INDICATIONS OF PRE-ASCUS RECOMBINATION IN NEUROSPORA CROSSES

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IN heterothallic species (those regularly showing mating-type reaction) of Neurospora, two modes of fruiting body initiation have been recognized. Parent *A* may form incipient fruiting bodies which are then fertilized by parent *a*, or the reverse. DODGE (1935), working with *N. sitophila* and SANSOME (1946), with *N. crassa*, observed that hyphae growing out from fertile, immature perithecia expressed the phenotype of one or the other parent rather than that expected of a heterokaryon constituted by vegetative interaction of the two. The behavior of a maternally inherited character in *N. crassa* (MITCHELL and MITCHELL 1952) also suggested that heterokaryon formation did not regularly precede the initiation of perithecia. The observations to be reported here are in accord with this but suggest that, although it may be less common and may usually be accompanied by one or both of the other two possibilities, initiation of perithecia by heterokaryons also occurs. The point of primary interest is the possibility suggested that such a situation may, by increasing the opportunity for vegetative recombination, lead to events which have heretofore been interpreted in terms of mutation.

METHODS

With a few exceptions, crosses were made on slants of Difco (B114) cornmeal agar and incubated in the dark at 25°C for about 14 days. Spontaneously released spores and spores removed from asci were heat shocked, incubated and classified, according to colony forms produced, on plates of minimal agar medium as described earlier (MITCHELL 1959, 1960). Stock cultures were usually maintained on essentially the same medium with 0.25 percent Difco yeast extract added. The strains used will be described as they are introduced in the following section.

RESULTS

Cross A: Progenies of Neurospora crosses are rather often found to include occasional segregants with mutant phenotypes not expressed by the parents and not predicted as recombinants. These have long been referred to, informally, as "wandering mutants," and have been thought of as new mutations. When individual perithecia are analyzed, it is not uncommon to find a wandering mutant as an infrequent and sometimes erratic segregant in asci of the majority, but showing regular 4 m:4m^+ segregation in those of an occasional perithecium.

An illustration of this behavior is provided by a cross of the temperature-sensitive colonial mutant, C102, in linkage group IV (*cot*-8025-1*a*, MITCHELL 1960) to a morphological marker in linkage group I (author, unpublished). The latter mutant *csf*(C170 R2 *A*, corkscrew), was found by DR. H. K. MITCHELL. It is characterized, in young colonies, by slower than average growth and fine, curly hyphae. The cross was an unusually satisfactory one from the standpoint of the proportion of normal asci produced, germination of ascospores, unambiguous expression and regular segregation of phenotypes. From 12 perithecia tested, the numbers of completely germinated octets were as follows: 11, 11, 3, 7, 9, 14, 28, 17, 66, 47, 71, 22. The conspicuous wandering mutant observed was a clearly expressed auxotroph (*au*), the average length of hyphae produced by an ascospore germinated on minimal medium (25°C) being about that of the spore. When such a spore was transplanted on complete medium, the hyphae grew slowly as if partially inhibited. The specific growth requirement, if there is one, has not been determined. The *au* character was expressed in two spore pairs of one ascus from the seventh perithecium, in one spore pair of one ascus from the ninth, but in two spore pairs of each of the seventy-one asci from the eleventh.

From a cross of an *au csf*$^+$ *cot*$^+$ strain to the *csf* parent, 129 complete octets showed 4 *au*:4 *au*$^+$ segregation and no indication of linkage. A similar backcross to the *cot* parent was unsuccessful, but the appearances of *cot* among *au*$^+$ segregants of the exceptional (eleventh) cross-A perithecium indicated no linkage.

In this case appearances of the wandering mutant could be attributed to three occurrences of the mutation, *au*$^+$ to *au*, in perithecium 11 prior to ascus initiation and in perithecia 7 and 9 after ascus initiation. However, a faint possibility of a relationship between the mutation and the *cot* strain is suggested by observations of wandering mutants of the same phenotype in two other crosses, one involving the *cot* parent of cross A and the other, a *csf cot* segregant from it.

