955), in analyzing the genetic composition of these rare recombinants, noted that a large proportion of the recombinational events detected in such a short genetic region could not be explained by *single* exchange of the conventional kind. This reopened the question of the nature of the exchange events taking place in meiotic recombination.

The experiments reported here, designed to pursue this question, were performed with crosses between seven allelic cysteine-requiring mutants of *Neurospora crassa*. The results indicate that recombination within the cysteine gene is always nonreciprocal. In a cross between two cysteine mutants, cysteine-independent recombinants may arise from a nonreciprocal event at either of the parent mutant sites. About one half of these nonreciprocal recombinants for the cysteine gene are found in tetrads which show reciprocal recombination for outside markers, and in these tetrads the cysteine-independent recombinant is nearly always one of the two crossover strands. Certain aspects of the results reveal an asymmetry of the location of the cysteine mutants with respect to recombination. Thus, nonreciprocal ratios (other than 2:2) are more frequent at mutant sites on the left side of the cysteine locus than at those on the right. Reciprocal crossing over accompanying cysteine recombination nearly always results from an exchange at the left of the cysteine locus.

Finally, the bearing of these data on the general problem of recombination and its relationship to the organization of the genetic material is considered.

MATERIALS AND METHODS

The cysteine mutants were induced with ultraviolet light and isolated after filtration-enrichment from a stock of mating type *A* carrying the mutant genes

[1] This investigation was supported by a grant from the National Science Foundation and by State of Washington Initiative 171 funds for research in biology and medicine.

cot (C102t, colonial growth at temperatures above 31°C), *ylo* (Y30539y, yellow conidia), and *ad-1* (3254, adenine requirement). Ten cysteine mutants were picked up in this background, and all were crossed to a *cot lys-5* (DS6–85, lysine requirement) strain which was the product of six generations of backcrossing to the *cot ylo ad* strain. Seven of the ten cysteine mutants showed linkage to the other segregating mutants in these crosses and were found to lie in the same genetic region as the cysteine mutants *cys-1*, *cys-2*, *cys-c* and *cys-t* of previous studies (STADLER 1956, 1959a). All these genes are in the left arm of linkage group VI, and the order of the loci is *lys–cys–ylo–ad*–centromere. The other three cysteine mutants showed no linkage and were not studied further.

Progeny of the genotype *cot lys cys A* were isolated from these crosses and heterocaryons of the composition *lys* cys^a + cys^b *ylo ad* were built on minimal sorbose medium plus cysteine. Such "forced" heterocaryons were built for all 21 pairwise combinations of the seven mutants. Every heterocaryon had an absolute requirement for cysteine, demonstrating that these seven mutants are all located in the same cistron.

All subsequent crosses were made with progeny of these inbred strains, the *ad-1* mutant not being used further. All crosses were homozygous for *cot;* the small, dense colonies formed by *cot* strains at 33°C proved ideal for clean isolation of single spore colonies from plates containing dense populations of spores. Crosses between pairs of the cysteine mutants were constituted *cot* cys^a *ylo* × *cot lys* cys^b. The *cot cys ylo* strain was inoculated as protoperithecial parent into a slant of synthetic crossing medium supplemented with 500 μg/ml of L-methionine (which is more effective than cysteine in promoting fertility in these crosses). After five days at 25°C the cross was fertilized with conidia of the *cot lys cys* parent.

Three to five weeks after fertilization the crosses were analyzed for cysteine-independent recombinants among random ascospores. All the perithecia from the slant as well as shot spores on the agar or glass surface were immersed in 10 ml of water plus 0.1 percent agar in a small glass dish. The perithecia were teased apart with forceps and the entire mixture was poured into a screw-cap bottle and shaken vigorously. Filtration through a double layer of surgical gauze gave a heavy suspension of spores. The concentration was estimated by examining a sample in a blood-counting chamber. Suitable amounts were then added to flasks of water plus one percent agar at 59°C to give a concentration of about 2000 spores per ml. After 25 minutes at this temperature the suspension was pipetted in 4 ml aliquots onto plates of medium N plus lysine. The plates were incubated at 33°C for three days and then examined for colonies. These cys^+ colonies were isolated microscopically (to insure that no other spores were carried along) and grown up on slants of medium N plus lysine for later classification for the linked markers. In order to calculate the frequency of cys^+ recombinants, the total number of spores plated was determined by direct counts of sample areas from the plates.

Dissection of asci was performed on blocks of 4 percent agar in medium N plus lysine. The dissected asci were heat-shocked (30 minutes in a 60°C oven) on the block and incubated 24 hours at 33°C. Any ascus which contained one or more

spores showing wild-type growth was transferred to slants of complete medium for growth and classification. Dissections were also performed on crosses in which only one parent required cysteine. In this case the procedure was the same, except that the asci which were retained for further study were those which had more or less than two pairs of spores showing wild-type germination.

Recombination asci in crosses between cysteine mutants were also collected by the method of STRICKLAND (1960). The asci are not dissected. An agar surface is placed to catch the spores as they are ejected from ripe perithecia in the hope that they will come out in groups of eight, representing whole asci. In our experience the groups were not uniform, and we frequently found nine to twelve spores close together on the agar. Therefore, other unlinked markers were incorporated into the cross to confirm the identification of the members of an ascus. The eight putative members were accepted only if they segregated properly for mating type (linkage group I), *pdx-1* (39106p, pyridoxine requirement, linkage group IV) and *thi-4* (85902, thiamine requirement, linkage group III). The crosses were made in petri dishes. Shot spores were collected (starting about ten days after fertilization) by replacing the lid of the dish with another which contained a layer of 4 percent agar in medium N plus lysine, pyridoxine and thiamine. These collecting lids were rotated every few minutes and replaced after about four rotations, the frequency of rotation and replacement depending on the rate at which the spores were being ejected from the fruiting bodies. After exposure, the collecting lids were kept 24 hours at 25°C, then heat-shocked and incubated 24 hours at 33°C before being scanned microscopically for cys^+ spores. All the spores of any group (of seven to 14 spores) containing one or more such recombinants were transferred to slants for further study.

RESULTS

Table 1 shows the results of the analysis of random spores for cys^+ recombinants in crosses between various pairs of the cysteine mutants. There are 26 crosses in all, representing 19 of the 21 possible pairwise combinations. The crosses are listed in order of increasing frequency of cys^+ recombinants; the highest frequencies are about 150 times as great as the lowest. The event is called recombination, rather than mutation, because it occurs only in crosses between two different alleles at the cysteine locus. In control crosses in which each mutant was crossed to another derived strain carrying the same allele, no cys^+ spores occurred in populations of about 10^5 spores. (However, cys^+ progeny do occur with very low frequency even in "selfed" crosses, and it is possible that some of the cys^+ spores recovered from crosses 1, 2 and 3 of Table 1 resulted from this type of event.)

