GENETIC RECOMBINATION IN NEUROSPORA

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STUDIES of the genetic behavior of nutritional mutants of Neurospora have, in the past, been carried out with certain assumptions already in mind. These assumptions were based, in part, on conclusions drawn from the behavior of mutants of higher organisms, particularly Drosophila and maize. It has been assumed that nuclei of the vegetative mycelia are regularly haploid; that one to one segregation of mutant and wild counterparts in a small number of asci demonstrates, more probably, control of the mutant expression by a single locus; that unlinked characters recombine by reassortment of chromosomes, but that linked characters recombine through crossing over. In the course of the genetic experiments, situations encountered which appeared not interpretable in a straightforward way within the framework of these assumptions have been put aside with the hope that, as knowledge of the organism and the mutants increased, such observations would become understandable in terms of the basic assumptions. This does not appear to be taking place, however. Rather, it seems, from the observations now accumulated, that the assumptions are, in general, simply not applicable. Further, it seems that it may be possible to arrive at others which more nearly apply. Observations accumulated over a number of years will be discussed from this point of view.

Frequencies of recombinants among random spores

Counts given in Table 1, 2, 3 and 4 are of samples of spontaneously released ascospores, spread on minimal agar plates and classified and counted under the microscope about 12 hours after germination. (The spores of mature asci are shed with some force so that in a slant culture they are deposited on the wall of the tube opposite the perithecia.) Thus it can be ascertained that each individual arises from a single ascospore and the question of heterocaryosis does not arise unless the spores themselves are heterocaryotic (MITCHELL, PITTENGER and MITCHELL 1952). It may be pointed out that data obtained in this way from random spores of Neurospora have, perhaps, few parallels. The frequencies observed are, presumably, analogous to gamete frequencies.

As such data have been accumulated odd coincidences have increased in number to a point at which they are scarcely credible as coincidences. Some examples will be elaborated as illustrations. The mutant designations used are those assigned by BARRATT *et al.* (1954). In general, different numbers following the same symbol (*arg-3* and *arg-6*, for example) indicate the same or similar requirements thought to be due to mutations at different "loci."

Taking the map distance or recombination frequency between two markers as twice the percentage of germinated spores which show neither of the two mutant phenotypes has been the usual practice in mapping from random spore counts. Thus cross 51 gives the distance between *arg-3* and *lys-4* in linkage group I as 2 x 1.9 units. But cross 52 shows *arg-6* and *lys* (28815), previously assigned to positions in another part of group I, (BARRATT *et al.* 1954) also to be located 2 × 1.9 units apart. If these four mutants actually represent four different sites on the chromosome it is curious that *arg* should be located at the same distance from *lys* in each case. No *lys* mutant is known to be located near *arg-2* in group IV. However, it has recently been found that *pyr-3a* and *pyr-3b* will respond to lysine as a substitute for pyrimidine if ammonia is excluded from the basal medium. And curiously, crosses 64 and 65 show *pyr-3a* and *pyr-3b* to be located 2 × 0.62 and 2 × 2 units, respectively, from *arg-2*. Such coincidences in conjunction with the observations that *lys* mutants are inhibited by arginine (DOERMANN 1945), *arg* mutants are inhibited by lysine and both *arg* and *lys* mutants interact in unpredicted ways with *pyr-3a* and its suppressor (HOULAHAN and MITCHELL 1948; MITCHELL and MITCHELL 1952) suggest that the relationship between *arg* and *lys* mutants is not readily understandable in terms of entirely separate mutational effects.

Further examples of these coincidences are offered by crosses involving *ad* and *hist* mutants. Cross 77 gives the distance between *hist-4* and *ad-6* in group IV as 2 × 2.3 units which is rather near that obtained between several pairs of mutants in group I as follows: *hist-3* × *ad-3*, 2 × 2.2 (91); *hist-2* × *hist* (1710), 2 × 1.9 (79) and *hist-2* × *hist-3*, 2 × 2.2(84). Nor are these distances very different from those found from *hist-2* × *ad-3*, 2 × 3.3 and 2 × 3.4 (42 and 87) or from *hist* (1710) × *ad-3*, 2 × 1.2 (43). Cross 78, on the other hand, gave a different distance between *hist-4* and *ad-6*, 2 × 8.4, but this is almost the same as that between *ad-7* and *hist-1* in group V, 2 × 8.9 (44). Also, it is curious that cross 83, of *hist-3* × *sn hist-3*, not expected to give prototrophs at all, gave 7.9 percent, and one of the crosses of *hist-3* × *sn hist-3 ad-3* (94), instead of giving no prototrophs as expected, gave 17.8 percent which is very nearly the same as the frequency of prototrophs from *ad-7* in group V × *ad-6* in group IV, 17.3 percent (50) although the latter cross was expected to give 25 percent.