(An exceptional ascus of a different sort from cross A is of interest in connection with cross C below. Here *csf* appeared to have recombined reciprocally, the two recombinants having, respectively, fine, slow-growing, not-curly hyphae (*sf*) and coarse, curly hyphae growing at the wild-type rate (*c*). Phenotypes of the four spore pairs might, therefore, be written as follows: *c sf*; *c*$^+$ *sf*$^+$; *c*$^+$ *sf*; *c sf*$^+$. A *c sf*$^+$ segregant was crossed to the *c sf* parent and 100 asci from a single perithecium examined. All segregants had curly hyphae and all 83 completely germinated octets showed 4 *sf*:4 *sf*$^+$ segregation. This is the result expected if *csf* is a double mutant whose components are curly and slow-fine.)

Cross B: This cross gave, in some respects, less tidy results than cross A, but is of interest because of the very close linkage observed between the wandering mutant recovered and a mutant carried by one of the parents. The cross, *sn a* (C136, snowflake) × *cot A* has been, in part, described before (MITCHELL 1959). In each of 31 complete octets obtained from six perithecia, each marker showed 4 *m*:4 *m*$^+$ segregation, but the degree of expression, particularly of *sn*, was variable. The wandering mutant was a slow grower, *so*, not distinctive in phenotype but clearly distinguishable from *so*$^+$ in *sn*$^+$ segregants. The *so* phenotype appeared in one member of one *sn*$^+$ spore pair in each of four asci, two from one perithecium and

one from each of two others. In the eight octets from a fourth perithecium all 26 *sn*$^+$ segregants expressed *so* (six spores failed to germinate).

Asci were examined from two intra-ascus crosses of *sn* × *so* (an F_1 ascus from the exceptional (fourth) cross B perithecium, and an F_2 ascus). In 38 complete octets, *so* was expressed in each of the four *sn*$^+$ segregants. Also, in 51 partially germinated octets, *sn*$^+$ expressed *so*. Among 5445 random spores from these two and from one other intra-ascus cross (F_3) only 7 *sn*$^+$ *so*$^+$ segregants were recognized. Thus *so* appears to be quite closely linked to *sn*.

As in cross A one might conclude that chance mutations, this time of *so*$^+$ to *so*, were detected, these having occurred prior to ascus initiation in the exceptional perithecium and after initiation of the exceptional asci. The question arises, however, as to whether close linkage between the wandering mutant and a parent mutant may constitute a somewhat more substantial clue than was recognized in cross A, to a relationship between factors carried by the parents and the appearance of wandering mutants among the progeny.

Cross C: The parents of this cross were poky-3627-2*a* (MITCHELL and MITCHELL 1956) and the composite mutant strain, *csf* R2 *A*, a parent of cross A and shown there to be separable, by infrequent reciprocal recombination, into components expressing the phenotypes, *c sf*$^+$ and *c*$^+$ *sf*. In cross C, a relationship between factors carried by the parents and the appearance of unpredicted mutant segregants among the progeny, is suggested by the observation that the conspicuous wandering mutant recovered is phenotypically indistinguishable from the *c*$^+$ *sf* component of *csf*.

Since one of the parents expresses the maternally inherited character, poky, cross C is expected to give three different results with regard to recovery of the poky phenotype, depending upon whether the opportunity to produce protoperithecia is given to poky alone, to the not-poky parent alone or to both. The first situation would give perithecia with poky monotype asci only, the second, with not-poky monotypes only and the third, a mixture of these two types of perithecia. In the case of the mutants, *c sf*, carried by the not-poky parent, all three situations are expected to give the same result, namely that all asci of all perithecia will be balanced 4 *c sf*, 4 *c*$^+$ *sf*$^+$ ditypes (except for infrequent recombinations as found in cross A). These expectations have been realized throughout with respect to poky and also to *c sf*, when the cross is made by fertilizing preformed protoperithecia of the *c sf* parent with vegetative spores of poky. The unhandicapped cross (started from vegetative spores of both parents), on the other hand, gave repeatedly, among not-poky segregants, a conspicuously large number expressing the *c*$^+$ *sf* phenotype.

