Giant spore, a new developmental mutant of N. crassa

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Abstract
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Abnormal ascus mutants.

As reported by Srb and Basl (1969 Genet. Res. 13:303) large numbers of colonial mutants of N. crassa that affect morphogenesis of the ascus have been isolated. By a "zygote complementation test" the mutants so far analyzed have been shown to fall into seven functional groups. More recently, we have begun a search among colonial mutants obtained from the Fungal Genetics Stock Center to determine whether any of them affect ascus morphology and, if so, whether they correspond to mutants isolated by us. The following mutants have been found to have relevance to the purpose of our search:

1. colonial-2 (VII R): Early developmental stages of ascis initiated by zygotes homoallelic for col-2 are abnormal in that immature ascospores frequently show nonlinear arrangements and that occasional ascis show dichotomization. Thus for, intact mature ascis have not been obtained. In crosses with wild type, col-2 gives linear ascis, the mutant therefore being recessive with reference to its effect on the ascus. The mutant recombines freely with representatives of each of the seven groups of abnormal ascis mutants reported by us.

2. pile, colonial-IO (II L): Zygotes homoallelic for either of these mutants give rise to abnormal ascis morphologically similar to those produced by peak-2 (also called "biscuit") (Pincheira and Srb 1969 Am. J. Botany 56:846). Each mutant is recessive to wild type. By the zygote complementation test, col-10 and pl are functionally allelic. No wild type recombinants were found in a small number of progeny resulting from a cross between the two mutants. Both by recombination and complementation tests these mutants are distinct from members of the seven groups of abnormal ascus mutants reported by us.

3. remicolenial-9 (IV R): After a cross of the rmco-9 stock with wild type, two visibly distinguishable kinds of colonial mutants were found among the progeny. One kind behaved as a recessive abnormal ascus mutant, and the other did not. Whether the two categories represent separate morphological mutants or modified and unmodified forms of the same mutant remains to be determined.

4. spreading colonial-5 (VII): Mutant rmco-5 behaves as a recessive in determining abnormal ascis. Neither recombination nor complementation tests suggest alelism with mutants in the seven groups of abnormal ascus mutants reported by us.

5. clock (V R): The first efforts to obtain ascis initiated by zygotes homoallelic for clock (culture c126, kindly provided by A. S. Sussman) were unfruitful. After two backcrosses to our wild type strains, interfertile cl cultures were obtained, giving a abnormal ascis similar to those produced by pk-2. With reference to ascis morphology, the cl strains are recessive to wild type and functionally allelic with peak-2 and biscuit, the latter two mutants being members of functional group I of the abnormal ascis mutants reported by us. Wild type recombinants at a frequency of 0.06% were found in the progeny of a cross cl X pk-2.

Among other colonial mutants from the FGSC that have been examined by us, a substantial fraction appears not to affect ascus morphology in any obvious way. These mutants will be specified at a later date. Still other mutants remain to be resolved, because of inability to obtain mature ascis from homoallelic zygotes, a difficulty also encountered with certain colonial mutants isolated by us. Nevertheless, a significant number of mutants, mapping to different sites in the genome, are found to intervene in the development of the ascus. (Work supported by grant GM 12953, National Institute of Health, USPHS.)


In the course of a mutation run utilizing N-methyl-N'-nitro-N-nitrosoguanidine to induce colonial mutants of N. crassa, a colonial isolate was obtained that produced giant ascospores when colonials of opposite mating type were crossed. The original isolate turned out to be a double mutant, inasmuch as by recombination the alteration in spore size could be obtained independent of colonial morphology. Further genetic analysis of the mutant ascospore attribute, designated "giant spore" (grp), revealed that it segregates as a single gene difference. Linkage data indicate that grp is situated on the left arm of linkage group 1. Although a polygenic system controlling ascospore size has been reported for linkage group I by Lee and Pateman (1961 Heredity 14:223), these characteristics of grp appear to be distinct from those of the strain described by these workers.

With reference to phenotype expression, grp behaves as a zygote recessive; i.e., ascis produced by +/grp zygotes have the normal 8 spores while only 2 or 4, rather than 8, spores are produced by -/grp zygotes. The phenotype is variable. Perithecia resulting from the appropriate cross produce some ascis containing 8 normal-sized spores, ascis containing both normal-sized and one or more large spores, and ascis containing a single giant spore, approximately the size of the ascus. The ratio of normal to abnormal ascis from one perithecium to another appears to be irregular, even in the same crossing tube.