At the right in Table 1 the cys^+ recombinants are divided into four groups, according to which markers they carry. MITCHELL (1955) pointed out that if the event which produced the wild-type recombinant for the two middle sites were a single exchange, these recombinants should usually carry the same nonparental combination of markers. Her results, like those shown here, revealed all four

TABLE 1

Cysteine-independent recombinants among random ascospores from crosses between cysteine mutants

Cross	Groups	No. of spores screened	*cys*+ recombinants		Recombinants in the four marker classes			
					Parental		Recombinant	
			Number	Frequency per 10^5	P1 ++*ylo*	P2 *lys*++	R1 +++	R2 *lys*+*ylo*
A × *A*:								
1. *cys*^{7} *ylo* × *lys cys*^{4}	*A* × *A*	728,400	7	0.9	4	2	1	0
2. *cys*^{7} *ylo* × *lys cys*^{17}	*A* × *A*	992,000	10	1.0	6	1	3	0
3. *cys*^{4} *ylo* × *lys cys*^{17}	*A* × *A*	867,400	22	2.5	10	3	1	8
B × *B*:								
4a. *cys*^{38} *ylo* × *lys cys*^{15}	*B* × *B*	491,900	23	4.7	8	5	7	3
4b. *cys*^{15} *ylo* × *lys cys*^{38}	*B* × *B*	352,400	26	7.4	11	10	4	1
5a. *cys*^{15} *ylo* × *lys cys*^{64}	*B* × *B*	211,200	18	8.5	7	3	4	4
5b. *cys*^{64} *ylo* × *lys cys*^{15}	*B* × *B*	53,700	4	7.5	0	1	2	1
6. *cys*^{9} *ylo* × *lys cys*^{38}	*B* × *B*	682,200	83	12.2	19	26	21	17
7a. *cys*^{15} *ylo* × *lys cys*^{9}	*B* × *B*	359,200	60	16.7	16	21	7	16
7b. *cys*^{9} *ylo* × *lys cys*^{15}	*B* × *B*	128,800	24	18.6	5	5	9	5
8a. *cys*^{64} *ylo* × *lys cys*^{9}	*B* × *B*	169,000	53	31.4	14	9	12	18
		uncounted	116	...	41	20	18	37
8b. *cys*^{9} *ylo* × *lys cys*^{64}	*B* × *B*	58,700	24	40.9	6	3	9	6
		uncounted	113	...	17	23	54	19
9. *cys*^{64} *ylo* × *lys cys*^{38}	*B* × *B*	348,600	136	39.0	36	29	40	31
A × *B* (or *B* × *A*):								
10a. *cys*^{9} *ylo* × *lys cys*^{4}	*B* × *A*	170,700	166	97.4	16	72	59	19
10b. *cys*^{4} *ylo* × *lys cys*^{9}	*A* × *B*	96,000	100	104	36	9	9	46
11. *cys*^{7} *ylo* × *lys cys*^{64}	*A* × *B*	81,300	83	102	45	13	12	13
12a. *cys*^{17} *ylo* × *lys cys*^{64}	*A* × *B*	42,000	45	107	12	10	8	15
12b. *cys*^{64} *ylo* × *lys cys*^{17}	*B* × *A*	60,900	65	107	12	23	20	10
13. *cys*^{9} *ylo* × *lys cys*^{17}	*B* × *A*	267,700	297	111	44	109	100	44
14. *cys*^{7} *ylo* × *lys cys*^{9}	*A* × *B*	80,000	90	112	60	13	8	9
15a. *cys*^{4} *ylo* × *lys cys*^{15}	*A* × *B*	163,900	196	120	66	42	37	51
15b. *cys*^{15} *ylo* × *lys cys*^{4}	*B* × *A*	221,100	305	138	97	103	47	58
16. *cys*^{7} *ylo* × *lys cys*^{15}	*A* × *B*	106,200	135	127	55	27	27	26
17. *cys*^{15} *ylo* × *lys cys*^{17}	*B* × *A*	84,000	114	136	26	35	33	20
18. *cys*^{64} *ylo* × *lys cys*^{4}	*B* × *A*	120,700	159	132	28	54	47	30
19. *cys*^{7} *ylo* × *lys cys*^{38}	*A* × *B*	56,100	82	146	40	9	10	23

possible marker combinations in appreciable frequencies, and thus made the single exchange (crossover) hypothesis untenable. There is, however, a correlation of *cys*$^{+}$ progeny with recombination for the surrounding markers, a feature also pointed out by MITCHELL. In the present case, the frequency of recombination between *lys* and *ylo* in these crosses is 15 percent (*lys*–6–*cys*–9–*ylo*), while among *cys*$^{+}$ spores nearly 50 percent carry nonparental combinations of the *lys* and *ylo* loci.

In seven cases, the same pair of mutants has been employed in a second cross with the marker alignments reversed. If the presence of the markers does not

influence the nature or the frequency of the recombination event, and does not influence the viability of the different classes of progeny, the two crosses should give the same overall frequency of cys^+ recombinants. With regard to the different marker combinations, the ratios of the paired classes should be reversed. That is, P1/P2 in the first cross should equal P2/P1 in the cross with markers reversed. Similarly, R1/R2 in the first cross should equal R2/R1 in the second. The results fit these predictions quite satisfactorily.

A genetic map of the cysteine mutants, based solely on the frequencies of cys^+ recombinants, is shown in Figure 1. In those cases in which two crosses were made between the same two *cys* mutants, the results have been summed to determine the frequency. The mutants fall into two clusters, crosses between two members of the same cluster giving rare recombinants, while crosses between mutants from different clusters yield more frequent recombinants. To this extent the map "makes sense" and strengthens our confidence that the frequency of these recombinants is some kind of measure of the spatial separation of mutant sites. The cluster composed of cys^4, cys^7 and cys^{17} has arbitrarily been called the *A* group, and the four mutants of the other cluster make up the *B* group. Group *A* has been placed to the left of group *B* (distal to *B* with respect to the centromere) because in nearly all the $A \times B$ crosses, the more frequent nonparental marker combination among the cys^+ recombinants is that which could result from a single exchange if the order of sites were *lys–A–B–ylo*. This method cannot be employed to order the sites within a cluster, because the number of recombinants is too small. The sites have been arranged in the order which best fits the observed frequencies, but its accuracy is doubtful.

Frequencies of cys^+ recombination are expressed here in terms of *total* spores, while other investigators have used *germinated* spores as the denominator. We believe that, in our material at least, total spores give a more consistently accurate measure of the frequency at meiosis. When crosses have been matured for too short a time or at suboptimal conditions of temperature or nutrition, the number

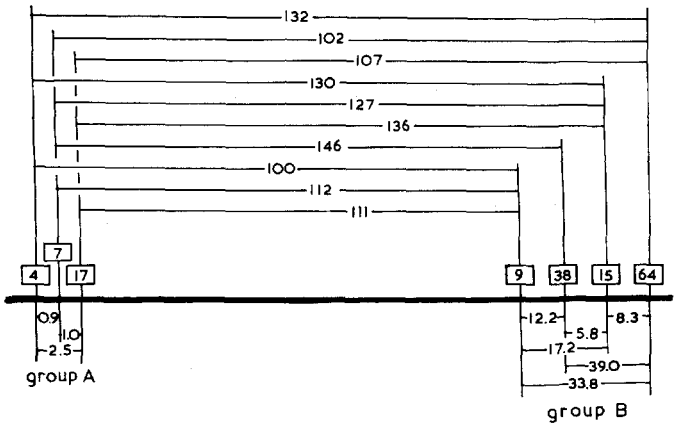

FIGURE 1.—Recombination map of the cysteine mutants. The intervals are measured in frequencies of cys^+ recombinants per 10^5 ascospores.

of cys^+ recombinants for a given number of spores remains almost as high as under optimal conditions, even though the fraction of spores capable of germination may drop appreciably. This suggests that the cys^+ spores are able to mature under a wider variety of conditions and are, therefore, more hardy than the mutants. The spores which fail to germinate probably include very few of the cys^+ genotype. Spore germination in the crosses reported in Table 1 ranged from 35 to 80 percent.

