THE INDUCTION OF RECESSIVE SUPPRESSOR MUTATIONS AND SOMATIC RECOMBINATION IN THE COMMON-AB DIPLOID OF SCHIZOPHYLLUM COMMUNE

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THESIS

This is to certify that the

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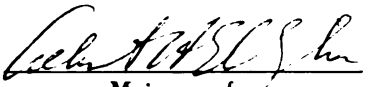

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ABSTRACT

THE INDUCTION OF RECESSIVE SUPPRESSOR MUTATIONS AND SOMATIC RECOMBINATION IN THE COMMON-AB DIPLOID OF SCHIZOPHYLLUM COMMUNE

By

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The objectives of this research were to: 1) induce recessive suppressors of auxotrophic mutations that would be effective for detecting recombinational events, 2) develop techniques for synthesizing stable diploid strains, and 3) use the recessive suppressors for detecting recombinational events in diploid strains of Schizophyllum commune.

Genetic analyses have been made to detect recessive suppressor mutations in twelve prototrophic strains of S. commune derived by treating an arginine dependent strain with either hydroxylamine, nitrosoguanidine or acridine red. The results indicate that one strain possesses a recessive suppressor, su-1, which maps outside the arg-2 locus and is capable of suppressing auxotrophy conferred by the arg-2 mutation. This suppressor is recessive in dikaryons and diploids and is incapable of suppressing auxotrophy conferred by eight other loci. Prototrophy in the remaining eleven strains resulted from either intragenic suppression, reversion, or

from a suppressor mutation that is closely linked to the arg-2 locus. Heterokaryotic allelic tests with the eleven strains indicate that the mutation to prototrophy is recessive. The mutation to prototrophy in each of the twelve strains segregates as a single gene through at least two generations of backcrossing to the parent auxotroph. No recessive suppressor mutations were detected among 764 prototrophs induced with ultraviolet light.

A technique was developed which allows for the recovery of stable vegetative diploid strains from the common-AB heterokaryon of S. commune. The common-AB diploid, which is heterozygous for auxotrophic mutations, is prototrophic and resembles the homokaryon in morphology. An analysis of the segregation of five biochemical markers and mating-type factors from a diploid X haploid cross closely fit the predicted values for triploid segregation. At least four linkage groups appear to be present in S. commune based on the segregation of mutant markers in segregants of an aneuploid from the diploid X haploid cross.

Somatic recombination in the common-AB diploid was studied using the recessive suppressor, su-1, to detect recombinational events. An analysis of spontaneous and ultraviolet induced recombinants indicate that diploid, aneuploid and haploid individuals were recovered. Results from preliminary experiments indicate crossing over is rare and haploidization proceeds via stages of aneuploidy.

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By

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DEDICATION

To Mary

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INTRODUCTION

A considerable effort has been expended to elucidate the mechanisms of somatic recombination in fungi (Pontecorvo 1956; Ellingboe and Raper 1962a; Sweizynski 1963). At least three mechanisms have been described. Pontecorvo (1956) described a mechanism of recombination in diploid strains of Aspergillus nidulans whereby haploidization of the diploid nucleus proceeds via stages of aneuploidy, with infrequent mitotic crossing over. Crossing over and haploidization are distinct in time and space and haploidization results in recombination of genes on different chromosomes.

The methods for detecting recombinational events in Aspergillus were not available to those studying somatic recombination in Schizophyllum commune and Coprinus spp. In the absence of stable diploid strains and recessive suppressors (which had been used to detect recombination in Aspergillus) studies of somatic recombination in S. commune and Coprinus spp. utilized the technique of incompatible dikaryotic-homokaryotic (di-mon) matings to detect recombinational events in vegetative mycelium. Since neither nucleus of the dikaryon was compatible with the nucleus of the homokaryon, dikaryotization of the homokaryon could result when recombination

between the two nuclei of the dikaryon produced a nucleus that was compatible with the homokaryon. Recombinants were detected as dikaryotic sectors in the homokaryotic mycelium.

The results obtained with S. commune using this technique suggested two new mechanisms of somatic recombination (Ellingboe and Raper 1962a; Ellingboe 1963). A meiosis-like mechanism of recombination in vegetative cells was postulated to account for the high frequency of recombination of linked and unlinked genes. A second mechanism, Specific Factor Transfer, was suggested to account for the transfer of only an incompatibility factor specificity from each of the two nuclei of the dikaryon into the nucleus of the homokaryon. The role of the intervening homokaryon in determining the mechanism of recombination in incompatible di-mon matings is not known. The transfer of the incompatibility factor specificities was confounded with the method used to detect recombinational events. New techniques for detecting recombinational events, therefore, are needed.

The purpose of this research was to develop new techniques for studying somatic recombination in S. commune. The specific objectives of the research were to: 1) induce recessive suppressors of auxotrophic mutations that would be effective for detecting recombinational events; 2) develop techniques for synthesizing stable diploid strains; and 3) use the recessive suppressors for detecting recombinational events in diploid strains of S. commune.

LITERATURE REVIEW

There are numerous examples in the literature where a second mutation, at a site different from a primary mutation, partially or completely restored the function lost by the primary mutation. These secondary mutations were termed suppressor mutations. Two types of suppressor mutations are known. Intragenic suppression is the restoration of a lost function by a second mutation which is located within the same gene as the primary mutation. Intergenic suppression is the restoration of a lost function by a secondary mutation that maps outside the site of the primary mutation. The mechanisms of intra- and intergenic suppression may be very diverse (Yanofsky, Horn and Thorpe 1964; Capecchi and Gussin 1965).

Suppressor mutations have been reported in bacteria and a variety of fungi including Aspergillus (Lilly 1965; Weglenski 1966; Gajewski and Litwinska 1968), Neurospora (Giles and Partridge 1953; Davis 1961; McDougal and Woodward 1966; Seale 1968), Coprinus (Lewis 1961; Morgan 1966) and Saccharomyces (Hawthorne and Mortimer 1963; Kakar 1963; Manney 1964). These investigations have revealed that suppressors may be dominant, semidominant or recessive (Lilly 1965; Weglenski 1966; Gajewski and Litwinska 1968). It

is not fully understood why suppressor mutations exhibit these different phenotypic responses. The following is a brief review of the work which has given some insight into how suppression of auxotrophy is believed to occur in the systems investigated in bacteria, fungi, and yeast.

Suppression has been most rigorously studied with the alkaline phosphatase gene of Escherichia coli (Garen and Siddiqi 1962; Weigert and Garen 1965; Weigert, Lanka and Garen 1967) and with the rII genes of the T4 bacteriophage (Benzer and Champe 1962; Stretton and Brenner 1965; Kaplan, Stretton and Brenner 1965). The amber triplet, UAG, and the ochre triplet, UAA, have been identified as nonsense triplets or chain terminating codons which do not code for any amino acids in suppressor-negative strains of E. coli (Sarabhai, Stretton, Brenner and Bolle 1964; Brenner, Stretton and Kaplan 1965; Weigert, Lanka and Garen 1967). That amber mutations result in the cessation of polypeptide chain propagation has been confirmed by demonstrating that only fragments of coat protein were found in T4 bacteriophage possessing amber mutations. The length of the polypeptide chain was determined by the position of the amber mutation from the end of the gene corresponding to the N-terminal end of the polypeptide chain (Sarabhai, Stretton, Brenner and Bolle 1964). Suppression of amber mutants in the alkaline phosphatase gene and the coat protein of T4 phage was by insertion of an amino acid at the site

of the amber mutation thus allowing for the propagation of the entire polypeptide chain (Kaplan, Stretton and Brenner 1965). Subsequent studies have implicated mutated transfer RNA in the mechanism of suppression. In a cell free system where amber suppressible messenger RNA is used as messenger, suppression would occur only when t-RNA from a suppressor strain of E. coli was present (Capecchi and Gussin 1965; Garen 1968). Amber suppressors which map anywhere in the genome suppress the amber mutation regardless of its location in the genome of the bacteria or bacteriophage. The suppressors of the am17 allele in the am (amination) locus, which codes for the primary structure of the NADP-linked enzyme glutamate dehydrogenase (GDH) in Neurospora crassa, were also capable of suppressing mutations present in three unrelated genes (Seale 1968). In this respect, the suppressors of am17 were analogous to the amber suppressors in E. coli and the allele specific super-suppressors in yeast (Hawthorne and Mortimer 1963; Gilmore and Mortimer 1966; Mortimer and Hawthorne 1966). Therefore, amber suppressors and super-suppressors are said to be allele specific and not gene specific.

Some ochre suppressors reported in E. coli map at genetically different loci from amber suppressors while others map very close to or within the same loci (Signer, Beckwith and Brenner 1965; Eggertsson and Adelberg 1965). The level of restored activity by ochre suppressors is very low (1-5%) in contrast to the level of

suppression by amber suppressors (30-63%) (Gorini and Beckwith 1966). Some ochre and amber suppressors are dominant over their wild type alleles (Eggertsson and Adelberg 1965; Gorini and Beckwith 1966; Seale 1968).

Mutations to missense result in the transformation of a codon specifying one amino acid into a codon specifying another amino acid. Suppression of a missense mutant, A36, in the structural locus for the tryptophan synthetase A-protein in E. coli, was by the production of a transfer RNA which was capable of introducing glycine into the polypeptide chain in response to the AGA triplet which normally codes for arginine (Carbon, Berg and Yanofsky 1966). Transfer RNA from strains carrying the suppressor of mutant A78 substituted glycine for cysteine in poly UG directed synthesis of a copolypeptide chain (Gupta and Khorana 1966).

An entirely different mechanism of suppression was suggested for pyr-3 (pyrimidineless) mutants in Neurospora crassa, prolineless mutants in Aspergillus nidulans and isoleucineless mutants in yeast. Suppression of pyr-3 mutants appeared to be through induction of an enzyme defect in the pathway leading to arginine synthesis which resulted in the diversion of precursors of arginine synthesis to the pool leading to pyrimidine synthesis (Davis 1961, 1962; Davis and Woodward 1962). Suppression by recessive or dominant suppressors of pro mutants in A. nidulans was suggested to result from the

alteration of an enzyme which permitted the synthesis of proline by an alternative pathway (Weglenski 1967). A similar mechanism of suppression was suggested for the nonspecific suppressors of isoleucine mutants of yeast (Kakar 1963).

The mechanism of suppression of methionine suppressors and purple color suppressors in Coprinus lagopus (Lewis 1961; Morgan 1966) and suppressors of the arg-2 locus in Schizophyllum commune (Mills and Ellingboe 1968) is not known.

Recessive suppressors, regardless of their mechanism of suppression, have been extremely useful tools for studying somatic recombination in Aspergillus nidulans (Pontecorvo and Kafer 1958; Kafer 1961) and Coprinus lagopus (Cowan and Lewis 1966). Diploid strains of A. nidulans were synthesized which were heterozygous for numerous auxotrophic mutations and the suppressor, but homozygous for the mutation upon which the suppressor acted. When the diploids were plated on a selective medium, either a recombinational event which lead to the production of a diploid homozygous for the suppressor or a haploidization event which yielded a haploid strain with the suppressor allele would allow normal growth.

An extensive analysis of somatic recombinants obtained from heterozygous diploids in A. nidulans has resulted in the description of the Parasexual Cycle (Pontecorvo 1956). The steps in the Parasexual Cycle are: 1) the fusion of unlike nuclei in the heterokaryon

2) mitotic crossing over may occur during the multiplication of the diploid nuclei and 3) haploidization of diploid nuclei via stages of aneuploidy (Pontecorvo 1956).

In the Parasexual Cycle, crossing over or haploidization of the diploid nuclei via stages of aneuploidy may yield recombinant nuclei. Crossing over occurs in approximately one out of 500 nuclear divisions. The rarity of crossing over is evident in that a clear case of crossing over in two different regions during the same division has never been observed. If a diploid nucleus were heterozygous for several markers on one chromosome, segregation in any one recombinational event would occur only for the markers distal to the crossover. Markers proximal to the exchange are unaffected. Because of these infrequent exchange crossovers, it was possible to order the genes on chromosomes using a "mitotic map" by observing the segregation of genes distal to the crossover. Markers that remained heterozygous were proximal to the crossover (Pontecorvo 1956).

Since the haploidization process proceeded via stages of aneuploidy, barring infrequent crossovers, whole chromosomes assorted recombining genes only on different chromosomes. When aneuploid strains were recovered as segregants from diploid strains, some chromosomes were disomic and heterozygous for all markers. Subsequent haploidization which yielded the chromosome monosomic

resulted in expression of the auxotrophic mutations (Pontecorvo 1956). This method of haploidization provided a convenient method for establishing linkage groups in A. nidulans.

Recessive suppressors of met (methionineless) in C. lagopus have been used to detect recombinational events in a dikaryon (Cowan and Lewis 1966). Though only 6 recombinants were recovered and analyzed, the recovery of haploid and disomic nuclei suggested a mechanism of haploidization proceeding via stages of aneuploidy, as expected with the Parasexual Cycle.

The methodology and techniques applied in the study of somatic recombination in A. nidulans were not available for a study of somatic recombination in Schizophyllum. In Schizophyllum commune and Coprinus spp., heterothallic basidiomycetes, compatibility is controlled by two unlinked mating factors, A and B. Matings between two strains with dissimilar A and B factors (i.e. A1 B1 X A2 B2) results in the establishment of the dikaryon, the secondary mycelium, which has two haploid nuclei per cell. The diploid phase is short in duration and normally is confined to the basidium. Meiosis proceeds immediately following the formation of the diploid nucleus. The four haploid nuclei migrate into the four basidiospores that are formed on the basidium (Raper 1966). Matings between strains with similar A factors (i.e. A1 B1 X A1 B2) or similar B factors (A1 B1 X A2 B1) result in the establishment of common-A or common-B heterokaryons,

respectively. A third type of heterokaryon, the common-AB heterokaryon, results from matings between strains with similar A and B factors.

That transient diploid nuclei exist in vegetative mycelium in higher fungi has been tacitly accepted since Quintanilha (1939) first demonstrated nuclear exchange in Coprinus. However, only recently have diploid strains been recovered in three different species of higher fungi. They have been recovered from common-A heterokaryons of Coprinus lagopus (Casselton 1965); from incompatible dikaryotic-homokaryotic (di-mon) matings in C. radiatus (Prud'homme 1965); and in S. commune from common-B heterokaryons (Parag and Nachman 1966), compatible dikaryons (Koltin and Raper 1968) and common-AB heterokaryons (Mills and Ellingboe 1969).

Casselton (1965) was able to obtain diploids from a common-A heterokaryon of C. lagopus heterozygous for complementing auxotrophic mutations in the two nuclei. Since the oidia are uninucleate, only diploid oidia which resulted from a fusion of two unlike nuclei could germinate and produce a colony on a nonsupplemented medium. The diploid strains were very stable. Only rare haploidization yielded intermediate aneuploids. The diploid nucleus was apparently unstable when in a dikaryon with a haploid nucleus and always underwent haploidization before the dikaryon fruited.

