*FURTHER EVIDENCE OF ABERRANT RECOMBINATION IN NEUROSPORA**

By Mary B. Mitchell

KERCKHOFF LABORATORIES OF BIOLOGY, CALIFORNIA INSTITUTE OF TECHNOLOGY, PASADENA, CALIFORNIA

Communicated by G. W. Beadle, September 13, 1955

Wild progeny from intercrosses of pyridoxine mutants have been found in tetrads showing irregular segregation of one or the other of the mutants.[1] These wild types therefore appear not to be true recombinants arising from crossing over between the mutant genes. Earlier, individuals of the genotypes expected to result from two- or three-strand double crossovers involving the regions adjacent to the locus of a pyridoxine mutant had been obtained with surprisingly high frequency from marked crosses between this mutant and its wild allele.[2] Since the frequency was very nearly that with which wild offspring were obtained from intercrosses, the possibility was considered that the apparent double-crossover recombinants might be due not to ordinary crossing over but to the mechanism which operates in the intercross. That these apparent recombinants also are found to accompany abnormal segregation is shown by the experiments reported here.

Apparent Double-Crossover Recombinants among Random Spores.—The mutants used here are as follows: *pyr 1* (263), pyrimidine; *pdx* (37803), pyridoxine; *pdxp* (39106), pyridoxine, pH-sensitive; *co* (70007), "colonial"; *arg* (33442), arginine; *pyr 3 b* (37815), pyrimidine, temperature-sensitive. The gene order[2] has been shown to be centromere—*pyr 1*—*pdx* or *pdxp*—*co*—*arg*—*pyr 3*.

Unexpectedly frequent double-crossover types were first observed from the crosses *pyr 1* + *co* × + *pdx* + and *pyr 1* + + × + *pdx co*. Later the same effect was found when *pdx* was replaced by *pdxp*. Frequencies of types classified among germinated spores on minimal agar plates are given in Table 1. (Pseudo-wilds[3] are counted as mutants here and throughout.) The single crossovers here give as a measure of the distance *pyr 1* to *pdx* or *pdxp* from 0.5 to 1.7 units. The distance *pdx* or *pdxp* to *co*, is not given but has been found to be 3–4 units in other crosses. Therefore, the appearance of nearly 0.1 per cent double-crossover progeny is surprising, particularly when this behavior is compared to that found in the crosses *co* + *pyr 3* × + *arg* + and + + *pyr 3* × *co arg* +. Here the distances found are somewhat shorter, *co* to *arg* being about 0.2 to 0.5 and *arg* to *pyr 3* about 2.5 to 3.5 units, but there is no indication of a coefficient of coincidence greater than 1, since, among 25,187 spores, only one double-crossover type appeared.

TABLE 1

	pyr and *pdx* or *pdxp*	"Double Crossovers"	Single Crossovers
pyr 1 + *co* × + *pdx* +	14,748	(+ + +) 13	(+ + *co*) 108
pyr 1 + + × + *pdx co*	16,523	(+ + *co*) 11	(+ + +) 140
pyr 1 + *co* × + *pdxp* +	15,060	(+ + +) 14	(+ + *co*) 38
pyr 1 + + × + *pdxp co*	12,792	(+ + co) 11	(+ + +) 72

In order to obtain the frequencies of apparent double crossovers in the two cases from the same cross, *pyr 1* + *co* + *pyr 3* × + *pdx* + *arg* + was examined. It was found that plating spores on different media and at different temperatures made it

possible to classify among random spores at least one recombinant resulting from single crossovers in each of the four marked regions and also from two-strand and one kind of three-strand double crossovers, except in regions 2 and 3 (*pdx* to *co* to *arg*). Frequencies of recombinants thus classified are shown in Table 2. Classi-

TABLE 2

		Per Cent
Region 1	+ + *co* + *pyr 3*	142 in 17,809 = 0.80
Region 2	*pyr 1* + + (*arg*) +	383 in 17,084 = 2.2
Region 3	+ *pdx* + + *pyr 3*	26 in 10,380 = 0.25
Region 4	*pyr 1* + *co* + +	191 in 10,881 = 1.8
Regions 1 and 2	+ + + (*arg*) +	31 in 17,084 = 0.17
Regions 1 and 4	+ + *co* + +	12 in 39,317 = 0.030
Regions 2 and 4	+ *pdx co* + +	6 in 11,342 = 0.053
Regions 3 and 4	+ *pdx* + + +	1 in 21,722 = 0.0046
Regions 1, 2, and 3	+ + + + *pyr 3*	1 in 28,189 = 0.0035
Regions 1, 2, 3, and 4	+ + + + +	0 in 50,412

fications of the recombinants among germinated spores were made under the following conditions.