In considering various hypotheses about the exchange events involved in recombination at the *cys* locus, an important facet of the data is the ratio of frequencies of the four different marker combinations among the cys^+ recombinants. In the $A \times A$ crosses (Table 1) the numbers are too small to give any accurate estimate of this ratio. A pattern of inequalities emerges in the $A \times B$ crosses, though there are exceptions which obscure the pattern. In these crosses the two marker combinations (one parental and one recombinant) which carry the marker from the cys^A parent on the right (*ylo* locus) tend to be equally-frequent majority classes; the two classes carrying the right-hand marker from the cys^B parent tend to be equally-frequent minority classes. Of the 13 $A \times B$ crosses, Number 15b is the only one that does not follow this pattern at all. The other 12 crosses all fit the pattern, if one ignores the R2 (*lys* cys^+ *ylo*) class in the crosses involving cys^7, in which this class is less frequent than would be predicted by the pattern. (The original cys^7 stock produced crosses of low fertility with many abnormally-shaped spores. Although crossing has produced a stock which is free of these gross effects, it is possible that the cys^7 stock still carries a second alteration of the chromosome which is modifying the recombination pattern in the *lys–cys–ylo* region.) In spite of the exceptions, we propose to defend the view that the pattern described above does represent the basic products of recombination in the $cys^A \times cys^B$ crosses and that a valid hypothesis should predict this pattern.

The numbers of cys^+ progeny studied in the $B \times B$ crosses are large enough to give some information on this point, and in most of these crosses there appears to be no pronounced or consistent departure from a 1:1:1:1 ratio. However, in the pair of crosses for which the data are most extensive (8a and 8b) there is an indication of a pattern similar to that observed in the $A \times B$ crosses.

Table 2 shows the analysis of asci containing cys^+ recombinants in crosses between pairs of the cysteine mutants. Among 1651 dissected asci (in which at least three spore pairs germinated) from the cross between cys^{17} and cys^{64}, there were 14 which contained cys^+ spores. Twenty-three asci with cys^+ recombinants were isolated from the undissected material of this cross, but nine of these were rejected because the eight spores making up the recombination ascus could not be identified with assurance. From the other three crosses analyzed by this method ($cys^{38} \times cys^4$, $cys^{38} \times cys^{64}$ and $cys^9 \times cys^{64}$), 40 clusters with cys^+ spores were isolated, but definite identification of the components of the recombination ascus was possible in only 17 of these. No counts were made of the numbers of spores examined in the collections of undissected asci.

The order of the spore pairs is not accurate for some of the asci shown in Table

TABLE 2

Analysis of asci with cysteine-independent recombinants in crosses between cysteine mutants

lys cys^{17} × cys^{64} (*A* × *B*)

1651 dissected asci:

miscopy at cys^{17} (ten asci):

lys	cys^{17}	+	*lys*	cys^{17}	+	*lys*	cys^{17}	+
lys	+	+	+	+	+	*lys*	cys^{64}	+
+	cys^{64}	*ylo*	*lys*	cys^{64}	*ylo*	+	+	*ylo*
+	cys^{64}	*ylo*	+	cys^{64}	*ylo*	+	cys^{64}	*ylo*
5 asci (Nos. 1–5)			4 asci (Nos. 6–9)			1 ascus (No. 10)		

miscopy at cys^{64} (four asci):

lys	cys^{17}	+	*lys*	cys^{17}	+	*lys*	cys^{17}	+	*lys*	cys^{17}	+
lys	cys^{17}	+	+	+	+	+	cys^{17}	+	*lys*	cys^{17}	+
+	+	*ylo*	*lys*	cys^{17}	*ylo*	+	+	*ylo*	+	+/cys^{64}	*ylo**
+	cys^{64}	*ylo*	+	cys^{64}	*ylo*	*lys*	cys^{64}	*ylo*	+	cys^{64}	*ylo*
1 ascus (No. 11)			1 ascus (No. 12)			1 ascus (No. 13)			1 ascus (No. 14)		

undissected asci:

miscopy at cys^{17} (six asci):

lys	cys^{17}	+	*lys*	cys^{17}	+	*lys*	+	+
lys	+	+	+	+	+	+	cys^{64}	+
+	cys^{64}	*ylo*	*lys*	cys^{64}	*ylo*	*lys*	cys^{17}	*ylo*
+	cys^{64}	*ylo*	+	cys^{64}	*ylo*	+	cys^{64}	*ylo*
3 asci (Nos. 15–17)			2 asci (Nos. 18, 19)			1 ascus (No. 20)		

miscopy at cys^{64} (eight asci):

lys	cys^{17}	+	*lys*	cys^{17}	+
lys	cys^{17}	+	+	cys^{17}	+
+	+	*ylo*	*lys*	+	*ylo*
+	cys^{64}	*ylo*	+	cys^{64}	*ylo*
6 asci (Nos. 21–26)			2 asci (Nos. 27, 28)		

lys cys^{38} × cys^{4} *ylo* (*B* × *A*) undissected asci:

miscopy at cys^{38} (one ascus):

lys	cys^{38}	+
lys	+	+
+	cys^{4}	*ylo*
+	cys^{4}	*ylo*
1 ascus (No. 29)		

miscopy at cys^{4} (five asci):

lys	cys^{38}	+	*lys*	cys^{38}	+	*lys*	cys^{38}	+
lys	cys^{38}	+	+	cys^{38}	+	+	cys^{38}	+
+	+	*ylo*	*lys*	+	*ylo*	+	+	*ylo*
+	cys^{4}	*ylo*	+	cys^{4}	*ylo*	*lys*	cys^{4}	*ylo*
2 asci (Nos. 30, 31)			2 asci (Nos. 32, 33)			1 ascus (No. 34)		

lys cys^{38} × cys^{64} *ylo* (*B* × *B*) undissected asci:

miscopy at cys^{38} (two asci):

lys	cys^{38}	+	*lys*	cys^{38}	+
lys	+	+	+	cys^{64}	+
+	cys^{64}	*ylo*	*lys*	+	*ylo*
+	cys^{64}	*ylo*	+	cys^{64}	*ylo*
1 ascus (No. 35)			1 ascus (No. 36)		

miscopy at cys^{64} (three asci):		
lys cys^{38} +		*lys* cys^{38} +
lys cys^{38} +		+ cys^{38} +
+ + *ylo*		*lys* + *ylo*
+ cys^{64} *ylo*		+ cys^{64} *ylo*
1 ascus (No. 37)		2 asci (Nos. 38, 39)
lys cys^{9} × cys^{64} *ylo* (*B* × *B*) undissected asci:		
miscopy at cys^{9} (three asci):		
lys cys^{9} +		*lys* cys^{9} +
lys + +		+ + +
+ cys^{64} *ylo*		*lys* cys^{64} *ylo*
+ cys^{64} *ylo*		+ cys^{64} *ylo*
1 ascus (No. 40)		2 asci (Nos. 41, 42)
miscopy at cys^{64} (three asci):		
lys cys^{9} +		*lys* cys^{9} +
lys cys^{9} +		+ cys^{9} +
+ + *ylo*		*lys* + *ylo*
+ cys^{64} *ylo*		+ cys^{64} *ylo*
1 ascus (No. 43)		2 asci (Nos. 44, 45)

* One spore of this pair was *cys*, the other *cys*+; the two spores were identical with regard to *lys* and *ylo*.

