GENETIC EVIDENCE FOR NUCLEAR MIGRATION IN BASIDIOMYCETES

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IN various fungi genetically marked nuclei implanted in a mycelium can be recovered centimeters from the point of implantation after an interval of hours or days. The transport of such nuclei (or their mitotic progeny) could occur by the dispersal of spores, by the intrusive growth of new hyphae, by the migration of whole nuclei through pre-existing hyphae, or by other means. In fact, such transport may occur by a variety of mechanisms in the same fungus.

Although the *idea* of nuclear migration in fungi has become widespread, PAPAZIAN (1958, pp. 44–45) has questioned the absolute validity of the *evidence* for migration in Basidiomycetes, and even in a recent article about migration, SWIEZYNSKI (1961) evaluates the evidence with the guarded comment that "The alternative hypothesis . . . is difficult to accept for various reasons." The original report of nuclear translocation by migration in fungi (BULLER 1931) involved *Coprinus lagopus* (Basidiomycetes) and was based upon: (1) kinetic data indicating the rate of nuclear migration is faster than the rate of hyphal-tip growth (p. 217), and (2) genetic data involving the detection and recovery of migrating nuclei from pre-existing, partially isolated hyphae (pp. 234–241). More recent studies of nuclear migration, including those of FULTON (1950, 1951), KIMURA (1954, 1958), SNIDER and RAPER (1958), and SWIEZYNSKI and DAY (1960 a,b), either have assumed that the type of transport studied was nuclear migration, on the basis of BULLER's original evidence, or have relied upon kinetic data to separate nuclear migration from intrusive growth. Too frequent reference to kinetic data (the more unreliable of BULLER's two types of evidence) and neglect of BULLER's genetic data (PAPAZIAN 1958; SWIEZYNSKI 1961) may account largely for the doubts expressed in the reviews and by other investigators (see below) about the conclusiveness of the evidence. The purpose of this report is to show that the definitive evidence for nuclear migration in Basidiomycetes depends upon genetic, not kinetic, data. Genetic data presented here confirm in full and extend the validity of BULLER's original conclusions.

If only kinetic data are considered, there are several possible, even if somewhat bizarre, explanations for transport other than by nuclear migration. Intrusive growth itself is not necessarily ruled out. Because such growth, in the thick mycelium where nuclear transport takes place, would be difficult or impossible

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to observe directly, the maximal rate of presumed intrusive growth is usually measured indirectly in another part of the culture or in a separate culture, in the absence of the recipient strain. The rate of the presumed intrusive growth is assumed to be equal to or less than the radial growth of the donor mycelium in the absence of the recipient mycelium, but the conditions of the two treatments are not strictly comparable, and there is thus no guarantee that the comparison is always reliable. That the conditions of intrusive growth could be growth-accelerating seems possible, even if remotely so, and this factor would increase the danger of confusing intrusive growth with nuclear migration. PAPAZIAN suggests (1958 and personal communication) that intrusive growth may be accomplished by rapidly growing hyphae, thinner in diameter than ordinary vegetative hyphae, which might grow preferentially appressed to the pre-existing vegetative hyphae of the "recipient" mycelium. Other possible explanations include translocation of fragmented nuclei or translocation of soluble DNA, which could bring about a type of transformation after translocation (GIRBARDT, personal communication) somewhat analogous to transformation in bacteria. Translocation of nuclear material smaller than the whole nucleus could take place inside a hypha in a manner similar to nuclear migration. Recent studies by GIRBARDT (1961) and by MOORE and MCALEAR (1961) raise the question of whether whole nuclei can actually pass through the relatively small septal pores (< 100 mμ) found in many fungi, including species of Basidiomycetes in which nuclear migration has been reported. None of these explanations accounts for the evidence submitted in this paper or in BULLER's original report.

MATERIALS AND METHODS

The method depends upon two essential steps: (1) the experiment is done under conditions where nuclear translocation and intrusive growth can be observed simultaneously in the same part of a mycelium, and (2) an analysis of meiotic products is made afterwards to determine what has been translocated. If the hyphae of the donor and recipient strains are sufficiently different and if the density of hyphae is not allowed to become too great, there would be no difficulty in determining the role of intrusive growth directly by microscopic inspection. Once the general means of transport is clear, evidence for normal meiosis would indicate either that whole nuclei were transported or that whole nuclei were reconstituted quite precisely after translocation; normal meiosis could not be expected if only parts of nuclei, soluble DNA, gene products, or cytoplasmic particles are translocated.