In general, with respect to mutants assumed to be linked, there seem to be certain preferred recombination frequencies. Of particular interest in this connection are crosses 56, 57 and 58 in which *rib-1* gave 8.2, 7.4 and 7.9 percent prototrophs with *cyt* (C117), *tryp-2* and *tryp* (B1312) respectively. But cross 61, of *rib-1* × *pyr-3d*, which have been supposed to show false linkage, gave 8.3 percent. It seems a strange coincidence that the false and true linkages should be so nearly the same. The false linkage was once thought to be associated with a reciprocal translocation carried by the original isolate of *pyr-3d*, but it was later found that this strain is characterized instead by an extra chromosome (MCCLINTOCK 1954; SINGLETON 1948).

It may be seen from Tables 2 and 4 that crosses involving the three "colonials,"

TABLE 1

Mutants crossed to wild

Cross	Prototrophs percent	Number of spores
1. Wild Abbott 4 A × *hist-2* I	42.4	823
2. Wild Abbott 4 A × *hist-3* I	33.0	2513
3. Wild Abbott 4 A × *hist-4* IV	38.6	956
4. Wild—5912–2 A × *tryp-1* III	43.5	1443
5. Wild—5912–2 A × *pdx* (37803) IV	46.6	1413
6. Wild—5912–2 A × *ad-7* V	49.3	1629
7. Wild—4323–1 a × *pdx* (37803) IV	49.4	1601

TABLE 2

Crosses of nutritional and visible mutants

Cross	Prototrophs percent‡ v^+	v	Number of spores
8. *col-4* IV × *tryp-1* III	41.9	16.4	1661
9. *col-4* IV × *pyr-3a* IV*	1.2	46.9	1717
10. *col-4* IV × *pyr-3b* IV†	0.73	49.5	4113
11. *col-4* IV × *pyr 3d* IV	4.5	50.8	2879
12. *col-4* IV × *arg-2* IV*	0.4	48.9	3253
13. *col-4* IV × *pdx* (37803) IV	2.3	44.2	915
14. *col-4* IV × *arg-3* I	28.8	14.5	743
15. *col-4* IV × *ad-1* VI	23.8	24.2	959
16. *cot* IV × *ad-5* I	19.0	26.8	868
17. *cot* IV × *hist-1* V	22.2	27.0	803
18. *cot* IV × *hist-3* I	21.4	28.5	586
19. *cot* IV × *hist-4* IV	5.1	46.4	994
20. *cot* IV × *pan* (34556) IV	1.8	50.1	1508
21. *cot* IV × *ad-6* IV	0.76	55.2	787
22. *cot* IV × *ad-6* IV	0.87	58.5	1379
23. *cot* IV × *pyr-2* IV	11.2	not counted	2621
24. *cot* IV × *pyr-2* IV	11.3	not counted	3068
25. *cot* IV × *pyr-3b* IV†	12.4	not counted	3027
26. *cot* IV × *pyr* (37709) IV	13.0	not counted	1913
27. *cot* IV × *ad-7* V (25° C)	22.9	19.3	647
28. *sn* (C136) I × *arg-3* I	1.6	49.6	4902
29. *sn* I × *arg-3* I	4.0	51.1	2305
30. *hist-2* I × *sn* I	0.77	49.7	5033
31. *sn* I × *hist* (1710) I	2.1	49.1	2129
32. *sn* I × *lys-4* I	1.8	50.1	1622
33. *sn* I × *sn* I	1.9	96.5	4434
34. *sn* I × *sn-b* (C132) I	0	100	4000 app.

* MITCHELL, PITTENGER and MITCHELL 1952.
† MITCHELL and MITCHELL 1954.
‡ The symbol, v, represents the appropriate visible mutant, *col*, *cot* or *sn*.

TABLE 3

Crosses between nutritional mutants

Cross	Prototrophs percent	Number of spores
35. *tryp-1* III × *nic-1* I	27.1	1102
36. *tryp 1* III × *pdx* (37803) IV	22.4	2450
37. *pdx* (37803) IV *tryp-1* III × *tryp-1*	0	4000 app.
38. *ad-6* IV × *tryp-1* III	45.0	650
39. *tryp-1* III × *hist-1* V*	28.7	1583
40. *hist-2* I × *tryp-1* III*	27.6	553
41. *hist-4* IV × *tryp-1* III	23.9	4891
42. *hist-2* I × *ad-3* I*	3.3	1712
43. *hist* (1710) I × *ad-3* I	1.2	2694
44. *ad-7* V × *hist-1* V	8.9	906
45. *hist-4* IV × *pyr-2* IV*	13.1	2448
46. *hist-4* IV × *pyr-3a* IV*	13.9	4891
47. *pyr-3b* IV × *hist-4* IV‡	14.3	3116
48. *hist-4* IV × *hist* (1710) I	23.3	2335
49. *hist* (1710) I × *hist-3* I	0.07	5926
50. *ad-7* V × *ad-6* IV	17.3	797
51. *arg-3* I × *lys-4* I	1.9	1950
52. *lys* (28815) I × *arg-6* I	1.9	1910
53. *inost* (83201) V × *pyr-3a* IV	34.4	3776
54. *rib-1* VI × *ad-1* VI	0.45	6783
55. *rib-1* VI × *un* (66204) VI	4.2	7417
56. *rib-1* VI-*cyt* (C117) VI†	8.2	2405
57. *rib-1* VI × *tryp-2* VI	7.4	1654
58. *rib-1* VI × *tryp* (B1312) VI	7.9	869
59. *rib-1* VI × *pyr-4*	24.9	1640
60. *rib-1* VI × *hist-1* V*	31.2	1228
61. *rib-1* VI × *pyr-3d* IV	8.3	3381
62. *rib-1* VI × *pyr-3d* IV	15.4	1155
63. *un* (66204) VI × *cyt* (C117) VI	3.8	743