2. (In the undissected asci the order could not be determined, and the dissected asci with a crossover to the right of *ylo* in the same chromosome arm had a different order than shown.) To simplify the table, all asci showing the same segregation and recombination for the *lys–cys–ylo* region have been grouped together. Several asci contained ungerminated single spores, and in each case such a spore has been assumed to have the genotype of its sister spore. Two asci (Nos. 4 and 5) each had an ungerminated spore pair; in these cases the genotype has been assumed to be the same as the fourth pair in other asci which had identical germinated pairs.

It may be noted that the frequency of cys^+ recombinants in the dissected asci of the cross between cys^{17} and cys^{64} is about twice as high as among random spores of the same cross (Table 1). Also, the relative frequencies of the different types of recombinant asci in the dissected material from this cross appear to be different from the frequencies among the undissected asci. We believe that the random spore analysis gives the most accurate information on the frequency of cys^+ recombinants as well as on the ratio of the different marker combinations. The random spores are a sample of the entire progeny of the cross after sufficient aging for maximum germination. In the dissection, on the other hand, a deliberate selection was made for asci with eight black spores. The undissected asci came from relatively young fruiting bodies which ejected spores at a higher rate than the older ones and gave more frequent groups of eight. These selective methods used in the collection of tetrad data could favor certain classes of cys^+ recombinants, and thus obscure the ratios of types produced in meiosis.

Interesting asci from the crosses in which one parent carried a cysteine mutant and the other carried its wild-type allele are listed in Table 3. Included are all asci in which a segregation other than 4:4 was detected at the *cys* locus. At least one member of each spore pair grew in every ascus listed in this table. The number of asci with more than four cys^+ spores (21) is much higher than the number

with more than four *cys* spores (one). Since the method favors the detection of extra cys^+ spores, it is improbable that this is the true ratio of these events. Germination of cys^+ spores in these asci was over 90 percent, and these spores were always clearly recognizable at the time of examination. Germination of *cys* spores was not as good, and the germ tubes of some were so short that it was impossible to tell if germination had occurred without transferring the spore to a slant. There were, in fact, numerous asci which had fewer than four cys^+ spores but not more than four spores that were clearly *cys*; that is, one or more spores were not detectably germinated. Such asci were not studied further, and it is probable that some asci with more than four *cys* spores were missed for this reason. Whether the frequency at meiosis of events giving extra cys^+ spores is actually greater than that of the reverse event cannot be determined from these data. Such an unequal ratio of corresponding events has been observed in a well-studied case in Sordaria (KITANI, OLIVE and EL-ANI 1962).

In each of the crosses listed in Table 3 there were about a dozen asci which had 3:5 segregation patterns (3 cys^+:5 mutant). These were picked up on slants of complete medium, but, in every case, the fifth mutant spore (the one which was paired with a cys^+ spore) failed to grow further. It was concluded that these aberrant asci resulted from postmeiotic mutation at other loci and they were not included in the table.

The tetrads listed in Table 2 show no evidence of reciprocal recombination within the *cys* locus. None of the asci with cys^+ recombinants were found to contain the reciprocal recombinant (cysteine double mutant).

Note: A rigorous search for the double mutant was made in the recombination asci from the *cys* × *cys* crosses using the backcross test of MITCHELL (1955). Representatives of each of the three cysteine-requiring spore pairs of such an ascus were crossed back to both parent strains. In this way the *cys* allele of each strain could be identified. The cross to the parent carrying the same allele gave no cys^+ progeny (in samples of about 10^5 ascospores) while the cross to the other parent gave cys^+ recombinants in the same frequency as the original cross. The double mutant should give no cys^+ recombinants when crossed to either parent. The validity of this method in Neurospora has been verified in a case in which double mutants were recovered (CASE and GILES 1958). The backcross test was performed in 40 of the 45 asci listed in Table 2, but in three asci the test was incomplete because a spore (in ascus No. 14) or a spore pair (in asci Nos. 4 and 5) which could have been the reciprocal recombinant failed to germinate. The backcross test was not performed on the remaining five asci (Nos. 9, 17, 19, 25 and 26). A specific suppressor of cys^{64} *(su64)* which is dominant in a heterocaryon appeared spontaneously in one of the stocks. This permitted a spot test to distinguish cys^{64} from cys^{17}. Condidia of the strain under test were placed on conidia of cys^{17} *su64* on a minimal sorbose plate. If the unknown was cys^{64}, growth ensued; if it was cys^{17} there was no growth. This test was used to classify the *cys* spores of five asci, and while it was much simpler to perform than the backcross test, it could not distinguish cys^{17} from a cysteine double mutant.

The 37 recombination asci which were fully classified were all alike in that none contained a cysteine double mutant; in every one of them there was a 2:2 segregation at one of the cysteine mutant sites and a 3:1 (3 cys^+:1*cys*) ratio at the other. However, it is important to note that in a given cross the nonreciprocal ratio (3:1) may occur at either mutant site. In this report a segregating site at which a ratio other than 2:2 occurs will be referred to as a "miscopy site."

TABLE 3

Asci with aberrant segregation ratios in crosses segregating for cysteine requirement

lys cys^{17} × *ylo*. 2240 dissected asci:					
lys cys +	*lys cys* +	*lys* + +	*lys* + +	*lys cys* +	
lys + +	+ + +	+ *cys* +	+ + +	+ *cys* +	
+ + *ylo*	*lys* + *ylo*	*lys* + *ylo*	*lys cys ylo*	+ *cys ylo*	
+ + *ylo*	+ + *ylo*	+ + *ylo*	+ + *ylo*	*lys* + *ylo*	
3 asci	9 asci	1 ascus	1 ascus	1 ascus	
lys × *cys*^{64} *ylo*. 1637 dissected asci:					
lys + +	*lys* + +	*lys* + +	*lys* + +		
lys + +	+ + +	*lys* +/*cys* +*	+ + +		
+ + *ylo*	*lys* + *ylo*	+ + *ylo*	*lys* +/*cys ylo**		
+ *cys ylo*	+ *cys ylo*	+ *cys ylo*	+ *cys ylo*		
3 asci	2 asci	1 ascus	1 ascus		

* One spore of this pair was *cys*, the other *cys*$^+$; the two spores were identical with regard to *lys* and *ylo*.

The random-spore analysis (Table 1) yields extensive information about the frequencies of different marker combinations accompanying *cys*$^+$ recombination, but there is no direct way of determining which parent mutant was involved in the nonreciprocal event. The recombination tetrads do give this information, so one can ask whether a particular marker combination is diagnostic of a nonreciprocal event at a particular mutant site. Table 4 gives the results of this analysis for the 45 asci in which recombination took place between two *cys* mutants. The striking result is that the right-hand marker (*ylo* locus) nearly always identifies the *cys* mutant which has segregated 3:1. Among 25 asci in which the *cys*$^+$ recombinant carried the right-hand marker from the *cys*^{1} parent, 24 resulted from a miscopy at the *cys*^{1} site. The same correlation prevails for *cys*^{2} in 18 out of 20 asci. (There is a distance of nine map units between *cys* and *ylo*, so it may be that the three exceptions in 45 asci are cases in which a second recombination event to the right of the *cys* locus has changed the marker alignment.) On the other hand, there is no evidence of any correlation between the left-hand markers (*lys* locus) and the site of the miscopy.