A common-B diploid in S. commune was found to be stable

as a homokaryon but was unstable when mated to a compatible strain (Parag and Nachman 1966). Germination and viability of progeny of a diploid X haploid cross were both very low. Although all the mutations present in the diploid and haploid were recovered in the progeny of this mating, the segregation of these mutations was not in accordance with triploid segregation.

Koltin and Raper (1968) discovered an unusual mating in which the product of the interaction of two compatible strains, each with different auxotrophic mutations, had the morphology of a haploid rather than a dikaryon. The product of this mating was also prototrophic and compatible with either parent. The authors concluded that the establishment of the dikaryon in S. commune is controlled by a dominant gene, dik^{+}. Interactions between strains with the recessive allele, dik, results in the formation of a diploid mycelium.

In the absence of diploids and recessive suppressors, the most complete analysis of somatic recombination in Coprinus spp. and Schizophyllum has been done using dikaryotic-homokaryotic (di-mon) matings for the detection of recombinational events (Quintanilha 1939; Papazian 1954; Crowe 1960; Ellingboe and Raper 1962a; Ellingboe 1963, 1964; Swiezynski 1962, 1963).

The dikaryotization of a homokaryon by a dikaryon was first studied in Coprinus lagopus by Buller in 1931 (Buller 1931). The process was later termed the "Buller Phenomenon" by Quintanilha

(1937). Three kinds of matings were shown to occur. They may be 1) compatible, where both nuclei of the dikaryon are compatible with the nucleus of the homokaryon (A1 B1 + A2 B2) X A3 B3, 2) hemicompatible, where only one nucleus in the dikaryon is compatible with the nucleus of the homokaryon (A1 B1 + A2 B2) X A1 B1, and 3) incompatible, in which neither nucleus of the dikaryon is compatible with the nucleus of the homokaryon (A1 B1 + A2 B2) X A1 B2. In the compatible and hemicompatible matings, a nucleus of the dikaryon which is compatible with the nucleus of the homokaryon migrates into the mycelium of the homokaryon and forms a new dikaryon (A1 B1 + A2 B2) X A3 B3 ⟶ (A1 B1 + A3 B3) or (A2 B2 + A3 B3) ⟶ and (A1 B1 + A2 B2) X A1 B1 ⟶ (A2 B2 + A1 B1). The incompatible mating is not expected to result in the dikaryotization of the homokaryon. An infrequent recombinational event leads to an "Illegitimate" dikaryotization of the homokaryon (A1 B1 + A2 B2) X A1 B2 (A2 B1 + A1 B2) (Quintanilha 1939; Papazian 1954). The mechanisms of recombination which ultimately result in the formation of a recombinant nucleus have been the subject of considerable investigation.

The preliminary work (Quintanilha 1939; Papazian 1954) demonstrated that there had been an exchange of genetic material between the nuclei of the dikaryon. Further evidence (Papazian 1954) for somatic recombination was derived from the fact that following two successive incompatible di-mon matings, hyphal tip isolation

yielded a nucleus of a genotype which at no point had been introduced into either of the di-mon matings. No evidence for disomy was observed.

Additional evidence for somatic recombination in incompatible di-mon matings has been presented (Ellingboe and Raper 1962a; Ellingboe 1963, 1964). A number of different auxotrophic mutations introduced into the cross in each of the three types of nuclei were used to determine the mechanism of recombination. One hundred and seventy eight recombinants of independent origin, selected only on the basis of recombinant mating-type factors, were analyzed from a total of 7,160 incompatible di-mon matings (Ellingboe and Raper 1962a). Two classes of individuals were described. Class I consisted of those individuals that possessed non-selective markers in various combinations from the nuclei of the original dikaryon. Class II consisted of those individuals that possessed, aside from the recombined mating-type factors, only the non-selective markers from the homokaryon. The recombination frequencies of linked and unlinked auxotrophic mutations of class I individuals were indistinguishable from frequencies observed via the standard sexual cycle, and was distinctly different from recombination expected via the Parasexual Cycle. There were no aneuploid recombinants observed and no indications that recombination and haploidization were separable in time and space. The mechanism of recombination of class II individuals involved some event

which transferred only incompatibility factors from the two nuclei of the dikaryon into the nucleus of the homokaryon. Subsequent experiments designed to study the mechanism of recombination of class II individuals confirmed the uniqueness of a second mechanism of somatic recombination in S. commune.

In a comprehensive series of experiments, Ellingboe (1963) demonstrated conclusively that incompatibility factor specificities of the dikaryon could be recovered in the genome of the homokaryon without concomitant transfer of closely linked auxotrophic mutations. This novel mechanism of recombination was termed Specific Factor Transfer. That only the incompatibility factors were transferred was demonstrated in the following manner. The A mating-type factor consists of two closely linked subunits, α and β (Raper, Baxter and Ellingboe 1960). Using incompatible di-mon matings, where one nucleus of the dikaryon possessed pab (para-aminobenzoic acid requiring mutation) situated between the α and β subunits while the other component possessed ade-5 (adenine-requiring mutation) which mapped 0.5 units from the Aβ subunit, it was shown that without concomitant transfer of these auxotrophic mutations, the α and β specificities from one nucleus and the B factor specificity from the other nucleus of the dikaryon were transferred into the genome of the homokaryon (Ellingboe 1963). Further evidence for Specific Factor Transfer was substantiated by the recovery of the x15 mutation

(unknown requirement), a mutation linked to Aβ and introduced into the mating in the homokaryon, in all of the recombinants. Furthermore, the recombinants possessed the xll mutation (unknown requirement) not linked to mating-type factors that was introduced into the cross only by the homokaryon. The author concluded that " The incorporation of specific genetic material into the nucleus [of the homokaryon] would have to assume a specificity of incorporation analogous to the incorporation of a temperate phage into a bacterium and the prophage's association with a specific site on a bacterial chromosome" (Ellingboe 1963). This unique mechanism of somatic recombination has not been observed in Coprinus spp. nor have these types of experiments been performed.

Further evidence for a meiosis-like mechanism of somatic recombination in S. commune was obtained with the recovery of a larger sample of recombinants from a single incompatible di-mon mating (Ellingboe 1964). The 61 recombinant nuclei detected on the basis of recombinant mating-type factors comprised 43 genotypes. The frequency of linked and unlinked genes was compatible with a meiosis-like process of reduction of a diploid nucleus to a haploid nucleus. That no class II recombinants were recovered among the recombinants of this dikaryon suggests that Specific Factor Transfer may be lacking in some incompatible di-mon matings.

Neither of the two recombinational mechanisms described

in S. commune have been acknowledged in incompatible di-mon matings in two species of Coprinus. Progeny analysis of derived dikaryons from incompatible and hemicompatible di-mon matings was a method used to detect somatic recombination in C. lagopus (Swiezynski 1962, 1963). Germination of the basidiospores was low in many derived dikaryons (2-71%) with 10 of the 18 derived dikaryons having less than 10% germination. The viability was low when spores did germinate and the segregation of mutant markers was abnormal for most dikaryons. The results were interpreted as indicative of a parasexual mechanism of recombination with haploidization proceeding via stages of aneuploidy.

Other techniques have been employed to detect somatic recombination in the Basidiomycetes. Somatic recombination has been detected in Coprinus radiatus using a triheterokaryon, i.e., a mycelium with three nuclear types (Prud'homme 1965). Two of the nuclear types, although dissimilar in mating-type factors, possessed a morphological mutation, col, which prevented dikaryosis and, therefore, none of the three possible combinations of two gave a dikaryon. Dikaryotic sectors which arose via a recombinational event were analyzed for their genetic constitution by progeny analysis and dedikaryotization by maceration. The conclusions drawn were 1) that haploidization proceeded via stages of aneuploidy since numerous aneuploid and some diploid strains were recovered 2) recombination

via crossing over is rare (none observed in 21 recombinant nuclei) and 3) there is in many cases, a parental association of independent non-selected markers.

An analysis of 14 prototrophic hyphal tips of a common-AB heterokaryon of S. commune which was nutritionally forced on minimal medium by non-allelic auxotrophic mutations indicated recombination in all 14 cases with evidence for disomy of certain chromosomes in 10 of 14 recombinants (Middleton 1964). The disomic chromosomes were unstable as indicated by the recovery of different markers in several crosses of each of the 14 isolates to wild type tester strains. These results were interpreted to favor a mitotic mechanism of haploidization as described in the Parasexual Cycle.

Recombinants were obtained from five prototrophic hyphal tips isolated from an aged culture of a heterokaryon of S. commune formed between compatible homokaryotic strains, each of which possessed a modifier mutation (thereby yielding the heterokaryon incapable of fruiting) (Raper and Raper 1964). Three were recombinant homokaryotic prototrophs and two were recombinant dikaryons. No evidence of aneuploidy was observed.

Somatic recombination has been observed in the Uredinales (Watson 1957; Ellingboe 1961) and in the Ustilaginales (Holliday 1961) using spore color, pathogenicity or nutritional markers to detect recombinational events. When equal proportions of a urediospore

mixture of 2 isolates of Puccinia graminis tritici, one having red spores and few genes for virulence, the other orange spores and many genes for virulence, were used to inoculate wheat seedlings, 4 virulent red races were recovered as a result of somatic recombination (Watson 1957). Fifteen recombinants were obtained from mixtures of 2 dikaryons of Puccinia graminis tritici when spore color and virulence were the selective techniques used to detect recombination in a subsequent study (Ellingboe 1961).

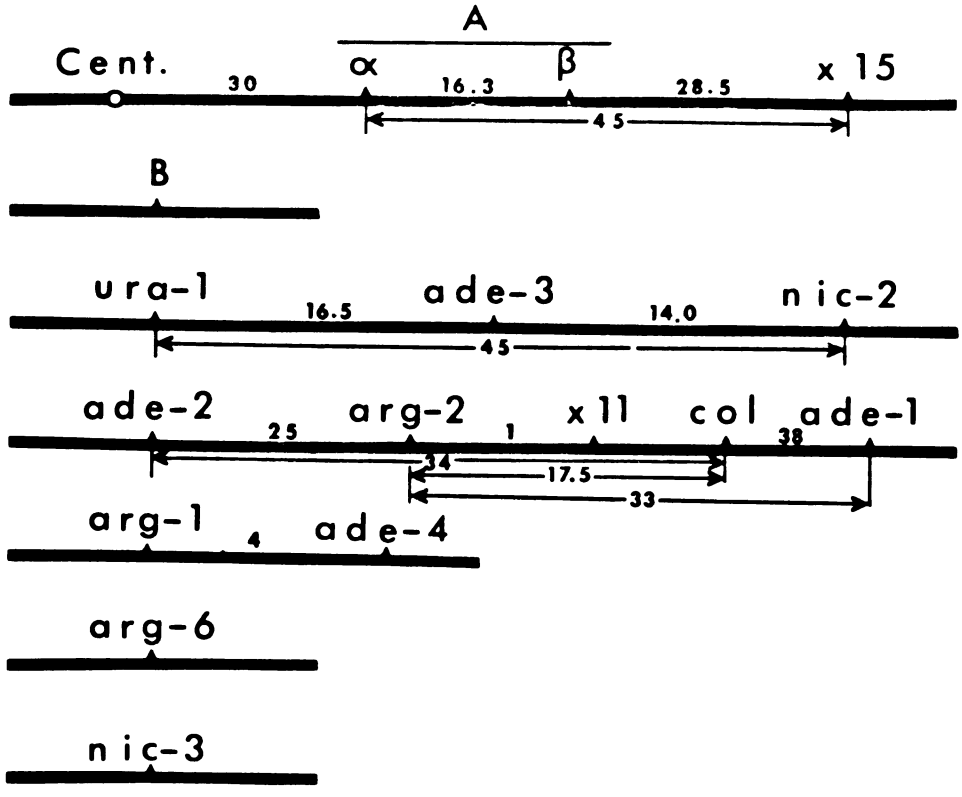
Holliday (1961) studied induced mitotic crossing over in Ustilago maydis using diploid strains heterozygous for auxotrophic mutations. Two types of segregation were observed; crossing over and haploidization. Following irradiation with ultraviolet light, induced mitotic recombination was detected by the acquisition of auxotrophy by previously prototrophic diploid cultures. That these auxotrophic recombinants were diploid was shown by subsequent segregation of other auxotrophic markers following a second treatment of UV. The results were best explained by a mechanism of mitotic crossing over and haploidization as described in the Parasexual Cycle.

MATERIALS AND METHODS

Schizophyllum cultures: All cultures used in this study had a common background genome, strain 699 (Ellingboe and Raper 1962a, 1962b). The auxotrophic mutations and their linkage relations as determined from meiotic products, are given in Figure 1. The symbols used are as follows: ade-1, ade-2, ade-3 and ade-4 (unlinked adenine-requiring); arg-1, arg-2 and arg-6 (unlinked arginine-requiring); nic-2 and nic-3 (unlinked nicotinic acid-requiring); su-1, su-2 .... su-12 (recessive suppressors of arg-2).

Tester strains: A large number of tester strains which possessed a single auxotrophic mutation were synthesized by crossing selected auxotrophs with strain 699. Tester strains which possessed two mutant markers were derived from crossing two compatible strains, each of which possessed a different mutation. The auxotrophic progeny from these crosses were subsequently tested for the mating-type factors they possessed. A number of prototrophic tester strains with different A and B factors were synthesized. These strains were established by crossing strain 699 with a number of other wild type strains that possessed different A and B factors. The mating-type factors of the progeny from these crosses were

Figure 1. Genetic map of Schizophyllum commune. Linkage relations adopted from Ellingboe and Raper (1962a) and Middleton (1964).


A
Cent.
30
α
16.3
β
28.5
x 15
45
B
ura-1
16.5
ade-3
14.0
nic-2
45
ade-2
25
arg-2
1
x11
col
38
ade-1
34
17.5
33
arg-1
4
ade-4
arg-6
nic-3

determined by mating reactions with tester strains for which the A and B factors were known. An example of how the mating types of the progeny from the cross A41 B41 X A42 B42 are determined is given below:

	Testers			
	A.	B.	C.	D.
Expected genotypes	A41 B1	A42 B1	A1 B41	A1 B42
A41 B41	F	+	B	+
A41 B42	F	+	+	B
A42 B41	+	F	B	+
A42 B42	+	F	+	B

+ = compatible reaction

F = flat reaction

B = barrage reaction

A second series of prototrophic tester strains was synthesized to detect intra-A factor recombinants. The A factor is composed of two linked subunits, Aα and Aβ. From a cross of A41α 1- β1 X A42 α 3- β 5, the recombinant progeny Aα 3-β 1 and Aα 1- β 5 are compatible with all four tester strains in the example used above. Progeny that scored as having recombinant A factors were mated to compatible stock tester strains known to possess A α3- β1 or A α1-β 5. The "flat" reaction with one of these testers

established the genotype of each of the recombinant progeny. Once the intra-A factor recombinants were identified they were mated to compatible stock cultures which possessed a B factor (B47) which was different from all cultures used in this study, and recombinant progeny were saved for stock cultures. These cultures were of mating type A α1- β5 B47 or A α3- β1 B47.

