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[1] Laki, K., *Arch. Biochem. Biophys.*, **32**, 317 (1951).

[2] Lóránd, L., *Nature*, **167**, 992 (1951).

[3] Steiner, R. F., and Laki, K., *Arch. Biochem. Biophys.*, **34**, 24 (1951).

[4] Shulman, S., and Katz, S., *Fed. Proc.*, **11**, 286 (1952).

[5] Shulman, S., and Ferry, J. D., *J. Phys. Coll. Chem.*, **55**, 135 (1951).

[6] Ehrlich, P., Shulman, S., and Ferry, J. D., *J. Am. Chem. Soc.*, **74**, 2258 (1952).

[7] Katz, S., Gutfreund, K., Shulman, S., and Ferry, J. D., Reported at the 121st Meeting of the American Chemical Society, April 3, 1952.

[8] Foster, J. F., Samsa, E. G., Shulman, S., and Ferry, J. D., *Arch. Biochem. Biophys.*, **34**, 417 (1951).

[9] Scheraga, H. A., and Backus, J. K., *J. Am. Chem. Soc.*, **74**, 1979 (1952).

[10] Ferry, J. D., and Shulman, S., *ibid.*, **71**, 3198 (1949).

[11] Shulman, S., and Ferry, J. D., *J. Phys. Coll. Chem.*, **54**, 66 (1950).

[12] Mihályi, E., *Acta Chem. Scand.*, **4**, 351 (1950).

[13] Debye, P., *J. Phys. Coll. Chem.*, **53**, 1 (1949).

[14] Hartley, G. S., *Aqueous Solutions of Paraffin Chain Salts*, Hermann & Cie, Paris, 1936.

[15] Bailey, K., Bettelheim, F. R., Lorand, L., and Middlebrook, W. R., *Nature*, **167**, 233 (1951).

[16] Waugh, D. F., and Livingstone, B. J., *J. Phys. Coll. Chem.*, **55**, 1206 (1951).

[17] Ferry, J. D., and Morrison, P. R., *J. Am. Chem. Soc.*, **69**, 388 (1947).

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*PSEUDO-WILD TYPES IN NEUROSPORA CRASSA**

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In Neurospora the test for non-allelism which is usually considered conclusive consists of the recovery of the double mutant from a cross between the two mutants in question and the demonstration that the two parent mutants segregate in asci from a cross of the double mutant to wild type.[1] Carrying out this test is usually a simple matter if fertile crosses can be obtained and if close linkage between the two mutants does not exist. Crosses between two pyrimidine mutants to be considered here, however, gave no complete asci and only a small percentage of phenotypically wild progeny. Since the mutants are not distinguishable by known physiological tests, the double mutants, if they occurred, could presumably be detected only by out-crossing mutant progeny at random and examining asci. This procedure seemed impracticable, and, instead, phenotypically wild progeny were studied with the possibility in mind that they might be found

to differ from standard wilds in a way which would indicate that they did not arise from crossing-over between the mutant loci. Such a difference was readily demonstrated by crossing these strains to standard wild, since these crosses regularly gave about 45% pyrimidine mutant progeny. Strains which behaved in this fashion have been termed pseudo-wilds, since they appear to be wild phenotypically, but give mutant progeny. Pseudo-wilds from several other crosses have been isolated and studied in an attempt to explain the origin and behavior of these strains.

