THE ROLE OF SEXUAL INCOMPATIBILITY FACTORS IN SOMATIC RECOMBINATION IN SCHIZOPHYLLUM COMMUNE

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Major professor

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ABSTRACT

THE ROLE OF SEXUAL INCOMPATIBILITY FACTORS IN SOMATIC RECOMBINATION IN SCHIZOPHYLLUM COMMUNE

By

Carl Stephen Frankel

This research was undertaken to determine whether the compatibility relationship of diploid cultures of Schizophyllum commune affects the type of somatic recombination which occurs in those diploids. Two types of somatic recombination were considered: "meiotic-like", characterized by direct breakdown from diploid to haploid with meiotic levels of crossing-over, and parasexual, characterized by breakdown to haploid via stages of aneuploidy and very little crossing-over.

There were two additional objectives: (1) generation, by mutagenesis, of sufficient different biochemical mutations to facilitate this study, and (2) determination, from the somatic haploidization data, of the number of independently segregating chromosomes represented in the known meiotic linkage groups of this organism.

N-methyl-N'-nitro-N-nitrosoguanidine was used as a mutagen and seventy-four mutations were induced. Twenty-two of these were of usable "new" genes and fourteen mapped to existing linkage groups. Twenty-five proved to be alleles of the other new or stock mutations.

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Three sets of diploids were obtained. Each set comprised cultures which were almost identical genetically but differed in their compatibility relationship. Four common-AB diploids were isolated as spontaneous sectors from nutritionally forced common-AB heterokaryons. Five compatible diploids were isolated from among the progeny of crosses of the common-AB diploids with appropriately marked haploids. (No usable common-A or common-B diploids were obtained.)

Two of the sets of diploids were homozygous for arg2 and heterozygous for su1, a recessive suppressor of arg2. Haploid and aneuploid products were selected on arginineless medium as rapidly growing sectors produced when haploidization caused loss of the chromosome bearing arg2 without its suppressor. The third set of diploids was heterozygous for ad4 and ad9, linked "pink" adenine mutations in repulsion. Haploid and aneuploid products were selected as sectors of non-diploid morphology and/or pink colour.

Two of the compatible "diploids" in one set proved to contain a high number of dikaryotic cells. One of these, Culture 19, possessed a B mating-type factor with anomalous behaviour in that it repressed the expression of certain other B factors, including the other B factor of Culture 19, and showed a marked tendency to non-disjoin.

Haploid and/or aneuploid sectors were recovered from all the diploids, including those partially dikaryotic.

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Carl Stephen Frankel

Haploids from the common-AB and normal compatible diploids showed almost no evidence of crossing-over. Haploids from the partially dikaryotic cultures included a significant frequency of crossover genotypes. It appears that the somatic recombination process in diploids is classically mitotic (parasexual). The diploid cells of the partially dikaryotic cultures can undergo mitotic haploidization, but their nuclei give evidence of a previous history of meiotic-like behaviour involving crossing-over. The tendency toward meiotic-like somatic recombination is apparently a property of the dikaryotic state rather than of compatibility *per se*.

Analysis of segregation of markers on separate meiotic linkage groups during haploidization of the diploids indicated that each linkage group corresponds to a separate chromosome. There are at least seven chromosomes in *S. commune*.

THE ROLE OF SEXUAL INCOMPATIBILITY FACTORS IN SOMATIC RECOMBINATION IN *SCHIZOPHYLLUM COMMUNE*

By

Carl Stephen Frankel

A THESIS

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1974

DEDICATION

To the memory of
Joe Treiger,
a great and good friend

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I would also like to thank Joe Martin for the photography and Ray Hancock for technical assistance and both of these honourable gentlemen for the good times, even while in despair at the recalcitrance of my fungus.

My gratitude also to Le Club de Hockey Canadien and to Michael Kalin and Joe Vechter, as promised, for being partial answers to the existential dilemna.

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TABLE OF CONTENTS

LIST OF TABLES

Table

Table | Page

Table

Table | Page

Table

41. Hapl
P-1

42. Pair
stra

43. Soma
link
are

Figure

1. Linka

2. Diploi

3. Compat
argini

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LIST OF FIGURES

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INTRODUCTION

The first description of a mechanism, other than heterokaryosis, to account for somatic recombination in fungi was presented by Pontecorvo (1953). The mechanism described, i.e. mitotic haploidization of diploid nuclei via stages of aneuploidy, was characterized by infrequent crossing-over in the Ascomycete Aspergillus nidulans. Haploidization was separated in space and time from crossing-over. This process was defined as an integral part of what was termed the "parasexual cycle."

Experiments with the Basidiomycetes Schizophyllum commune and Coprinus spp. utilized the illegitimate (or incompatible) dikaryotic - homokaryotic (di-mon) mating (e.g. [A1B1 + A2B2] x A1B2) for detecting somatic recombination. Only a recombinational event which recombined mating types from the dikaryon nuclei gave a nucleus that was compatible with the nucleus of the homokaryon (e.g. [A1B1 + A2B2] x A1B2 → [A2B1 + A1B2]). In one series of such experiments (Ellingboe and Raper 1962) two novel types of somatic recombination were described: a meiosis-like event in which there was a high frequency of crossing-over between linked genes, and Specific Factor Transfer in which only an incompatibility factor from each of the two nuclei of the dikaryon was transferred into the genetic background of the homokaryon nucleus.