Media: All matings were made on migration-complete medium (Snider and Raper 1958). Scoring for nutritional requirements was made either on minimal medium (Raper and Miles 1958) or drop-out medium. Drop-out medium is minimal medium supplemented with all but one of the growth factors required by the cultures being tested. The omitted growth factor is specified in the description of the media; e.g., arginine drop-out medium contains no arginine. All growth factors were supplemented at 10^{-4} M concentration. The heterokaryotic allelic tests were performed on minimal medium. Progeny from crosses were germinated on migration-complete medium, yeast medium or yeast medium supplemented with arginine. Yeast medium is made from minimal medium by substituting yeast extract and bacto peptone at 2 g/liter for asparagine. All stock cultures were maintained on yeast medium in bottles at 4° C.

Single spore isolation and determination of germination percentage: The progeny from all of the crosses were obtained by removing an agar plug bearing the mature fruiting body from the

established dikaryon and placing it on the inside of the cover of a petri dish containing migration-complete medium. When the cover was placed back on the petri dish, the fruiting body assumed an inverted position over the agar in the dish. The spores abjected from the basidia were collected on the agar. The basidiospores were spread over the surface of the agar in a drop of sterile water with a small bent glass rod. The spores were allowed to germinate at 22° C for 20-30 hr. The percentage of germination was estimated from counts of a minimum of 100 spores in several fields of vision in a dissecting microscope with magnification of 48X. The well separated germlings of 20-30 hr were removed, together with a small agar block, with the aid of a sharpened isolating needle. Each germling was placed either in a stock bottle or in petri plates and incubated at 32° C until macroscopic growth was evident.

Purification of cultures: A number of experiments in this study involved the isolation of prototrophic strains from sectors that arose in an auxotrophic culture. Inoculum from each prototrophic sector was transferred to fresh medium identical to that from which it was removed and incubated at 32° C for 2 days. With the aid of a dissecting microscope and an isolating needle, 3 to 5 hyphal tips consisting of 2-4 cells were removed from the growing edge of each culture. The hyphal tips were transferred to migration-complete medium and incubated at 32° C for two days.

Scoring for nutritional requirements: In crosses where a single auxotrophic mutation was segregating, a small agar block containing mycelium was transferred from each of the progeny to migration-complete and minimal media and incubated at 22° C for 3 days. In crosses where more than one gene was segregating for nutritional requirements, transfers were made to several types of drop-out media that would identify the growth factors required by each of the progeny. Scoring for nutritional requirements in homokaryons on minimal or drop-out media was done at 3 days.

Heterokaryotic allelic tests: The genotypes of progeny from crosses where more than one gene was segregating for a particular growth factor were inferred from heterokaryotic allelic tests. For example, there could be three genotypes, ade-2 ade-4$^+$, ade-2$^+$ ade-4, and ade-2 ade-4 which would require adenine among progeny of a cross involving ade-2 and ade-4. Each of the adenine requiring progeny was mated to two compatible strains, each of which possessed a single adenine mutation, either ade-2 or ade-4. The matings were incubated at 32° C for 3 to 4 days to establish dikaryons. Transfers from each dikaryon were plated on migration-complete and minimal media and incubated at 22° C for 5 days.

Dikaryons synthesized from progeny of unknown genetic constitution and ade-4 tester strains that failed to grow on minimal medium must have been homoallelic for the mutation carried by the

tester strain. Since the tester strain carried the ade-4 mutation, the other component of the dikaryon, the nucleus from the adenine-requiring progeny, of necessity, must also have possessed the ade-4 mutation. Dikaryons derived from adenine-requiring progeny and ade-4 testers that grew on minimal medium indicated that the wild type allele was present at the ade-4 locus in the strain being tested. Progeny that failed to produce dikaryons which would grow on minimal medium with either ade-2 or ade-4 tester strains must have had both adenine mutations.

Experiments with ultraviolet light: Cultures of six different auxotrophic strains were transferred to petri dishes that contained minimal medium plus the appropriate growth factor supplemented at a concentration that would allow very limited growth. After incubation at 22° C for 5 days, cultures were irradiated for 10, 20, 30, 40, 50, or 60 sec with a General Electric G-30T8 germicidal lamp (short wave length, 254 mu) at a distance of 12 inches. Irradiated cultures were incubated in the dark at 22° C for 3 days to minimize photoreactivation. After 10-14 days, sectors in which growth was more rapid than the parent culture, were removed from the dishes, transferred to migration-complete medium and incubated at 32° C for 2 days and then transferred to minimal medium. Cultures which grew on minimal medium were purified by hyphal tip isolation and transferred to minimal and migration-complete media. Prototrophic strains that

possessed normal morphology were placed in stock bottles for subsequent tests.

Experiments using growth factor analogues: Two adenine-requiring strains, two nicotinic acid-requiring strains and a wild type strain, 699, were tested for their ability to grow in the presence of growth factor analogues. Five different kinds of media were prepared, each consisting of minimal medium with one growth factor analogue supplemented at 10^{-4} M concentration. Three nicotinic acid analogues, pyridine-3-sulfonic acid, picolinic acid and acetyl pyridine and two base analogues, benzimidazole and 2, 6 diamino purine SO_4 were tested. Growth responses on migration-complete and minimal medium served as controls.

Induction of mutations to prototrophy with hydroxylamine, N-methyl-N'-nitro-N-nitrosoguanidine and acridine red: Experimentation with hydroxylamine (HA): Strain 10209 (A41 B41 arg-2) was grown for 5 days on migration-complete medium at 32° C and then fragmented in a Waring Blendor with 25 ml sterile distilled water for one min. Two ml of the suspension of mycelial fragments were added to 25 ml migration-complete liquid medium in a 300 ml Erlenmeyer flask. Sterilized HA was added to a final concentration of 1×10^{-3} mg/ml. After incubation at 22° C for 48 hr, the contents of each flask were fragmented in a Waring Blendor for 15 sec. One ml was pipetted onto each of 25 plates containing minimal medium. The

plates were examined for prototrophic colonies 9 to 10 days later. Each culture that developed on minimal medium was transferred to migration-complete medium and incubated for 2 days at 32° C, then transferred to minimal and migration-complete media. Hyphal tips were removed from cultures that were growing on minimal medium and transferred to stock bottles to be used in further tests.

Experimentation with nitrosoguanidine (NG): Experiment I: A culture of each of 6 auxotrophic strains grown on migration-complete medium for 5 days at 32° C was fragmented in a Waring Blendor with 25 ml sterile distilled water for one min. Five ml of macerate of each strain were pipetted into a 300 ml Erlenmeyer flask containing 20 ml of liquid migration-complete medium and incubated for 4 days at 32° C. The contents of each flask were fragmented in a Waring Blendor for 1 min, centrifuged in a Servall Model ss-1 clinical centrifuge at 6,500 X g for 20 min. The pellet was resuspended in 25 ml of 0.2 M citrate buffer pH 6.0, and nitrosoguanidine was added to a final concentration of 1 mg/ml. Following an exposure time of 45 min, the fragments were centrifuged at 6,500 g for 10 min and the pellet was resuspended in 25 ml sterile distilled water. One ml of the treated fragments was pipetted onto each of 20 petri dishes containing minimal medium. Prototrophic colonies were removed from the dishes 10-20 days after treatment

Experiment II: Cultures of each of the 6 strains grown on

migration-complete medium for 5 days at 32° C were fragmented in a Waring Blendor for 1 min with 25 ml sterile distilled water. Five ml aliquots of the fragments were pipetted into 300 ml Erlenmeyer flasks containing 20 ml liquid migration-complete medium and NG at a total concentration of 5×10^{-3} mg/ml. After 3 days incubation at 22° C, the contents of each flask were centrifuged in a clinical centrifuge at 6, 500 X g. The pellet was resuspended in 25 ml sterile distilled water and fragmented in a Waring Blendor for 10 sec. One ml of the mycelial suspension was pipetted onto each of 25 petri dishes containing minimal medium. Prototrophic colonies were pruified and bottled for subsequent tests.

Experimentation with acridine red (AR): Strain 10204 (A47 B47 arg-2) was incubated for 5 days on migration-complete medium at 32° C, fragmented in a Waring Blendor with 25 ml sterile distilled water, and 4 ml of the mycelial suspension from each culture were pipetted into 300 ml Erlenmeyer flasks containing 20 ml migration-complete medium. One ml from a sterilized stock solution of acridine red (AR) was added to bring the final concentration to 5×10^{-3} mg/ml. After 48 hr incubation at 22° C, the contents of each flask were fragmented for 60 sec in a Waring Blendor. One ml portions of the treated mycelial fragments were pipetted onto petri dishes containing minimal medium. Prototrophic colonies were removed, purified and transferred to stock bottles.

Tests of the prototrophs recovered following treatment with mutagens: All prototrophs were mated to a strain which possessed different mating-type factors and the arg-2 mutation, e.g., either 10204 (A47 B47 arg-2) or 10201 (A42 B42 arg-2). After 4 days incubation at 32° C, small pieces of the newly synthesized dikaryons were transferred to minimal and migration-complete media. If the mutation to prototrophy were the result of the induction of either a dominant suppressor or an arg-2 revertant, the dikaryon should grow on minimal medium. If the mutation to prototrophy were the result of the induction of a recessive suppressor, the dikaryon formed by mating a prototrophic culture with an arginine dependent culture should be homoallelic for arg-2, heteroallelic for the suppressor and, therefore, require arginine for growth on minimal medium. Progeny were collected from those dikaryons that did not grow on minimal medium and tested for the segregation of prototrophy. Prototrophic progeny that had the normal morphology were mated to the parent auxotroph and the newly synthesized dikaryon again was tested for arginine dependence. After two or three generations of backcrossing to the auxotrophic parent, prototrophic progeny were again mated to the auxotrophic parent and the dikaryon was tested for arginine dependence. Failure of the dikaryon to grow on minimal medium even though one of the two components of the dikaryon has no requirement has been considered evidence for the presence of a suppressor which is capable of suppressing

the arginine requirement in the homokaryon but which is recessive to its homologous wild type allele in the other component of the dikaryon. The cultures used in subsequent genetic tests were recovered as prototrophic progeny from at least two generations of backcrossing to the auxotrophic parent. Similar procedures were used for the induction of prototrophy in strains possessing mutations other than arg-2.

Synthesis and isolation of diploid strains: Common-AB heterokaryons, heteroallelic for complementing auxotrophic mutations, were synthesized on migration-complete medium at 32° C. After 4 days, small pieces of inocula from the area of confluence of the two mated cultures were transferred, one plug per plate, to 50 petri dishes containing minimal medium. Incubation was at 22° C. Inocula from common-AB heterokaryons which were heteroallelic for su-1 and complementing auxotrophic mutations were transferred to minimal medium supplemented with arginine. Diploid strains were recovered from dense, rapidly growing, white, fan-shaped sectors that arose from a background of sparse, irregular mycelia typical of the morphology of the common-AB heterokaryon. All suspected diploid strains were tested for nutritional dificiences on minimal medium and drop-out medium.

Diploid X haploid mating: One putative diploid strain, D-1, was mated to a compatible wild type strain (A42 B42) to determine if mating-type factors and nutritional markers would show trisomic

segregation. Spores were collected from fruiting bodies which developed on the haploid side of the mating. The single spore isolates which were collected were plated one per plate, to migration-complete medium. When growth was macroscopically visible on the plate, the agar plug bearing the germling was transferred to a stock bottle and stored at 4° C. Sectors which developed on each plate were tested for nutritional requirements and mating-type factors. All single spore isolates plus any sectors which developed on the plates were first tested for nutritional requirements by plating on drop-out media. Those progeny and sectors with nutritional requirements were subsequently mated to tester strains with the appropriate mutations and the heterokaryotic allelic tests were performed to determine which mutations conferred auxotrophy. The primary sectors were subsequently plated to fresh migration-complete medium and secondary or tertiary sectors were isolated and analyzed as described above for primary sectors.

Testing for mating-type factors of progeny and segregants of the diploid (D-1) X haploid cross: The mating-type factors of the progeny and segregants from the D-1 X haploid cross were determined by mating with 6 tester strains. All progeny and segregants were first mated to four tester strains with the following mating-type factors: (A41 B41), (A42 B41), (A41 B42), and (A42 B42). All cultures and sectors which scored compatible with A41 and A42

were tested further by mating them with recombinant A tester strains, (A α3- β1 B47) and (A α1-β 5 B47) (Raper, Baxter and Middleton 1958; Raper, Baxter and Ellingboe 1960). Strains which possessed a recombinant A factor on a monosomic chromosome were incompatible with one of the recombinant A tester strains. Cultures and segregants disomic and heterozygous for the A factor, i.e., A41/A42 or A α3- β1/A α1- β5, were compatible with all A factors present in the tester strains. Distinction between strains disomic and heterozygous either for intra-A factor recombination or A41/A42 was made by test mating haploid segregants from the cultures with the tester strains.

Detection of somatic recombinational events in common-AB diploids: Method I: Inocula from a common-AB diploid strain, homozygous for arg-2 but heterozygous for su-1 and other auxotrophic mutations were plated onto minimal medium supplemented with adenine and nicotinic acid. The ensuing growth was sparse because the diploids were heterozygous for su-1, homozygous for arg-2, a genotype that resulted in very limited suppression of auxotrophy conferred by arg-2. Recombinational events which yielded a diploid homozygous for su-1, or a haploidization event which yielded a haploid strain which carried su-1 were detected as dense white sectors.

Method II: The appearance of pink colored sectors in a background of white mycelium provided a second method for detecting

recombinational events. Haploid strains that carry the ade-4 mutatuon in the absence of the ade-2 mutation, and dikaryons homoallelic for ade-4 and heteroallelic for ade-2, accumulate a pink substance in their hyphae. Dikaryons heteroallelic and diploids heterozygous for ade-4 do not accumulate this substance and remain white.

Statistical analysis: Chi square tests were used with 5% level of significance.

RESULTS

Growth responses in the presence of growth factor analogues: Recessive mutatuons to drug resistance have proved to be useful tools for detecting recombinational events in Aspergillus nidulans (Pontecorvo and Kafer 1958). A limited study was conducted to determine whether growth of wild type strains would be inhibited in the presence of growth factor analogues or if these analogues could be substituted for the growth factors required by auxotrophic strains of S. commune. The analogues were incorporated into minimal medium at concentrations ranging from 4.6-8.4 X 10^{-4} M. The adenine-requiring strains were unable to utilize the adenine analogues tested and neither could the nicotinic acid-requiring strains utilize the niacin analogues (Table 1). The wild type strain was insensitive to any of these growth factor analogues at the concentrations used.

Prototrophs isolated following treatment with ultraviolet light: Six auxotrophic cultures were exposed to UV radiation from a General Electric G-30T8 germicidal lamp at a distance of 12 inches. No attempt was made to determine the per cent kill because of the difficulty in obtaining accurate data from irradiated cultures growing on an argar surface. The experiments were designed to

Table 1. Growth of four auxotrophic strains and a wild type strain on minimal medium supplemented with the following growth factor analogues: A) pyridine-3-sulfonic acid; B) picolinic acid HCl; C) acetyl pyridine; D) benzimidazole; E) 2, 6 diamino purine SO_4.

