in the progenies. This result was offered in support of a two-phase model of crossing-over.

Preliminary observations of the present authors had shown that aging had little or no effect at the late developmental stages at which somatic crossing over occurs, and, in an extensive series of tests, Schwartz's experimental findings were not confirmed. These results do not disprove the two-phase hypothesis but show that the method chosen to provide supportive evidence will not do so.

The size-class distribution of mosaic spots seems to be one of the most consistent features of somatic crossing-over. Aging may somewhat increase the size of the spots.

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[1] D. Schwartz, *Genetics*, **38**, 251–260, 1953.

[2] D. Schwartz, *ibid.*, **39**, 692–700, 1954. This paper should be consulted for the relationship of Schwartz's to Belling's ideas on crossing-over.

[3] C. Stern, *Genetics*, **21**, 625–730, 1936.

[4] The diagrams by Schwartz (see n. 2) help materially in visualizing the various types of crossovers and their products.

[5] S. W. Brown and Aloha Hannah, these PROCEEDINGS, **38**, 687–693, 1952.

[6] Aloha Hannah, *J. Exptl. Zool.*, **123**, 523–560, 1953.

[7] The freezing method was adopted at the suggestion of Mr. George Brosseau and was checked by tests of yellow, singed, and wild-type flies as well as various sorts of gynandromorphs. There was no evidence that freezing altered the appearance of the bristles in any way.

[8] In *Zea mays*, Barbara McClintock, *Genetics*, **23**, 315–376, 1938; in *Nicotiana tabacum*, K. R. Stino, *J. Heredity*, **31**, 19–24, 1940.

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*ABERRANT RECOMBINATION OF PYRIDOXINE MUTANTS OF NEUROSPORA**

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Giles[1] has reported that crosses between certain inositol mutants of *Neurospora* give infrequent wild recombinants, and from the segregation of linked markers it appeared that multiple crossovers might sometimes be involved. The present author subsequently observed that crosses between two closely linked or allelic pyrimidine mutants, one of which carried a "colonial" marker, gave occasional genetically wild offspring, but roughly half of these carried the "colonial" marker and half did not. This was surprising, since the marker appeared to be located only two or three units from the pyrimidine locus or loci, and, if the wild recombinants arose from crossovers between the two pyrimidine mutants, all would be expected to be alike with respect to the marker unless, in half the cases, a second crossover

occurred between the pyrimidine locus and the locus of "colonial." These observations will be described in detail elsewhere. Unfortunately, a closely linked marker on the other side of pyrimidine is not known, and, moreover, crosses between these mutants are regularly semisterile, so that it is not possible to observe complete asci. However, another pair of mutants, requiring pyridoxine, also closely linked to "colonial," was found by Dr. T. H. Pittenger (personal communication) to exhibit the same behavior, and these mutants give fertile crosses. When a suitable marker was located[2] on the other side of pyridoxine, a study of recombinants among random spores and in complete asci was undertaken.

Recombinants among Random Spores.—The markers used are *co* (70007), "colonial," and *pyr 1* (263), pyrimidine. The pyridoxine mutants are *pdxp* (39106), pH-sensitive,[3] and *pdx* (37803), not pH-sensitive. Data showing the location of the markers with respect to *pdx* have been presented.[2] The "colonial" marker is located about 10–13 units from the centromere in linkage group D; *pyr 1*, about 4–5 units nearer the centromere; and *pdx*, between these two, 0.5–1 unit from *pyr 1*. Data from more recent crosses are consistent with the location of *pdxp* at approximately the same position as *pdx*. The double mutant *pyr 1 pdx* was obtained from an ascus from the cross *pyr 1* + *co* × + *pdx* +, and *pdxp co* was found among random spores from *pyr 1* + *co* × + *pdxp* + by isolating spores producing mycelium which grew on minimal plus pyridoxine and showed the "colonial" growth habit. These were then tested for pyridoxine requirement.