More recent work on S. commune was based on a selective system to detect haploidization and/or recombination within linkage groups in common-AB (fully incompatible) diploids (Mills and Ellingboe 1971). This is a

system analogous to that used with Aspergillus. The recombinants obtained appeared to have originated via a mitotic parasexual process.

These two systems used to detect somatic recombination differ in one fundamental respect. In the diploids, compatible mating type factors, which are ordinarily necessary for meiosis, are absent. In the di-mon matings they are present. Indeed, a recombinational event must produce a nucleus compatible with the nucleus of the homokaryon if it is to be detected. Thus the question can be posed: are the differences in somatic recombination mechanisms that were detected in these two systems due to differences in incompatibility factor relationships, or to some mechanistic or selective idiosyncracies?

The former seems to be a reasonable alternative in light of the numerous published accounts of genes, in several organisms, affecting recombination in some way, of the known role of incompatibility factors in initiating meiosis (Raper 1966), and of the popular view of the incompatibility factors as "master switches" that control cascading arrays of genes and many developmental pathways (Raper 1966). Thus this research was initiated with the objective of (1) creating diploid strains of S. commune identical in genetic background and marking but differing in the compatibility relationship of their constituent nuclei, and (2) to observe whether these incompatibility differences lead to different modes of somatic recombination detectable upon haploidization.

A mutagenesis and mapping project was undertaken first to obtain new mutations marking the linkage groups of S. commune. This was done with two goals in mind: (1) generation of many different auxotrophic mutations so that extensive complementation tests were not necessary to analyse crosses between multiply marked strains, and (2) generation of sufficient markers in linkage groups to detect crossing-over with confidence.

Finally, there was another goal of this research which would be obtainable as a by-product of analysis of haploidization. Meiotic map distances in S. commune can vary widely under a variety of circumstances and considerable doubt exists concerning the linkage relationships of known markers. Thus diploid strains were constructed of genotypes such that analysis of marker segregation during haploidization, together with meiotic linkage relationships, would give the necessary data to unambiguously assign the markers of S. commune to independent chromosomes.

LITERATURE REVIEW

Investigation of somatic recombination in fungi may be said to have begun with the study of dikaryotization of a homokaryon by a dikaryon in Coprinus lagopus by Buller (1931). This process was later termed the "Buller Phenomenon" and shown to comprise three different kinds of mating: (1) compatible, in which both nuclei of the dikaryon are compatible with the homokaryon: (A1B1 + A2B2) x A3B3, (2) hemicompatible, in which only one nucleus of the dikaryon is compatible with the homokaryon: (A1B1 + A2B2) x A1B1, and (3) incompatible, in which neither nucleus of the dikaryon is compatible with the homokaryon (A1B1 + A2B2) x A1B2 (Quintanilha 1937). Dikaryotization of the homokaryon in the third type of mating is made possible only by a recombinational event. Such "illegitimate (incompatible) di-mon matings" in C. lagopus provided the first demonstration of genetic exchange between the nuclei of a dikaryon (A1B1 + A2B2) → (A1B2 + A2B1). Such matings used the appearance of dikaryotic sectors in homokaryotic mycelium as the selective system for detecting recombination. A later study (Papazian 1954) used a variation of this basic technique. Two successive illegitimate di-mon matings were made and hyphal tips that were isolated were found to contain nuclei of novel genotypes. No evidence of disomy was observed.

Significant advance beyond this point was made possible by the development of a technique for production of stable diploid strains of filamentous fungi (Roper 1952). Pontecorvo (1956) used diploids of

Aspergillus nidulans to characterize a system of somatic recombination he termed the parasexual cycle. The cycle consists of three basic phases: (1) fusion of unlike nuclei of a heterokaryon, (2) propagation of diploid cells during which mitotic crossing-over may occur, and (3) haploidization of the diploid by successive random loss of chromosomes, i.e., via stages of aneuploidy. It became readily apparent that mitotic crossing-over was a rare event. Mitotic map distances were only a small fraction of meiotic distances and the two were not regularly proportional. The rarity of mitotic crossing-over means that genes on the same chromosome generally segregate together. Analysis of somatic recombination is thus a useful tool for assigning genes to their chromosomes. When a crossover occurs on a chromosome marked by a series of heterozygous genes, all genes distal to the crossover can become homozygous. Thus genes on the same chromosome can be placed in linear order and the position of the centromere can be determined.

This method was used together with a selective system based on recombination to homozygosity of heterozygous recessive suppressors of auxotrophic mutations. If the fungus was growing sparsely on a medium deficient in the requirement of the auxotrophic gene (which was homozygous), recombination causing homozygosity of the suppressor would create a rapidly growing sector. These techniques made possible extensive mitotic mapping of Aspergillus nidulans and extensive study of somatic recombination (Pontecorvo and Kafer 1958) (Kafer 1961).

The discovery of parasexuality stimulated further work with di-mon matings in basidiomycetes in an effort to understand the nature of the recombinational process in this system. Crowe (1960) studied compatible di-mon matings in S. commune and found dikaryotic sectors in the

homokaryon which arose by migration of both nonrecombinant nuclei of the dikaryon through the homokaryon, and other sectors that resulted from association of the homokaryon nucleus with a nucleus from the dikaryon which may or may not have been recombined. The recombinant nuclei from the dikaryotic sector sometimes showed evidence of crossing-over, but the data were insufficient for Crowe to conclude whether she was observing parasexuality or a precocious form of meiosis.