	Growth factor analogue[a]					Controls	
Culture	A	B	C	D	E	MC	MIN
699 wild type	+[b]	+	+	+	+	+	+
10505 nic-2	-	-	-			+	-
10810 nic-3	-	-	-			+	-
10915 ade-2				-	-	+	-
101717 ade-4				-	-	+	-

[a] A, B and C are analogues of niacin; D and E are analogues of adenine.

b + = growth, - = no growth.

determine the exposure time most effective for inducing prototrophic colonies that could be tested for the presence of recessive suppressors. An exposure of 30 sec was found most effective for inducing prototrophic colonies (Table 2). Nearly twice as many prototrophs were isolated following the 30 sec UV treatments as were isolated following the 20 or 40 sec UV treatments. UV treatments of 60 sec appeared to inhibit the formation of prototrophic colonies.

An examination of the number of colonies isolated as prototrophs from each of the six cultures exposed to UV treatment indicate that UV is extremely effective for inducing prototrophy in cultures with the arg-2 mutation but relatively ineffective for inducing mutations to prototrophy of the cultures with nic-3 or ade-4. One-half the total colonies isolated as prototrophs (815/1, 631) were obtained following UV treatment of the arg-2 auxotroph. As indicated by the number of prototrophic colonies isolated from strains carrying ade-2, arg-6 or nic-2, radiation is equally effective for inducing prototrophy in these strains.

Upon purification of these 1, 631 colonies it was apparent that many of the prototrophs merely represented adaptations because when the cultures were returned to minimal medium they would not grow. The prototrophic strains that possessed good morphology after single hyphal tip isolation were mated to the appropriate tester strains and heterokaryotic allelic tests were performed to

Table 2. Total number of colonies isolated as prototrophs following treatment of six auxotrophic cultures with UV irradiation.

	Exposure time in seconds						
Culture	10	20	30	40	50	60	Total
10915 *ade-2*	38	33	134	24	5	0	234
101716 *ade-4*	13	9	42	13	2	0	79
10204 *arg-2*	76	159	298	188	86	8	815
101422 *arg-6*	25	51	53	47	46	0	222
10505 *nic-2*	44	57	51	45	33	1	231
10810 *nic-3*	5	9	21	11	4	0	50
Totals	201	318	599	328	176	9	1,631

determine whether any of these strains possessed a recessive suppressor (Table 3). None of these strains possessed a recessive suppressor.

Induction of recessive suppressor mutations with acridine red: An attempt was made to recover prototrophs from an arg-2 strain following treatment with acridine red (AR). Twenty-one arginine independent cultures isolated in one experiment were tested for the presence of a recessive suppressor in heterokaryotic allelic tests (Table 4). Two backcrosses to the parent auxotroph were made in an effort to rid these two prototrophs of any additional undesirable mutations that may have been induced with AR and to determine if the mutation to prototrophy segregated as a single gene. Prototrophs with normal morphology were selected from the progeny of each backcross to be used in subsequent crosses. The mutations to prototrophy in strains AR 6 and AR 17 segregated as a single gene through two generations of backcrossing (Table 5). Prototrophic progeny were selected from the last backcross of AR 6 and AR 17 with the arg-2 culture and heterokaryotic allelic tests performed to reaffirm the presence of a recessive suppressor.

Each prototroph was mated to 1) an arg-2 culture, 2) an arg-2^{+} culture and 3) a compatible sister prototrophic culture. Growth of these three dikaryons on minimal medium was compared to the growth of a dikaryon homoallelic for the arg-2 mutation.

Table 3. The number of colonies obtained following UV irradiation that were tested in heterokaryotic allelic tests.

	Exposure time in seconds						
Culture	10	20	30	40	50	60	Total
10915 *ade-2*	11	13	116	9	2	0	151
101716 *ade-4*	6	3	32	2	2	0	45
10204 *arg-2*	45	79	132	64	67	2	389
101422 *arg-6*	18	25	24	20	24	0	111
10505 *nic-2*	11	11	5	9	5	1	42
10810 *nic-3*	2	4	16	4	0	0	26
Totals	93	135	325	108	100	3	764

Table 4. Growth of cultures isolated from minimal medium following treatment of mycelial fragments of an arg-2 culture with acridine red.

No. of cultures tested	Growth of isolated cultures on minimal medium			
	as homokaryons		as dikaryons (Mutant + arg-2)	
	-[a]	+	-	+
22	1	21	2	19

[a] - = no growth on minimal medium; + = growth on minimal medium.

Table 5. Segregation of the mutations to prototrophy of two cultures recovered following treatment of an arg-2 culture with acridine red when backcrossed to an arg-2 strain.

Strain	f_1	b_1	b_2
AR 6	17/50[a]	17/50	24/50
AR 17	26/50	21/50	23/50

[a] Prototrophs/total progeny.

The growth responses of AR 6 and AR 17 after 2 backcrosses to an arg-2 parent are shown in Figure 2 D and E. The mutation conferring prototrophy in the homokaryon is recessive since dikaryons homoallelic for the mutation exhibit no nutritional requirement on minimal medium while those dikaryons heteroallelic for the mutation are arginine dependent.

Recessive suppressor mutations induced with N-methyl-N'-Nitro-N-nitrosoguanidine: Six auxotrophic strains were treated with nitrosoguanidine (NG) using two different methods of exposure. With the first method, mycelial fragments from each of the 6 strains were exposed to NG for 45 min, washed and pipetted onto minimal medium. The second method allowed mycelial fragments of each strain to grow in the presence of NG in liquid complete medium for 48 hr before plating onto minimal medium. Twenty-three prototrophic cultures were isolated from the nicotinic acid-requiring, adenine-requiring and the arg-6 auxotrophic strains using both methods of exposure (Table 6). None of these 23 cultures possessed a recessive suppressor mutation as indicated by heterokaryotic allelic tests.

The arg-2 strain yielded 69 prototrophic cultures that were tested for recessive suppressor mutations; 54 cultures following treatment using Method I and 15 cultures following exposure by Method II (Table 6). Two strains scored as having recessive

Figure 2. Growth responses of suppressed strains in heterokaryotic allelic tests. In each set of dishes, five day old dikaryons growing on migration-complete (left) and minimal media (right). In all dishes; top row, dikaryons homoallelic for arg-2, heteroallelic for suppressor; row two, homoallelic for arg-2; row three, homoallelic for arg-2 and suppressor; bottom row, suppressor X wild type. A) su-1 (HA41); B) su-3 (HA 61); C) su-6 (HA 1034); D) su-9 (AR 6); E) su-10 (AR 17); F) su-12 (NG 18).

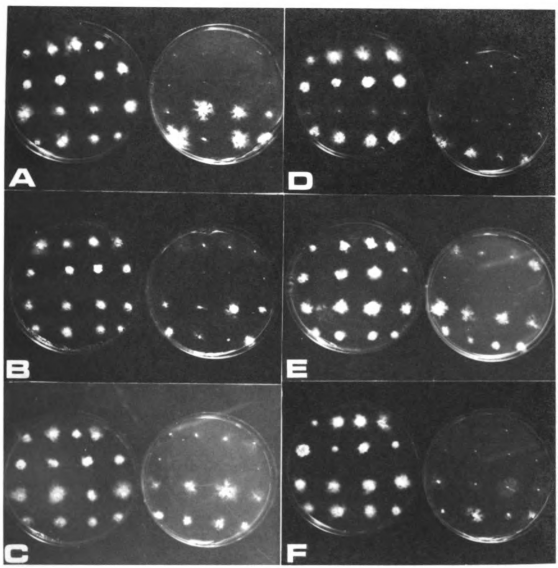

A
D
B
E
C
F

Table 6. Growth of cultures isolated from minimal medium following treatment of mycelial fragments of six auxotrophic cultures with nitrosoguanidine.

Auxotroph treated with NG	No. of cultures tested	Growth of isolated cultures on minimal medium			
		as homokaryons		as dikaryons (Mutant + arg-2)	
		-[a]	+	-	+
10915 ade-2	9	7	2	0	2
101716 ade-4	11	7	4	0	4
101422 arg-6	9	3	6	0	6
10505 nic-2	14	13	1	0	1
10810 nic-3	35	25	10	0	10
10204 arg-2					
Method I	168	144	54	1	53
Method II	18	3	15	1	14

[a] - = no growth on minimal medium; + = growth on minimal medium.

suppressor mutations in heterokaryotic allelic tests. The mutation to prototrophy in each of these strains (NG 18 and NG 21) segregated as a single gene through two generations of backcrossing to the auxotrophic parent (Table 7). Heterokaryotic allelic tests performed with the progeny from the second backcrossing reaffirmed that each strain carried a recessive suppressor of arg-2. Growth responses of NG 18 in heterokaryotic allelic tests are shown in Figure 2 F.

Recessive suppressor mutations obtained following treatment with hydroxylamine: Two hundred thirty arginine independent cultures were isolated from an arg-2 strain in four separate experiments (Table 8). Each culture was mated with an arg-2 strain and the dikaryon resulting therefrom was transferred to minimal medium. Eight of the 230 dikaryons did not grow on medium lacking arginine. The mutation conferring prototrophy in these eight strains segregated as a single gene through at least two generations of backcrossing to the parent auxotroph (Table 9). The heterokaryotic allelic tests performed with progeny from the second and third backcross generations confirmed that each of these 8 strains carried a recessive suppressor of the arg-2 mutation. Heterokaryotic allelic tests with 3 strains induced with HA are shown in Figure 2 A-C. The growth responses of some of the suppressed strains as homokaryons that were induced with either HA, AR or NG are given in Figure 3. The morphology and growth characteristics of strain HA 41 on minimal

Table 7. Segregation of the mutations to prototrophy of two cultures recovered following treatment of an arg-2 culture with nitrosoguanidine when backcrossed to an arg-2 strain.

Strain	f_1	b_1	b_2
NG 18	25/50[a]	21/50	22/50
NG 21	25/50	29/50	47/100

[a] Prototrophs/total progeny.

Table 8. Growth of cultures isolated from minimal medium following treatment of mycelial fragments of an arg-2 culture with hydroxylamine.

Experiment No.	No. of cultures tested	Growth of isolated cultures on minimal medium			
		as homokaryons		as dikaryons (Mutant + arg-2)	
		-[a]	+	-	+
1	164	20	144	3	141
2	63	28	35	1	34
3	41	4	37	3	34
4	15	1	14	1	13
Totals	283	53	230	8	222

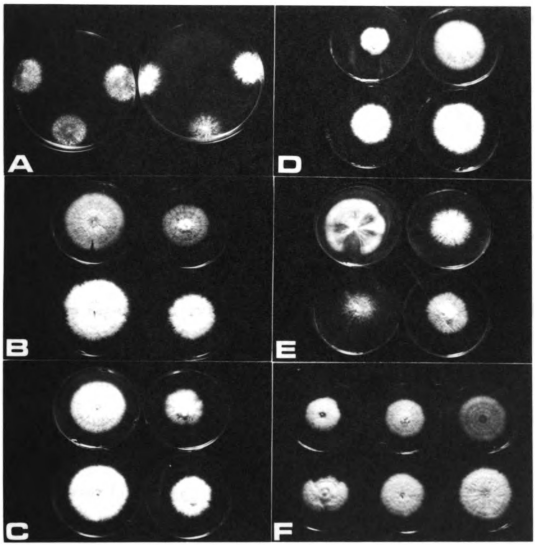
[a] - = no growth on minimal medium; + = growth on minimal medium.

Table 9. Segregation of the mutations to prototrophy of eight strains following treatment of an arg-2 culture with hydroxylamine when backcrossed to an arg-2 strain.

Strain	f_1	b_1	b_2	b_3
HA 41	26/50	-----[a]	24/50	25/50
HA 31	18/50	21/50	25/50	
HA 61	23/50	-----[a]	23/50	26/50
HA 132	27/50	26/50	23/50	
HA 134	-----[a]	27/50	28/50	
HA 1034	18/50	-----[a]	17/50	
HA 1068	23/50	25/50	23/50	
HA 1049	44/50	24/50	29/50	

[a] Exact number of prototrophs not recorded. One prototroph was selected for backcrossing to the arg-2 strain.

Figure 3. Growth of suppressed homokaryons on migration-complete (MC) and minimal (MM) media. A) su-1 (HA 41) MC, left, and MM, right; B-F) top row of plates is MC, bottom row is MM. B, su-3 (HA 61); C, su-6 (HA 1034); D, su-9 (AR 6); E, su-10 (AR 17); F, su-12 (NG 18).


A
D
B
E
C
F

medium as a homokaryon and in dikaryons homoallelic for arg-2 but either homoallelic or heteroallelic for the suppressor, were considered ideal for the study of somatic recombination.

Prototrophy in the remaining 222 mutants tested was assumed to be due either to back mutation at the arg-2 locus (to wild type) or to a dominant suppressor mutation. Prototrophs obtained following treatment with any mutagenic agent, including HA, which did not possess a recessive suppressor were not tested further.

Recombination between the suppressor mutations and the arg-2 locus: An attempt was made to recover the arg-2 mutation from each of the 12 prototrophic strains which initially scored as having a recessive suppressor mutation. Each strain was crossed to a compatible wild type strain in an attempt to isolate recombinant progeny which would possess the arg-2 mutation. With random recombination between the suppressor locus and arg-2, one quarter of the progeny should require arginine for growth. Linkage of the suppressor to the arg-2 locus should result in fewer auxotrophic progeny. The 8 prototrophs induced with HA were mated with a compatible wild type strain, the arg-2 mutation was recovered in the progeny from only one of the 8 crosses (Table 10). Failure to recover any arg-2 recombinants from the other 7 crosses indicated that the mutation to prototrophy occurred either in close proximity to, or at, the arg-2 locus. If the mutation to prototrophy did occur within

Table 10. Recovery of arginine dependent progeny from crosses of suppressed strains X wild type strains on minimal medium.

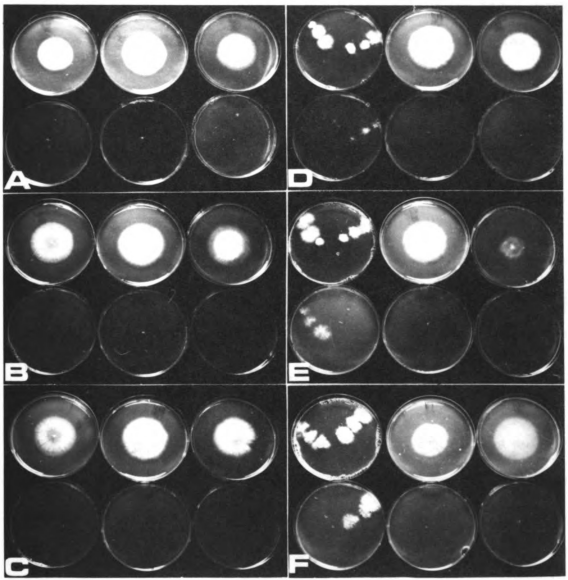
Strains crossed with wild type cultures		Total progeny	No. arginine requirers	No. progeny which produced auxotrophic dikaryons with arg-2 testers
HA 41	(su-1)	87	19	6
HA 31	(su-2)	141	0	0
HA 61	(su-3)	96	0	0
HA 132	(su-4)	136	0	0
HA 134	(su-5)	154	0	0
HA 1034	(su-6)	137	0	0
HA 1068	(su-7)	139	0	0
HA 1049	(su-8)	99	0	0

the arg-2 locus, it did not score as a reverse mutation. The recessive nature of two of these 7 strains when scored in the heterokaryotic allelic test is shown in Figure 2 B and C. All 7 of these prototrophs score similarly to strain su-1 in the heterokaryotic allelic tests. Therefore, either they possess a recessive suppressor or a mutation within the arg-2 locus which confers the wild type phenotype, but which is recessive to the mutant arg-2 allele.