Germinated spores from the crosses *pdx* × *pdxp*, *pdx* × *pdx*, and *pdxp* × *pdxp* were counted on minimal agar supplemented with pyrimidine. The results are given in the accompanying tabulation. Pittenger[4] has shown that pseudo-wilds

	pdx or *pdxp*	+ *co*	+ +
+ *pdxp co* R 1 a × *pyr pdx* + R 1 A	7,255	6	8
+ *pdxp co* R 1 a × *pyr pdx* + R 7 A	14,278	15	15
+ *pdx co* R 2 a × + *pdx* + R 4 A	13,554	0	0
+ *pdx co* R 2 a × *pyr pdx* + R 7 A	8,271	0	0
+ *pdxp co* R 1 a × + *pdxp* + R 1 A	22,747	0	0

are found from *pdx* × *pdxp* crosses, and they were also observed here. With practice, however, these can be distinguished from true wilds by their growth habit on the agar plates, and they are not included in the counts given. Of the forty-four not *pdx* or *pdxp* progeny counted, twelve + *co* and twenty + + were isolated and tested for pyrimidine requirement. Among these were the following types: five + + *co*, seven *pyr* + *co*, seven + + +, and thirteen *pyr* + +. Two of each of the four types were crossed to wild-type protoperithecia. Random spores from these crosses were of the types expected if the recombinants have the genotypes corresponding to their phenotypes.

The distance between *pyr* and *co* in the heterozygous crosses, checked by plating spores on minimal plus pyridoxine, was about 4–5 units. Counts were as follows:

	pyr +, + *co*, *pyr co*	+ +
+ *pdxp co* × *pyr pdx* + R 1	3,844	85
+ *pdxp co* × *pyr pdx* + R 7	2,330	64

Recombinants among Segregants in Asci.—Dissections of asci from the two heterozygous crosses were done on plates of minimal agar supplemented with pyrimidine.

The spore pairs were separated, so that after germination they could be transferred to slants, and the plates were then heat-treated and incubated at 25° C. After about 17 hours the plates were examined under a microscope, and the spore pairs of asci which contained recombinants were transferred to slants of complete medium. The cultures thus obtained were tested on media suitable for the classification of all segregants. Among 988 asci dissected, 585 gave complete germination (at least one member of each spore pair germinated), and among these were found four showing recombinants. The segregants contained in these were as shown in the accom-

	Spore Pairs			
Ascus	1	2	3	4
1	+ *pdxp co* A	+ + *co* A	*pyr pdxp* + a	*pyr pdx* + a
2	*pyr pdx* + a	*pyr pdx* + a	+ + *co* A	+ *pdxp co* A
3	+ + + a	+ *pdxp co* a	*pyr pdx* + A	*pyr* + *co* A
4	+ *pdxp co*	+ + *co*	*pyr pdx* +	*pyr pdx* +

panying tabulation. It will immediately be seen that if the double mutant *pdx pdxp* is present in these asci it must have the phenotype of *pdx* in asci 2 and 4, but of *pdxp* in ascus 1, and in ascus 3 its phenotype must be that of *pdxp* in spore pair 2 and that of *pdx* in spore pair 3. Although, in the author's experience with *Neurospora* mutants, no double mutant has been observed to exhibit such behavior, backcrosses to the two parent types were made in order to test for the presence of the double mutant. Since the crosses *pdx* × *pdx* and *pdxp* × *pdxp* failed to give recombinants, it would be expected that *pdx pdxp* would not give recombinants when crossed either to *pdx* or to *pdxp*. Random spores from crosses of all *pdx* and *pdxp* segregants from asci 1, 2, and 3 to *pdx* and to *pdxp* were plated on minimal plus pyrimidine and examined. Recombinants were found among spores from all *pdx* × *pdxp* crosses but not among those from *pdx* × *pdx* and *pdxp* × *pdxp* crosses. Spore counts are given in Table 1. It appears, then, that these asci did not contain a conventional double mutant such as would be expected to arise from a crossover between *pdx* and *pdxp* if they represent conventional independent loci.

The not *pdx* or *pdxp* segregants from the same three asci, when crossed to wild protoperithecia, gave, as far as could be seen, no *pdx* or *pdxp* progeny but only such progeny as would be expected from + + +, + + *co* and *pyr* + *co* strains.