This procedure is similar to BULLER's *second* method in principle, but there are important differences. The fungus selected here produces no asexual spores; more genetic loci are involved; individual hyphae of the donor and recipient strains are morphologically distinct; the distances of transport are greater; and the genetic data are sufficiently extensive to erase possible doubts about statistical significance. Quite aside from these differences, however, BULLER's genetic evidence remains essentially sound.

Schizophyllum is ideally suited for this experiment for two reasons: (1) asexual spores are unknown in *S. commune*, and (2) the morphological mutant puff (*p*) (RAPER and MILES 1958) in this species constitutes uniquely favorable material for the first part of the experiment. Puff affects the morphology of individual hyphae and differs from wild type in two essential characters: (1) a puff hypha resembles a string of beads (Figure 1a), each bead, or puff, formed by extensive dichotomous branching of a single lateral branch from the original hypha, and (2) a puff mycelium can be grown with mature hyphae so well separated from one another that single hyphae often extend for several centimeters without touching other hyphae. Into such a mycelium of puff, intrusive growth of wild-type hyphae cannot proceed unnoticed.

The experiment was done on migration complete medium (SNIDER and RAPER 1958) in ten cm petri plates. The puff mutant, which was used as the recipient strain, was first inoculated in the plates as a thin, five-cm strip of inoculum placed across the center of the plate. At least 25–50 cultures were required for critical observations. After the puff hyphae had grown two to three cm laterally, the donor strain, morphologically wild type, was implanted near and parallel to the center line of the now-expanded strip of puff mycelium. Progressive initiation of wild-type dikaryotic growth along a linear series of puffs on a single hypha served as a visual indicator of translocation. There is probably a time lag between

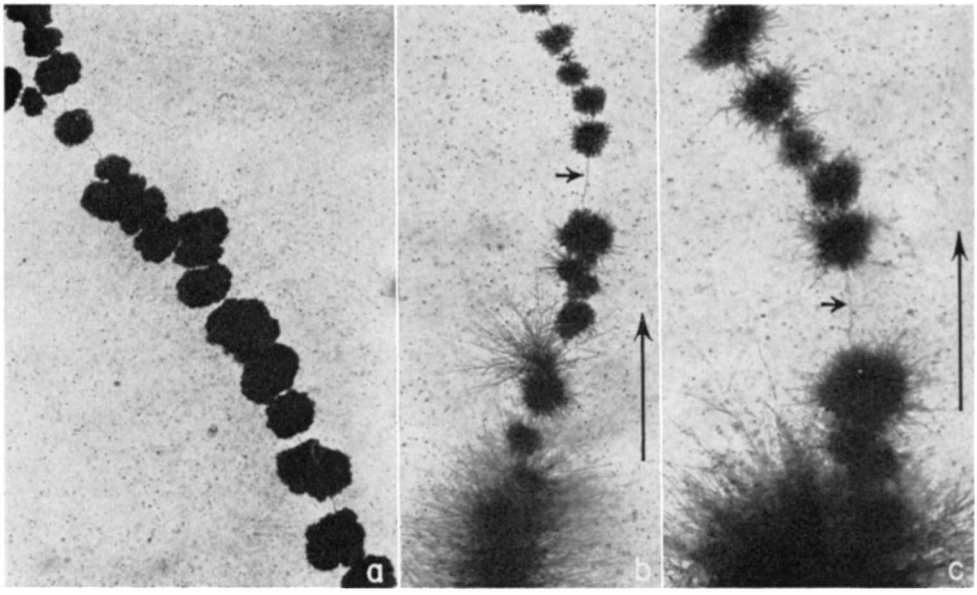

FIGURE 1.—Visual indicators of translocation in puff. Long arrow indicates direction of growth and translocation; short arrow, position of a long hyphal bridge. (a) A single hypha of puff (control); (b) wild-type growth responses indicating translocation up to a long bridge (most of puff mycelium and donor strain implant out of view at bottom of photo); (c) a later view of the same responding puff with indicators now present on the terminal segment distal to the bridge. See text.

transport to a given point and the occurrence of a visible response at that point, but the length of the lag, if it exists, was not critical in this study and was not investigated. Only a few recipient hyphae responded in the early part of the experiment, and these were located by scanning the cultures at 30X. Each responding hypha was then examined at 200X, and only those observations were recorded as critical in which a response was obtained on the far side of an acceptable hyphal bridge on a suitably isolated terminal segment of a puff hypha (Figure 1b,c). From a number of hyphae giving critical responses, the most distal puff to initiate wild-type growth was isolated, cultured and fruited, in order to obtain basidiospores, which are the direct haploid products of meiosis in Schizophyllum.