* HAAS *et al.* 1951.
† MITCHELL, MITCHELL and TISSIERES 1953.
‡ MITCHELL and MITCHELL 1954.

col-4, *cot* (C102, colonial, temperature-sensitive) and *sn* (C136, snowflake), with nutritional mutants linked to them show, in general, rather similar map distances. The three mutants are similar in phenotype, the abnormality consisting of excessive branching of the hyphae. At about 32°C the growth habit of *cot* is very like that of *sn* at all temperatures, whereas *col-4* differs from them both in that its hyphal branches are not straight, but gently curved. In several crosses (between nutritional mutants) in which *sn* was heterozygous and which gave low frequencies of *sn* prototrophs (particularly crosses involving *hist* and *ad*) it was noticed that some of these prototrophs were so like *col-4* that, had *col-4* been present in the cross they would have unhesitatingly been so classified. It was not a complete surprise, then, when a cross of *sn* × *lys arg sn* (not shown in the table) gave the following frequencies of prototrophs: *sn col-4*$^{+}$, and *sn col-4*, 52

TABLE 4

Crosses involving three mutants

Cross	Prototrophs percent‡ v+	Prototrophs percent‡ v	Number of spores
64. *col-4 arg-2* × *pyr-3a* IV	0.60	0.018	5608
65. *col-4 arg-2* × *pyr-3b* IV	2.0	0	4542
66. *arg-2* × *col-4 pyr-3d* IV*	0.11	0.94	2653
67. *col-4 arg-2* × *pyr* (49001) IV	1.1	0	7674
68. *col-4 pyr-1* × *pyr-3b* IV	1.2	1.8	2898
69. *col-4 pyr-3b* × *pyr-1* IV†	0.75	0.23	2611
70. *col-4 pyr-3b* × *pyr-1* IV	1.7	1.4	4309
71. *col-4 pyr-3d* × *pyr* (67011) IV	0.9	0	3789
72. *col-4 pyr-3b* IV × *sn* I	23.7	0.35 *col sn+*	
		25.6 *col+ sn*	
		0.31 *col sn*	2287
73. *col-4 pyr-1* × *pdxp* (39106) IV	0.036	0.14	2729
74. *cot* × *col-4 pyr-2* IV	1.42	35.9 *col+ cot*	
		7.2 *col cot+*	
		7.2 *col cot*	1829
75. *pan* (34556) × *cot ad-6* IV†	0.89	0.29	3027
76. *cot hist-4* × *pan* (34556) IV†	0.67	0.04	9773
77. *cot hist-4* × *ad-6* IV†	1.1	1.2	4290
78. *hist-4* × *cot ad-6* IV	3.9	4.5	1690
79. *sn hist-2* × *hist* (1710) I	1.8	0.06	10915
80. *hist-2* × *sn hist-2 hist* (1710) I	0	0	2000 app.
81. *hist* (1710) × *sn hist-2 hist* (1710) I	0	0	3000 app.
82. *sn hist-3* × *hist* (1710) I	0.006	0.003	59000 app.
83. *hist-3* × *sn hist-3* I	5.9	2.0	1818
84. *sn hist-2* × *hist-3* I	2.0	0.17	8978
85. *hist-2* × *sn hist-2 hist-3* I	0	0	3000 app.
86. *hist-3* × *sn hist-2 hist-3* I	0	0	5000 app.
87. *sn hist-2* × *ad-3* I	3.3	0.12	4927
88. *hist-2* × *sn hist-2 ad-3* I	0	0	6000 app.
89. *hist* (1710) × *sn hist-2 ad-3* I	0	0	1000 app.
90. *hist-3* × *sn hist-2 ad-3* I	12.0	0.6	1463
91. *sn hist-3* × *ad-3* I	2.1	0.05	9558
92. *sn ad-3* × *hist-3* I	0.21	4.2	2418
93. *hist-3* × *sn hist-3 ad-3* I	19.0	2.7	1608
94. *hist-3* × *sn hist-3 ad-3* I	16.0	1.8	1279
95. *sn hist-2* × *arg-3* I	1.8	0.3	8414
96. *arg-3* × *sn hist-2* I	2.3	0.5	7915
97. *sn hist-2* × *arg-3 hist-2* I	0	0	4000 app.
98. *sn* × *arg-3 hist-2* I	0.09	53.9	3544
99. wild × *sn arg-3 hist-2* I	46.3	0.018	5674
100. *sn hist-2* × *lys-4* I	0.6	0.18	11175
101. *sn hist-2* × *lys-4* I	4.0	0.18	5400 app.
102. *sn arg-3* × *lys-4* I	2.6	5.4	6679
103. *sn* × *arg-3 lys-4* I	0.12	50.3	2449
104. wild × *sn arg-3 lys-4* I	36.7	0.11	1756
105. wild × *sn arg-3 lys-4* I	44.0	0.039	2800
106. *ad-5* × *sn arg-3* I	0.13	0	6041