Analysis of individual perithecia from the unhandicapped cross revealed three types rather than two. One of the expected types was represented, in the samples examined, by five perithecia from which 34 of the complete octets obtained were monotype with respect to poky, and balanced ditype with respect to *c sf* and *c*$^+$ *sf*$^+$. The remaining two were 2 *c sf*, 2*c sf*$^+$, 2 *c*$^+$ *sf*, 2 *c*$^+$ *sf*$^+$ tetratypes. Complete octets from the remaining ten perithecia were all not-poky monotypes. Those from six were, with one exception, balanced *c sf*, *c*$^+$ *sf*$^+$ ditypes, but the

other four perithecia yielded three frequent classes: two balanced ditypes, *c sf*, c^+ sf^+ and *c sf*, c^+ *sf*, and a tritype, 4 *c sf*, 2 c^+ *sf*, 2 c^+ sf^+. Table 1 shows the numbers of these classes obtained from the ten not-poky perithecia.

Few individual perithecia have been analyzed from the handicapped crosses, since observations of random spores gave no indication that asci other than the expected ditypes would be found with appreciable frequencies. The cross of *c sf* protoperithecia to poky conidia gave, predominantly, not-poky *c sf* and c^+ sf^+ segregants. Counts of *c sf*, c^+ sf^+ and c^+ *sf* in two samples, in which germination was nearly 100 percent, were as follows: 115, 123, 0 and 267, 251, 3. (No *c* sf^+ recombinants were found.) Of 41 complete octets from three perithecia, 40 were balanced *c sf*, c^+ sf^+ ditypes. The remaining one was ambiguous in that one segregant, expected to be *c sf*, expressed a phenotype intermediate between *c sf* and c^+ *sf*. Non-germination of spores has been more common from poky protoperithecia × *c sf* conidia, but among the segregants obtained (all poky) both *c sf* and c^+ sf^+ were frequent, and c^+ *sf* was infrequent.

A simple interpretation of the frequent octet classes from the unpredicted cross-C perithecia could be made by supposing a second *sf* to have arisen which was not linked to *c sf-1* and not recognizable in *c sf-1 sf-2*. The results of a few of the many possible testcrosses do not clearly exclude this possibility, but there are ambiguities. For example, crosses C-2 and C-3 (Table 1), involving segregants from one of the frequent tritype octets from cross C, might be expected to give like results, since one pair of *c sf* segregants must, on the above assumptions,

TABLE 1

Phenotypes composing complete octets from cross C, one backcross, two intra-ascus crosses and one inter-ascus cross, all unhandicapped

	Cross C *c sf* R2 *A* × poky *a*	C-1 *c sf* R2 *A* × c^+ *sf 7 a*	C-2 *c sf 3 A* × c^+ *sf 7 a*	C-3 *c sf 1 a* × c^+ sf^+ 5 *A*	C-4 *c* sf^+ *A* × c^+ *sf 7 a*
Ditypes					
4 *c sf* 4 c^+ sf^+	53	12	9	20	3	..
4 *c sf* 4 c^+ *sf*	..	7	6	21	19	..
4 *c* sf^+ 4 c^+ *sf*	..	..	..	..	..	9
4 c^+ *sf* 4 c^+ sf^+	..	1	..	..	..	4
2 *c sf* 6 c^+ sf^+	..	1	..	..	..	..
Tritypes						
4 *c sf* 2 c^+ *sf* 2 c^+ sf^+	..	20*	32	71	76	..
4 c^+ *sf* 2 *c* sf^+ 2 c^+ sf^+	..	..	..	..	..	31
4 c^+ *sf* 2 *c sf* 2 c^+ sf^+	..	..	..	..	1	..
4 c^+ *sf* 2 *c sf* 2 *c* sf^+	..	..	..	..	1†	..
Tetratype						
2 *c sf* 2 *c* sf^+ 2 c^+ *sf* 2 c^+ sf^+	1	..	..	..	..	..
Ambiguous‡	..	4	1	2	6	..
Total octets	54	45	48	114	106	44
Fraction of tested perithecia	6/15	4/15	4/4	7/7	13/13	5/5