Dry weights of mycelium were obtained from 125-ml. flask cultures of all segregants from asci 1, 2, and 3 and of nine recombinants from random spores—one + + +, four + + *co*, one *pyr* + *co*, and three *pyr* + +. The media used were minimal at pH 5.1, with and without a supplement of pyridoxine, and minimal at pH 7.1. All flasks were supplemented with pyrimidine. The *pdx* and *pdxp* isolates showed no significant differences from the parent strains. The not *pdx* or *pdxp* recombinants gave no response to pyridoxine.

Since most of the asci not showing recombinants were not transferred from the plates, classifications of *pdx*, *pdxp*, and *pyr* could not usually be made, but, if the plates were examined after 40 hours, *co* could be scored, due to the slow growth on minimal agar of *pdx* and *pdxp*. There were 123 second-division segregations in 584 asci, giving a centromere distance of 11 units. In some cases, at least, asci containing + *pdxp* + could be detected, since *pdx* grows more slowly on minimal agar than *pdxp*, and when *co* is removed from *pdxp* this is more obvious. Of six asci isolated and tested, five were of the following constitution:

+ *pdxp* + + *pdxp co* *pyr pdx co* *pyr pdx* +,

indicating a crossover between *pdxp* and *co*. One was of the constitution

$$+\ pdxp\ co \qquad pyr\ pdx\ co \qquad +\ pdxp\ + \qquad pyr\ pdx\ +,$$

indicating a crossover between the centromere and *pyr* as well. These asci do not give a measure of the frequency of crossing-over in the region *pdxp* to *co*, since not all asci which appeared to contain + *pdxp* + were tested; but they serve to show that these crosses give apparently normal asci of the constitution expected to result from such a crossover.

TABLE 1

BACKCROSSES OF *pdx* AND *pdxp* SEGREGANTS FROM ASCI 1, 2, AND 3

									pdx or *pdxp*	+ *co*	+ +
+	*pdx*	+	a	×	+	*pdxp*	*co*	1-1 A	6,437	5	3
+	*pdxp*	*co*	a	×	+	*pdxp*	*co*	1-1 A	3,238	0	..
+	*pdx*	*co*	A	×	*pyr*	*pdxp*	+	1-3 a	4,860	2	3
+	*pdxp*	+	A	×	*pyr*	*pdxp*	+	1-3 a	4,175	..	0
+	*pdxp*	+	A	×	*pyr*	*pdx*	+	1-4 a	7,036	..	10
+	*pdx*	*co*	A	×	*pyr*	*pdx*	+	1-4 a	4,106	0	0
+	*pdxp*	+	A	×	*pyr*	*pdx*	+	2-1 a	2,276	..	3
+	*pdx*	*co*	A	×	*pyr*	*pdx*	+	2-1 a	5,269	0	0
+	*pdxp*	+	A	×	*pyr*	*pdx*	+	2-2 a	3,438	..	5
+	*pdx*	*co*	A	×	*pyr*	*pdx*	+	2-2 a	3,795	0	0
+	*pdx*	+	a	×	+	*pdxp*	*co*	2-4 A	3,234	1	3
+	*pdxp*	*co*	a	×	+	*pdxp*	*co*	2-4 A	3,085	0	..
pyr	*pdx*	+	A	×	+	*pdxp*	*co*	3-2 a	2,240	2	3
+	*pdxp*	+	A	×	+	*pdxp*	*co*	3-2 a	3,836	0	0
+	*pdxp*	*co*	a	×	*pyr*	*pdx*	+	3-3 A	2,588	4	0
+	*pdx*	*co*	a	×	*pyr*	*pdx*	+	3-3 A	4,656	0	0

Discussion.—But for the apparent absence of *pdx pdxp* double mutants in the asci, the frequencies of the four recombinant types among random spores and the segregations in asci could, perhaps, be explained by assuming that *pdx* and *pdxp* are independent loci and that in the region *pyr* to *co* multiple crossovers occur as frequently as single crossovers. There is, however, the fact that data[2] from other crosses involving this region indicate a much higher frequency of single crossovers, and, to the author's knowledge, multiple crossovers have not been found in asci. It therefore seems desirable to attempt to account for the recombinants on the basis of some mechanism other than crossing-over.