Several genetic markers were included in addition to puff. In one experiment, the genotype of the recipient strain was *A*^{2} *B*^{1} *ura-1 p* [*A* and *B* are incompatibility factors and *ura-1* is uracilless (RAPER and MILES 1958)]; in another experiment the genotype of the recipient strain was *A*^{2} *B*^{1} *ura-1 p nic-2* (*nicotinic-acidless*). The genotype of the donor strain in all instances was *A*^{4} *B*^{4} *ura-1*$^+$ *p*$^+$ *nic-2*$^+$. Previous data indicate that the majority of the genetic markers used should show independent assortment in small samples of random spores, and linkage should be detectable only between the *ura-1* and *nic-2* loci, which have a recombination frequency of 28.3 percent (nonparental/Total = 94/332) (RAPER and MILES 1958).

RESULTS

A total of seven critical observations were made in the two pairings. Six of these were observed in the first pairing, which involved four genetic markers, and the seventh was observed in the second pairing which involved all five genetic markers. The distance from the edge of the implant of the donor strain to the distal ends of the puff hyphae of the recipient strain was two to four cm, and at 32°C the visual indicators of translocation in the puff hyphae appeared in 70–100 hours after implantation. In a typical responding hypha, a clearly visible response had occurred on the near side of a bridge where a critical observation could be made 84 hours after implantation (Figure 1b), and the response had reached the terminal puff on the same hypha six hours later at a distance of approximately 30 mm from the edge of the implant (Figure 1c). In the same interval of 90 hours, the intrusive growth of wild-type hyphae was no more than three mm. As a general impression, the extent of intrusive growth was inversely proportional to the concentration of the hyphae in the puff mycelium; intrusive growth became significant (more than five mm), however, in only a few exceptionally wide gaps in the puff mycelium. During the 100 hours that the cultures were closely observed, less than one percent of the puff hyphae gave evidence of translocation. Many more hyphae had responded after several more days. Only a few of the responding hyphae were appropriate for critical observation, e.g., (1) possessed a hyphal bridge of sufficient length and (2) had the portion of the hypha on the far side of the bridge sufficiently isolated from all other hyphae. The low frequency of the responding hyphae within the first 100 hours and the

morphological restrictions required for critical observations account for the small number of such observations, in spite of the large number of cultures used.

The most distal responding puff on the far side of the hyphal bridge of each critical observation was isolated and fruited in separate cultures. By the time of isolation, the wild-type hyphae developing from the terminally responding puffs were forming morphologically normal clamp connections, and they continued to do so after transfer to fruiting medium. The clamps were expected, since the pairings involved fully compatible combinations for mating type (see RAPER and MILES 1958, for details) and should form dikaryotic mycelia after the transport of nuclei, irrespective of the means of transport. Tests for normal meiosis were made with most of the isolates. Abundant sporulation was obtained from all the crosses, and spore germination ranged from 80–90 percent. Progeny from four of the fruit bodies involving four genetic markers were tested for normal meiosis, and segregation and linkage relationships were normal in every instance. Data concerning segregation and linkage among the progeny of one of the fruits are shown in Table 1. The evidence for single gene segregation was significant at the one percent level for each locus, no evidence was found for linkage in any of the possible combinations of two loci, and the 16 expected F_1 genotypes were obtained in approximately equal frequency. The minimum number of progeny per class was ten, the maximum 28, and mean 20 ($\chi^2 = 18.65$: $P = 0.25$).

The remaining cross involved the additional genetic marker *nic-2*, which is known to be linked to *ura-1*. The results of this cross will not be given in detail as they were in every way similar to the results described above except for the involvement of the additional locus. The segregation of *nic-2* in this last cross was 42:68 (P of 1:1 = 0.02), and the *nic-2* locus showed evidence of linkage with only the *ura-1* locus, as expected. The recombination frequency between *nic-2*

TABLE 1

Test for normal meiosis at four nonlinked loci
Presumed migrant nuclei × resident nuclei
A^4B^4 ura-1$^+$ p^+ × A^2B^1 ura-1 p

A. Segregation

Markers	No. progeny	χ^2	P of 1:1 ratio
p^+ : p	165:151	0.62	0.43
u^+ : u	176:140	4.10	0.043
A^4 : A^2	139:177	5.06	0.024
B^4 : B^1	149:167	1.02	0.31

B. Linkage

Markers	Non-parental:Parental	χ^2	P of 1:1 ratio
A to *B*	158:158	0	1.00
A to *u*	152:164	0.456	0.50
A to *p*	159:157	0.012	0.92
B to *u*	155:161	0.114	0.75
u to *p*	160:156	0.050	0.82

and *ura-1* was 23.6 percent (nonparental/Total = 26/110). Thirty-one of the 32 possible genotypes in this last cross were recovered in 110 progeny.