The 19 arginine requiring progeny collected from the cross HA 41 (su-1) X 699 (wild type) were mated to a compatible arg-2 tester strain. The newly synthesized dikaryons were plated on minimal and migration-complete media. Failure of these dikaryons to grow on minimal medium was considered proof that the arg-2 mutation was recovered (Table 10). Growth responses of 9 of the 19 progeny on migration-complete and minimal media are shown in Figure 4 A-C. The 19 progeny are interesting because only 6 exhibit a non-leaky requirement for arginine in the heterokaryotic allelic tests whereas all 19 failed to grow on minimal medium as homokaryons. Heterokaryotic allelic tests performed with progeny, selected at random from the 19, are shown in Figure 4 D-F. A clear explanation for these differences in scoring is not available at this time.

The recovery of arginine dependent progeny from crosses of suppressed strains induced with AR or NG with wild type strains,

Figure 4. Growth of arginine requiring progeny, derived from the cross su-1 (HA 41) X 699 (wild type), as homokaryons and dikaryons with an arg-2 tester strain. A-C, Nine progeny growing on migration-complete (MC), top row, and minimal media (MM), bottom row. D-F, In each photograph, first petri dish shows growth of dikaryons derived from mating the two arginine requiring progeny shown in the second and third dishes with an arg-2 tester strain. Top row, MC; bottom row, MM. The dikaryon formed between homokaryon in the middle dish and the arg-2 tester is plated on the right side of the first petri dish in each photograph. The dikaryon formed with homokaryon of petri dish on far right and the arg-2 tester is plated on the left side of first petri dish in each photograph.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as determined by heterokaryotic allelic tests, was not obtained (Table 11). Ten progeny from AR 6 X wild type exhibited a requirement for arginine when scored as homokaryons at 3 days. However, growth was detected in 5-7 days as homokaryons and the arg-2 mutation was not recovered in heterokaryotic allelic tests. Similar results were obtained with one of the progeny from NG 18 X wild type and 3 progeny from NG 21 X wild type.

Supersuppressibility tests with su-1 and eight different auxotrophic mutations: The ability of su-1 to suppress auxotrophy conferred by mutations other than arg-2 was tested by examining progeny of crosses su-1 by eight different doubly auxotrophic strains. Each of the 8 doubly auxotrophic strains possessed the arg-2 mutation plus one other auxotrophic marker. For example, progeny were obtained from a cross of su-1 arg-2 ade-1$^+$ X su-1$^+$ arg-2 ade-1. The four genotypes expected are: su-1$^+$ arg-2 ade-1$^+$, su-1$^+$ arg-2 ade-1, su-1 arg-2 ade-1$^+$ and su-1 arg-2 ade-1. If su-1 does not suppress ade-1, all four classes of progeny should be identifiable by plating on two media, one lacking arginine, the other lacking adenine. The last two classes should be indistinguishable on these media if su-1 also suppresses ade-1. The progeny from crosses of su-1 arg-2 with strains carrying the adenine or nicotinic acid mutations were scored on adenine or nicotinic acid drop-out media. The data show one recombinant class of individuals which has no requirement for

Table 11. Recovery of arginine dependent progeny from crosses of suppressed strains X wild type strains on minimal medium.

Strains mated to wild type cultures	Total progeny	No. arginine requirers	No. progeny which produced auxotrophic dikaryons with arg-2 testers
AR 6 (su-9)	98	10	0
AR 17 (su-10)	152	0	0
NG 21 (su-11)	147	3	0
NG 18 (su-12)	93	1	0

arginine but possessed requirements for either adenine or nicotinic acid (Table 12). These data demonstrate that su-1 is incapable of suppressing auxotrophy resulting from these adenine or nicotinic acid mutations.

All progeny from crosses of strain su-1 arg-2 by the double auxotroph arg-2 arg-6 were tested for their ability to grow on minimal medium. Those progeny that displayed a requirement were subsequently mated to three tester strains which had the following genetic constitutions: A) arg-2 su-1 arg-6$^+$, B) arg-2 su-1$^+$ arg-6$^+$ and C) arg-2$^+$ su-1$^+$ arg-6 (Table 13 A). The recombinant progeny which possess only the arg-2 mutation should produce dikaryons with testers A and B which will not grow on minimal medium (Figure 5 A). Those progeny which possess only the arg-2 and arg-6 mutations should produce dikaryons with testers A, B and C which will not grow on minimal medium (Figure 5 B). Those progeny which possess su-1 and the arg-2 and arg-6 mutations should produce dikaryons with testers B and C which will not grow on minimal medium (Figure 5 C). Only those auxotrophic progeny which possess su-1, arg-2 and arg-6 produce dikaryons with tester A that will grow on minimal medium (Figure 5 C and Table 13 A). Similar results were obtained when progeny were examined from crosses of strain su-1 by the double auxotroph arg-2 arg-1 (Table 13 B). The three tester strains used in the heterokaryotic allelic tests with these progeny

Table 12. Progeny identified from crosses of su-1 (arg-2 su-1) with eight doubly auxotrophic strains, each of which possessed arg-2 and one additional mutation.

	Genotypes of progeny									
	arg-2	su-1	+	arg-2	+	X[a]	arg-2	+	+	arg-2 su-1 X[a]
arg-2 su-1 X arg-2 ade-1	30			20			27			15
arg-2 su-1 X arg-2 ade-2	29			26			13			24
arg-2 su-1 X arg-2 ade-3	26			17			21			21
arg-2 su-1 X arg-2 ade-4	26			20			30			29
arg-2 su-1 X arg-2 nic-2	30			29			6			19
arg-2 su-1 X arg-2 nic-3	25			19			12			22
arg-2 su-1 X arg-2 arg-1	20			24			17			30[b]
arg-2 su-1 X arg-2 arg-6	25			23			17			30[b]

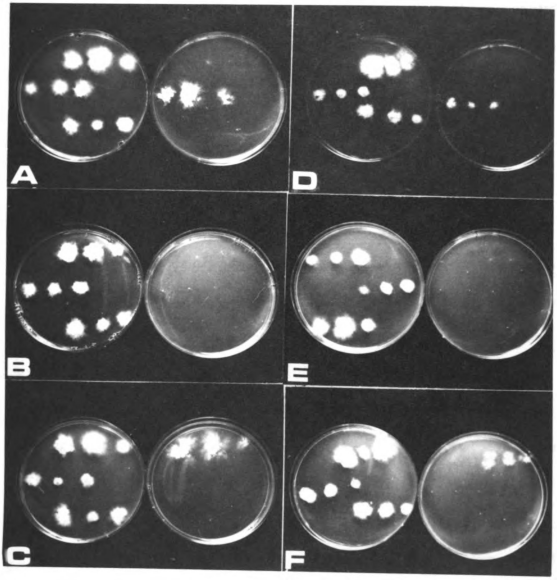
[a] Mutation being tested for suppressibility by su-1.
[b] Genotype deduced from heterokaryotic allelic tests.

Table 13. Growth of dikaryons derived from crossing arginine dependent progeny from two different crosses with tester strains.

	Tester strains								
	A			B			C		
	arg-2	su-1	+	arg-2	+	+	+	+	arg-6
A. arg-2 su-1 X arg-2 arg-6									
arg-2 + +	-[a]			-			+		
arg-2 + arg-6	-			-			-		
arg-2 su-1 arg-6	+			-			-		
	A			B			C		
	arg-2	su-1	+	arg-2	+	+	+	+	arg-1
B. arg-2 su-1 X arg-2 arg-1									
arg-2 + +	-			-			+		
arg-2 + arg-1	-			-			-		
arg-2 su-1 arg-1	+			-			-		

[a] - = no growth on minimal medium; + = growth on minimal medium.

Figure 5. Growth of dikaryons derived from crossing three different arginine dependent progeny from two crosses with three different testers. A-C. Progeny from the cross: arg-2 su-1 arg-6$^+$ X arg-2 su-1$^+$ arg-6. In each set of plates, top row, tester A, arg-2 su-1 arg-6$^+$; bottom row, tester B, arg-2 su-1$^+$ arg-6$^+$; middle row, tester C, arg-2$^+$ su-1$^+$ arg-6. D-F. Progeny from the cross: arg-2 su-1 arg-1$^+$ X arg-2 su-1$^+$ arg-1. In each set of plates, top row, tester A, arg-2 su-1 arg-1$^+$; bottom row, tester B, arg-2 su-1$^+$ arg-1$^+$; middle row, tester C, arg-2$^+$ su-1$^+$ arg-1. For each set of plates, plate at left contains migration-complete medium; plate at right contains minimal medium.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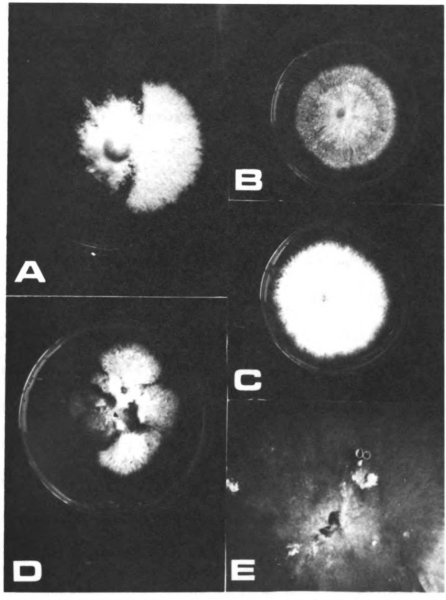
were the same as those described above except that the tester strain possessing arg-6 was replaced with a tester strain carrying arg-1. Again the recessive nature of the suppressor and the three genotypic classes of progeny that exist among progeny exhibiting a requirement for arginine is evident (Figure 5 D-F and Table 13 B). Since the frequency with which the recombinant genotypes arg-2 su-1 arg-6 and arg-2 su-1 arg-1 is high (30/88 and 30/90 respectively, Table 12), it is concluded that su-1 does not suppress auxotrophy conferred by either the arg-1 or arg-6 mutations.

Recovery of diploid strains from connon-AB heterokaryons: Four rapidly growing sectors were isolated from common-AB heterokaryons, heteroallelic for 5 complementing auxotrophic mutations, growing on minimal medium. These strains resembled the homokaryon in morphology, were prototrophic, and remained stable through repeated transfers to migration-complete and minimal media (Figure 6 A-C). None of these strains has sectored or acquired a nutritional requirement.

One presumptive diploid was mated with a haploid, allowed to fruit, and 200 germinated basidiospores were collected and plated onto individual petri plates. Growth from 140 germlings was macroscopically visible within 14 days. Colonial growth of 13 germlings was detected only after one month of incubation at 22° C. Many of the original germlings developed colonies with abnormal morphology

Figure 6. Diploids of Schizophyllum commune. A. Fan-shaped diploid sector arising from the common-AB heterokaryon on minimal medium. B. Diploid strain, D-1, on migration-complete medium and C. on minimal medium. D. Sectors arising from a presumptive aneuploid derived from the diploid X haploid cross. E. Fruiting bodies on a compatible diploid strain isolated as a single spore from the diploid X haploid cross.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which eventually produced many sectors (Figure 6 D). Many of the colonies which sectored were later shown to be aneuploids, disomic for some of the markers that were present in the common-AB heterokaryon. Some germlings developed colonies which never sectored and resembled the homokaryon in morphology. Strains which scored in the initial heterokaryotic allelic tests as auxotrophic, were not considered auxotrophic if sectors were subsequently recovered which possessed the wild type allele for the mutation in question. In these cases the initial scoring was considered to be made on haploid segregants from strains which were originally disomic for the chromosome in question. All mutant markers present in the common-AB heterokaryon were recovered among the progeny of the diploid X haploid cross. The expected values for triploid segregation assume that all products of meiosis are recovered among the progeny of the diploid X haploid cross and that each of the mutant markers should be expressed in one-sixth of the progeny. Progeny exhibiting a nutritional requirement should be monosomic for the chromosome carrying that particular auxotrophic mutation.

Segregation of auxotrophic mutations and mating-type factors from a diploid X haploid cross: The frequency of recovery of three mutations, ade-2, arg-2 and arg-6, closely fit the frequency predicted for trisomic segregation, while two mutations, ade-4 and nic-2, were recovered in excess (Table 14).

Table 14. The frequency of recovery of mutant markers in 153 progeny of the common-AB diploid X haploid.

	Expected	Observed	Difference^2/expected	$\Sigma d^2/e$
ade-2	25.6	29	.5	
ade-2$^+$	127.4	124	.1	
				.6
ade-4	25.6	44	13.2	
ade-4$^+$	127.4	109	2.7	
				15.9*
arg-2	25.6	24	.1	
arg-2$^+$	127.4	129	.02	
				.12
arg-6	25.6	26	.006	
arg-6$^+$	127.4	127	.001	
				.007
nic-2	25.6	42	10.5	
nic-2$^+$	127.4	111	2.1	
				12.6*

* Significant deviation from expected at the 5% level.

The segregation of mating-type factors also shows a reasonably close fit to the frequency predicted for a diploid X haploid mating. The A42 and B42 mating-type factors that were introduced via the haploid component were recovered in excess of the expected frequency whereas recovery of disomics A41/A42 and B41/B42 was less than expected (Table 15). Eight intra-A factor recombinants were also detected among the progeny. Since the frequency of recombinant A factors is extremely variable, (Raper, Baxter and Middleton 1958) no attempt was made to determine their frequency. Analysis of the segregation of the A factors with the B factors is given in Table 16. The results indicate trisomic segregation, with the exception that the number of individuals A42 B42 is greater than expected and individuals of mating type A41/A42 B41 is less than expected.

Some progeny that were disomic for chromosomes carrying dissimilar A and B mating-type factors, i.e., A41/A42 B41/B42, developed fruiting bodies (Figure 6 E). Basidiospores were collected from one culture and were analyzed for nutritional deficiencies. Viability was low (67%) but the results were compatible with two genes segregating for adenine dependence, two genes for arginine dependence, and one gene segregating for nicotinic acid dependence (Table 17). Recovery of four progeny that had no requirements substantiates the premise that this diploid was heterozygous for all markers. All

Table 15. The expected and observed frequencies of mating-type factors among progeny from a diploid X haploid cross based on trisomic segregation.

	Segregants	% among progeny	Number among progeny Expected	Observed
I. The A factor	A41	33.3	77	77
	A41/A41	16.7		
	A41/A42	33.3	51	37
	A42	16.7	25	31
II. The B factor	B41	33.3	77	72
	B41/B41	16.7		
	B41/B42	33.3	51	46
	B42	16.7	25	35
III. Recombinant A factor	Ax*	----	--	8

* Ax indicates an intra-A factor recombinant.