Swiezynski (1962, 1963) studied recombination in incompatible and hemicompatible di-mon matings in C. lagopus. He experienced difficulty in analysing the genotype of the derived dikaryons due to poor germination of the basidiospores and low viability of those spores that germinated. Segregation ratios were mostly abnormal. The results obtained suggested a parasexual type of recombination with haploidization proceeding via stages of aneuploidy.

A study of 178 recombinant dikaryons from 7,160 incompatible di-mon matings in S. commune (Ellingboe and Raper 1962) made use of several non-selective markers, one of which was linked to the A incompatibility factor. With these it was possible to classify the recombinants obtained into two major groups: class I, in which non-selective markers represented various combinations of markers from the original dikaryon, and class II, in which non-selective markers were exclusively those of the homokaryon. There was no evidence of aneuploidy. Class I types most likely arose from familiar meiotic-like events in the original dikaryon, but the authors had to suggest two successive recombinational events, segregation from a triploid nucleus, or "some heretofore unknown mechanism" to account for individuals of class II.

Additional tests were performed with the use of two additional non-selective markers that were each less than one crossover unit from the Aα and Aβ loci, respectively (Ellingboe 1963). Twenty of eighty-six derived dikaryotic cultures from several incompatible di-mon matings were class II types in which an A factor from the dikaryon was transferred to the background genome of the homokaryon without transferring the closely linked markers. Whatever process was operative had a specificity of incorporation analogous to that of a temperate phage into a bacterium. It constituted a new, third mode of somatic recombination and was termed "Specific Factor Transfer."

Sixty-one well-marked recombinants from a single incompatible di-mon mating were shown in another study to be of forty-three different genotypes, none of which were class II types (Ellingboe 1964). This raised the possibility that Specific Factor Transfer may be a feature of only certain matings and therefore under a specific form of control.

A more recent study of S. commune yielded over three hundred derived dikaryotic sectors from incompatible di-mon matings (Shalev et al 1972). Three different dikaryons were used in these matings but their constituent nuclei were from genotypically identical sibling strains and all three dikaryons were mated to the same homokaryon. The genotypes of the derived dikaryotic sectors were only partially analysed; auxotrophic markers shown to be heterozygous were not tested to show which nucleus of the derived dikaryon contained the wild-type or the mutant allele. However, the analysis was sufficient for the authors to conclude that the sectors had arisen in at least three ways: (1) migration of both nuclei of the original dikaryon, (2) production of a recombinant nucleus from fusion and and haploidization of the nuclei of the original dikaryon and

mating of this nucleus with that of the homokaryon, and (3) production of a recombinant nucleus from fusion and haploidization of the homokaryon nucleus with a common-A nucleus of the dikaryon and mating of this nucleus with the other of the dikaryon. This third process had not been previously reported, as such, in the literature. The recombinant genotypes revealed no evidence of Specific Factor Transfer, and, more interestingly, showed that although crossing-over was of essentially meiotic frequency where it occurred, it occurred only in the regions between mating-type factor subunits. The authors interpreted this as evidence for a mechanism controlling frequency of recombination in different parts of the genome.

During this period data on somatic recombination in Basidiomycetes were also obtained from systems which did not have the selective bias of the incompatible di-mon mating system. Raper and Raper (1964) obtained five recombinant prototrophic hyphal tips from an old S. commune heterokaryon which had been formed from homokaryons carrying a modifier mutation which eliminated fruiting. Three were homokaryotic, two were dikaryotic and there was no evidence of aneuploidy.

A common-AB heterokaryon of S. commune has been produced by mating two homokaryons with complementing auxotrophic mutations on minimal medium (Middleton 1964). Fourteen hyphal tips isolated from the heterokaryon were prototrophic and stable, presumably diploids. When crossed to wild-type homokaryons, ten gave progeny that were auxotrophic for one or more requirements. The combinations of auxotrophic mutations segregating in each cross were consistent with what would be expected if the hyphal tips had been diploid, the diploid underwent mitotic hyploidization (i.e. reassortment of whole chromosomes), and the resultant haploid nucleus participated in the mating with the wild-type homokaryon.

An attempt was made to use recessive suppressors as a selective system to detect recombinational events in C. lagopus dikaryons (Cowan and Lewis 1966). The recombinational mechanism which produced rapidly growing sectors appeared to be parasexual. The total sample size was only six so it was impossible to draw any conclusions.

A different selective system was used to detect recombination in a triheterokaryon of Coprinus radiatus (Prud'homme 1965). Two of the three nuclei were compatible with each other but possessed a morphological mutation which prevented dikaryosis. The third nucleus did not have the morphological mutation but was incompatible with the other two. Thus a recombinational event was necessary to establish a dikaryon. The dikaryotic sectors which arose were genetically characterized by progeny analysis and dedikaryotization by maceration. Three conclusions were drawn: (1) haploidization proceeded via stages of aneuploidy, since numerous aneuploid and some diploid strains were recovered, (2) crossing-over is rare (none occurred in twenty-one recombinant nuclei), and (3) there was in many cases a parental association of independent non-selective markers. This last observation may be interpreted as evidence either for a mechanism similar to Specific Factor Transfer or for mutation to the wild type allele.

Somatic recombinants have been obtained from Uredinales (Puccinia graminis tritici) using mixed inoculation of two dikaryotic urediospore isolates on wheat seedlings (Watson 1957) (Ellingboe 1961). In both cases spore colour and virulence were used as markers. In neither case was it possible to determine the mechanism of recombination.