If the replication of chromosomes in meiosis were strictly semiconservative, and if recombinants were formed in replication, each of the four chromatids of a

TABLE 4

Correlation of markers with the site of miscopy in cys$^+$ *recombinants. Forty-five asci from crosses of the form:* lys cys^1 × cys^2 ylo

Genotype of *cys*$^+$ recombinant	Site of miscopy	
	cys^1	*cys*^{2}
lys + +	14	0
+ + *ylo*	1	12
lys + *ylo*	1	6
+ + +	10	1

TABLE 5

Third-division segregation in recombination at the cysteine locus

	Site of miscopy	Number of asci tested	Number with 3rd-division segregation
Dissected:	*cys*^{17}	17	0
	cys^{64}	8	3
Undissected:	*cys*^{17}	5	0
	cys^{64}	11	0
	cys^{38}	2	0
	cys^{4}	2	0
	cys^{9}	3	0
	Total	48	3

tetrad would include an "old" half which would necessarily carry a parental combination of genes. None of the four products of meiosis could be a pure recombinant, and recombinants could segregate only in the third (first post-meiotic) division. In Neurospora this would result in spore pairs with one parental genotype and one recombinant. Third-division segregation has been detected at the *cys* locus in only three of the asci listed in Tables 2 and 3.

Note: Forty-eight of the 67 asci were analyzed in such a way as to detect this type of segregation, had it occurred (Table 5). In the early dissections the spores were germinated and picked up in pairs, rather than individually as was done in the later work. This meant that a pair including one *cys*$^+$ spore and one *cys* spore would be scored as *cys*$^+$. Eight of the recombination asci were handled in this manner. The other 11 cases in which third-division segregation could not be checked were mostly among the undissected asci, in which it was frequently difficult to isolate the two *cys*$^+$ spores separately for technical reasons.

DISCUSSION

The following results of the current study should be accounted for by any hypothesis for the mechanism of recombination: 1. Recombination at the *cys* locus is always nonreciprocal. 2. In a given cross, *cys*$^+$ recombinants may arise by a nonreciprocal event at either of the parent mutant sites. 3. The recombinants are nearly always pure at the conclusion of meiosis and do not segregate in a post-meiotic division. 4. Half (36 out of 67) of the *cys*-recombination asci have a crossover (reciprocal recombination) between *lys* and *ylo*. 5. The *cys*$^+$ recombinant is nearly always (18 out of 21) one of the two crossover strands. (This information can be obtained only in the asci from the *cys* × *cys* crosses, because the cysteine recombinant cannot be identified in the *cys* × *cys*$^+$ crosses.) 6. The *cys*$^+$ recombinant is strongly linked to the marker at the *ylo* locus. It carries the allele which came into the cross with the mutant at the miscopy site. (This enables us to identify the miscopy site in the *cys*$^+$ recombinants harvested as random spores.) 7. In a cross between a mutant in the *A* cluster and one in the *B* cluster, the majority of the *cys*$^+$ recombinants result from miscopy at the distal (*A*) site. 8. In all the crosses the two types of *cys*$^+$ recombinants carrying the

same marker at the *ylo* locus occur with approximately equal frequency. This means the *cys*$^+$ recombinants are unlinked to the *lys* locus.

Points 1, 2, 3, 4, 5 and 6 come from the tetrad data (Tables 2 and 3). Having established point 6, we are able to derive points 7 and 8 from the random-spore data of Table 1.

MITCHELL (1955) pointed out that while the recombination between closely-linked mutant sites did not have the characteristics of crossing over, it was strongly correlated with crossing over between the surrounding markers. She concluded that gene conversion, the term previously applied in the case of irregular segregations in yeast by LINDEGREN (1953), and crossing over were separate events and were correlated only because both were favored by the same special conditions (localized pairing, perhaps). FREESE (1957), on the other hand, proposed that gene conversion and crossing over could result from the same event. Several studies have subsequently shown that there is no rigid correlation between gene conversion and crossing over (MITCHELL 1957; ROMAN and JACOB 1958; STADLER 1959a, b). However, the pattern which has emerged from the tetrad analysis in the present study persuades us to attempt to design a model to account for both types of recombination.

There have long been two general theories about the mechanism of genetic recombination. One holds that recombinants are formed by breakage and reunion of two of the four chromatids of the meiotic tetrad. The other theory holds that recombinants are formed in the course of chromosome replication by a "copy-switch" (i.e., an exchange of templates) by the two homologous new strands. The occurrence of a segregation ratio other than 2:2 suggests an error in copying, and when this occurs at or near the site of recombination, it argues for the copy-switch theory in a modified form. The copy-error hypotheses to be described below hold that recombination results from an exchange of templates during replication, but the point of exchange does not have to be precisely the same for the two replicating strands. Thus both may copy the same template for a short distance. In the present study the correlation of nonreciprocal events at the *cys* locus with recombination for surrounding markers has led us to conclude that some form of the copy-error theory will give the most satisfactory account of this recombination.

In our discussion of possible mechanisms for the recombination at the *cys* locus we shall speak of the two old, or parental, strands (chromatids) and the two new strands formed in meiosis. This terminology assumes a conservative replication in the recombinational event (for the cysteine gene, if not the whole chromosome), and it seems to be justified by the rarity of third-division segregation among the recombinants at this locus (Table 5).

We shall first examine three copy-error models which have been designed by other authors to see if they can account for the observations at the *cys* locus.

Switch Hypothesis: FREESE (1957) proposed that each of the two new strands formed in meiosis duplicates the information along its own template strand until it comes to a "region of switching" (possibly a region of very close pairing). Here a duplicating strand may switch to the corresponding point on the other template

and proceed to copy it. After a short distance it may switch back to the original template, possibly switching three or more times in a short interval. The second new strand need not make all of the switches corresponding to those of the first but merely emerge from the switch region on the unengaged template (see Figure 2).

The switch hypothesis predicts that when mutant sites *a* and *b* are close together (Figure 2A) all four marker combinations may have similar frequencies among the recombinants. As the mutant sites *a* and *b* move farther apart (Figure 2B) the frequencies of the four recombinant types should diverge. The two parental combinations (A ++ A and B ++ B) should always be equal in frequency and should increase to a certain maximum rate which remains constant with further increase in the distance between *a* and *b*. This maximum rate would be the chance of a double switch around any point site. The B ++ A class should continue to increase until it includes nearly all of the recombinants. A ++ B should decrease and become zero when the distance between *a* and *b* exceeds the length of a switch region.

While these trends are not seen in our data ($A \times B$ crosses of Table 1), there is a loophole which should be mentioned. The trends are expected to be clear only when the distance between the mutant sites becomes large as compared with the length of a switch region. It is possible that even the most distantly spaced of the *cys* mutants are not far enough apart for this demonstration.