Cytological investigation of grp/grp ascis suggests that the variant phenotype is accounted for by disruption of the normal timing of nuclear divisions vis-a-vis the initiation of arcorpore wall formation. Mature mutant ascis seem always to include the normal number of 16 nuclei, but spore walls may be formed when only 2 or 4 rather than 8 nuclei are present.

A dominant round spore mutant and both dominant and recessive ascus mutants have been isolated following dimethyl sulfate mutagenesis of N. tetrasperma conidia. The characteristics of the dominant round spore mutant are similar to those of the round spore mutant of N. crosse described by M. Mitchell (1966 Neurospora News, 10:6). Crosses in which both parents carry the round spore mutation are sterile. Each spore from a four-spored ascus germinates from two germ pores. In the infrequent case of a three-spored ascus, the exceptionally large round spore has four germ pores and presumably con germinate from all four pores. Germination from more than two pores has been observed in multi-porated spores derived from single-spored* tetrasperma asc i and also in the "giant" spore (gsp) mutant of N. crosse described in the note above by Leary and Srb.

Unlike the N. crosse round spore which Cameron has mapped as one of the outermost mutants in I R, segregating independently of mating type (1967 Neurospora News, 11:6), N. tetrasperma round spore is in linkage group I and shows close linkage to the mating type locus (approx. 12 map units). However, the linkage of round spore to mating type in N. tetrasperma need not be taken to mean that round spore is located closer to the mating type in N. tetrasperma than in N. crosse nor can it be assumed that more than one gene on linkage group I, when mutated, is capable of producing round spores. These reservations are based on evidence that in N. tetrasperma crossing over is greatly reduced, at least in linkage group I. Adenine and several other N. tetrasperma linkage group I markers obtained in our lab, have never shown recombination with the mating type locus.

Normal N. tetrasperma spores average 16μ x 31μ while "round" spore dimensions average 18μ x 21μ. Round spores show a slight elongation near the germ pores and the 21μ is measured along a line drawn between the two germ pores. Unlike normal spores, round spores usually do not fill the length of the ascus, yet the volume of a round spore is calculated to be about 85-90% that of a wild type spore.

An occasional non-genetic reversal of dominance occurs, that is, in a cross heterozygous for round spores, one or two asc i in a given peritheium may contain four phenotypically normal spores. However, given the absence of secondary division segregation in such exceptional asc i, each of the normal heterocaryotic spores upon germination give rise to a self-fertile mycelium which produces perithecia containing round-spored asc i. Asc i have never been observed to contain mixtures of round and normal spores. Thus the dominance effects are observed for the ascus as a whole and not for individual spores.

Ascus mutants: The vegetative mycelium of both the dominant and the recessive abnormal ascus mutants is colonial. The dominant ascus mutant has the effect of producing abnormal asc i when crossed to a wild type parent, whereas with the recessive, only mutant x mutant crosses have an effect on the ascus. The type of ascus produced by these mutants is similar to that produced by the peak-2 (pk-2, also called bis) mutant isolated in N. crosse (Pinheira and Srb 1969 Am. J. Botany 56:846).

The dominant N. tetrasperma ascus mutant is allelic to the pk-2 N. crosse ascus mutant. Allelism could be tested for directly since the pk-2 gene has been transferred from N. crosse into N. tetrasperma. The recessive N. tetrasperma ascus mutant is not allelic with pk-2 but this is not surprising since recessive mutants affecting ascus morphology in N. crosse have been found for at least seven different loci (Srb and Basi 1969 Genet. Res. 13:303). It is interesting to note that at present all dominant ascus mutants so far obtained in N. crosse, map at the pk-2 locus.

The transfer of genes between two evolutionarily distinct species of Neurospora is a valuable tool and has lead to interesting observations. One cannot accurately predict that the same mutant will have on identical expression in a pseudohomothallic species (N. tetrasperma). The pk-2 N. crosse ascus mutant has undergone fifteen backcrosses to the N. tetrasperma wild type parent. Although in N. crosse pk-2 has an effect on the ascus only when homozygous, in N. tetrasperma the mutant has a partial dominant effect; that is, although the ascus produced in a pk-2 x wild (N. tetrasperma) cross are linear, a high frequency of them contain more than four spores. Only 1-2% of the ascus in the corresponding wild type N. tetrasperma cross contain more than four spores. (DNR is supported by Grant GM 1035, the research program by Grant GM 12953, National Institutes of Health, USPHS) - - Section of Genetics, Development and Physiology, Cornell University, Ithaca, New York 14850.