Mutant Strains from Which Pseudo-wilds Were Derived.—The three pyrimidine mutants which were used in these experiments are *pyr-3a* (37301),[2, 3] *pyr-3b* (37815)[2] and *pyr-3d* (45502).[4, 5] Mutants *pyr-3a* and *-3d* require pyrimidine at any temperature, but *pyr-3b* has an absolute requirement only at temperatures above about 32°C., being phenotypically wild at 25°C. There is no evidence at present that these three mutants are non-allelic. The formation of pyrimidine-independent heterocaryons in mixed cultures of *pyr-3a* and *-3d* has not been observed although several isolates of each mutant have been tested. Pseudo-wilds have been found from *3a* × *3d* and *3b* × *3d* but not, so far, from *3a* × *3b*. Two other mutants, closely linked to these, have been used, the "colonial," *co* (70007),[6] and the arginine mutant, *arg* (33442).[7] The isolate of *pyr-3d* first studied carried a translocation[4, 8] to which the mutant gene is linked. Isolates of this mutant with and without the translocation have been used. When the translocation is absent, crosses to wild and to certain other mutants give predominantly normal asci, but when it is present many asci are found with defective spores. Some contain only defective spores and others contain both normal and defective spores in pairs. The mutant without the translocation was found in an ascus from a cross of *pyr-3d* T to *co*. The strain carries mutant *co* and is designated *co pyr-3d*. The absence of the translocation has not been demonstrated cytologically but is deduced from the observations that asci showing defective spores in pairs are rare from crosses involving this strain but were found very frequently from those involving strains from two other spore pairs from the same ascus. Also, the centromere distance of *pyr-3d* calculated from 19 asci from *co pyr-3d* × *co* is 13 units, whereas that calculated from 104 asci[5] from crosses involving *pyr-3d* T is one unit. A value for *pyr-3a* based on 401 asci[5] (an unpublished data) is 14 units.

With a few exceptions progeny of crosses reported here were analysed by classifying and counting random spores plated on minimal medium, or minimal supplemented with cytidine or arginine. Spores were collected from the wall of the test tube in which the cross was made, suspended in 1 ml. of minimal medium which was then spread on the agar surface. The spores were heat-treated on the plates and incubated at 25°C., except those from crosses involving *pyr-3b* which were incubated at 34°C. After 12 to

20 hours the germinated spores were classified under the microscope. The *arg* and *pyr* spores can be distinguished from wild type because of the limited growth of their hyphae on minimal medium. "Colonial" can be classified by its growth pattern which is quite different from that of wild type. It can be classified on supplemented medium when it is combined with *arg* or *pyr*. From crosses involving the five mutants, counts of spores plated in minimal medium are given below.

	CROSS	WILD AND/OR PSEUDO-WILD	*pyr* AND/OR *arg*	*co*
1	*co* × *pyr-3a*	21 = 1.2%	891	805
2	*co* × *pyr-3d* T	265 = 4.7%	2,613	2693
3	*co* × *arg*	13 = 0.4%	1,648	1592
4	*arg* × *pyr-3b*	12 = 0.6%	2,125	..
5	*pyr-3a* × *pyr-3b*	0	20,888	..
6	*pyr-3a* × *pyr-3d* T	60 = 4.6%	1,259	..
7	*pyr-3b* × *pyr-3d* T	57 = 3.8%	1,448	..
8	*pyr-3b* × *pyr-3b*	0	16,607	..
9	*pyr-3a* × *co pyr-3d*	81 = 1.0%	8,034	0
10	*pyr-3b* × *co pyr-3d*	4 = 0.14%	2,913	0
11	*arg* × *co pyr-3d*	7 = 0.15%	4,549	49

Crosses 1, 3, 4, 5, 8 and 11 gave many asci with eight normal-appearing spores but germination was not always good. Cross 2, as already mentioned, gave many asci with aborted spores (pale, or small and colorless). Asci with eight normal-appearing spores have not been observed from crosses 9 and 10. Most of the spores appear to be of normal size but many do not darken normally. The degree of darkening, even among members of pairs, is variable and those which are not fully darkened rarely germinate. Spores from crosses 6 and 7 exhibit this type of abnormality as well as the spore abortion accompanying the translocation. Of the *pyr* mutants only *pyr-3b* could be crossed to itself successfully. Mutants *pyr-3a* and *pyr-3d*, when selfed, formed perithecia but normal spores were not observed. Spores from the crosses *pyr-3a* × *pyr-3b* and *pyr-3b* × *pyr-3b* were incubated at 34°C. for 2 or 3 days before they were counted.