Table 16. Segregation of A and B mating-type factors among progeny of the diploid X haploid cross.

Mating types recovered	Expected	Observed
A41 B41	38.2	37
A41 B42	12.5	16
A42 B41	12.5	14
A42 B42	4.3	9
A41 B41/B42	25.5	24
A42 B41/B42	8.5	8
A41/A42 B41	25.5	16
A41/A42 B42	8.5	7
A41/A42 B41/B42	17.0	14
Ax B41*	----	5
Ax B42	----	3
Ax B41/B42	----	0

* Ax indicates an intra-A factor recombinant.

Table 17. Segregation for nutritional dependence among 67 progeny derived from an A41/A42 B41/B42 diploid.

Nutritional requirement	No. expected among progeny	No. observed among progeny
adenine	50*	47
arginine	50*	49
nicotinic acid	33.5**	41

* Assuming segregation at two unlinked loci. ** Assuming segregation of one gene.

markers present in the common-AB heterokaryon have been recovered in segregants of this compatible diploid, except for the ade-4 mutation.

Segregation of the A mating-type factors with auxotrophic markers is given in Table 18. The expected number of progeny of A41 genotype includes both those disomic A41/A41 and those monosomic A41. The expected number of progeny of A42 is based on the recovery of only monosomics, since only one dose of A42 entered the cross.

Results of the segregation of nutritional markers with B mating-type factors are presented in Table 19. The expected number of progeny with the B41 and B42 mating-type factors was determined with the same method as used for the A factor in Table 18.

An analysis of segregants isolated from a sectoring aneuploid, disomic for the A mating-type factor was used to determine linkage between the markers present in the diploid X haploid cross. Segregants from this strain possess only the B42 mating-type factor believed to be carried on a monosomic chromosome. Since the B42 mating-type factor went into the cross in the haploid parent, a crossover between the centromere and B42 plus proper alignment at metaphase II would be necessary to yield a disomic chromosome homozygous for B42. If the mechanism of recombination is similar to the Parasexual Cycle (Pontecorvo 1956), mitotic crossing over

Table 18. Segregation of A mating-type factors with auxotrophic markers among 153 progeny from the diploid X haploid mating.

Genotype	Expected	Observed
A41 ade-2	17.0	18
A41 ade-4	17.0	22
A41 arg-2	17.0	9
A41 arg-6	17.0	14
A41 nic-2	17.0	15
A42 ade-2	4.3	8
A42 ade-4	4.3	8
A42 arg-2	4.3	5
A42 arg-6	4.3	2
A42 nic-2	4.3	5

Table 19. Segregation of B mating-type factors with auxotrophic markers among 153 progeny from the diploid X haploid mating.

Genotype	Expected	Observed
B41 ade-2	17.0	13
B41 ade-4	17.0	20
B41 arg-2	17.0	11
B41 arg-6	17.0	14
B41 nic-2	17.0	18
B42 ade-2	4.3	9
B42 ade-4	4.3	9
B42 arg-2	4.3	6
B42 arg-6	4.3	6
B42 nic-2	4.3	8

would be infrequent and haploidization would proceed via stages of aneuploidy recombining genes on nonhomologous chromosomes. The data from these segregants indicate that ade-2, ade-4 and arg-2 were heterozygous in culture 144 (Table 20). Segregant 144A, A41 B42, is very likely monosomic for chromosomes carrying mating-type factors, but is apparently disomic for chromosomes carrying ade-2, ade-4 and arg-2 since expression of these markers occurs in secondary sectors 144A1 and 144A2. Segregant 144C, A42 B42, is very likely monosomic for chromosomes carrying the mating-type factors and chromosomes carrying ade-4, arg-6 and nic-2, but disomic for chromosomes carrying ade-2 and arg-2 since the latter two mutant markers are expressed in 144 C1, a secondary segregant. All segregants possessed the arg-6 and nic-2 markers.

Synthesis of multiply auxotrophic strains: For a study of somatic recombination in common-AB diploid strains of S. commune, there was a need for a large number of homokaryotic strains with different mating-type factors and auxotrophic mutations. Various combinations of these strains were used to synthesize common-AB heterokaryons that were heteroallelic for a number of different, complementing auxotrophic mutations. A systematic series of crosses was made which ultimately resulted in two groups of multiply mutant, auxotrophic, homokaryotic strains that possessed different mating-type factors. The auxotrophic mutations present in each

Table 20. Recovery of mutations present in segregants of an aneuploid derived from the diploid X haploid cross.

		Mutations recovered in segregants				
Segregant	Mating type	ade-2	ade-4	arg-2	arg-6	nic-2
144A	A41 B42				yes	yes
144A1	A41 B42		yes		yes	yes
144A2	A41 B42	yes	yes	yes	yes	yes
144B	A42 B42	yes		yes	yes	yes
144C	A42 B42		yes		yes	yes
144C1	A42 B42	yes	yes	yes	yes	yes
144D	A42 B42	yes	yes	yes	yes	yes
144E	A42 B42	yes	yes	yes	yes	yes
144F	A42 B42	yes	yes	yes	yes	yes

group were unique to that group except that the arg-2 mutation was present in all strains of both groups and the recessive suppressor, su-1, was present in some strains of both groups.

The crosses were designed so that a minimum number of auxotrophic mutations were introduced into each of the crosses. This method enabled the detection of morphological changes which may have occurred upon introducing two different mutations into one strain. A second advantage was that the genotypes of the auxotrophic progeny from many of the crosses could be determined on drop-out media and did not necessitate the use of heterokaryotic allelic tests.

Four strains, each of which carried the arg-2 mutation and one other auxotrophic mutation were first mated with a strain which possessed the recessive suppressor, su-1, and arg-2 (Table 21). The recombinant progeny in the first three crosses were identified on arginine drop-out medium and medium lacking the second growth factor, e.g., in the first cross, adenine. Recombinant progeny that had no requirement for arginine but possessed a requirement for the second growth factor were transferred to stock bottles for subsequent experiments.

The genotype of the progeny from the cross involving arg-6 did require the use of heterokaryotic allelic tests. Those progeny that displayed a requirement for arginine were subsequently mated to three tester strains of the following constitutions:

Table 21. Recombinant progeny derived from crossing strains possessing two auxotrophic mutations with a suppressed strain, arg-2 su-1.

Cross	Desired recombinant genotype	No. recombinants / total progeny
1. arg-2 su-1 + / X / arg-2 + ade-3	arg-2 su-1 ade-3	21/85
2. arg-2 su-1 + / X / arg-2 + nic-3	arg-2 su-1 nic-3	22/78
3. arg-2 su-1 + / X / arg-2 + ade-4	arg-2 su-1 ade-4	29/105
4. arg-2 su-1 + / X / arg-2 + arg-6	arg-2 su-1 arg-6	30/92
5. arg-2 su-1 + / X / arg-2 + ade-1	arg-2 su-1 ade-1	15/92
6. arg-2 su-1 + / X / arg-2 + nic-2	arg-2 su-1 nic-2	19/84
7. arg-2 su-1 + / X / arg-2 + ade-2	arg-2 su-1 ade-2	24/92
8. arg-2 su-1 + / X / arg-2 + arg-1	arg-2 su-1 arg-1	30/91

A) arg-2 su-1 arg-6$^+$, B) arg-2 su-1$^+$ arg-6$^+$, and C) arg-2$^+$ su-1$^+$ arg-6. Those progeny which possessed arg-2, su-1 and arg-6 produced dikaryons with tester A that would grow on minimal medium but dikaryons with testers B and C would not grow on minimal medium (Figure 5 C).

The second group of multiply auxotrophic homokaryons was synthesized using the same procedure. The initial matings (crosses 5-8) are given in Table 21. Heterokaryotic allelic tests were performed on all arginine dependent progeny derived from the cross where arg-1 was present. The progeny were mated to the following three tester strains: A) arg-2 su-1 arg-1$^+$, B) arg-2 su-1$^+$ arg-1$^+$ and C) arg-2$^+$ su-1$^+$ arg-1. Only those progeny which were arg-2 su-1 arg-1 produced dikaryons with tester A that would grow on minimal medium (Figure 5 F).

The next four crosses made between the 8 newly synthesized strains are given in Table 22. The procedure eliminated the necessity of using heterokaryotic allelic tests to distinguish between strains carrying the ade-3 and ade-4 mutations of the first 2 crosses and the ade-1 and ade-2 mutations of crosses 3 and 4. The recombinant progeny in each cross were identified on adenine, nicotinic acid or arginine drop-out media. Arginine dependency among progeny from the second cross and the fourth cross resulted from the arg-6 and arg-1 mutations respectively. The progeny of each

Table 22. Recombinant progeny derived from crossing suppressed strains, each of which possessed a single different auxotrophic mutation.

Cross	Desired recombinant genotype	No. recomb. total
1. arg-2 su-1 ade-3 + / X / arg-2 su-1 + nic-3	arg-2 su-1 ade-3 nic-3	30/108
2. arg-2 su-1 ade-4 + / X / arg-2 su-1 + arg-6	arg-2 su-1 ade-4 arg-6	13/86
3. arg-2 su-1 ade-1 + / X / arg-2 su-1 + nic-2	arg-2 su-1 ade-1 nic-2	20/98
4. arg-2 su-1 ade-2 + / X / arg-2 su-1 + arg-1	arg-2 su-1 ade-2 arg-1	9/71

cross possessed arg-2 and su-1. The cross made between recombinant progeny derived from crosses 1 and 2 in Table 22 is shown in Table 23 A. Because the arg-2 mutation was suppressed by su-1 in all of the progeny, any arginine requirement was attributed to the arg-6 mutation. The strains that possessed a requirement for adenine, nicotinic acid and arginine were mated to two adenine-requiring testers; one that possessed the ade-3 mutation and one that carried the ade-4 mutation. The strains which failed to grow as dikaryons on minimal medium with both of these testers possessed the ade-3 and ade-4 mutations. The multiply auxotrophic homokaryons which comprised the second group were derived from crossing the recombinants of crosses 3 and 4 in Table 22 and are presented in Table 23 B. Heterokaryotic allelic tests were performed on all progeny of each group to determine the mating-type factors of each.

A similar series of crosses was performed to yield strains which possessed the auxotrophic mutations of each group; however, none possessed the suppressor. Therefore, common-AB matings between homokaryons from the different groups were heteroallelic for all auxotrophic mutations except arg-2 which was always homoallelic. The suppressor, su-1, in these matings was either homoallelic or heteroallelic depending on the cross.

Verification of diploid strains which possess su-1: To circumvent the laborious task of demonstrating trisomic segregation

Table 23. Recombinant progeny derived from crossing suppressed strains, each of which possessed two different auxotrophic mutations.

Cross	Desired Recombinant Genotypes	Recombinants Total
A.		
arg-2 su-1 ade-3 nic-3 + + X arg-2 su-1 + + ade-4 arg-6	arg-2 su-1 ade-3 nic-3 ade-4 arg-6	5/103
	arg-2 su-1 ade-3 nic-3 ade-4 +	11/103
	arg-2 su-1 ade-3 nic-3 + arg-6	4/103
	arg-2 su-1 + nic-3 ade-4 arg-6	3/103
B.		
arg-2 su-1 ade-1 nic-2 + + X arg-2 su-1 + + ade-2 arg-1	arg-2 su-1 ade-1 nic-2 ade-2 arg-1	5/138
	arg-2 su-1 ade-1 nic-2 ade-2 +	3/138
	arg-2 su-1 ade-1 nic-2 + arg-1	11/138
	arg-2 su-1 + nic-2 ade-2 arg-1	15/138

each time a putative diploid strain was isolated, two criteria were adopted as evidence for the presence of diploidy. Strains that were presumed to be diploid were plated onto arginine, adenine and nicotinic acid drop-out media and minimal medium supplemented with all growth factors. Strains which were prototrophic, except that they grew very slowly on arginine drop-out media, were considered to be diploid. Strains that were subsequently used in the study of somatic recombination were mated to a compatible wild type strain. Spores were collected from a single fruiting body that developed on the haploid side of the mating, germinated on yeast medium supplemented with arginine, and the viable germlings were plated to drop-out media, where auxotrophic requirements were determined. The mutations conferring auxotrophy in the progeny were inferred from heterokaryotic allelic tests. The recovery of all of the mutations present in the common-AB heterokaryon among the progeny of a cross between the putative diploid and a haploid wild type strain was considered evidence that the one parent was diploid and that the diploid was heterozygous for all auxotrophic mutations. The recovery of auxotrophic progeny derived from two diploid X haploid crosses is given in Table 24. All mutant markers present in each common-AB heterokaryon from which these diploid strains were derived were recovered among the progeny of each cross.

Somatic recombination in common-AB diploids: One diploid

Table 24. Recovery of auxotrophic mutations present in the progeny derived from diploid X haploid crosses.

	Number of progeny which possessed:								
	ade-1	ade-2	ade-3	ade-4	arg-1	arg-2	arg-6	nic-2	nic-3
D-9 X 605	-----	11	16	14	12	12	-----	15	9
D-27 X 201	3	15	6	7	15	35	15	7	5

strain, D-9, provided most of the recombinants that were analyzed during this study. D-9 produced a mycelium which was very sparse on arginine drop-out medium. Recombinants were derived from dense, white sectors which were conspicuous within 10-20 days after the plates were inoculated (Figure 7). Inocula obtained from these sectors were plated to arginine drop-out medium and after 48 hr growth at 32° C, hyphal tips were isolated. The cultures derived from the hyphal tip isolations were plated onto adenine, arginine and nicotinic acid drop-out media and to minimal medium supplemented with all growth factors. Cultures that possessed nutritional requirements were mated to appropriate auxotrophic testers, and the auxotrophic mutations were inferred from heterokaryotic allelic tests. Cultures that exhibited no nutritional requirements were transferred to either migration-complete or yeast medium and examined periodically for sectors.

Isolation and characterization of recombinants derived from common-AB diploids: The results from three separate experiments with D-9 are given in Table 25. The genetic constitution of D-9 with established linkage groups is also given.

Recombinant progeny have been placed in three separate classes based on their state of ploidy. The first class is comprised of 129 recombinants which in all respects appear to be haploid. All of these individuals possessed the nic-2 mutation. None of the

Figure 7. Sectors from common-AB diploid, D-9, on arginine drop-out medium. Recombinants were isolated from dense, white sectors. A-B, colonies 10 days after plating. C-D, colonies 21 days after plating.