Holliday (1961) induced mitotic recombination in Ustilago maydis with ultraviolet light (UV). Heterozygous prototrophic diploids

exhibited crossing-over by becoming auxotrophic after irradiation. That these auxotrophs were still diploid was shown by a second UV treatment which initiated segregation of other auxotrophic markers. The results indicated mitotic crossing-over followed by haploidization, similar to what would be expected with the parasexual cycle.

It is apparent that the information about somatic recombination in basidiomycetes, which has come from a variety of techniques, is not consistent with any general theory. Recombination studies in basidiomycetes which used the techniques applied so successfully to Aspergillus had to await the development of stable diploid strains.

The notion of transient diploidy in vegetative mycelium gained acceptance with the demonstration of genetic exchange between the nuclei of a dikaryon (Quintanilha 1939). The first persistant diploids in basidiomycetes were produced from heterokaryons. They were stable as homokaryons but unstable when mated to a haploid (Casselton 1965) (Parag and Nachman 1966). In the former case Casselton made a common-A heterokaryon in C. lagopus with complementing auxotrophs. Oidia that could grow when plated on minimal medium were taken to be diploid since oidia are uninucleate. The instability in dikaryons was manifested as haploidization before fruiting. In the latter case, the authors used a common-B heterokaryon of S. commune. The diploid, when mated with a compatible haploid, produced progeny which germinated poorly and had low viability. All mutations present in both parents were recovered, but genotypic frequencies were not in accord with triploid segregation. Diploids were also produced in C. radiatus (Prud'homme 1965) as sectors from a triheterokaryon.

A stable diploid of S. commune was picked up serendipitously as a prototrophic product of the mating of two compatible, differently auxotrophic strains (Koltin and Raper 1968). The product had the morphology of a homokaryon instead of a dikaryon. The ability of the original homokaryons to produce a diploid when mated appeared to be controlled by a simple recessive gene. The authors named this gene dik, and suggested that the dik+ allele was necessary for the establishment and maintenance of a dikaryon.

Finally, diploids of S. commune were found to arise in a nutritionally forced common-AB heterokaryon (Mills and Ellingboe 1969b). These diploids were the first constructed expressly for the study of somatic recombination. They were heterozygous for a recessive suppressor of the arg2 mutation and homozygous for arg2 (Mills and Ellingboe 1969a). They thus comprised a system for examining somatic recombination exactly analogous to that used so successfully in A. nidulans (Pontecorvo and Kafer 1958).

These diploids produced 154 sectors which grew rapidly on arginine deficient medium. Of these, 129 were apparently haploid, the remaining 25 aneuploid and diploid. Haploidization occurred via stages of aneuploidy. Since none of the recombinants showed evidence of crossing-over, the recombination mechanism appeared to be typically parasexual, i.e., mitotic.

There seems no absolute pattern to the occurrence of the three modes of somatic recombination - parasexuality, meiosis-like, and Specific Factor Transfer. However, the most extensive studies (Pontecorvo 1956) (Ellingboe and Raper 1962) (Ellingboe 1963) (Ellingboe 1964) (Middleton 1964) (Mills and Ellingboe 1971) (Shalev et al 1972) seem to suggest that

parasexuality might be expected in a homothallic fungus such as A. nidulans or a basidiomycete with common-AB mating type factors, while meiosis-like recombination would occur in basidiomycetes with compatible mating type factors. Indeed, the incompatible di-mon mating system selects for recombination to compatibility between nuclei. (Specific Factor Transfer, of course, can only be detected in the presence of compatible mating type factors.) This pattern in turn suggests a role of the incompatibility factors in determining modes of recombination.

The incompatibility factors are known to control specific sequences of events leading to the establishment of a dikaryon and thence to meiosis and fruiting (Raper 1966). Obviously such complex activities are unlikely to be the direct result of the products of only a few genes. Therefore, it is supposed that these factors are regulatory in nature, and that they control the activity of numerous other genes. It can be postulated that among the genes controlled by the incompatibility factors are ones which initiate the events of meiosis.

There are precedents for gene effects specific to somatic or meiotic recombination, but not both. Jansen (1970) described a UV-sensitive mutant in A. nidulans, uvsB10, which when homozygous led to abnormally high frequency of mitotic recombination while meiotic recombination frequency remained unchanged. The author suggested that the high recombination activity of the ascus primordia may mask the effect of the gene, but that is only one possible explanation. Minute mutants in Drosophila melanogaster, known to have no effect on meiotic crossing-over, were shown to raise the frequency of mitotic crossing-over (Kaplan 1953). The meiotic mutant c(3)G of D. melanogaster virtually abolishes meiotic crossing-over but was shown to have no effect on somatic recombination

(Le Clerc 1946). Finally, a UV-sensitive mutant uvsB in A. nidulans was shown to increase mitotic recombination more than ten-fold while not affecting meiotic recombination at all (Shanfield and Kafer 1969).

Specific Factor Transfer is a more difficult problem to deal with. That its occurrence or non-occurrence may be under genetic control is indirectly suggested by its absence in some studies (Ellingboe 1964) (Shalev et al 1972) which employed the same experimental methods and selective system as studies in which it was detected (Ellingboe 1963) (Ellingboe and Raper 1962). If the phenomenon is considered to be one of highly localized and regulated crossing-over there is ample precedent for its genetic control. Catcheside (1968) described genes which specifically control recombination frequencies in the am locus of Neurospora crassa.