Pseudo-wilds from pyr-3a × *pyr-3d T.*—From two crosses involving different isolates of *pyr-3a* and *-3d* T, 40 phenotypically wild spores were transferred from minimal plates to slants of minimal medium. The resulting strains were subcultured on minimal medium and on minimal with cytidine. Ten strains from one cross were tested in 125-ml. flasks containing 20 ml. of liquid minimal medium. Each strain was tested after being subcultured twice on minimal and twice on minimal with cytidine. Mycelial pads were weighed after a growth period of 4 days at 25°C. The lowest, highest and average dry weights, in mg., for each set of 10 cultures are given below

	LOWEST	HIGHEST	AVERAGE
Subcultured on minimal	39	74	62
Subcultured on cytidine	34	80	66

A second group of 10 strains from the other cross was tested after being subcultured four times on minimal. Dry weights after a 3-day growth period were as follows: lowest—21; highest—49; average—41. At the same time corresponding dry weight determinations were made from flask cultures in which the minimal medium was supplemented with 1 mg. of cytidine sulfate per flask. These weights did not differ significantly from those given above. Thus, in their growth on minimal medium and in their failure to be stimulated by cytidine, these strains resemble closely wild types isolated at random. In other experiments, however, 12 strains derived from phenotypically wild, germinated spores which were transferred to Westergaard's basic medium[9] supplemented with an autolysate of wild-type Neurospora mycelium, and subcultured on this medium, showed stimulation by cytidine in the early stages of growth in liquid culture. This behavior suggested that the strains were not genetically pure and that a pyrimidine-dependent component was selected.

Crosses of the 40 strains mentioned above (kept on minimal medium) to standard wild types, 7A and 8a, were attempted. In every case perithecia were formed with one or the other mating type, but in 25 cases no mature spores, or very few, were observed. Spores from the 15 more fertile crosses (7 of the strains involved were among those tested in flasks) were plated and counted with the following results:

WILD × PSEUDO-WILD	WILD	MUTANT	% MUTANT
7A × 6 R 22	988	804	45
8a × 6 R 31	740	589	44
7A × 6 R 32	759	625	45
8a × 6 R 36	916	660	41
7A × 6 R 38	666	489	42
7A × 6 R 41	715	578	45
8a × 6 R 42	711	556	44
8a × 6 R 43	713	542	43
7A × 6 R 45	468	444	49
7A × 6 R 46	236	156	40
7A × 6 R 48	460	428	48
8a × 6 R 50	796	691	46
8a × 6 R 55	887	695	44
7A × 6 R 56	422	323	43
8a × 6 R 57	908	747	45
	10,385	8327	44

From each cross 10 mutant-appearing spores were isolated and cultured on minimal with cytidine. Five did not grow further but the remaining

145, when tested in liquid culture, grew when supplied cytidine and failed to grow when not so supplied. Spores from a small number of asci (26 in all) from three crosses were isolated and tested. From each ascus four spores gave rise to *pyr* strains and four to wild type.

Pyrimidine mutant spores were recovered from crosses to wild of 5 of the 12 strains kept on the autolysate medium. Randum spores from these 5 crosses were plated but not counted.

Pseudo-wilds from pyr-3b × pyr-3d T.—Since *pyr-3a* and *-3d* are not distinguishable from each other, it was not possible to discover whether both parent mutants were recovered from out-crosses of pseudo-wilds. In order to answer this question, attempts were made to obtain pseudo-wilds from crosses involving *pyr-3b*. As shown above, phenotypically wild progeny have not been observed from *pyr-3a × pyr-3b*, but have been obtained from *pyr-3b × pyr-3d* T. Six strains from the latter cross were isolated and cultured on Westergaard's minimal. (There was no reason for using this medium instead of Fries, except that it was convenient.) On minimal medium dry weights, in mg., obtained from flask cultures kept at 34°C. for 3 days were as follows: 7 R 1—63; 7 R 3—68; 7 R 4—60; 7 R 5—49; 7 R 7—48; 7 R 9—75. When crossed to wild types 1400-4A and 1347-2a, each strain formed perithecia with only one mating type but three produced few spores. Spores from the three fertile crosses were plated and incubated at 25° and at 34°C. Counts appear below:

	WILD	MUTANT	% MUTANT
Wild a × 7 R 1 A 25°C.	857	799	48
Wild a × 7 R 1 A 34°C.	865	873	50
Wild a × 7 R 4 A 25°C.	550	549	50
Wild a × 7 R 4 A 34°C.	910	959	51
Wild a × 7 R 9 A 25°C.	1500 (approx.)	0	..
Wild a × 7 R 9 A 34°C.	1212	1241	50

Ten mutant spores isolated from the 7 R 1 cross proved to be phenotypically like *pyr-3d* and 14 from the 7 R 9 cross, plated at 34°C., were like *pyr-3b*. From this it appears that either one or the other of the parent mutants was recovered from these pseudo-wilds. Examination of asci from the three crosses showed the presence of defective spores in pairs in the 7 R 1 and 7 R 4 asci but not in asci from 7 R 9.

Pseudo-wilds from co pyr-3d Crosses.—It seemed possible that the pseudo-wilds might arise from an effect of the translocation. Therefore, when the *co pyr-3d* strain was obtained, phenotypically wild strains from *pyr-3a × co pyr-3d* were examined. When six such strains were isolated and cultured on Westergaard's medium it was observed that, although they had not been phenotypically *co* on the agar plate, as they grew they began to show this character in what may be called a diluted condition. The extent of

expression of *co* varied in different isolates and in different subcultures of the same isolate. The six strains gave the following dry weights, in mg., from flask cultures kept at 25°C. for 4 days: 9 R 2—48; 9 R 4—58; 9 R 6—55; 9 R 7—55; 9 R 8—54; 9 R 9—39. Crosses of the six isolates to wild were all fertile; spores were plated on minimal agar and classified as follows:

	WILD	*pyr*	*co*	% *pyr*
Wild A × 9 R 2 a	742	629	14	46
Wild A × 9 R 4 a	738	517	4	41
Wild a × 9 R 6 A	1006	789	12	44
Wild A × 9 R 7 a	788	557	4	41
Wild a × 9 R 8 A	717	532	2	42
Wild A × 9 R 9a	719	624	3	46

Germination of spores on these plates was not especially good, which may account for low percentages of *pyr* mutants.

The origin of pseudo-wilds, then, does not depend upon the presence of the translocation, but the behavior of these strains with respect to *co* suggests that they differ from those isolated from crosses involving the translocation. The mutant form of *co* is undoubtedly present since typically *co* spores were recovered, but the phenotype of the strains themselves was clearly not typically *co*. Examination of spores from crosses of the pseudo-wilds to *co* showed that although they were quite possibly pure with respect to *pyr* they were not pure with respect to *co*. Spores were plated on minimal and on minimal supplemented with cytidine. Those which were phenotypically wild, *pyr* and *co* on these two media are recorded below.

	MINIMAL			CYTIDINE		
	WILD	*pyr*	*co*	WILD	*co*	% WILD
co A × 9 R 2 a	8	873	864	136	1627	8
co A × 9 R 4 a	72	2002	1857	510	921	36
co a × 9 R 6 A	16	1035	980	409	2695	13
co A × 9 R 7 a	30	931	830	1044	1491	41
co a × 9 R 8 A	21	1370	1482	630	1074	37
co A × 9 R 9 a	1	1173	1216	52	1329	4

From two of these crosses 69 asci from 13 perithecia were dissected and the spores were allowed to germinate on minimal with cytidine. The asci were found to contain either eight *co* spores of four *co* and four not-*co* spores, but in no case were both types of asci obtained from the same perithecium.