The phenomenon usually referred to as back mutation hardly seems to furnish a suitable explanation because of the stability of *pdx* and *pdxp* in vegetative culture and the failure to find recombinants among spores from *pdx* × *pdx* and *pdxp* × *pdxp* crosses, in contrast to the rather high frequency of recombinants among spores from *pdx* × *pdxp* crosses. Also, in ascus 1, it would be necessary to assume a two-strand double crossover between *pyr* and *co*, as well as back mutation of *pdx*, in order to account for the segregants observed.

In order to explain abnormal segregations which cannot be accounted for by polyploidy or chromosome loss in yeast asci, Lindegren[5] has revived the theory of gene conversion by means of which Winkler[6] once sought to account for all genetic recombination. Conversion of a dominant gene to its recessive allele partly, at least, through the influence of the recessive was suggested to explain asci in which, for example, three spores carried the recessive gene and only one the dominant, although another linked gene pair segregated normally. If it is assumed that *pdx* and *pdxp* are alleles, and in the author's opinion there is no satisfactory evidence that they are not, the formation of not *pdx* or *pdxp* recombinants may be pictured

as resulting from some sort of interaction between the two alleles, perhaps analogous to gene conversion. Since the two mutants are phenotypically different and since they are capable of forming a pyridoxine-independent pseudo-wild, they might be considered to represent different defects at the same locus. Hence, together they may collaborate to obliterate the pyridoxine requirement, not only in a pseudo-wild, but also occasionally at meiosis when the chromosomes are being duplicated. If each mutant carries in the normal condition the part of the locus which is defective in the other and if the chromosome strands are closely associated at the time they are being duplicated, a recombinant might arise as a result of the normal part of one allele being duplicated twice and incorporated into each new strand. This scheme is diagrammed in Figure 1 for each of the three different asci found. If such abnormal duplication actually occurs, segregation ratios of three