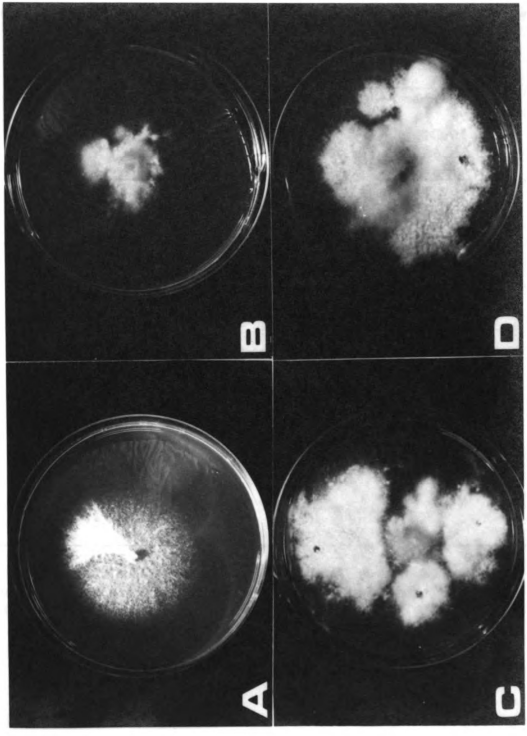
A
B
C
D

Table 25. Spontaneous recombinants recovered from rapidly growing sectors of the common-AB diploid, D-9.

Common-AB Diploid, D-9	Number of recombs.
$\frac{A41\ B41\ arg\text{-}2\ +\ ade\text{-}2\ \ +\ \ nic\text{-}2\ \ arg\text{-}1\ +\ \ +}{A41\ B41\ arg\text{-}2\ su\text{-}1\ +\ \ ade\text{-}3\ +\ \ +\ \ ade\text{-}4\ nic\text{-}3}$	
I. Haploids:	
$arg\text{-}2\ su\text{-}1\ ade\text{-}2^{+}\ ade\text{-}3^{+}nic\text{-}2\ arg\text{-}1^{+}\ ade\text{-}4\ nic\text{-}3$	69
$arg\text{-}2\ su\text{-}1\ ade\text{-}2^{+}\ ade\text{-}3^{+}nic\text{-}2\ arg\text{-}1^{+}\ ade\text{-}4\ nic\text{-}3^{+}$	60
II. Aneuploids:	
$arg\text{-}2\ su\text{-}1\ ade\text{-}2^{+}\ ade\text{-}3^{+}\ nic\text{-}2\ \frac{arg\text{-}1\ +}{+\ ade\text{-}4}\ nic\text{-}3^{+}$	2
$arg\text{-}2\ su\text{-}1\ ade\text{-}2^{+}\ \frac{ade\text{-}3\ +}{ade\text{-}3\ nic\text{-}2}\ arg\text{-}1^{+}\ ade\text{-}4\ nic\text{-}3^{+}$	1
III. Diploids:*	
$\frac{arg\text{-}2\ su\text{-}1\ ade\text{-}2\ \ +\ \ nic\text{-}2\ \ arg\text{-}1\ +\ \ +}{arg\text{-}2\ su\text{-}1\ +\ \ ade\text{-}3\ +\ \ +\ \ ade\text{-}4\ nic\text{-}3}$	12

* Assumed to be homozygous for su-1 since none exhibit a requirement for arginine; some may be homozygous for wild type alleles at other loci.

recombinants possessed the arg-1 mutation or the arg-2 mutation without su-1, which is to be expected since recombinants were selected on arginine drop-out medium. Furthermore, ade-2 which is linked to arg-2 and is in repulsion with su-1, was not recovered among these recombinants. The nic-3 mutation segregated independently of all auxotrophic mutations. No crossing over between linked markers was detected in these strains. To demonstrate that these individuals were haploid, three cultures were mated to a compatible wild type strain and progeny were collected from single fruiting bodies that developed on the wild type side of the cross. The results indicated no evidence for triploid segregation (Table 26).

The aneuploid recombinants consisted of three strains. Two exhibited no requirement for arginine or adenine although both strains possessed the nic-2 mutation (Table 25). These strains were assumed to be aneuploid, at least for the arg-1 ade-4 chromosome, although their genetic constitution has not been ascertained. They may be recombinant for these genes. They were plated onto yeast medium and examined periodically for sectors. Neither strain has sectored.

The third recombinant aneuploid was assumed to be aneuploid for only the ade-3 nic-2 chromosome. The ade-3 mutation was detected twice while the nic-2 mutation was detected only once in two separate heterokaryotic allelic tests. The recombinant was

Table 26. Segregation of auxotrophic mutations in the progeny derived from crossing haploid spontaneous recombinants selected at random and one aneuploid recombinant with a wild type strain.

Genotype of recombinants inferred from heterokaryotic allelic tests	Requirers / Total sample arginine	adenine	nicotinic acid
A. Haploid:			
arg-2 su-1 ade-2$^+$ ade-3$^+$ nic-2 ade-4 arg-1$^+$ nic-3$^+$	0/63	27/63	37/63
arg-2 su-1 ade-2$^+$ ade-3$^+$ nic-2 ade-4 arg-1$^+$ nic-3$^+$	0/77	31/77	41/77
arg-2 su-1 ade-2$^+$ ade-3$^+$ nic-2 ade-4 arg-1$^+$ nic-3	0/62	30/62	53/62
B. Aneuploid:			
arg-2 su-1 ade-2$^+$ $\frac{\text{ade-3 nic-2}}{\text{ade-3 +}}$ ade-4 arg-1$^+$ nic-3$^+$	0/102	75/102	19/102

mated to a compatible wild type strain and progeny were collected from a single fruiting body. The observed number of nicotinic acid-requiring progeny (19/102) was compatible with the expected number for trisomic segregation (17/102) assuming only one dose of nic-2 entered the cross. Segregation of the ade-3 mutation was confounded with the segregation of ade-4. There are two possible genotypes for the ade-3 mutation on a disomic chromosome. If ade-3 were heterozygous, a mating between this recombinant and a wild type strain, where only ade-3, ade-4 and nic-2 mutations are depicted is given below:

$$\frac{\text{ade-4}}{+} \quad \frac{\text{ade-3}\quad +}{+\quad \text{nic-2}}$$

$$\times$$

$$\frac{+}{+} \quad \frac{+}{+\quad +}$$

The expected number of adenine-requiring progeny would be 59/102. If ade-3 were homozygous and disomic, two doses of ade-3 and one dose of nic-2 would enter the cross as indicated below:

$$\frac{\text{ade-4}}{+} \quad \frac{\text{ade-3}\quad +}{\text{ade-3}\quad \text{nic-2}}$$

$$\times$$

$$\frac{+}{+} \quad \frac{+}{+\quad +}$$

The expected number of adenine-requiring progeny would be

76.5/102. The number of adenine-requiring progeny recovered was 75/102. A mitotic crossover between ade-3 and nic-2 would be required to yield a disomic homozygous for ade-3, since ade-3 and nic-2 were in repulsion in the D-9 diploid. If the frequency of crossing over in triploids of S. commune is reduced, as is the case in Drosophila, (Bridges and Anderson 1925; Redfield 1930, 1932), the mating between the aneuploid homozygous for ade-3 and a wild type strain should yield progeny that always have the adenine requirement, conferred by ade-3, when the nicotinic acid requirement is expressed. However, if ade-3 were heterozygous and disomic, 8.5/102 progeny would be expected to have a nicotinic acid requirement in the absence of an adenine requirement. The observed number of progeny which possessed only a nicotinic acid requirement was 8. These data are compatible with ade-3 being heterozygous and disomic even though the number of adenine-requiring progeny (75/102) was higher than expected (59.5/102, Table 26).

The third group of recombinants was also selected on the ability to grow normally in the absence of arginine (Table 25). None exhibited requirements for adenine or nicotinic acid. These strains appeared to be homozygous for su-1 and arg-2 but heterozygous for the remaining auxotrophic mutations. These strains were plated onto yeast medium where some sectoring was observed. Segregants of each strain were tested for nutritional requirements on drop-out

media and auxotrophic mutations were inferred from heterokaryotic allelic tests. Two of the 12 strains of this recombinant group sectored and an analysis of the segregation of biochemical mutations is given in Table 27. The ade-3 and arg-1 mutations were not recovered among secondary segregants of recombinant 128. Segregant 128f is the only individual that possesses the ade-2 mutation and the arg-2 mutation in the absence of su-1. This would suggest that 128 was not homozygous for su-1 or that nuclei still heterozygous for su-1 were transferred with the nuclei homozygous for su-1 in the original transfer to yeast medium. The segregation of auxotrophic mutations in the progeny of matings between 128e and 128f and a wild type strain reaffirmed that these recombinants are haploid (Table 28).

Segregants obtained from recombinant 194 possessed only the nic-2 mutation (Table 27).

Recombinants were also recovered from diploid D-9, following 10, 20 or 30 sec exposure to UV irradiation (Table 29). One recombinant genotype differed from those obtained from spontaneous recombination. The suppressor was not prosent in this recombinant and this was the only recombinant isolated that possessed the ade-2 mutation. Since ade-2 and su-1 were in repulsion, a crossover between su-1 and ade-2 was required to allow them to segregate as a unit in any given nucleus. The results suggest that this recombinant, isolated from an arginine deficient medium, was

Table 27. Growth of primary and secondary segregants derived from the D-9 diploid.

Segregant	Test media: Drop-out and fully supplemented A[a]	B	C	D	Growth on minimal medium of dikaryons derived by mating the segregants with auxotrophic testers. Tester strains: ade-2	ade-3	ade-4	arg-1	arg-2	nic-2	nic-3
128	+[b]	+	+	+	+	+	+	+	+	+	+
128a	±	+	+	+	+	+	+				
128b	+	+	-	+						+	-
128c	+	±	+	+							
128d	+	+	-	+						+	-
128e	-	+	-	+	+	+	-	+	±	-	+
128f	-	-	-	±	-	+	-	+	-	-	-
128g	+	+	-	+	+	+	+	+	+	-	+
194	+	+	+	+							
194a	+	+	-	+						-	+
194b	+	+	-	+						-	+
194c	+	+	-	+						-	+

[a] Test media: A = adenine drop-out; B = arginine drop-out; C = nicotinic acid drop-out; D = fully supplemented media. [b] + = growth, - = no growth on the indicated medium.

Table 28. Segregation of auxotrophic mutations in the progeny derived from crossing secondary segregants from a prototrophic recombinant with a wild type strain.

	Genotype of recombinants inferred from heterokaryotic allelic tests	Requirers / Total sample arginine	adenine	nicotinic acid
128e	arg-2 su-1 ade-2$^+$ ade-3$^+$ nic-2 arg-1$^+$ ade-4 nic-3$^+$	0/61	24/61	27/61
128f	arg-2 su-1$^+$ ade-2 ade-3$^+$ nic-2 arg-1$^+$ ade-4 nic-3	56/104	74/104	89/104

Table 29. Recombinants recovered from fast growing sectors following treatment of strain D-9 with UV irradiation.

Common-AB Diploid, D-9

$$\frac{\underline{A41}\ \underline{B41}\ \underline{arg\text{-}2}\ +\ \underline{ade\text{-}2}\ +\ \underline{nic\text{-}2}\ \underline{arg\text{-}1}\ +\ +}{A41\ B41\ arg\text{-}2\ su\text{-}1\ +\ ade\text{-}3\ +\ +\ ade\text{-}4\ nic\text{-}3}$$

	No. of recombinants following UV exposure of:		
	10 sec	20 sec	30 sec
I. Haploid recombinants:			
$\underline{arg\text{-}2}\ \underline{su\text{-}1}\ \underline{ade\text{-}2}^{+}\ \underline{ade\text{-}3}^{+}\ \underline{nic\text{-}2}\ \underline{arg\text{-}1}^{+}\ \underline{ade\text{-}4}\ \underline{nic\text{-}3}$	3	1	3
$\underline{arg\text{-}2}\ \underline{su\text{-}1}\ \underline{ade\text{-}2}^{+}\ \underline{ade\text{-}3}^{+}\ \underline{nic\text{-}2}\ \underline{arg\text{-}1}^{+}\ \underline{ade\text{-}4}\ \underline{nic\text{-}3}^{+}$	4	5	14
$\underline{arg\text{-}2}\ \underline{su\text{-}1}^{+}\ \underline{ade\text{-}2}\ \underline{ade\text{-}3}^{+}\ \underline{nic\text{-}2}\ \underline{arg\text{-}1}^{+}\ \underline{ade\text{-}4}\ \underline{nic\text{-}3}^{+}$	0	0	1
II. Prototrophic diploid recombinants:			
$\frac{\underline{arg\text{-}2}\ \underline{su\text{-}1}\ \underline{ade\text{-}2}\ +\ \underline{nic\text{-}2}\ \underline{arg\text{-}1}\ +\ +}{arg\text{-}2\ su\text{-}1\ +\ ade\text{-}3\ +\ +\ ade\text{-}4\ nic\text{-}3}$	4	11	10

probably carried over in the original inoculum transferred from the rapidly growing sector. Hyphal tip isolation resulted in the purification of a recombinant nuclear type that would not be expected to be detected using these methods. The other two haploid genotypes were common among spontaneous recombinants, and displayed a pink color. The single recombinant that possessed the ade-2 as well as the ade-4 mutation was white.

The recombinants that exhibited dense growth on all drop-out media were assumed to be diploids, homozygous for su-1. This group consisted of 25 individuals (Table 29 II). These strains were maintained on yeast medium and examined periodically for segregants. Fifteen of the 25 strains sectored on yeast medium (Table 30). A total of 27 sectors analyzed for nutritional requirements on drop-out medium exhibited a requirement for nicotinic acid. Heterokaryotic allelic tests indicated that 26 possessed only the nic-2 mutation, one sector was a double mutant, having nic-2 and nic-3, but none of the sectors possessed only the nic-3 mutation (Table 30).

The appearance of colored sectors in the common-AB diploid culture on yeast medium provided another method for the detection of recombinational events. Only a limited number of recombinants were recovered and analyzed from a single diploid strain, D-25. Strain D-25 was assumed to be diploid and heterozygous for all auxotrophic mutations since it was repeatedly

Table 30. Nicotinic acid-requiring segregants from the prototrophic sectors that were isolated following treatment of D-9 with UV irradiation.

Time D-9 was exposed to UV	No. of prototrophic recombinants that sectored	No. of sectors analyzed	No. of sectors with: Single mutation nic-2	Single mutation nic-3	Double mutation nic-2	Double mutation nic-3
10 sec	6	7	6	0	1	
20 sec	5	15	15	0	0	
30 sec	4	5	5	0	0	

transferred from the stock bottle to drop-out media. It possessed only a requirement for arginine.

The recombinant sectors derived from D-25 are presented in Table 31. Since there was no selection against arginine-requiring recombinants, it should be possible to recover both the arg-2 and arg-6 mutations. The second recombinant genotype listed in Table 31 must have arisen as the result of a crossover between su-1 and ade-2 as only the arg-2 mutation was recovered. The third recombinant nuclear type listed possessed the ade-3 mutation. ade-3 and nic-2 went into the diploid in repulsion and it is considered significant that when ade-3 was recovered, nic-2 was not. The fourth recombinant possessed the arg-6 requirement and was isolated from a sector of very restricted growth.

Table 31. Spontaneous recombinants derived from a common-AB diploid, D-25, which were isolated from yeast medium as pink sectors or in areas of the mycelium that exhibited sparse growth.