Simchen (1967) found that crossing-over between the α and β subunits of the A incompatibility factor in S. commune appeared to be under control of a major gene, linked to the A factor, and several minor genes. This conclusion was later modified to a more general form by Simchen and Stamberg (1969) who suggested that (1) recombination in unlinked chromosome segments is regulated by different components of a control system, (2) the effects of certain loci on recombination seems localized to short segments, (3) probably at least some loci control recombination in more than one region, and (4) a specific relationship likely exists between controlling genes and their segments. A gene controlling recombination frequency between the α and β subunits of the B incompatibility factor in S. commune was mapped as being linked to the B factor itself, and described as having no effect on recombination in an unlinked region or a region contiguous with the B factor (Koltin and Stamberg 1973).

This information, in addition to the description of the specifically localized crossing-over between mating type factor subunits (Shalev et al 1972), can be considered evidence for a process which, if not exactly Specific Factor Transfer, is mechanistically identical to it and under tight genetic control.

METHODS AND MATERIALS

Schizophyllum cultures: All cultures used had a common background genome, strain 699 (Ellingboe and Raper 1962). Auxotrophic mutations and their linkage relationships were either previously known and published (Ellingboe and Raper 1962) or induced and characterized in this study. No markers were extensively used which affected morphology, were very leaky, slow growing, or which did not segregate normally.

Various tester strains with single auxotrophic mutations in the 699 background were available, or, in the case of the new mutations, were prepared by backcrossing to 699. Multiply marked strains were synthesized by crossing single mutation stocks, their doubly marked progeny, etc.

Prototrophs representing most of the possible combinations of five A and five B incompatibility factors (A1, A2, A41, A42, A47, and B1, B2, B41, B42 and B47) were maintained as mating type testers. Most auxotrophs were available in the nine possible combinations among A41, A42, A47, and B41, B42, and B47.

The subunit composition of the A factors is as follows:

A41 = Aα1β1 A42 = Aα3β5 A47 = Aα3β5

Intra-A factor recombinants among these A factors were of three possible types: Aα3β1, Aα1β5, and Aα1β4. Tester strains of each type were available with various B factors. No recombination between subunits of B factors was observed.

Media: All matings were made on migration complete medium (Snider and Raper 1958). Stock cultures were maintained on yeast medium in bottles at 4°. Yeast medium is made from minimal medium (Raper and Miles 1958) by substituting yeast extract and bacto-peptone at 2g/liter for asparagine. Growth on yeast medium was used as a criterion of viability in all tests for auxotrophic mutations. The tests for auxotrophy were made on minimal medium and minimal supplemented with all possible nutrients necessitated by the mutations in the experiment except one, and called variously "adenineless", "sulphateless" etc. according to the missing supplement.

Supplements were kept in stock solutions. All nucleic acid bases and amino acids were used at a final concentration of 10^{-4}M, except cysteine and methionine at 1.5×10^{-4}M and leucine, tyrosine, and phenylalanine at 2×10^{-4}M. Vitamins were used at the following concentrations: inositol .04%, pantothenate, ascorbate, and choline .02%, niacin .01%, pyridoxine and riboflavin .005%, para aminobenzoic acid .001%, and biotin .0004%.

Mutagenesis with N-methyl-N'-Nitro-N-nitrosoguanidine (NG): Wild type strain 699 was grown on three plates of minimal medium for five days at 32° and then macerated in sterile Waring Blendors for one minute with 25 ml sterile distilled water. Five ml samples of the macerate were pipetted into 300 ml Erlenmeyer flasks that each contained 20 ml liquid migration complete medium with 5×10^{-3} mg/ml NG. Fourteen flasks were prepared and placed on a shaker at 22° for three days, after which the contents of each flask were centrifuged in a clinical centrifuge at 6500 g for 3 minutes. Each pellet was resuspended in 25 ml sterile distilled water and macerated in a Waring Blendor for 20 seconds. One ml

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of each suspension was pipetted onto each of four petri plates of yeast medium. The mycelial fragments were allowed to grow on these plates for 24 hours at 22°, after which they were examined under a dissecting microscope. Blocks of agar containing viable fragments were removed and placed on fresh plates of yeast medium, 16 to a plate. Twelve such plates were made from each of the fourteen original flasks. The plates were incubated at 22° for four days, by which time most of the fragments had produced macroscopically visible colonies. Small pieces of each colony were replicated onto yeast and minimal media and allowed to grow for three days at 22°. Auxotrophs were selected by their inability to grow on minimal medium. Several mutations affecting colony morphology were also isolated.

Testing the requirement of new auxotrophs: Newly acquired auxotrophs were plated on three types of media: minimal to which had been added, respectively, a solution of the five nucleic acid bases, a solution of nine vitamins, and vitamin-free casein hydrolysate.

Those that failed to grow only in the absence of nucleic acid bases were plated on minimal medium supplemented with either adenine, guanine, thymine, cytosine, or uracil. Requirements were, therefore, determined directly. Those that responded to the vitamins were placed on "pool plates" - minimal with three supplements as follows:

	#1	#2	#3
#4	riboflavin	pyridoxine	inositol
#5	niacin	pantothenate	ascorbic acid
#6	para aminobenzoic acid	choline	biotin

An auxotroph which grew, for example, only on pools #3 and #6 would appear to require biotin, the only supplement the two pools had in common. This would be verified by testing for growth on minimal plus biotin.

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Auxotrophs which responded to casein hydrolysate were tested on amino acid pool plates - minimal with either four or five supplements as follows:

	#1	#2	#3	#4	#5
#6	alanine	aspartic acid	leucine	serine	trytophan
#7	isoleucine	cystine	phenylalanine	glycine	threonine
#8	cysteine	lysine	glutamic acid	tyrosine	arginine
#9	histidine	methionine	proline	glutamine	valine

An auxotroph which grew, for example, only on pools #4 and #6 would appear to require serine, the only supplement the two pools had in common. This would be verified by testing for growth on minimal plus serine.