* MITCHELL, PITTENGER and MITCHELL 1952.
† MITCHELL and MITCHELL 1954.
‡ The symbol, v, represents the appropriate visible mutant, *col, cot* or *sn.*

percent; *sn*+ *col-4*, 4.2 percent; *sn*+ *col-4*+, 12.8 percent. A few of the auxotrophs (31 percent) were isolated and found to respond to arginine plus lysine. In this connection it is of interest that *sn*, which is of spontaneous origin and has been found as an infrequent segregant in several crosses, was first isolated from a cross involving *col-4*. It seems that *sn* and *col-4*, and possibly *cot*, may be related to one another in a way not elucidated by supposing them to be mutations at three different sites, two linked and one unlinked.

If one considers the crosses of auxotrophs to prototrophs, expected to give 50 percent prototrophs, and crosses between supposedly unlinked mutants, from which are expected 25 percent of progeny showing neither mutant phenotype, one again encounters the numbers which recur as frequencies of recombination of linked mutants. But here they represent deviations from the expected values of 50 or 25 percent. The more frequent deviations are around 2 or 3 and 7 or 8. In the past such discrepancies have been dismissed as possible reflections of differences in viability, since the germination is usually not 100 percent. Yet if one dismissed the 7.6 percent excess of mutants in cross 1 as due to failure of the wild spores to germinate as frequently as the mutants, then, to be consistent, one would need to dismiss the difference between 1.42 per cent *col-4*+ *cot*+ *pyr*+ and 7.2 percent of both *col-4 cot*+ *pyr*+ and *col-4 cot pyr*+ in cross 74. But if this is done, there remains no reason for supposing *col-4*+ *cot*+ *pyr*+ to result from double crossovers and the other two classes of prototrophs from single crossovers; hence the basis for assigning the gene order, *col-4 cot pyr*, then disappears. If the differences in frequency of these three classes are not disregarded, then it may be of interest to attempt to find an explanation of the fact that the 1.72 percent excess of *pyr*+ progeny so nearly parallels the frequency of *col-4*+ *cot*+ *pyr*+ (1.42 percent).

The demonstration of 1:1 segregation by analysis of whole asci has often been taken as evidence of the 1:1 relationship between mutants and their wild counterparts. However, the number of asci examined per cross has usually been small, around 20 to 40, which represents, of course, 160 to 320 spores. Because of the sterility of many of the crosses (to be discussed below) even a small number of complete asci is often not easily found and may represent, therefore, a highly selected sample, giving a limited picture of events in the cross.

Assignment of gene order

Some of the data in Tables 2 and 4, from crosses involving mutants of group IV, have been published more fully along with a map of this group (MITCHELL and MITCHELL 1954). When this map was being prepared there was, in several instances, some uncertainty about the order assigned. To consider first the crosses 75, 76 and 77, if a linear order is given to *ad-6*, *pan*, *cot* and *hist-4*, two of the three nutritional mutants must, of course, be on the same side of *cot*. Hence, one of the three crosses should give a low frequency of either *cot*+ or *cot* prototrophs since one of these would have to arise as double crossovers. It might have been supposed that the *cot* prototrophs from *cot hist* × *pan* (76) were double crossovers and that the order was *ad cot hist pan*, although if single crossovers in this cross occurred between *cot* and *hist* with about the same frequency as in *cot hist* × *ad* (77) the frequency of double crossovers would have been too high. It was noticed, however, that both *cot hist* × *pan* and *cot hist* × *ad* gave two types of cot^+ prototrophs, "typical" and "atypical," but that *pan* × *cot ad* gave only the "atypical" type. The atypical cot^+ prototrophs resembled, in their growth habit on agar plates, the pseudowilds found from other crosses (MITCHELL, PITTENGER and MITCHELL 1952; PITTENGER 1954) although usually with a lower frequency. The pseudowilds had been found to behave as heterocaryons from which both parent mutants of the same mating type could be recovered by isolating single conidia. Samples of the "atypical" wilds were therefore tested and found, indeed, to behave as heterocaryons. It was then supposed that cot^+ progeny from *pan* × *cot ad* were not products of crossing over and could be disregarded, so that the gene order, *ad pan cot hist*, could be assigned. More recently it has been observed that from cross 3, of wild × *hist-4*, one third of the prototrophs were "atypical" and from wild × *hist-3* (2) about two thirds were "atypical." Cross 19, *cot* × *hist-4*, was then found to give not only at least two types of cot^+ $hist^+$, but also two types of *cot* $hist^+$ progeny, more extreme and less extreme, or "dilute," occurring with very nearly the same frequency. A similar situation was found in *cot* × *hist-3* (18) and *cot* × *hist-1* (17) although in the latter cross the two types of cot^+ $hist^+$ and *cot* $hist^+$ progeny were less sharply defined, as if there were more than two types in each case. These results make it evident that the gene order, *ad pan cot hist*, is not adequately demonstrated.