DISCUSSION

Several methods, variable in reliability, have been applied or are plausible in obtaining evidence for nuclear migration in fungi. They may be based upon visual, kinetic, autoradiographic, or genetic evidence. Direct visual evidence in living material appears to be the most difficult to obtain. Kinetic evidence, the type most frequently reported, may be ambiguous and, as experience indicates, will be questioned repeatedly. No method based upon autoradiography is known to be operational, and in the presence of proved genetic methods, the development of an autoradiographic method appears to depend more upon a sufficient need than upon technical feasibility. The specific genetic method used here is unique at present in the fungus Schizophyllum, although mutants similar to puff might be usable in other fungi.

The microscopic observations in this paper give definitive evidence that something from a donor strain able to convert puff hyphae of Schizophyllum into wild-type hyphae was translocated three cm *within* preformed single hyphae of the puff recipient strain. The genetic analysis made after the microscopic observations demonstrates definitively that the translocated material is genetic and can participate normally in meiosis. In the absence of direct visual observation of nuclei passing through septa, genetic evidence, such as presented here, appears to be the strongest possible basis for a valid conclusion that nuclear migration exists. To obtain visual or genetic evidence involves reasonably laborious procedures, and once such evidence is established in a given fungus, the more conveniently obtained kinetic data should be acceptable in many types of experimentation, if such data are cautiously applied.

Visual evidence for migration of whole nuclei appears to be more firmly established in Ascomycetes than in Basidiomycetes, although a few incidental observations have been noted about possible visual evidence in Basidiomycetes (cf. MacDonald 1949) and in an imperfect fungus with undoubted affinity to Basidiomycetes (Sanford and Skoropad 1955). Shatkin and Tatum (1959) have published an electron micrograph showing a nucleus of Neurospora halfway through a septal pore (100–200 mμ in diameter) in a vegetative hypha; the nucleus was presumably fixed in the act of migrating from one cell into the next. Unidirectional streaming of the *entire* protoplasmic contents of certain hyphae is not difficult to observe in various Ascomycetes with coarse hyphae, as the present author was able to demonstrate to his own satisfaction in Neurospora, Gelasinospora, and Ascobolus, as have numerous other observers. Such observations of streaming, and the electron micrograph, constitute convincing visual evidence of nuclear migration in Ascomycetes. Similar attempts to observe intercellular streaming of particles in Schizophyllum by phase microscopy have been strikingly unsuccessful. If protoplasmic streaming and nuclear migration are associated in Schizophyllum (the simplest hypothesis by analogy with Ascomy-

cetes), the processes in typical mycelia are evidently limited in time and space to a few, well-scattered hyphae. There is independent evidence suggesting the active hyphae are well-scattered (SNIDER and RAPER 1958). The puff mutant of Schizophyllum offers one possibility for overcoming the problem of locating hyphae in which migration is or recently has been occurring. Unlike those of Neurospora and other Ascomycetes with coarse hyphae, the hyphae of Schizophyllum are small enough in diameter so that nuclei are easily visible by phase microscopy. The puff technique thus suggests an approach that may be technically feasible for seeing nuclei migrate in living cells and checking for the presence or absence of an associated protoplasmic streaming.

Alternative conclusions excluded in this paper deserve brief consideration. The genetic analyses appear to exclude the possibility of a growth-stimulating substance diffusing through the agar. Transport by asexual spores was excluded by the selection of *S. commune* as the test fungus, since this species is not known to produce asexual spores. Even if the production of some type of asexual spore at low frequency has escaped attention in Schizophyllum, transport by dispersal of asexual spores would be expected in many instances first to initiate responses in the ends of the puff hyphae distal to the implant, with the consequent generation of a wave of response toward the proximal ends. In fact, the wave of response in all instances originated immediately adjacent to the donor implant and traveled distally.