One prediction of the switch hypothesis, however, is not so easily excused. The

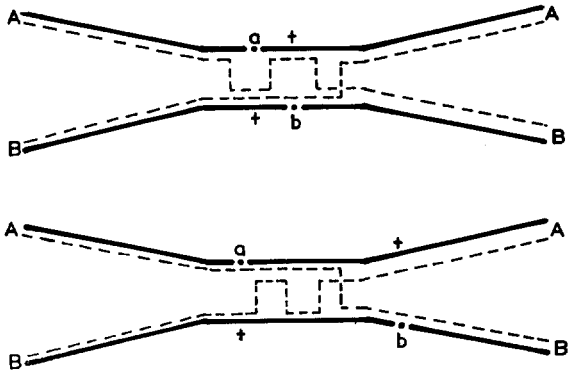

FIGURE 2.—Recombination by the Switch Hypothesis. The closely-linked mutant sites are *a* and *b*. The linked markers of the *a* parent are both designated *A*, and the markers linked to *b* are both called *B*. Thus the four products of the tetrad in the upper figure are:

A a+ *A*
A ++ *B*
B +*b A*
B +*b B*

The members of the tetrad shown in the lower figure are:

A a+ *A*
A a b B
B ++ *A*
B +*b B*

frequencies of the two parental marker combinations among the *cys*$^+$ recombinants should be *equal*, regardless of the distance between the mutant sites. This prediction is clearly violated by our data as well as by other extensive data in Neurospora (MURRAY 1960, 1963) and in Aspergillus (SIDDIQI 1962). In all three cases a consistent pattern of inequality for the parent types has demanded some modification of the switch hypothesis.

It was suggested in an earlier study (STADLER 1959a) that inequality of the two parental marker combinations among *cys*$^+$ recombinants might be explained by different sizes of the altered regions in the two *cys* mutants, and a detailed hypothesis based on this assumption was developed by EPHRUSSI-TAYLOR (1961) to explain recombination frequencies in transformation of Pneumococcus. Such an explanation hardly seems tenable with the present results from a larger series of mutants, since it would require a constant linear arrangement of mutant sites according to size. Furthermore, ELEANOR R. PIKER (unpublished) has found that all the *cys* mutants of the current study behave like point mutants in that they will revert when treated with ultraviolet irradiation, and her genetic analysis of these revertants points toward back-mutation rather than suppression.

The switch hypothesis makes one other prediction that is not substantiated by our data—that recombination between any pair of mutants will sometimes be reciprocal. Reciprocal recombination has been observed between mutants in the same functional gene in tetrad analyses of yeast (ROMAN 1958) and Neurospora (CASE and GILES 1958). However, these cases were rare (a total of three), and the much more extensive information on this point in Ascobolus (LISSOUBA, MOUSSEAU, RIZET and ROSSIGNOL 1962) strongly suggests that the chromosome is made up of a series of regions such that recombination between sites in the same region is never reciprocal. We prefer such an interpretation for recombination within the *cys* gene.

Fixed Pairing Regions: STAHL (1961) and MURRAY (1961) have pointed out that the disparity between the two parental marker combinations among the recombinants would be expected if one of the parent mutants were close to the end of the "effectively paired region." On this model, as in the switch hypothesis, recombination results from multiple exchanges within a very short segment. However, it imposes the restriction that the positions of such segments are not randomly distributed, but that they correspond to some fixed structural discontinuity along the chromosome. Such a scheme was considered by PRITCHARD (1960), but he found no support for it in his observations on recombination between adenine mutants in *Aspergillus nidulans*. STAHL's model involves the assumption that the chromosome is constructed of a series of circles, but he has pointed out (personal communication) that many of the same predictions can be made assuming a linear structure with fixed pairing regions. Such a model is demonstrated in Figure 3.

In a cross between two *cys* mutants, consider the pairing region containing the cysteine locus to be made up of three segments: Segment 1 includes the interval from the left end of the paring region to the left-hand mutant; Segment 2 is the interval between the two mutants; Segment 3 extends from the right-hand

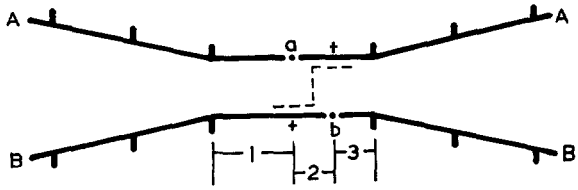

FIGURE 3.—Recombination between mutant sites in the same Fixed Pairing Region. The selected recombinants must have odd-numbered exchange in Segment 2, as shown. The markers carried by the recombinant will be determined by whether or not exchange occurs in Segment 1 and in Segment 3.

mutant to the right end of the pairing region. When the region is paired, there is a fixed probability of exchange per unit length along its entire extent. The STAHL model is not specifically a copy-error model, as he does not discuss the question of reciprocal and nonreciprocal recombination. However, we will assume that only one of the two new strands in a tetrad engages in switching and miscopying within the region spanned by the *cys* mutants. In this situation recombination will always be nonreciprocal.

The selected type of recombination (a^+ b^+) occurs only when an odd number of exchanges takes place in Segment 2.

The frequencies of the four marker-types among the recombinants will all be equal if the frequencies of odd-numbered exchange in Segments 1 and 3 are both one half.

The pattern of frequencies of marker types among cys^+ recombinants in the $A \times B$ crosses of Table 1 may be accounted for by assuming that the frequency of odd-numbered exchange in Segment 3 is less than that in Segment 1. The nonlinkage of recombinants to the left-hand marker means that in Segment 1 this frequency is one half. The average *number* of exchanges in Segment 1 could be one half or it could be greater.

The overall frequency of odd-numbered exchange in Segment 2 is measured by the frequency of cys^+ recombinants. However, it is impossible to determine the frequency of exchange in Segment 2 when the region is paired because we have no direct way of measuring how often it is paired.

The Fixed Pairing Region model accounts for the pattern of frequencies in the $A \times B$ crosses, but it would predict as great a departure from a 1:1:1:1 ratio of the recombinant types in the $B \times B$ crosses. This prediction is not clearly fulfilled by our results. Most of the $B \times B$ crosses give a close approximation to a 1:1:1:1 ratio. The pair of crosses with the most extensive data (8a and 8b) do show a pattern of inequality which is similar to the pattern seen in the $A \times B$ crosses and indicates that the mutant site of cys^9 is at the right of cys^{64}. However, this tells us only that these two mutant sites may not be close enough together to provide a critical test of the FPR model on this point. Furthermore, the recent study of recombination at the *paba-1* locus of *Aspergillus nidulans* by SIDDIQI and PUTRAMENT (1963) shows extensive data which bear critically on this very point, and, as the authors point out, the results argue strongly against an FPR model.

The Polaron: LISSOUBA, MOUSSEAU, RIZET and ROSSIGNOL (1962) have made an extensive analysis of tetrads with recombination between closely-linked mutants in Ascobolus. They have explained their results by proposing that there is a polarization of copy-errors in fixed recombination regions called polarons.

The only recombinational event which can occur within a polaron is a *single* switch by one of the new strands, resulting in a nonreciprocal ratio from that point to the terminal end of the polaron. Recombination in a cross between two mutants in the same polaron is thus always nonreciprocal, and the miscopy always takes place at the distal mutant site (left-hand site in Figure 4). If the mutants are in separate polarons, reciprocal recombination can take place. The authors concluded that the junction between two polarons ("linkage structure") was the site of reciprocal recombination. They also observed that copy-errors which occurred along the length of a polaron might sometimes be corrected at the linkage structure, but these corrections took place rather infrequently in the crosses which they studied. This abundance of uncorrected copy-errors throws doubt on the general applicability of the model (STADLER 1963).

It is somewhat awkward to explain the tetrad data of the present study by the polaron hypothesis. Recombination was consistently nonreciprocal, as expected when the mutants are in the same polaron. However, in any given cross either mutant could be the site of miscopy. This observation, which requires that the mutants be in separate polarons, was made even in the $B \times B$ tetrads (Table 2).