The obstacles encountered in assigning order to the markers closely linked to *col-4* have been described (MITCHELL and MITCHELL 1954; MITCHELL 1955a,b, 1956). If one class of prototrophs, diagnosed as pseudowilds, was disregarded, the more plausible order appeared to be *pyr-1 pdx col-4 arg-2 pyr-3*, but crosses involving the first three markers gave an unexpectedly high frequency of progeny which would have had to arise as double crossovers or through some mechanism other than crossing over. These progeny were found to behave as if they were genetically pure with respect to the phenotypes they showed and could not, therefore, be dismissed as pseudowilds. Upon finding these recombinants in asci which showed 3:1 segregation of pdx^+ and *pdx* but 2.2 segregation with respect to the other two markers, *pyr-1* and *col-4*, it was supposed that a mechanism other than crossing over was, indeed, at work. It was assumed that the mechanism involved was analogous to gene conversion, or mutation in heterozygotes (DE SERRES 1956; GILES 1956; ST. LAWRENCE 1956). There appeared to be an association with crossing over, however, since the frequency of the "aberrant recombinants" varied with the frequencies of the "crossover recombinants" in crosses involving different isolates of the mutants.

Prototrophs from *pdx* × *pdxp* (regarded as an allele of *pdx*) were attributed to the same mechanism when they were found in asci which appeared to represent irregular segregations of either *pdx* or *pdxp* because of the absence of the expected *pdx pdxp* segregants from asci in which pdx^+ $pdxp^+$ recombinants were found. The test for the double mutant, *pdx pdxp*, consisted of backcrossing the phentotypically *pdx* and *pdxp* segregants from these asci to each parent mutant. Neither *pdx* nor *pdxp* had been observed to give prototrophs when crossed to itself, hence the double mutant would be expected to give no prototrophs when crossed to either parent. By this test each of the mutant segregants behaved as a single mutant by giving prototrophs when crossed to the parent phenotypically unlike itself.

An attempt to find other cases of "aberrant recombination" has involved some closely linked mutants in linkage group I and has led to ambiguities not only in assigning gene order but also in distinguishing between double and single mutants. A recombinant tetrad from *sn hist-2* × *hist-3* (84) at first appeared entirely regular. By the backcross test (The two *hist* mutants are identical in phenotype (HAAS *et al.* 1952).) it appeared to be of the following constitution:

sn hist-2 hist-3
sn hist-2 hist-3$^+$
sn$^+$ *hist-2*$^+$ *hist-3*
sn$^+$ *hist-2*$^+$ *hist-3*$^+$

With the gene order given above, this result is consistent with the occurrence of a single crossover between *hist-2 and hist-3*. The appearance among random spores of 0.17 percent *sn hist-2*$^+$ *hist-3*$^+$ progeny was disturbing, however, not only because, with this gene order, they would have had to arise as double crossovers or as "aberrant recombinants" but because they appeared with the same frequency as the "aberrant recombinants" from *pdx* × *pdxp*. An essentially identical result was obtained with a recombinant ascus from cross 79 of *sn hist-2* × *hist* (1710), from which 0.06 percent *sn hist-2*$^+$ *hist* (1710)$^+$ segregants were found among random spores.

Recombinant asci from *sn hist-2* × *ad-3* (87) and *sn hist-3* × *ad-3* (91) were, from the standpoint of phenotypes of the segregants, conventional. These phenotypes were as follows:

sn hist-2 ad$^+$		*sn hist-3 ad*$^+$
sn hist-2 ad		*sn hist-3 ad*
	and	
sn$^+$ *hist-2*$^+$ *ad*$^+$		*sn*$^+$ *hist-3*$^+$ *ad*
sn$^+$ *hist-2*$^+$ *ad*		*sn*$^+$ *hist-3*$^+$ *ad*$^+$

But not only did the questionable recombinants, *sn hist-2*$^+$ *ad-3*$^+$ and *sn hist-3*$^+$ *ad-3*$^+$, again occur with nearly the same frequencies as in the *pdx* case (0.12 percent and 0.05 percent), the backcross test, as applied in the cross *hist-3* × *sn hist-3 ad-3* (93 and 94) gave results contrary to those expected, namely 21.7 and 17.8 percent prototrophs instead of none; and cross 90, *hist-3* × *sn hist-2 ad-3* appears to give, with respect to *hist-3* and *ad-3*, a different gene order from that found in *sn hist-3* × *ad-3*. From the cross involving all four markers one would suppose *hist-3* to be distal to *ad-3* since *sn*$^+$ *hist-2*$^+$ *hist-3*$^+$ *ad-3*$^+$ is the more frequent prototroph. Three other crosses, intended as checks on the behavior of the *hist-3* strain did nothing to clarify the situation. (The same *hist-3* isolate was used in crosses 2, 18, 83, 84, 86, 90, 93, 94 and in the cross to *sn* which produced the *sn hist-3* parent of crosses 83 and 91. Random spores from *sn* × *hist-3* were not counted but 23 asci gave an apparently conventional result, consistent with *hist-3* being 7.5 units distal to *sn*). In cross 83 *hist-3* behaved unconventionally by giving 7.9 percent prototrophs when crossed to *sn hist-3*, a descendent of itself; in cross 2 it behaved as a double mutant, giving 67 percent mutant progeny when crossed to wild; and in *cot* × *hist-3* (18) it behaved as a single mutant since 49.9 percent prototrophs were found (Peculiarities of the latter cross have been mentioned.).