These results suggest that each of these pseudo-wild strains contains at least two types of nuclei, of the constitutions, *co pyr* and + *pyr*, and that, in each perithecium, one or the other of these types functioned as the parent nucleus contributed by the pseudo-wild. From the crosses to wild, spores which were *co* + could be attributed to cross-overs in zygote nuclei of the

constitution, *co pyr*/ ++. From crosses to *co*, spores which were phenotypically wild on minimal medium could result from crossing-over in *co* +/+ *pyr* zygote nuclei. Some of the latter spores may have been pseudo-wild, however, as will appear below. Since both *co pyr* and + *pyr* were recovered, it seems most probable that both *pyr-3a* and *pyr-3d* were recovered from the same strain.

The cross of *co pyr-3d* to *arg* was made to obtain the triple mutant so that *arg* might be introduced as a second marker in crosses of *pyr-3a* to *pyr-3d*. The small percentage of wilds observed suggested that *arg* may be located between *co* and *pyr* and that, since wilds from crossing-over would then be expected to be very infrequent, the wilds observed might, therefore, be pseudo-wilds. Two of these, when transferred to Westergaard's minimal, showed the dilute "colonial" character, as had pseudo-wilds from *co pyr-3d* × *pyr-3a*. The two strains were crossed to standard wilds, 1400-4A and 1347-2a, and spores were plated on three media, minimal, minimal with cytidine and minimal with arginine. The spores were thus classified and counted as follows:

	MEDIUM	WILD	*pyr-3d* AND/OR *arg*	*co*
Wild A × 11 R 1 a	minimal	1062	1050 = 50%	0
Wild A × 11 R 1 a	cytidine	1269	1208 = 48%	29
Wild A × 11 R 1 a	arginine	1511	66 = 4%	2
Wild a × 11 R 2 A	minimal	588	557 = 48%	3
Wild a × 11 R 2 A	cytidine	1006	368 = 19%	527
Wild a × 11 R 2 A	arginine	992	372 = 27%	8

From this it appears that both + *arg* + and *co* + *pyr-3d* were recovered from each strain. This was confirmed by isolating 20 mutant-appearing spores from each minimal plate, culturing them on minimal with cytidine and arginine and testing them for their response to these compounds. From the 11 R 1 cross, two responded only to cytidine and 18 only to arginine and from the 11 R 2 cross 10 responded to cytidine and 10 to arginine. Those which responded to cytidine were *co;* word those which responded to arginine were not-*co*.

Pseudo-wilds from co × *pyr-3d T.*—From this cross five spores which appeared phenotypically wild on minimal plates were isolated and cultured on Westergaard's minimal. Four of these remained phenotypically wild in slant culture, but the fifth, 2 R 8, became distinguishable from *co*. In flasks, after 4 days growth at 25°C., they gave the following dry weights, in mg.: 2 R 6—52; 2 R 7—56; 2 R 8—40; 2 R 9—52; 2 R 10—52. When crossed to wild each strain gave fertile perithecia with one mating type and no reaction with the other. Spore counts from plates of minimal and minimal with cytidine are given below.

	MINIMAL			CYTIDINE	
	WILD	*pyr*	*co*	WILD	*co*
Wild a × 2 R 6 A	814	824	0	927	0
Wild a × 2 R 7 A	576	488	0	635	0
Wild A × 2 R 8 a	726	0	478	. . .	. .
Wild a × 2 R 9 A	302	281	0	654	0
Wild a × 2 R 10 A	588	598	0	813	0

These crosses, including the one involving 2 R 8 showed the type of sterility associated with the translocation. Since 2 R 8 was not phenotypically *co* on the agar plate, but later showed this character, it seems possible that it was, at least originally, pseudo-wild. These strains resemble pseudo-wilds from the *pyr-3d* T × *pyr-3b* cross in that one or the other but not both parental types were recovered.