The Modified Polaron: A copy-error model which accounts for all the major points in the results of the current study can be achieved by making two modifications of the polaron model of LISSOUBA *et al.* In their scheme each copy-error resulted from a single switch and always persisted to the terminal end of the polaron. We propose that, while this sometimes happens, many of the copy-errors are corrected by a second switch only a small fraction of a polaron-length beyond the first (see Figure 5). This modification permits us to account for those aspects of the results which were not easily explained by the FPR model and the polaron model. (It should be pointed out that this "double switch" modification could be applied to the FPR model as well as the polaron model. Our reason for preferring a polaron scheme is based on another aspect of the results and will be developed below.)

The FPR model did not predict the close approximation to equality of the four recombinant types observed in some of the $B \times B$ crosses. It would have led us to anticipate a pattern of frequencies similar to that observed in the $A \times B$ crosses.

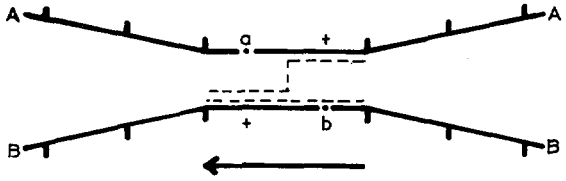

FIGURE 4.—The Polaron Model—recombination between two mutant sites in the same polaron. Recombination tetrads must *always* have a miscopy at the left-hand site.

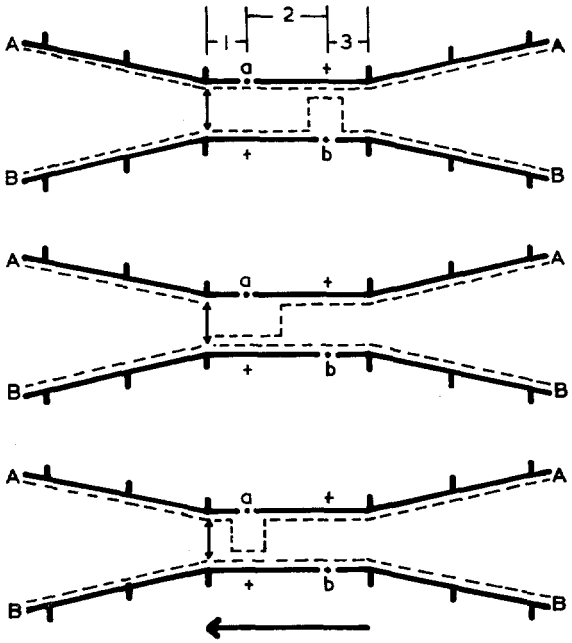

FIGURE 5.—The Modified Polaron Model. In the upper figure recombination results from a miscopy at *b* with a double exchange in Segments 2 and 3. In the middle figure a single exchange in Segment 2 gives a miscopy at *a*. In the lower figure a double exchange in Segments 1 and 2 results in miscopy at *a*. The doubleheaded arrow at the left end of the polaron signifies the crossover option at this point.

In the terms of the model, the observed result means that exchange in Segment 2 (the event required for cys^+ recombination) is more likely to be accompanied by exchange in Segment 3 in the $B \times B$ crosses than in the $A \times B$ crosses. This result is anticipated by the modified polaron model, with its double switches over very short intervals. Among the recombinants of the $B \times B$ crosses, with a short Segment 2, a large proportion will have double exchanges in Segments 2 and 3, while in the $A \times B$ crosses, with a much longer Segment 2, only a small fraction of the exchange events will be of that type.

When recombination occurs in a cross between mutants in the same polaron, the polaron hypothesis requires that the distal mutant must always be the site of miscopy. In the current study we observed that either mutant site might be miscopied even in crosses in which the mutants were very closely linked. Rather than postulate a series of polarons within the *cys* locus, we prefer a model which anticipates this result for mutants in the same polaron. The double switch proposal accomplishes this (see Figure 5). Recombination results from miscopy of the proximal (right-hand) mutant site by a double exchange in Segments 2 and 3. Recombination results from miscopy of the distal mutant site by either a single exchange in Segment 2 or a double exchange in Segments 1 and 2.

Double switches must be rather more frequent than single switches to account

for the approach to equality of the four recombinant types in the $B \times B$ crosses. However, when Segment 2 is elongated in the $A \times B$ crosses, single exchanges in this segment become the predominant source of recombinants. As Segment 2 is extended, an increasing majority of the recombinants result from miscopy at the distal mutant site. (In the Ascobolus work the distal mutant site was *always* the site of miscopy. This observation might be compatible with the modified polaron model if the two mutants in a cross were far enough apart for a considerable amount of single exchanges to take place between them. Perhaps the relative frequencies of single and double switches differ in Ascobolus and Neurospora.)

If the results of the current study are to be explained by an FPR model without polarity, we must conclude that the *cys* mutants span only a small fraction of the recombination region. This is revealed when we consider the restoration of 2:2 segregation in those tetrads in which the cys^+ recombinant has undergone and odd number of switches. There must be a compensating switch somewhere in the vicinity by one of the strands of the homolog. The data of Table 2 tell us that this compensating switch never occurred between the two *cys* mutant sites, and it occurred to the left of the mutant sites in 16 out of 18 tetrads (those in which the cys^+ spores had nonparental combinations of outside markers). If this is an event which can occur anywhere in the recombination region with an equal probability, it means that the major part of the region lies to the left of these two clusters of mutant sites, and it probably includes more than one functional gene. The polaron models, on the other hand, allow us to retain the appealing notion that the ends of the functional gene also serve as ends of the recombination region. For this reason we choose to account for the exchange to the left of the *cys* mutant sites as a regular event occurring at the terminal end of a polaron in recombination. Thus our second modification of the polaron model states that the occurrence of exchange (or perhaps the occurrence of pairing) in a given polaron sets up a "crossover option" at the terminal end of that polaron. The *cys*-recombinant strand has an equal chance of joining either homolog at this point, and the miscopies which have persisted up to this point are corrected. This results in the random distribution of left-hand markers among the recombinants.

The modified polaron is a formal genetic model designed to account for our observations, but it can be useful only if it is compatible with the known facts about meiosis. Among these there are three well-known impediments to speculation on copy-error (or copy-choice) models: (1) the chemically-detectable DNA synthesis in meiosis takes place before the synapsis of homologous chromosomes; (2) recombinants are usually pure at the completion of meiosis, rather than segregating at the first postmeiotic division as would be expected if they were produced in a semiconservative replication; (3) recombination can occur in all four products of a tetrad, rather than being restricted to the two "new strands." All these facts may be accounted for by a scheme in which most of the chromosome material does undergo semiconservative replication before synapsis, but there is a small amount of later synthesis which is directly involved in recombination. A representation of modified polaron recombination on this basis is shown in Figure 6.