* *c sf 1 a*, *c sf 3 A*, c^+ sf^+ 5 *A*, c^+ *sf 7 a* from one ascus.
† *c* sf^+ *A* from this ascus.
‡ Including one or more segregants with intermediate phenotypes.

be *c sf-1 sf-2*, and the other *c sf-1 sf-2*$^+$. Since C-2 gives a result similar to that of the backcross, C-1, it would seem to represent *c sf-1 sf-2*$^+$ × *c*$^+$ *sf-1*$^+$ *sf-2*. Hence C-3 would represent *c sf-1 sf-2* × *c*$^+$ *sf-1*$^+$ *sf-2*$^+$. But C-3 would then indicate negative linkage between *c sf-1* and *sf-2* because the nonparental ditype appears more frequently than the parental ditype. Also, one might be led to somewhat novel conclusions by the results of a cross (C-4) between the *c*$^+$ *sf* (*c*$^+$ *sf-1*$^+$ *sf-2*) parent of C-2 and a *c sf*$^+$ (*c sf-1*$^+$ *sf-2*$^+$) segregant from an exceptional cross C-3 tritype octet. To account for the tritypes and *c*$^+$ *sf*, *c*$^+$ *sf*$^+$ ditypes observed, one could assume that, while *c* is usually recognizable in *c sf-1 sf-2*, *c sf-1 sf-2*$^+$ and *c sf-1*$^+$ *sf-2*$^+$, it is not recognizable in *c sf-1*$^+$ *sf-2*. Thus it would appear that the new mutant, *sf-2*, which arises in a cross of *sf-1* and which has the same phenotype, is not only not recognizable, but is also unable to produce its effect on *c*, in the presence of *sf-1*. Results of several other test crosses, also unhandicapped, from which only random spores have been observed, do not immediately suggest more attractive alternative explanations. The question of the relationship between *c sf* and the unpredicted *c*$^+$ *sf* segregants seems best left open, pending further study.

DISCUSSION

The point thought to be worthy of emphasis now, in connection with cross C, is the indication that *c*$^+$ *sf* appears as a frequent segregant in perithecia initiated after the parents have had an opportunity to interact vegetatively. It is easier to visualize such interaction as favoring recombination than to suppose that it directly influences mutation. Therefore, it seems permissible to consider the possibility that vegetative recombination, possibly analogous to that reported by WEIJER and DOWDING (1960), may occur prior to fruiting body initiation and may be reflected, among ascospore segregants, by the appearance of wandering mutants or by novel recombination patterns of markers being followed. The frequency of such recombination might be expected to depend, first upon the opportunity and capacity for vegetative interaction (possibly equivalent to heterokaryon formation), and then upon the capacity of factors present to recombine under whatever restrictions may apply. Multiplication and selection of recombinants during vegetative growth preceding initiation of het-type perithecia (i.e., perithecia assumed to arise from heterokaryons) might also influence the final result.

The sequence of events following initiation of perithecia and culminating in ascospore formation is imperfectly understood. However, if, as it is often supposed, formation of a heterokaryon precedes ascus initiation, then the opportunity for vegetative recombination would exist here, not only in het-type but also in hom-type perithecia (i.e., perithecia assumed to arise from protoperithecia produced by one homokaryotic parent and fertilized by nuclear contributions from the other). Thus recombinations occurring subsequent to ascus initiation, during the production of spores, might involve not only parental associations but also recombinants or even recombinants of recombinants.

Some aspects of this proposal may be applied, in a general way, to the recov-

ery of the wandering mutant, *au*, from cross A (an unhandicapped cross). One may assume *au* to be a double mutant, such as *csf* appears to be, whose closely linked components have entered the cross in repulsion. The separate components may be assumed to produce inconspicuous mutant phenotypes as, for example, does the suppressor of *pyr-3a* (DAVIS 1962), but to be readily detectable when combined. To put it another way, each may be thought of as an enhancer of the other. The *au* components may then be assumed to have become associated by recombination prior to initiation of the exceptional perithecium, prior to initiation of one exceptional ascus and after initiation of the other.