Asci Containing Pseudo-wilds.—From the *co* × *pyr-3d* T cross asci which contained part normal and part defective spores were examined in an attempt to learn whether pseudo-wilds came from these. (Aberrant segregations of *pyr* have not been observed in asci with 8 normal spores.) Of 13 asci which had two normal spore pairs in one half of the ascus and two defective pairs in the other, four contained pseudo-wilds. From two of these asci (1296 and 1320) both normal pairs were pseudo-wild. From the other two (1358 and 1360) one pair was pseudo-wild and the other was *co* +. The remaining nine asci of this type contained one + *pyr* pair and one *co* + pair. Asci of this type, from mature perithecia, were rather infrequent;[8] it was usually necessary to examine several perithecia in order to find one. Another type of ascus which is very frequent contains one normal and one aborted pair in each half. Only eight of this type were examined and no pseudo-wilds were found. The pseudo-wilds isolated were not tested in flasks, but they behaved as wild when tested in small tubes of liquid minimal. The six strains remained phenotypically not-*co* in slant cultures and *pyr* was recovered from crosses of each one to standard wild. Spores from crosses involving 1320-1 and -2 were not counted but counts from the four other crosses appear below.

	WILD	*pyr*	*co*	% *pyr*
Wild a × 1296-1A	609	489	0	44
Wild A × 1296-2a	685	541	0	44
Wild a × 1358-1A	1278	964	0	43
Wild a × 1360-4A	1242	945	0	43

Asci from each of these crosses showed patterned spore abortion but this was not true of those from crosses of the phenotypically *co* + strains 1358-2A and 1360-3A to wild. Spores from the latter crosses were plated but not counted. Pyrimidine mutant spores were not observed.

Studies of asci containing normal and defective spores, from crosses not involving the translocation, are in progress. Asci of this type are more

difficult to find from these crosses, which may reflect the fact that phenotypically wild progeny were less frequent, at least among germinated spores. One ascus of the same type as 1296 was among those dissected from a cross to *co* of pseudo-wild 9 R 4 (from *pyr-3a* × *co pyr-3d*). Other asci from the same perithecium contained both *co* + and + *pyr* spores, hence, presumably, the parent nuclei were of these constitutions. From this ascus one member of each of the two normal pairs germinated; both appeared phenotypically wild on the plate but subsequently showed the dilute "colonial" character. One strain grew very slowly on minimal and was finally discarded. The other was crossed to *co* and a few asci were dissected. Five asci from one perithecium contained only *co* + spores but 6 asci from 3 other perithecia contained both *co* + and + *pyr* spores. From *pyr-3a* × *co pyr-3d* an ascus containing one pseudo-wild spore pair was found. The spores of the other pair occupying the same half of the ascus were defective; the four spores in the other half appeared normal but failed to germinate. These observations suggest the possibility that, whether the translocation is present or absent, spores which give rise to pseudo-wilds occur in asci which also contain defective spores.

Discussion.—The occurrence of pseudo-wild serves to emphasize the questionable nature of the conclusion, based solely on the appearance of phenotypically wild progeny, that crossing-over between two mutant genes has taken place. Studies which will be reported later, on other mutants, have shown that the ability to produce pseudo-wilds is not confined to pyrimidine mutants or to mutants in the *pyr* linkage group. Hence, it seems desirable that this phenomenon be considered in attempting to evaluate data from crosses between Neurospora mutants which are closely linked or possibly allelic.

A satisfactory explanation of the origin of pseudo-wilds must account for their growth on minimal medium, for the types of progeny recovered from out-crosses, for the difference in their behavior depending upon the presence of the translocation in the parent cross, and for the development of the dilute "colonial" phenotype. Tentative explanations which have been considered are as follows: that suppression of the mutant character is effected by chance combinations of a number of genes; that back-mutation at a *pyr* locus results in the formation of a pyrimidine-independent heterocaryon in which *pyr* mutant nuclei predominate; that $n + 1$ nuclei, which arise during meiosis as a result of non-disjunction of the *pyr* chromosome pair, account for pyrimidine-independence but are not usually transmitted in crosses; that a heterocaryon, capable of wild-type growth, is established by the inclusion of genetically different nuclei in an ascospore.