Purine requirers were tested for growth response to inosine, xanthine, and 4-amino-5-imidazole-carboxamide, all intermediates of the adenine - guanine synthetic pathway. All these supplements were at a final concentration of 10^{-4} M.

Heterokaryotic allelic tests: New mutants with the same requirements as existing stocks were tested for allelism. They were mated to the tester stocks and incubated at 32° for four or five days until a dikaryon was well established. Three small pieces of each dikaryon were removed and replicated onto minimal medium and minimal plus the appropriate supplement. Growth of the dikaryon on unsupplemented minimal medium would occur if the mutants were non-allelic and complementing. Homoallelism was inferred if no growth occurred on minimal medium.

Different mutations implicated in this way to be allelic to the same stock strain were crossed with each other, and a heterokaryotic allelic test was performed with the two new mutations. Similarly, those mutations with the same requirement, but which were not allelic to any stock strain, were tested against each other. These tests were performed only after backcrossing the mutant strains to wild type to generate compatible mating types.

Heterokarytic allelic tests were also used to determine genotypes of progeny from crosses which involved markers with common requirements. For example, if a strain bearing ni2 were crossed with one bearing ni3, niacin requiring progeny would be mated to ni2 and ni3 testers to determine whether their requirement for niacin were the result of carrying ni2, ni3, or both

Assigning mutations to their linkage groups and mapping: New auxotrophs were mated with tester strains carrying, respectively, ad3, arg2, arg1, arg6, and ni3, each marking one of the known linkage groups of S. commune. The necessity of heterokaryotic allelic tests was avoided when possible by the use of other genes in the linkage group, i.e. ad3 strains could be substituted by ni2 strains, arg2 by ad2, and arg1 by ad4. Samples of 32 progeny spores were collected and tested for evidence of linkage. Each of these crosses also afforded an opportunity to check for linkage to the A or B incompatibility factors.

Auxotrophs which did not show linkage to any of the tester markers were tested against other markers of the same linkage groups and/or each other. When results of these tests were inconclusive larger samples of spores were collected and analysed.

Once assigned, the new markers were placed in correct order with respect to the other markers of their linkage groups by 3-, 4-, or 5-point crosses. Each new marker was crossed in this way with all or most of its linked markers. Gene distances were averaged from all available data.

Crossing, single spore isolation, percent germination: Crosses were made by placing small mycelial plugs of compatible strains about 5 mm apart on migration complete medium. Each plate contained duplicate

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matings. They were incubated at 32° for four or five days or until fruit body initials formed. Chunks of agar bearing mycelium with the largest fruit body initials were cut out and placed away from the mating on the plates. Plates were incubated upside down at 22°. When basidiospores began to be produced, the agar chunk was removed, placed on the cover of a yeast medium plate, and allowed to drop spores onto the medium. After sufficient spores had collected, they were spread over the medium in several drops of sterile distilled water with a bent glass rod.

The spores were allowed to germinate for one day at 22°. The number of germlings vs number of ungerminated spores in three or more microscopic fields (at least 50 total sample) was counted, and the percent germination was calculated.

Small agar blocks with single germlings were removed with a small, flattened needle and transferred to yeast medium, sixteen to a plate. An effort was made to cut out every germling, regardless of size, in a microscopic field to assure a random sample. However, germlings growing too close together to separate with confidance were omitted. The germlings were incubated for four days at 22°.

Scoring for requirement: When isolated germlings had grown into macroscopically visible colonies they were replica plated individually with a dissecting needle onto minimal medium, minimal supplemented with all possible requirements for that cross, and the various test media in which all supplements except one were present. Yeast medium was frequently substituted for the completely supplemented control plates. In crosses involving only a single requirement, yeast and minimal media were used. Scoring for requirement was generally done after three days of incubation at 22°.

Scoring for mating type: Four testers were generally used to determine the mating types of progeny from a cross. For example, the progeny of a cross of A41B41 x A42B42 would be tested with A41B1, A42B1, A2B41, and A2B42. The first two testers were used to determine A mating types, the last two to determine B mating types. The reactions are as follows:

Genotype of Progeny	Testers A41B1	A42B1	A2B41	A2B42
A41B41	-[a]	+	-	+
A41B42	-	+	+	-
A42B41	+	-	-	+
A42B42	+	-	+	-
AxB41[b]	+	+	-	+
AxB42	+	+	+	-

a + = compatible, - = incompatible
b Ax = recombinant A factor

Recombinant A factors were sometimes further typed to determine the subunit alleles. From the example above, since A41 is Aα1β1 and A42 is Aα3β5, recombinant A factors would be tested with two strains such as Aα3β1B1 and Aα1β5B1, only one of which should be compatible with a recombinant of crossing A41 by A42.

Synthesis of common-AB diploids: Small mycelial plugs of selected strains with complementing auxotrophic mutations, but with the same mating type, were placed approximately 1 cm apart on migration complete medium, two pairs per plate, and incubated at 32° for five to seven days. A strip of agar about 2 mm wide was removed from the region of confluence and divided into thin slices. Each slice was placed in the center of a plate of minimal medium (or minimal plus arginine for common-AB heterokaryons homozygous for arg2 but heterozygous for the recessive suppressor sul).

Thus only nutritionally-forced common-AB heterokaryons could grow. The plates were kept at 22°. Rapidly growing sectors with good morphology (presumptive diploids) were purified and analysed.