It seems, then, that *hist-3* can be regarded as a single-gene mutant assignable to a position in a lineal map only if the results of certain crosses are rejected, but there seems no justification for rejecting these crosses and accepting others. Nor does rejection of *hist-3* on the grounds that it is a "peculiar" mutant seem justified or even very helpful. The behavior of *hist-2* in crosses with *lys-4* and *arg-3* is not easily explained in conventional terms. An attempt to analyze complete asci from *sn hist-2* × *lys-4* (101) had to be abandoned because of the appearance of segregants which, although they germinated, were unable to grow on minimal medium supplemented with lysine and histidine. (Complete medium could not be used since it inhibits growth of *hist-2*.) About one in five asci were found to contain these segregants which, it appeared, could not be *hist lys* double mutants since they were not accompanied, in the same ascus, by *hist*$^+$ *lys*$^+$ segregants. Neither parent showed a requirement other than its own, nor did either behave as a double mutant when crossed to *sn* (30 and 32). Yet it seemed that they could recombine with each other in such a way as to produce an additional requirement. With respect to crosses of *hist-2* and *arg-3* (95, 96, 97, 98 and 99) it may be seen that 98 gave a conflicting result. In 99, in which *sn arg-3 hist-2* is crossed to *sn*$^+$ *arg-3*$^+$ *hist-2*$^+$, the two auxotrophs appear to have recombined much as they did in repulsion (95 and 96) but in 98, *sn arg*$^+$ *hist*$^+$ × *sn*$^+$ *arg hist*, they gave, instead of more than 50 percent auxotrophs as expected, an excess of prototrophs which is nearly the same as the excess of auxotrophs from *sn*$^+$ *arg*$^+$ *hist*$^+$ × *sn arg hist*.

Curiously, quite a similar situation is seen in crosses of the *arg-3 lys-4* double mutants. Crosses of *sn arg lys to sn*$^+$ *arg*$^+$ *lys*$^+$ (104 and 105) gave more than 50 percent auxotrophs as expected, but *sn arg*$^+$ *lys*$^+$ × *sn*$^+$ *arg lys* gave a little more than 50 percent prototrophs. The unpredicted behavior of *sn arg*$^+$ *lys*$^+$ × *sn arg lys* has already been mentioned. This cross not only gave 69 percent prototrophs although it was expected to give less than 50 percent; it gave 16 percent *sn*$^+$ progeny although it was supposedly homozygous with respect to *sn* and, further, 4.2 percent of the progeny were phenotypically indistinguishable from *col-4*, a mutant similar to *sn* but assigned to another linkage group. Another cross (33) in which *sn* was homozygous, involved the *sn arg*$^+$ *lys*$^+$ parent of the above cross (This was the *sn* parent in crosses 28, 29, 30, 32, 98 and 103 also.) and an *sn arg*$^+$ *lys*$^+$ segregant from 102 and gave, not only 1.9 percent *sn*$^+$ progeny, but also 1.6 percent auxotrophs.

Growth responses

A number of cases are known in which nutritional mutants appear to undergo changes in their growth responses as a result of recombination. One of the more carefully examined cases was reported by HASKINS and MITCHELL (1952) and concerned a mutant (39401) which responds to either trytophan or nicotinamide. In certain crosses this mutant showed frequent modifications such that its growth responses became more like those of mutants thought to be at other loci and to control other steps in the biosynthetic pathway concerned. Similar situations found with arginine, lysine and pyrimidine mutants have already been referred to (HOULAHAN and MITCHELL 1948; MITCHELL and MITCHELL 1952). The latter studies culminated in the finding that the original isolate of the pyrimidine mutant involved (37301) could, under certain culture conditions, utilize either lysine or pyrimidine to satisfy its growth requirement. Certain recombinants of this *pyr* mutant with lysine mutants appeared to have acquired a requirement for arginine although an inhibition by arginine had characterized one, or both parents. It has also been observed that crosses of *pyr* to a proline mutant (21863) appeared to give at least two kinds of double mutants, one responding, and the other failing to respond to proline plus pyrimidine. Another case, observed long ago, was that of two thiamin mutants (17084 and 56501) crossed to a third (50005). From these crosses any ascus containing wilds was found also to contain an equivalent number of segregants which did not respond to thiamin. Presumably these were the double mutants but they were not analyzed genetically nor was their requirement determined.