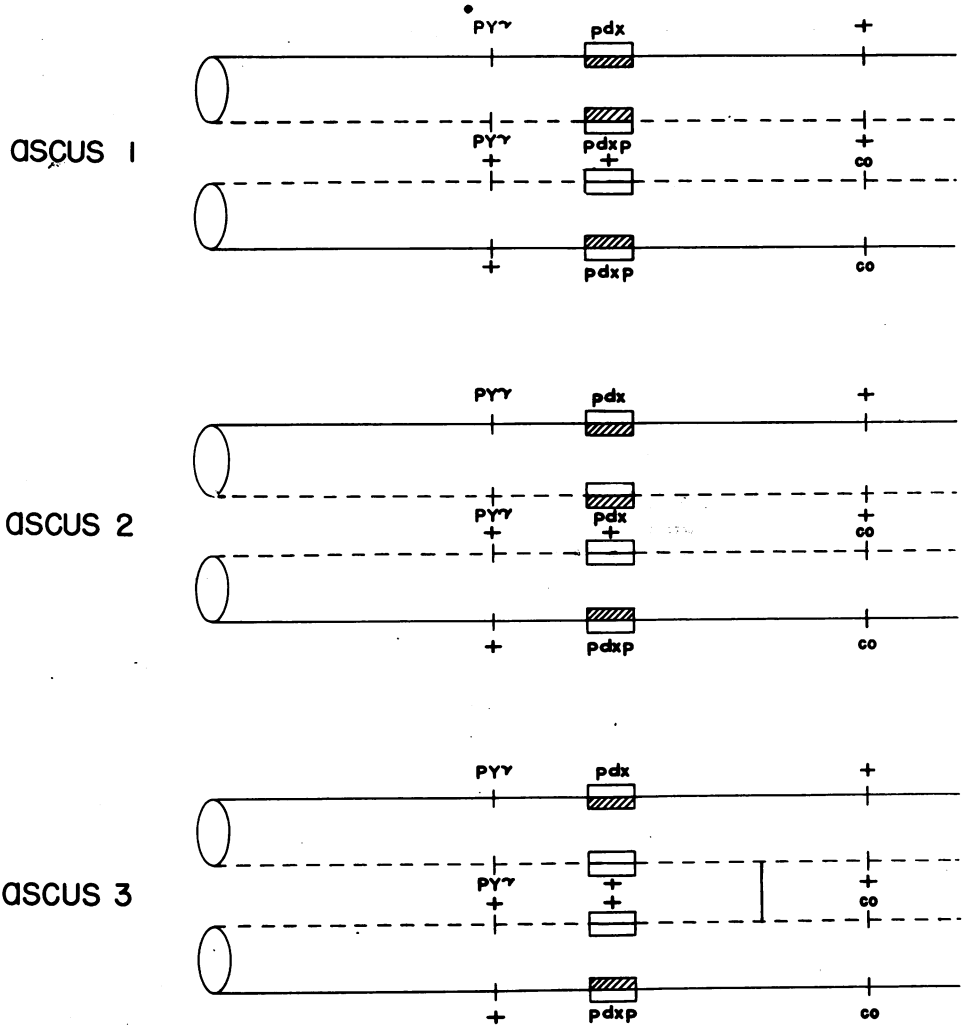

FIG. 1.—Hypothetical scheme to account for origin of not *pdx* or *pdxp* segregants in asci by repeated duplication of one or both normal parts of the locus and failure of corresponding defective parts to be duplicated. Newly formed chromosome strands are represented as broken lines. Parts of the locus assumed to be defective are shaded.

pdx to one *pdxp* and the reverse would be expected to result from repeated duplication of the defective parts of the locus. These might be detected if asci were examined on minimal at pH 7, but this has not been tried. Also, segregants carrying both defective parts of the locus would be expected and might behave as the double mutant in backcrosses, but they might not be distinguishable from *pdx* by any other test.

In order to explain the high frequency of recombinants showing recombination of *pyr* and *co*, it might be supposed that a crossover in the vicinity of the *pdx*, *pdxp* locus brings about a more intimate association of the duplicating strands and thereby increases the probability of abnormal duplication. It might even be supposed that the event which gives rise to crossing-over took place in each case very near the *pdx*, *pdxp* locus but that this event results in an actual exchange in, statistically, only half the cases.

The above scheme is, of course, purely speculative, but it serves to suggest a way in which the recombinants might arise as a result of abnormal meioses. The frequency of such abnormalities might be expected to vary a good deal with different loci, depending, perhaps, on the degree of structural difference between the wild and mutant alleles. This might be one reason for previous failure to observe abnormal segregations in *Neurospora* asci; but another reason may be that such segregations have been dismissed as mistakes because of the absence of suitable markers in many crosses observed.

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[1] Norman H. Giles, *Cold Spring Harbor Symposia Quant. Biol.*, **16**, 283, 1951.

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

[3] J. L. Stokes, J. W. Foster, and C. R. Woodward, Jr., *Arch. Biochem.*, **2**, 235, 1943; Bernard S. Strauss, *Arch. Biochem.*, **30**, 292, 1951.

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

[5] Carl C. Lindegren, *J. Genetics*, **51**, 625, 1953.

[6] H. Winkler, *Biol. Zentr.*, **52**, 163, 1932; C. Stern, *Biol. Zentr.*, **52**, 367, 1932.

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*SPLITTING OF VALUATIONS IN EXTENSIONS OF LOCAL DOMAIN II**

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In a previous note[1] Zariski and the author have proved that, if (R, M) is a regular local domain of dim $s > 1$, such that the quotient field K and the residue field R/M of R have equal characteristics, and, if K^* is a finite separable extension of K, then there exist infinitely many real discrete valuations of K having center M in R which split in K^*.[2] The purpose of this note is to generalize this result by relaxing the regularity condition on R and drawing, at the same time, a stronger conclusion. We state this generalization as our