Purification of cultures: Presumptive diploids, or any other culture in this study which arose as a sector in different genetic background, were purified by isolation of hyphal tips. Four samples were generally taken from a sector and placed on medium identical to that from which they had been removed. Wherever possible the samples were taken from the growing edge. Note was made of the sector's mycelial morphology if it was visibly different from the background. The samples were incubated at 32°, usually for two days. Hyphal tips of two to four cells were then cut out with a flattened isolating needle and transferred to fresh medium. Care was taken to select tips of the longest hyphae, or those with the correct morphology, whichever applied. The process was repeated after another two days of growth at 32°. The final tip cultures were kept at 32° until macroscopically visible colonies developed.

Diploid x haploid matings: Presumptive diploids were crossed with wild type haploids. Spores were collected from fruiting bodies which developed on the haploid side of the mating. All progeny were tested for nutritional requirement. Recovery of all markers of the original common-AB heterokaryon was taken as proof that the presumptive diploid was, in fact, diploid. Allele frequencies consistant with trisomic segregation were expected. The original inoculum of each germling onto the yeast control plates was transferred to an individual plate of yeast medium. The subsequent growth was examined for sectoring. Sectors were purified and tested for requirement as described previously. The process was continued until sectoring no longer occurred.

Mating type tests for progeny of diploid x haploid matings: Mating type tests for diploid x haploid matings were carried out in a manner analogous to those for regular matings. Six testers were used, four for the parental A and B factors and two for the recombinant A factors. The latter two testers helped to distinguish between individuals disomic for the chromosome containing the A factor and those with a recombinant A factor.

When a compatible diploid parent was used in diploid x haploid matings six tester strains were needed, three each for parental A and B factors. Progeny compatible with more than one A mating type tester were mated with two appropriate strains to determine whether they possessed a recombinant A factor.

Recovery of progeny diploids from diploid x haploid matings: Experiments were initiated in an effort to obtain common-A, common-B, and compatible diploids marked identically to the parental common-AB diploids by crossing the common-AB diploids to haploid strains containing all or most of the markers in the diploid. The matings were such that prototrophic progeny (or progeny requiring only arginine in the case of diploids homozygous for arg2 and heterozygous for sul) had to be diploid with all markers heterozygous, aneuploid, or haploid and recombinant.

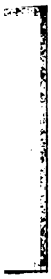
The probability of obtaining progeny diploids with all markers heterozygous was calculated on the basis of four assumptions: (1) the pairing and distribution of homologous chromosomes during meiosis in a diploid x haploid cross is totally random, (2) the linkage relationships of the markers reflect real chromosomal constitution, (3) crossing-over is usually of no effect upon chances of acquiring the desired progeny genotype and can be ignored, and (4) all progeny are, at least initially, equally viable.

While these assumptions taken together are almost certainly false, they had some heuristic value. The calculations provided a rough estimate of the number of spores to be collected in order for there to be a realistic chance of recovering the desired diploids.

Spores were spread at a density of about 100 per plate on minimal medium (or minimal plus arginine where appropriate). Each plate was checked daily, under a dissecting microscope, for six to eight days for thriving germlings. Spores germinating close to each other were separated in an attempt to avoid dikaryotization among progeny. The germlings which grew best under these selective conditions were isolated. Those which sectored spontaneously (presumptive aneuploids) were eliminated. Those kept were stored in bottles of yeast medium.

Verification and analysis of progeny diploids: Presumptive diploids from the diploid x haploid matings were checked for mating type. Strains showing compatibility to both parental A factors and to recombinant A factors, those compatible with both parental B factors, and those compatible with all the testers were assumed to be diploid, or stable aneuploid, and were saved for further analysis. They were then crossed to wild type homokaryons for a determination of markers present.

There was a possibility that those scoring as compatible diploids were actually dikaryons, having arisen undetected on the original germling plates. (It was highly unlikely that diploids scoring as common-B were actually heterokaryotic since they were maintained on unselective medium which would allow them to dedikaryotize rapidly.) Each strain was stained with a simplified modification of the Feulgen technique and the number of nuclei per cell was observed. The staining procedure was as follows: Pieces of mycelia were fixed in 3:1 ethanol - acetic acid for twenty



minutes, hydrolysed in 1N NCl at 60° for ten minutes, transferred to Schiff Reagent and stored in the dark for forty minutes at 22°, then removed and teased apart in a drop of 10% acetic acid on a slide, pressed firmly under a cover slip, and mounted with Permount.

With this technique nuclei were visible in only a fraction of the cells. Appearance of two nuclei in 10% or more of those with visible nuclei was arbitrarily taken as sufficient indication that the "diploids" were actually dikaryotic.

Detection of somatic recombination: Diploids homozygous for arg2 but heterozygous for sul, a recessive suppressor of arg2, were plated on minimal medium supplemented with all the requirements of the constituent haploid strains except arginine. These diploids could grow slowly and thinly without arginine. Somatic crossing-over could lead to homozygosity for sul. Haploidization could produce a cell line carrying both arg2 and sul. In both cases the arginine requirement is eliminated and rapid, dense sectors would be expected.

Occasionally, slow growing sectors - even slower than the background mycelium - were detected. These were predicted as the product of somatic crossing-over that led to elimination of sul (the reciprocal product of the crossover which led to homozygosity of sul) or haploidization and recovery of arg2 without sul.