The belated discovery that the *pyr* mutant can utilize lysine suggests the possibility that if more exhaustive growth tests were applied, growth responses of the mutants might, in general, appear less simple and straightforward. The apparent acquisition of new growth requirements calls to mind the fact that the way in which many of the genetic tests have been performed did not take into account the possibility that new requirements might arise. Crosses designed to test for 1:1 segregation of mutant and wild alleles in asci have often been examined by establishing cultures from single ascospores on complete medium and then testing these by subculturing on minimal medium. This, of course, only demonstrates a requirement for something in the complete medium. Crosses between mutants having the same requirement were regularly examined in the same way and, in some cases, when two requirements were involved, the double mutants were identified merely by their failure to respond to either growth factor alone. Thus we do not know to what extent the growth responses reappear unchanged from crosses. It can be said, however, that in the relatively few crosses for which minimal medium with restricted supplements was used to establish cultures, segregants with unexpected requirements were by no means rare. The appearance of such segregants from *hist-2* × *lys-4* (101) has been mentioned above, as has the *sn* × *sn* cross (33) which gave 1.6 percent auxotrophs. Cross 32, *sn* × *lys-4* gave 1.2 percent auxotrophs among 1027 spores plated on minimal plus lysine. From cross 91, *sn hist-3* × *ad-3* about one ascus in ten contained segregants which did not grow on the combined supplement of adenine and histidine. When random spores were plated on this medium 4.1 percent among 552 failed to grow. Cross 48, *hist-4* × *hist* (1710) gave 1.9 percent auxotrophs among 940 spores on minimal plus histidine. Among nine asci from cross 38, *ad-6* × *tryp-1* (which gave 45 percent prototrophs among random spores) three were found to contain segregants which grew very little on minimal plus adenine and trytophan but grew well on complete. Curiously, these segregants were albino on the minimal medium but normally colored on complete. Their requirement appeared partially satisfied by adenine plus methionine but the albino character was expressed on this medium, too.

Sterility

It is well known that very many of the Neurospora crosses are characterized by frequent ascus and spore abortion. A cross from which as many as 20 percent of the asci contain eight normal-appearing spores is considered rather fertile. The sterility is not usually traceable to any simple cause. More often it appears and disappears in an unpredictable fashion, even in crosses involving closely related strains. A fairly general approach to this difficulty is that if one can get some spores or asci to examine, one examines them, without undue concern about those that failed to appear.

Recently an attempt has been made to recheck some of the linkage map results by starting again with some highly fertile strains which had not been intercrossed. For this purpose the original isolates of several mutants were obtained from conidia lyophilized in 1944, soon after the mutants were isolated (BEADLE and TATUM 1945). These included *pyr-3a* (37301), *pyr-3b* (37815) and *lys* (37811), derived from the same wild parents, one of which (25a) was also obtainable from an old lyophilized culture. The results of crosses with these strains were disappointing. No highly fertile crosses were obtained. That of *pyr-3a* to *lys* was completely sterile and the others gave around 20 percent of asci with normal-appearing spores but perhaps as many as five percent had only four spores. This had not been noticed previously.

The four-spored asci were of at least two types, one long enough to have contained eight spores and the other just long enough for four. Germination of spores from these asci was poor but in two from Wild 1A × *lys* there was 1:1 segregation of *lys* and *lys*+. A cross of one of the *lys* segregants to a wild from a four-spored ascus of Wild 1A × Wild 25a seemed somewhat less fertile than either parent cross and still gave both four- and eight-spored asci.

The cross of *pyr-3b* × *pyr-3a* had previously been found to give 1:1 segregation in complete asci (HOULAHAN, BEADLE and CALHOUN 1949), but when random spores were now examined at 25° C (Mutant *pyr-3b* is temperature-sensitive and not fully expressed at 25° C) only 25 percent among 1050 spores were classifiable as *pyr-3a*.

The cross more thoroughly examined was that of the original isolate of *inost* (83201, derived from Wild Abbott 4A × 25a), also temperature-sensitive, to *pyr-3a*. At 35° C it gave 34.4 percent prototrophs instead of the expected value of

25 percent (53) and at 25° C, only 28 percent of progeny classifiable as *pyr-3a* instead of 50 percent as expected. Asci with eight mature spores (about 10 to 20 percent) appeared fairly regularly to show 1:1 segregation of two spore sizes. Of 33 asci dissected only one germinated completely. Segregation in this one was as follows:

pair 1	$inos^+$	pyr^+
pair 2	$inos^+$	pyr^+
pair 3	*inos*	*pyr*
pair 4	*inos*	*pyr*

Four crosses of these segregants showed no increased fertility, 1 × 3 and 1 × 4 being less fertile than the parent cross. Among spores incubated at 25° C three types could be classified, one presumably representing $inos^+$ *pyr* and *inos pyr;* the other two types were prototrophs, one slow-growing and the other "typical," perhaps representing *inos* pyr^+ and $inos^+$ pyr^+. The proportions of the three types are given in Table 5. It is unlikely that these frequencies represent chance deviations from the expected 50, 25 and 25 percent. Nor does the idea that the different phenotypes differ in their ability to germinate satisfactorily explain the deviations.