The behavior of pseudo-wilds from crosses involving the translocation may be explained in terms of back mutation of *pyr* and, possibly, of *co* in the case of 2 R 8, if it is supposed that, for some reason, nuclei carrying

the back-mutation remain greatly in the minority. Suppression of the mutant character by a chance combination of several genes also seems an adequate interpretation of these cases except that it does not account for the change in phenotype of 2 R 8, from not-*co* to *co* unless, perhaps, mutation of a gene of the suppressor complex is assumed. The origin of these strains from heterocaryotic spores seems less probable because of the lack of evidence from out-crosses that pseudo-wilds from *pyr-3b* × *pyr-3d* T and *co* × *pyr-3d* T were hetercaryotic. From the latter cross a pyrimidine-independent heterocaryon from which *pyr* could be recovered would presumably also contain nuclei carrying the normal allele of *pyr* but such nuclei would, most commonly, carry the mutant allele of *co*. Since, when *pyr* was recovered, *co* was not recovered, it would be necessary to suppose that back-mutation of *co* or crossing-over between *co* and *pyr* accompanied the formation of a heterocaryotic spore in each case studied, or that *co* + nuclei were present but so infrequent that no *co* + spores were found among the progeny of the eight crosses. Also, the types of asci from which the strains have been obtained are not those which would be expected to contain heterocaryotic spores. Since the nucleus functions in cutting out the spore[4, 10] there would seem to be no reason for the appearance of the full complement of eight spores if two nuclei were included in each of two or four spores. If additional nuclear divisions prior to spore formation are invoked to remove this difficulty there would appear no reason for two or four of the eight spores to be defective.

With regard to pseudo-wilds from *co pyr-3d* × *pyr-3a* and *co pyr-3d* × *arg*, back-mutation seems an improbable explanation, since, in the first case, two, and in the second, three, back-mutations per strain would be required. Because of the recovery of both parental types from the same strain the action of multiple-gene suppressors need not be considered. Derivation from heterocaryotic spores, on the other hand, remains a possible explanation since these strains behaved as if they were heterocaryotic and, at least in connection with those involving *arg*, there is no reason to doubt that a heterocaryon thus established would be capable of wild type growth on unsupplemented medium.

The explanation based on the origin of pseudo-wilds from ascospores with $n + 1$ nuclei can be made to appear consistent with the behavior of both types of pseudo-wilds if, in addition to the assumptions that such nuclei account for pyrimidine-independence but do not usually function in crosses, a further assumption is made. This is that in mitotic divisions $n + 1$ nuclei give rise to haploid nuclei by some mechanism such as somatic non-disjunction[11] and that it is the haploid nuclei which usually function in out-crosses. It may be pointed out that there are available no means of evaluating the justification for these suppositions. They can, however, be used as a basis on which to account for the fact that pseudo-wilds from

crosses involving the translocation gave, among progeny from out-crosses, only one parental type, whereas those from crosses without the translocation gave both. For this purpose it may be supposed that, in isolates tested from *pyr-3d* × *pyr-3d* T and *co* × *pyr-3d* T, the original disomic nucleus contained one normal and one translocated member of the *pyr* chromosome pair, and that, in the first case, *pyr-3b* and *pyr-3d*, and in the second, *co* + and + *pyr-3d* were thus both present in the same nucleus. If, for example a translocated chromatid from the other chromosome pair (linkage group B) involved in the translocation were included in the disomic nucleus, then a haploid nucleus which arose from it would be viable if it contained the translocated members of the *pyr* pair, but inviable if it contained the corresponding normal chromosome. Hence, if disomic nuclei did not function in the out-cross, only the mutant gene carried by the translocated *pyr* chromosome would appear among the progeny. Which mutant appeared in a given case would depend upon whether recombination of the mutant genes and the translocation occurred as a result of crossing-over and upon the way in which the chromatids of the B chromosome pair segregated. In the absence of the translocation there would be no such restriction on the type of viable haploid nuclei which could be formed and both members of the *pyr* pair could, therefore, be recovered from one strain. The process of formation of hapolid nuclei could serve also to explain the change in phenotype, with respect to *co*, of 2 R 8 and of the pseudo-wilds from crosses of *co pyr-3d* to *pyr-3a* and *arg*. It would, of course, be necessary to suppose that, at least in pseudo-wilds from which only one *pyr* mutant type was recovered, disomic nuclei persist in vegetative culture and that the pseudo-wilds tested are heterocaryons containing disomic as well as haploid nuclei. This explanation is perhaps the more convenient one of those suggested but the authors would like to emphasize that there is no direct evidence to support it and that it can probably be established with finality only by the cytological demonstration of the presence of disomic nuclei.