The evidence for normal meiosis argues strongly against the participation *solely* of soluble nucleic acids, of randomly selected, irregular fragments of chromosomes, or of anything less than whole nuclei. Objections to the possibility that nuclei can pass through the minute apertures in and the complex membranes adjacent to the septa of dikaryotic hyphae in Basidiomycetes (MOORE and MCALEAR 1961) have included the suggestion that the pores in homokaryotic hyphae, where nuclear migration has been demonstrated, may be larger in diameter and simpler in structure. Current electron microscopic comparisons of the structure of septal pores in homokaryotic and dikaryotic hyphae of Schizophyllum have indicated to date no difference in the size or structure of these pores (SNIDER, unpublished data). Through how small a pore nuclei of fungi can pass is not known; nor is it known to what extent intracellular membranes associated with pores can act as barriers. Although nuclei could in theory be translocated in fragments (the results of the type of genetic analysis reported here require only that the nuclei be whole after translocation), the simplest explanation of the genetic evidence is unquestionably that nuclear migration in Basidiomycetes involves the translocation of whole nuclei. The ability of particles to squeeze through small pores and the flexibility or elasticity of intracellular membranes may be somewhat greater than is generally recognized.

SUMMARY

Nuclear migration in fungi, the extensive intercellular translocation of nuclei through pre-existing hyphae, can be distinguished experimentally from several other means of transport. By a method described in this paper, puff, a uniquely

suitable morphological mutant, and several other genetic markers are used to obtain definitive genetic evidence for nuclear migration in *Schizophyllum commune*, a representative species of the Basidiomycetes. The evidence indicates that nuclei can migrate through a single pre-existing hypha for distances in the range of centimeters. The reliability of evidence obtained for nuclear migration by other methods is discussed briefly, and the present results are submitted to dispel the possible ambiguities that appear to have delayed the unconditional acceptance of the evidence for migration as a means of nuclear transport in Basidiomycetes.

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LITERATURE CITED

BULLER, A. H. R., 1931 *Researches on Fungi. IV.* Longmans, Green, and Co. London.

FULTON, I. W., 1950 Unilateral nuclear migration and the interactions of haploid mycelia in the fungus *Cyathus stercoreus*. Proc. Natl. Acad. Sci. U.S. **36**: 306–312.

1951 Nuclear migration and the interaction of haploid mycelia in *Cyathus stercoreus*. Ph.D. thesis. Univ. of Indiana, Bloomington, Indiana.

GIRBARDT, M., 1961 Licht- und elektronenmikroskopische Untersuchungen an *Polystictus versicolor*. II. Die Feinstrucktur von Grundplasma und Mitochondrien. Arch. f. Mikrobiol. **39**: 351–359.

KIMURA, K., 1954 Diploidization in the Hymenomycetes. I. Preliminary experiments. Biol. J. Okayama Univ. **1**: 226–233.

1958 Diploidization in the Hymenomycetes. II. Nuclear behavior in the Buller phenomenon. Biol. J. Okayama Univ. **4**: 1–59.

MACDONALD, J. A., 1949 The heather rhizomorph fungus, *Marasmius androsaceus* Fries. Proc. Roy. Soc. Edinburgh, **63**: 230–241.

MOORE, R. T., and J. H. MCALEAR, 1961 Fine structure of mycota. 7. Observations on septa of Ascomycetes and Basidiomycetes. Am. J. Botany **49**: 86–94.

PAPAZIAN, H. G., 1958 Genetics of Basidiomycetes. Advan. Genet. **9**: 41–69.

RAPER, J. R., and P. G. MILES, 1958 The genetics of *Schizophyllum commune*. Genetics **43**: 530–546.

SANFORD, G. B., and W. P. SKOROPAD, 1955 Distribution of nuclei in hyphal cells of *Rhizoctonia solani*. Can. J. Microbiol. **1**: 412–415.

SHATKIN, A. J., and E. L. TATUM, 1959 Electron microscopy of *Neurospora crassa* mycelia. J. Biophys. Biochem. Cytol. **6**: 423–426.

SNIDER, P. J., and J. R. RAPER, 1958 Nuclear migration in the Basidiomycete *Schizophyllum commune*. Am. J. Botany **45**: 538–546.

SWIEZYNSKI, K. M., 1961 Migration of nuclei in tetrapolar Basidiomycetes. Acta Soc. Bot. Polon. **30**: 529–534.

SWIEZYNSKI, K. M., and P. R. DAY, 1960a Heterokaryon formation in *Coprinus lagopus*. Genet. Res. **1**: 114–128.

1960b Migration of nuclei in *Coprinus lagopus*. Genet. Res. **1**: 129–139.