TABLE 5

Crosses of inost *(83201) and* pyr-3a *incubated at 25° C. Parent cross* = inos pyr+ × inos+ pyr; *intraascus crosses* = inos+ pyr+ × inos pyr

	Types of progeny (percent)			Number of spores
	$inos^+$ pyr^+	*inos* pyr^+	$inos^+$ *pyr* / *inos pyr*	
Expected	25	25	50	
Observed				
Parent cross	36.0	35.7	28.3	294
Pair 1 × pair 3	34.1	29.8	36.0	211
Pair 1 × pair 4	37.4	29.3	33.3	392
Pair 2 × pair 3	29.2	27.2	43.6	1223
Pair 2 × pair 4	30.9	28.2	40.9	932

The further observation was made that, from the parent cross, among six asci in which at least one member of each spore pair germinated two showed irregular segregations as follows:

$inos^+$	pyr^+	*inos*	pyr^+
$inos^+$	pyr^+	*inos*	pyr^+
$inos^+$	*pyr*	$inos^+$	*pyr*
inos	pyr^+	*inos*	pyr^+

Although particular care was taken in the dissections, the possibility that the order was incorrectly recorded is not ruled out, since, in the first ascus listed only one member of each spore pair germinated and in the second, only one member of pairs 1 and 2.

Reverse mutation and somatic segregation

When nutritional mutants appear to have reverted in vegetative culture it has sometimes been possible to find, from outcrosses of the reverted strains to standard wild, frequent asci in which all segregants are prototrophs, as one would expect if reverse mutation had occurred. In other cases there are found asci from which more than four spores give rise to prototrophs but the segregations are those expected if mutation to a suppressor of the mutant character had taken place. Sometimes, however, the presence of wild nuclei in a reverted strain has been difficult to demonstrate.

The latter situation was found with reverted *pdx* (37803) and *pdxp* (39106) segregants from crosses between these two mutants (MITCHELL 1956). A rather high incidence of reversion was found if conidia from the mutant segregants were inoculated into minimal medium and left standing for several weeks. When the reverted isolates were backcrossed to the same mutant, unreverted, no prototrophs were found among 5,000 to 10,000 offspring even though the protoperithecia had been formed by the reverted strain on minimal medium. Prototrophs were obtained from such backcrosses only after repeated subculturing of the reverted strain on minimal medium. Yet if conidia from the original reverted cultures were plated on minimal medium many of them were able to grow. A similar situation had been found earlier with revertants of *thi-2* (9185) (unpublished data of DR. G. W. BEADLE and the author).

It now seems that an interpretation of these cases, not in terms of reverse mutation, but of somatic segregation may be more fitting. It seems that, through somatic segregation, nuclei may arise with a chromosome complement such that they confer upon a strain, pure, with respect to them, the capacity for independent growth. These nuclei might reappear from an outcross or a backcross. If, on the other hand, independent growth depends upon the interaction, in the resulting heterocaryon, of the new nuclei with the old, prototrophs might not reappear from backcrosses. The prototrophs found after repeated selection might be due to a secondary process.

DISCUSSION

It is, of course, difficult to consider, at this time, the possibilities that we have not demonstrated the nutritional mutants of Neurospora to be due to "single-gene changes" and that we have not been able to "locate" them satisfactorily on "linear maps." Yet it is hardly possible not to see that the inconsistencies and ambiguities as well as the unpredicted regularities observed in genetic behavior may have a common explanation, merely that the nuclear behavior in developing asci and the number of chromosomes in the complement are variable. If it is supposed that expression of the mutant characters is determined in a manner analogous to that in which sex is thought to be determined in Drosophila, that is, by the relative dosages of certain chromosomes, then it seems that flexibility sufficient to account for the actual observations may exist, particularly if similar but slightly different chromosomes have been introduced into the stocks from different wild sources. As MCCLINTOCK (1954) has already suggested, it seems futile to attempt a serious, detailed interpretation of any cross without knowledge of the nuclear behavior in *surviving* asci. The recurrence of certain prototroph frequencies suggests that there are in general certain patterns of nuclear behavior which lead to the formation of viable spores. Possibly a more precise analysis, both genetically and cytologically, of fertile crosses will reveal the nature of some of these patterns.

With regard to the growth requirements, it already appears likely that relationships between mutants having different requirements as well as between those having like requirements have been misunderstood. Perhaps a more thorough analysis, from a point of view which allows as much significance to be attached to the "modifiers" as to the "primary characters," may be revealing here too.

SUMMARY

1. Recurrence of certain frequencies of recombination of "linked" mutants was observed by classifying and counting random ascospores about 12 hours after germination. The more commonly recurring frequencies of various classes of prototrophs are between 0.5 and three percent and around seven and eight percent. These same numbers appear as frequent deviations from expected frequencies of 25 or 50 percent in crosses of "unlinked" mutants or of mutants to wild.

2. In several "map regions" analysis of "closely linked" mutants failed to produce convincing evidence of a "linear order" of the markers.

3. Results of backcross tests used to classify mutant segregants as "single" or "double" mutants suggest that this distinction is meaningless except in application to specific crosses. The same mutant isolate may give the result expected of a "double" mutant in one cross but behave as a "single" mutant in another.

4. It appears that in crosses of nutritional mutants the growth responses may undergo frequent changes and new requirements may arise as a result of recombination.

An expansion and revision of basic assumptions to make them more applicable to the actual observations seems indicated. It is tentatively suggested that if the phenotypic expressions are regarded as reflections of differences in dosages of the chromosomes, a more realistic picture may be obtained through detailed cytological and genetic studies.

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