Even if the occurrence of $n + 1$ nuclei or of heterocaryotic spores were established as the explanation of the pseudo-wilds, the question of the genetic relationship between *pyr-3a* and *pyr-3d* would remain unanswered. The possibilities that they represent mutations at different loci or different mutations at the same locus would have to be considered equally probable since no evidence exists that in either case the presence of both *pyr-3a* and *pyr-3d* in the same nucleus would not establish a wild phenotype. The same, of course, applies if they were present together in a heterocaryon. From a practical standpoint, then, it seems useless at present to consider the question of whether the mutants are allelic. However, the fact that phenotypically wild strains have been obtained from crosses of *pyr-3d* to *pyr-3b* but have not yet been observed from crosses of *pyr-3a* to *pyr-3b* suggests the possibility that *pyr-3a* and *-3d* differ in some way. It may

be possible, by studying strains from asci with both normal and defective spores, to show that the cross of *pyr-3a* to *pyr-3b* yields strains which are not phenotypically wild but from which both parent mutants can be recovered. If so, investigation in this manner of other *pyr* mutants which are phenotypically similar to *pyr-3a* and *-3d* may prove to be of interest in showing how many types there are with respect to the ability to produce, in intercrosses, strains of this kind which are phenotypically wild.

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[1] Horowitz, N. H., Bonner, D. M., and Houlahan, M. B., *J. Biol. Chem.*, **159**, 145 (1945).

[2] Mitchell, H. K., and Houlahan, M. B., *Fed. Proc.*, **6**, 506 (1947).

[3] Mitchell, M. B., and Mitchell, H. K., PROC. NATL. ACAD. SCI., **38**, 205 (1952).

[4] McClintock, B., *Am. J. Bot.*, **32**, 671 (1945).

[5] Houlahan, M. B., Beadle, G. W., and Calhoun, H. G., *Genetics*, **34**, 493 (1949).

[6] Kölmark, G., and Westergaard, M., *Hereditas*, **25**, 490 (1949).

[7] Srb, A. M., and Horowitz, N. H., *J. Biol. Chem.*, **154**, 129 (1944).

[8] Singleton, J. R., unpublished Ph.D. Dissertation, California Institute of Technology (1948).

[9] Westergaard, M., and Mitchell, H. K., *Am. J. Bot.*, **34**, 573 (1947).

[10] Dodge, B. O., *J. Agr. Res.*, **35**, 289 (1927).

[11] Lawrence, W. J. C., *J. Genetics*, **24**, 257 (1931).

THE GENOTYPE OF THE ENDOSPERM AND EMBRYO AS IT INFLUENCES VIVIPARY IN MAIZE

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The development of the maize seed is dependent on the orderly unfolding of events in which each component of the developing caryopsis has a particular role to play. The ultimate control of these events must depend upon numerous genes, which if altered will interfere with normal development. Many mutants of this type have been described, ranging from those which produce relatively slight alteration in the caryopsis to those which prevent practically all development. Among those producing relatively slight changes are mutants which give rise to premature germination. The seeds of these mutants develop normally until late in ontogeny. During the early dough stage the plumule begins to elongate, and the seeds germinate while still attached to the ear. Such mutants have been called viviparous.