In the case of cross B (also unhandicapped) one could assume that *sn*, which is, initially, a slow-growing strain, regularly carries *so*, the closely linked wandering mutant; in other words, that *sn* is actually *sn so* just as *csf* appears to be *c sf*. The exceptional perithecium could then be accounted for by supposing that, through vegetative recombination, the mycelium which was to initiate it became homozygous with respect to *so*. Thus asci which were actually monotype with respect to *so* could be produced. The question of the exceptional asci seems best left open, since tests for linkage between *sn* and *so* were not made with their *so* segregants. The infrequent sn^+ so^+ segregants among random spores could be attributed to rare reassociations of enhancers and suppressors.

The cross C situation seems too involved to be approached in such simple terms. It may be of interest, however, to mention certain parallels between this case and one in which exceptional wild segregants were recovered from crosses involving pyridoxin-requiring mutants (M. B. MITCHELL 1956; H. K. MITCHELL 1957). (For a recent discussion of similar cases and for references, see KITANI, OLIVE and EL-ANI 1962.) Intercrosses of these mutants yielded infrequent tritype asci which, on the basis of testcross results, were judged to be genotypically as well as phenotypically tritype. Hence they appeared to demonstrate non-reciprocal recombination or mutation. However, if the exceptional segregants found reflect, more often than not, two or more recombinational events separated in time, then it may be that the appearance of non-reciprocality is to be expected. In other words, if they represent vegetative recombinants further recombined so as to accomplish unlikely associations of partial suppressors, then, possibly, each recombination occurred reciprocally, but all products were recovered only from the last event.

SUMMARY

Three crosses are described whose progeny included segregants expressing unpredicted mutant phenotypes. In one case, the new mutant showed very close linkage to one of the parent mutants. In another, frequent appearance of the unpredicted mutant phenotype, which was indistinguishable from that of a mutant known to be a component of one of the parents, was found to coincide with utilization of culture conditions favoring vegetative interaction of the parent strains before the initiation of perithecia. The possibility is considered that recovery of the unpredicted mutant segregants may reflect vegetative recombination occurring prior to ascus initiation.

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LITERATURE CITED

DAVIS, R. H., 1962 A mutant form of ornithine transcarbamylase found in a strain of Neurospora carrying a pyrimidine-proline suppressor gene. Arch. Biochem. and Biophys. **97**: 185–191.

DODGE, B. O., 1935 The mechanics of sexual reproduction in Neurospora. Mycologia **27**: 418–438.

KITANI, Y., L. S. OLIVE, and A. S. EL-ANI, 1962 Genetics of *Sordaria fimicola*. V. Aberrant segregation at the *G* locus. Am. J. Botany **49**: 697–706.

MITCHELL, H. K., 1957 Crossing over and gene conversion in Neurospora. pp. 94–113. *The Chemical Basis of Heredity*. Edited by W. D. MCELROY and B. GLASS. The Johns Hopkins Press. Baltimore, Maryland.

MITCHELL, M. B., 1956 A consideration of aberrant recombination in Neurospora. Compt. Rend. Trav. Lab. Carlsberg (Ser. Physiol.) **26**: 285–298.

1959 Detailed analysis of a Neurospora cross. Genetics **44**: 847–856.

1960 Evidence of non-random distribution of ascus classes in fruiting bodies of *Neurospora crassa*. Genetics. **45**: 1245–1251.

MITCHELL, M. B., and H. K. MITCHELL, 1952 A case of "maternal" inheritance in *Neurospora crassa*. Proc. Natl. Acad. Sci. U.S. **38**: 442–449.

1956 A nuclear gene suppressor of a cytoplasmically inherited character in *Neurospora crassa*. J. Gen. Microbiol. **14**: 84–89.

SANSOME, E. R., 1946 Heterokaryosis, mating-type factors and sexual reproduction in Neurospora. Bull. Torrey Botan. Club **73**: 397–409.

WEIJER, J., and E. S. DOWDING, 1960 Nuclear exchange in a heterokaryon of *Neurospora crassa*. Can. J. Genet. and Cytol. **2**: 336–343.