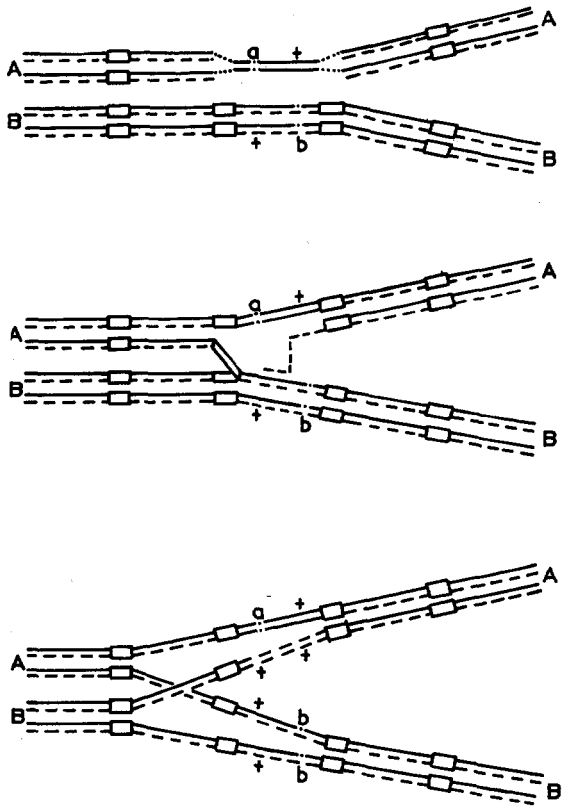

FIGURE 6.—Late replication of the recombinant on the Modified Polaron Model. The solid lines represent DNA strands which were present before meiosis. The dotted lines represent DNA strands synthesized during meiosis. In this tetrad a *cys*$^+$ recombinant has been produced by a miscopy at *a*, and a crossover has occurred at the terminal end of this polaron. Note that replication is semiconservative except in the polaron which is the site of recombination, but that recombinants are pure (for linked markers as well as *a* and *b* sites) at the completion of meiosis. Note that the unduplicated polaron in the *A* chromosome may establish connection with either of the two available double strands, thus accounting for the observation that any of the four members of a tetrad may participate in recombination.

We assume that, at the time of synapsis, synthesis of the new strands is complete except for an occasional polaron which has failed to duplicate. As the tetrad opens out it is held together at these points at which there are only three strands (Figure 6, top). Synthesis of the fourth strand then proceeds along the unoccupied template from the proximal end, with occasional copy-errors resulting from single or double switches to the nearby homolog (Figure 6, middle). The free end of the incomplete strand at the terminal end of the polaron is for some reason unable to initiate synthesis, so it competes with the homolog for an attachment, making a transitory three-way junction. This sets up the crossover option, which is resolved only when the fourth strand completes synthesis to permit the separation of the tetrad into two pairs (Figure 6, bottom).

Polarity is here assumed to result from polarized synthesis of these late-formed segments. This might be caused by the gross movements of the chromosomes. The *cys* locus and the centromere are close together (about ten map units) so that usually they will not be separated by a crossover. Therefore, at the *cys* locus the opening out at diplotene may be expected to commence from the centromere side, and the tardy synthesis of a polaron could commence from the side which opens out first. This would agree with our model for recombination at the *cys* locus, since the centromere is at its right. The same scheme could apply to recombination at the *me-2* locus of Neurospora (MURRAY 1960, 1963) and the *paba-1* locus of *Aspergillus nidulans* (SIDDIQI and PUTRAMENT 1963). Both of these studies deal with centromere-linked loci in which a pattern of recombinants emerges which is similar to that observed in our work. In each case the "unlinked" marker is the distal one.

Studies of *in vitro* synthesis of DNA (KORNBERG 1960) suggest that the synthesis of the two helices of opposite polarity should progress in opposite directions. If synthesis can proceed in only one direction, as envisaged here, it may be that a single-stranded DNA is temporarily formed. The subsequent synthesis of the complementary strand might copy the information from its partner, resulting in *conservative* replication of this molecule (see Figure 6). Alternatively, synthesis of both new strands of a replicating molecule may move along in the same direction, as was elegantly demonstrated in bacterial DNA by CAIRNS (1963). Even so, these two new helices might bind to each other, yielding a conservative molecule, if the formation of chromatids had proceeded to such a point that the strands which would have been their complements were no longer available. TAYLOR, HAUT and TUNG (1962) have proposed that supplementary DNA synthesis is responsible for the rejoining and repair of broken chromosomes, and they have pointed out that a similar mechanism could account for miscopy in recombination.

Probably the most awkward aspect of the process shown in Figure 6, in the light of our knowledge of the molecular behavior of DNA, is the accurate switching of a replicating strand from one template molecule to another, *in a different chromosome*. However, it is difficult to design a more palatable scheme for getting chromosomal recombination correlated with short segments of miscopy (3:1 segregation). One possibility is that short pieces of DNA copied from both homologs can be fitted together into a molecule (or a gene) after synthesis. Such a process would seem to require the production of some unused DNA.

If the scheme for recombination in Figure 6 is correct, the distinction between breakage and copy-choice becomes obscure. We have chosen to design a model employing copy-choice because breakage alone cannot account for miscopy. However, in order to account for the crossing over correlated with miscopy, we have found it necessary to invoke an event (the crossover option) which is operationally indistinguishable from breakage.

According to the switch hypothesis of FREESE, the recombination behavior of any point-mutant should be the same as that of any other. According to hypotheses of fixed recombination regions (Fixed Pairing Region, Polaron, Modified

Polaron) different mutants will behave differently during recombination according to their positions in their respective recombination regions. Evidence of such differences based on the location of the mutants is presented in this report as well as in other studies in Neurospora (MURRAY 1960, 1963), Aspergillus (SIDDIQI 1962) and Ascobolus (LISSOUBA, MOUSSEAU, RIZET and ROSSIGNOL 1962). We feel that the evidence is compelling for some sort of mechanism based on fixed recombination regions.

Whether it is necessary to invoke polarity in the recombination event remains doubtful. Clear evidence for or against polarity should come from extensive analysis of the recombination of an array of mutants spaced throughout a recombination region. On the polaron model, miscopy should be rare for mutant sites near the proximal end, and the frequency should increase all the way to the distal end. On the FPR model the frequency of miscopy should be low at *both* ends and higher toward the middle. In crosses between pairs of mutants in the same recombination region, the polarity should be consistently unidirectional on the polaron models, while in an FPR system the *apparent* polarity would be reversed in some crosses, depending on whether the mutant to the left or the one to the right were more centrally located. If the FPR model is correct, we must conclude that none of the studies to date has dealt with an array of mutants covering more than one half of a recombination region.

SUMMARY

The analysis of 67 tetrads with recombination at a cysteine locus in Neurospora reveals that: (a) recombination within this gene is always nonreciprocal; (b) one half of the cases of recombination at this locus are accompanied by reciprocal recombination (crossing over) of the surrounding markers; the cysteine recombinant is nearly always one of the two members of the tetrad participating in the crossover; (c) in a cross between two mutants at this locus, recombination can result from a nonreciprocal event (miscopy) at either mutant site; (d) in nearly all cases the linked marker on the right identifies the site of miscopy of a particular recombinant.

The analysis of recombinants among random spores from 26 crosses between pairs of mutants at the *cys* locus has resulted in a map of this locus in which the seven mutants fall into two clusters. The mutants show differences in recombination properties depending on their location. In a cross between a mutant in the *A* cluster and one in the *B* cluster, the majority of the recombinants always result from miscopy at the *A* site. This behavior can be explained if: (1) the chromosome is composed of a series of recombination regions in fixed positions; (2) recombination in a given region occurs in only a small fraction of tetrads, but when it does occur it frequently involves multiple (rather than single) exchange; (3) the two clusters of *cys* mutants are nonsymmetrically placed in the same recombination region. Certain features of the results suggest that the recombination event is polarized and always proceeds away from the centromere. However, further studies will be required to determine whether there is actual polarity in this process or not.

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