+ + *co* + *pyr 3:* Minimal at 25° C. At this temperature *pyr 3* grows, without added pyrimidine, almost as rapidly as wild.

pyr 1 + + (*arg*) +: Minimal plus arginine at 35° C. Mutant *pyr 1* grows more on minimal than does *pdx* or *pyr 3*, and sufficiently so that *co* can be classified. Tests for *arg* were not made, since no *pyr 1* + + + + recombinants were found among 10,881 spores on minimal without arginine at 35° C.

+ *pdx* + + *pyr 3:* Minimal plus pyridoxine at 25° C. Progeny which showed a wild or nearly wild phenotype were isolated and tested for the presence of *pdx* and *pyr 3*.

pyr 1 + *co* + +: Minimal at 35° C.

+ + + (*arg*) +: Minimal plus arginine at 35° C. Tests for *arg* were again not made, since no + + + + + recombinants were found among 50,412 spores.

+ + *co* + +: Minimal and minimal plus arginine or pyridoxine at 35° C. Those found on supplemented media were isolated and tested.

+ *pdx co* + +: Minimal plus pyridoxine at 35° C. These were isolated and tested for *pdx*.

+ *pdx* + + +: Minimal plus pyridoxine at 25° and 35° C.—isolated and tested.

+ + + + *pyr 3:* Minimal plus arginine or pyridoxine at 25° C.—isolated and tested.

From the frequencies of these recombinants the coincidence was calculated as shown in Table 3. The apparent difference in behavior in regions 1 and 2 as com-

TABLE 3

	Double Crossovers (Per Cent)				Double Crossovers (Per Cent)		
Regions	Expected	Observed	Coincidence	Regions	Expected	Observed	Coincidence
1 and 2	0.096	0.34	3.5	2 and 4	0.18	0.11	0.6
1 and 4	0.076	0.060	0.8	3 and 4	0.02	0.0092	0.5

pared to that in regions 3 and 4 seemed large enough to justify a search for the + + + *arg* + type in complete tetrads.

Apparent Double-Crossover Recombinants in Tetrads.—From the crosses + *pdx* *co* × *pyr 1* + + and + *pdx* + × *pyr 1* + *co*, about 379 asci had previously been examined,[2] but no "double-crossover" types were found in 153 asci in which germination was complete. From *pyr 1* + *co* + *pyr 3* × + *pdx* + *arg* +, 326 asci were dissected, and germination was complete in 246. Among these, two found to contain + + + *arg* + were as shown in Table 4. From each tetrad the three segregants not showing *pdx* were crossed to wild in order to see whether the mutant was present and suppressed, but *pdx* was not recovered from any of the crosses. Nor can the failure of *pdx* to appear, as expected, in two of the four spore pairs be explained in terms of ordinary back-mutation, since, among 39,651 spores from the cross *pdx* × *pdx*, none was found to be wild with respect to *pdx*.

TABLE 4

Spore pair 1	+ + + *arg* + a	*pyr 1* + *co* + *pyr 3* A
Spore pair 2	+ *pdx* + *arg* + A	*pyr 1* + *co* + *pyr 3* A
Spore pair 3	*pyr 1* + *co* + *pyr 3* a	+ + + *arg* + a
Spore pair 4	*pyr 1* + *co* + *pyr 3* A	+ *pdx* + *arg* + a

The fact that in both tetrads found to contain + + + *arg* + there had been a change from *pdx* to + suggests that the bulk of the apparent double-crossover recombinants found among random spores may be accounted for not by double crossing over but by this change.

Eighty-four of the above 246 tetrads were tested in such a way that those showing single crossovers in regions 1, 2, 3, and 4 could be detected. Three showed a crossover in region 1, six in region 2, one in region 3, and four in region 4. These were isolated and tested and found to be completely regular with respect to segregation of all five markers.

Conclusions.—It has been found that, in the intercross *pdxp* × *pdx*, *pdx* and *pdxp* appear to become wild with a frequency of about 0.2 per cent. In the crosses *pdx* × *pdx* and *pdxp* × *pdxp*, on the other hand, they either do not become wild or do so much less frequently. From the results reported here it appears that *pdx* becomes wild, or is "converted"[4] to wild, in + × *pdx* and that this is likely to be true of *pdxp* also, although the change has not been observed in tetrads. It also appears that the near-by mutant *arg* either does not become wild in + × *arg* or does so with a much lower frequency than *pdx*.

A discussion of these observations will be given elsewhere, in connection with some further results from the intercrosses.

* This work was supported by funds from the United States Atomic Energy Commission.

[1] Mary B. Mitchell, these PROCEEDINGS, **41**, 215, 1955.

[2] M. B. Mitchell and H. K. Mitchell, these PROCEEDINGS, **40**, 436, 1954.

[3] Thad H. Pittenger, *Genetics*, **39**, 326, 1953.

[4] C. C. Lindegren, *Science*, **121**, 605, 1955.