Common-AB Diploid, D-25

$$\frac{A42\ B41\ arg\text{-}2\ +\ ade\text{-}2\ +\ nic\text{-}2\ arg\text{-}1\ +\ ade\text{-}1\ +\ +}{A42\ B41\ arg\text{-}2\ su\text{-}1\ +\ ade\text{-}3\ +\ +\ ade\text{-}4\ +\ nic\text{-}3\ arg\text{-}6}$$

Recombinant genotypes	No. of recombinants
arg-2 su-1 ade-2$^+$ ade-3$^+$ nic-2 arg-1$^+$ ade-4 ade-1$^+$ nic-3 arg-6$^+$	1
arg-2 su-1$^+$ ade-2$^+$ ade-3$^+$ nic-2$^+$ arg-1$^+$ ade-4$^+$ ade-1$^+$ nic-3$^+$ arg-6$^+$	1
arg-2 su-1 ade-2$^+$ ade-3 nic-2$^+$ arg-1$^+$ ade-4 ade-1$^+$ nic-3$^+$ arg-6	1
arg-2 su-1 ade-2$^+$ ade-3$^+$ nic-2$^+$ arg-1$^+$ ade-4$^+$ ade-1$^+$ nic-3$^+$ arg-6	1

DISCUSSION

Ultraviolet light and chemical mutagens were employed in an intensive search for recessive suppressors in Schizophyllum commune. Of 764 prototrophic colonies obtained by treatment of 6 auxotrophic strains with UV, none possessed a recessive suppressor. If suppressors were present among these prototrophs, they were either partially dominant or dominant and, were of no value for detection of recombinational events in somatic cells.

Twelve cultures possessing suppressors of the arg-2 mutation were isolated following treatment of mycelial fragments with chemical mutagens. Eight strains were isolated following treatment with hydroxylamine (HA), two strains were isolated following treatment with nitrosoguanidine (NG) and two after treatment with acridine red (AR). No suppressors were obtained for loci other than arg-2. The recovery of the arg-2 mutation from one prototrophic strain induced with HA indicated that the mutation to prototrophy did not occur at the arg-2 locus. The recovery of the arg-2 mutation from the other eleven suppressors has not been accomplished although relatively small numbers of progeny have been tested. The mutations could have occurred within the arg-2

locus in each of the eleven strains as a result of intragenic suppression. However, dikaryons synthesized between these eleven prototrophic strains and an auxotroph possessing arg-2 failed to grow on minimal medium. The data suggest that if the mutations to prototrophy occurred at the arg-2 locus, they are recessive to the arg-2 allele or that prototrophy may have resulted from a recessive suppressor which is very closely linked to the arg-2 locus.

The two mutations which suppress auxotrophy conferred by arg-2 which were induced with either NG or AR appear to be different from those induced with HA. In heterokaryotic allelic tests on medium lacking arginine, comparisons were made between dikaryons that were heteroallelic for the suppressor but homoallelic for arg-2 and dikaryons that are homoallelic for both arg-2 and the suppressor. Differences in growth rates were easily detected up to 4 days but were not discernible after 7-8 days. These suppressors may correspond to the semidominant suppressors reported by other investigators (Weglenski 1966; Gajewski and Litwinska 1968). In contrast, dikaryons homoallelic for arg-2 but heteroallelic for the suppressors induced with HA are clearly distinguishable after several weeks on arginine-less medium from dikaryons homoallelic for the suppressor and arg-2.

It is unclear how su-1 suppresses auxotrophy conferred by arg-2. If su-1 were analogous to amber suppressors in bacteria

(Sarabhai, Stretton, Brenner and Bolle 1964) or the super-suppressors in yeast (Hawthorne and Mortimer 1963) it may have been possible to suppress auxotrophy at other loci. No biochemical data are available which would indicate the type of mutation present within other auxotrophic loci in S. commune. It is entirely possible that none of these loci possess amber mutations. That mutations to prototrophy which map near to, or within, the arg-2 mutation were induced with HA, NG and AR suggests that the arg-2 locus does not possess a nonsense mutation. Hydroxylamine appears to react specifically with cytosine producing one-way base transitions whereby the original guanine-cytosine base pair is replaced by an adenine-thymine base pair (Brown and Schell 1961; Freese, Bsutz-Freese, and Bautz 1961). In Neurospora, HA was shown to produce reversions only in strains which revert by base-pair substitution (Malling 1966). Reversion studies of amber and ochre mutations within the rII genes of bacteriophage T4 (Champe and Benzer 1962; Brenner, Stretton and Kaplan 1965) indicate that neither mutation can be induced to revert by HA. Acridine dyes appear to cause deletions or insertions of base-pairs in the DNA which result in frameshift mutations (Brammar, Berger, and Yanofsky 1967). These frameshift mutations have been postulated as a mechanism for intragenic suppression. The specific action of NG is thought to be a methylation of guanine which ultimately leads to mispairing and base substitution

(Singer, Fraenkel-Conrat, Greenberg and Michelson 1968). Because all three mutagens induce mutations which map at, or very close to, the arg-2 locus, they may act on the same mutated site. If the mutation to auxotrophy at the arg-2 locus were by the induction of an ochre or amber triplet, no transition of the type induced by HA would restore prototrophy. All the 230 prototrophs recovered following treatment with HA would have necessarily been suppressors (Table 8). A more plausible interpretation would be that both NG and HA are capable of causing base-pair substitutions within the arg-2 locus which lead to prototrophy and that the mutation which had lead to auxotrophy was not a mutation to a nonsense codon.

The results shown in Table 10 and Figure 6 are surprising. Only 6 of the 19 arginine-requiring progeny obtained from the cross HA 41 (su-1) X 699, failed to grow as dikaryons with an arg-2 tester. These data suggest that 13 of these progeny carried a suppressor but the arginine requirement was poorly suppressed in the homokaryotic state. Other examples of these differences in scoring were observed in strains induced with NG and AR (Table 11).

Regardless of how su-1 suppresses auxotrophy conferred by arg-2, it is ideally suited for experiments on somatic recombination. It is incapable of suppressing auxotrophy at 8 loci tested thus far and it maps within one of the few established linkage groups in S. commune.

The technique used to obtain diploid strains was rewarding.

The experimental results clearly show that stable vegetative diploid strains of S. commune have been isolated from a common-AB heterokaryon. All mutant markers that are present in both components of the common-AB heterokaryon can be recovered from diploid strains (Table 14 and 24).

The segregation of mating-type factors and nutritional markers among progeny of a diploid X haploid cross follow the predicted ratios of triploid segregation assuming recovery of all types of meiotic products.

Two mutant markers, ade-4 and nic-2 (each of which was contributed by a different nucleus), were recovered with a frequency greater than expected by trisomic segregation (Table 14). It is conceivable that the diploid nucleus is not stable prior to the formation of the fusion nucleus in the basidium, as reported in Coprinus lagopus (Casselton 1965). The fusion of an aneuploid nucleus with the haploid nucleus prior to meiosis could account for the high frequency of recovery of ade-4 and nic-2. Another possible explanation for the high frequency of recovery of ade-4 and nic-2 is that haploidization involving chromosomes with these markers occurred very soon after basidiospore germination, and that some strains originally disomic for certain markers were scored as haploids. That haploidization of some disomic chromosomes is occurring early is substantiated by results obtained for the segregation of mating-type

factors. Although the observed values agree reasonably well with the expected for trisomic segregation (P= .2-.05), it can be seen that disomic A and B factors occur less frequently than expected (Table 15 and 16). Another explanation would be that there is selective pressure against individuals disomic for chromosomes possessing the ade-4 and nic-2 markers in the heterozygous condition, and that these individuals were among those non-viable germlings.

Progeny from the diploid X haploid cross that have disomic chromosomes apparently undergo haploidization via stages of aneuploidy with reassociation of whole chromosomes, as in the Parasexual Cycle (Pontecorvo 1956). From a limited number of primary and secondary segregants, at least four linkage groups are shown to occur. Recovery of arg-6 and nic-2 among all segregants of culture 144 indicates that these markers very probably are not on chromosomes known to be disomic, i.e., chromosomes carrying the A mating-type factors and chromosomes carrying mutant markers, ade-2, ade-4 and arg-2. The genetic map of S. commune (Ellingboe and Raper 1962a) places ade-2 and arg-2 in the same linkage group approximately 25 cross-over units apart. The data obtained from segregants of culture 144 is consistant with this linkage arrangement. Mutant markers ade-2 and arg-2, entered the diploid X haploid cross in the cis-arrangement and they segregated as a unit among segregants of culture 144 (Table 20). For culture

144, recombinationhad not occurred between arg-2 and ade-2 in meiosis. Segregation of ade-4 was independent of ade-2 and arg-2 and the A mating-type factors and is interpreted as belonging to another linkage group. It remains to be established whether arg-6 and/or nic-2 are linked to the B factor or whether they belong to separate linkage groups or to the same linkage group.

The recovery of strains which were disomic and homozygous or heterozygous for mating-type factors, from the diploid X haploid mating, provides a way for obtaining commom-A, common-B or fully compatible diploids for a study of somatic recombination. The stability of these diploids is uncertain but at least eight strains which scored as compatible diploids have failed to fruit or sector.

The common-AB diploids heterozygous for su-1 provided a method, analogous to that used in Aspergillus nidulans (Pontecorvo and Kafer 1956), for studying somatic recombination. The greatest number of spontaneous recombinants (129/154) obtained from D-9 were haploids (Table 25). None of the 129 haploid strains appear to have recombined genes via crossing over. The failure to recover nic-2 and ade-3 in equal frequency is not understood. The nic-2 mutation which is in the same linkage group with ade-3 and in repulsion in D-9, was recovered in all of the 129 haploid recombinants. Since nic-2 was in repulsion with su-1, their being in the same linkage group is excluded since segregation as a unit would require

crossing over in each of the 129 recombinants. It is conceivable that the high frequency of recovery of nic-2 results from selective pressure against haploid strains which carry the ade-3 mutation. Since selection for recombination is based on detection of rapid growth, differential growth rates between haploids carrying ade-3 or nic-2 may account for selection. There may be selective pressure for strains carrying the nic-2 marker. There was also a higher frequency of recovery of nic-2 in the diploid X haploid cross (Table 14).

The recovery of ade-4 in all of the somatic segregants is easier to explain. Linkage data obtained from fruiting bodies of common-AB heterokaryons and compatible matings (Middleton 1964) place ade-4 and arg-1 in the same linkage group, approximately 4 to 8 crossover units apart. Recombinants were recovered on an arginine drop-out medium which excludes those recombinants that possess an arginine requirement. Since arg-1 and ade-4 were in repulsion, (barring crossing over between the markers, which could result in segregation of the wild type allele at each locus), all haploid strains should carry the ade-4 mutation.

None of these haploid recombinants show recombination between linked genes. These results suggest that haploidization proceeds via stages of aneuploidy, thereby recombining genes only on different chromosomes.

The segregation of ade-3 and nic-2 in the progeny of a

cross of a putative aneuploid X wild type strain was compatible with trisomic segregation. The recovery of 8 progeny with the nic-2 mutation in the absence of an adenine requirement was compatible only with ade-3 and nic-2 being heterozygous and disomic.

Further evidence for haploidization via stages of aneuploidy was observed in genotypes of secondary segregants derived from a prototrophic primary segregant of D-9 (Table 27). The recovery of the ade-2 and arg-2 mutation apparently without su-1 indicates that although the original prototrophic segregant, 128, appeared to grow well on arginine drop-out medium, it may still have been heterozygous for su-1.

Analysis of recombinants obtained following treatment with UV light revealed no increase in the incidence of crossing over (Table 29). One recombinant genotype was unique in that it was isolated from arginine drop-out medium but it did not possess su-1. Examination of the nicotinic acid-requiring segregants of prototrophic sectors isolated following UV treatment indicated strong selection pressure for the chromosome carrying nic-2 (Table 30).

Experiments designed to select for recombinants on the basis of colored segregants provided a means whereby arginine-dependent segregants could be recovered. This is possible because pink color is present in haploid strains that carry ade-4 in the absence of ade-2, and in diploids homozygous for ade-4 but

heterozygous for ade-2. Thus, any recombinational event leading to either of these two situations is detectable on yeast medium. Although no biochemical data are available about the product that accumulates or the position of the block in ade-4 or ade-2 mutants, it appears that the ade-2 mutation affects an earlier step in the adenine biosynthetic pathway than ade-4. With this method, only 4 segregants were recovered and analyzed, a sample too small to draw definite conclusions. The arg-6 mutation was recovered in 2 of the 4 cases. Modifications of this technique may provide an efficient way to obtain segregants.

The limited data on somatic recombination in common-AB diploids indicate a parasexual mechanism. The techniques used would not have detected Specific Factor Transfer since the A and B factors were similar. The methodology for detecting recombinational events in diploids is now available for the critical experiments that will test the role of mating-type factors in somatic recombinational events. It should now be possible to synthesize common-A, common-B and compatible diploid strains for the purpose of examining somatic recombination.

In earlier experiments (Ellingboe and Raper 1962a; Ellingboe 1963), the origin of class I recombinants was explained by meiosis-like recombination within the dikaryon of the incompatible di-mon mating. Using a compatible diploid heterozygous for the

recessive suppressor, experiments can be conducted to determine whether the Parasexual Cycle, Specific Factor Transfer, or a meiosis-like mechanism of recombination are indeed the mechanisms by which genetic material is exchanged in somatic cells or if these mechanisms are simply a reflection of the technique used to detect recombination.

SUMMARY

Recessive suppressor mutations of the arg-2 locus have been isolated in Schizophyllum commune by treating mycelial fragments of an arg-2 strain with hydroxylamine, nitrosoguanidine or acridine red and selecting for prototrophs. Each mutation to prototrophy segregated as a single gene through two or three generations of backcrossing to the arg-2 parent. One suppressor, su-1, maps outside the arg-2 locus and is incapable of suppressing auxotrophy conferred by mutations at eight other loci. su-1 is recessive in the dikaryon and common-AB diploid. Prototrophy in the remaining eleven strains may have resulted from intragenic suppression or from a mutation in a suppressor that is very closely linked to the arg-2 locus. If the suppression is intragenic, all are recessive to the arg-2 allele.

A technique was developed which permitted the recovery of stable vegetative diploid strains from common-AB heterokaryons of S. commune. Common-AB diploid strains heterozygous for auxotrophic mutations are prototrophic and resemble the homokaryon in morphology. Those strains homozygous for arg-2 but heterozygous

for su-1 and other auxotrophic mutations exhibit very slow growth on arginine drop-out medium. Segregation of five auxotrophic mutations and mating-type factors of a diploid X haploid cross closely fit the predicted values for triploid segregation. Segregation of mutant markers among somatic segregants of an aneuploid derived from the diploid X haploid cross indicated that at least four linkage groups are present in S. commune.

The recessive suppressor, su-1, was used to detect somatic recombination in common-AB diploids of S. commune. Recombinants were recovered from dense fast-growing, sectors on arginine drop-out medium. The majority of recombinants analyzed in this study were haploid. Aneuploid and diploid strains were also recovered and analyzed. Genetic analysis of spontaneous recombinants indicated that crossing over is rare and that haploidization proceeds via stages of aneuploidy. Recombinants derived following treatment with UV light showed no increase in the frequency of crossing over. The preliminary results favor a parasexual mechanism of recombination in common-AB diploids.

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