Some of the diploids constructed for this study contained, in repulsion, ad4 and ad9, the two loci for the enzyme controlling the last step in adenine synthesis. Adenine requirers containing ad4 or ad9 grown on supplemented or complete medium turn pink due to accumulation of an adenine precursor. Thus these diploids grown on medium supplemented with all possible requirements of their constituent haploids produced pink

sectors as a result of either haploidization or recombination to homozygosity of either marker.

These diploids also produced sectors of variant morphology - slow or rapid growth, thinner or irregular hyphae - with or without pink colouration. Thus recombinants from diploids heterozygous for ad4 and ad9 were not selected only on the basis of colour.

Interpretation of recombinant sectors: All sectors were purified and tested for nutritional requirement (and mating type when from other than common-AB diploids). Auxotrophy for non-selective markers was taken as one indication of haploidization. Recombinant sectors were isolated from completely supplemented minimal medium, but transferred to both completely supplemented minimal and yeast media. They were grown on these media and allowed to either produce further sectors or demonstrate stability (presumably due to complete haploidization) by lack of sectoring.

The genotypes of the haploid products revealed the occurrence of crossing-over in their parent diploids. The various diploids were compared, in this way, for frequency of somatic crossing-over.

Somatic sectoring in a dikaryon: Dikaryons bearing all or most of the desired heterozygous markers were accidentally formed in a cross of haploid x common-AB diploid heterozygous for ad4 and ad9 in repulsion. They were plated and examined for somatic sectoring in exactly the same way as were the diploids.

Mitotic mapping: The mutations used in this study marked all seven known linkage groups. The compatible diploids and dikaryons heterozygous for ad4 and ad9 in repulsion each had all seven linkage groups marked. This provided an opportunity to determine whether the meiotic linkage groups correspond to actual chromosomes. Mutations on the same chromosome would be predicted to segregate together during haploidization.

The common-AB and compatible diploids proved to have very low levels of somatic crossing-over and were thus ideal for this purpose. The common-AB diploids had no nutritional markers in the A factor or B factor linkage groups, but in compatible diploids the incompatibility factors themselves could be used as markers for their linkage groups. Thus a mitotic map of all seven meiotic linkage groups was obtained. No attempt was made to determine mitotic gene distances.

Statistical analysis: Variability in gene distances, when apparent, was calculated as standard deviation among samples. The comparisons among diploids and dikaryons for frequency of somatic crossing-over was done by Contingency Chi Square with a 5% level of significance.

RESULTS

Mutagenesis with N-methyl-N'-nitro-N-nitrosoguanidine (NG): NG proved to be a very effective mutagen. A total of 2,688 colonies were isolated, 192 from each incubation flask. It is likely that many of the colonies were originally plated as mycelial fragments of more than one viable nucleus, in which case mutant cells would have been hidden by growth of prototrophs. Thus the apparent rate of mutation, excluding lethals, (74 colonies of 2,688, or 2.8%) is a conservative estimate.

Requirement of the mutations: The initial characterization of the mutations into five classes is summarized in Table 1. Twenty-five mutations were originally of undefined requirement, but eight of these were classified after being backcrossed to wild type.

The requirement of each mutation, in so far as it was determined, is shown in Table 2. All but two of the sixty nutritional mutations were assigned specific biochemical requirements, although six of them had to be first backcrossed and/or tested on pool plates with double the normal concentration of supplement.

Several nucleic acid base requirers are peculiar in responding equally (and poorly) to all five bases. Strains bearing thy grow perhaps half as well given cytosine as they do with thymine, while strains bearing x31 respond somewhat to almost anything, but best to pyrimidines. Growth of pur strains is promoted almost as well by guanine as by adenine, which, in terms of the direct purine synthetic pathway, should

Table 1. Numbers of mutations of five general classes obtained by mutagenesis with NG in liquid culture flasks.

Flask #	Mutation Class					
	amino acid	vitamin	nucleic acid base	unknown	morpho-logical	total
1	0	1	0	0	1	2
2	1	4	1	1	0	7
3	1	0	1	2	1	5
4	1	1	1	1	0	4
5	1	0	0	0	2	3
6	0	1	1	4	2	8
7	0	1	1	1	1	4
8	0	1	2	0	3	6
9	0	0	4	0	2	6
10	1	2	2	2	1	8
11	2	1	0	1	1	5
12	0	2	3	1	0	6
13	0	2	2	2	1	7
14	0	1	1	0	1	3
Total	7	19	17	17	14	74

Table 2. Symbol, linkage group, segregation ratio (mutant/total) in a cross with wild type, requirement, and other characteristics of mutations obtained from mutagenesis with NG.

Name	Linkage Group	Segregation	Requirement	Description
ad2-b	IV	33/63	adenine	
ad2-c	IV	32/63	adenine	
ad3-b	III	28/60	adenine	
ad3-c	III	29/62	adenine	
ad4-b	V	29/58	adenine	pink
ad5-b	I	31/60	adenine	tighter than ad5
ad5-c	I	28/59	adenine	tighter than ad5
ad6	?	34/64	adenine	
ad7*	?	29/63	adenine	poor response
ad7-b	?	21/61	adenine	poor response
ad8	?	32/62	adenine	
ad9	V	32/63	adenine	pink
ad10	?	35/64	adenine	
ad11	I	29/62	adenine	
ad12	IV	34/63	adenine	
arg1-b*	V	29/59	arginine	
arg1-c	V	30/64	arginine	
arg2-b	IV	28/63	arginine	
aro	VII	26/57	aromatic amino acids	poor response to tryptophan
cho-b	IV	33/63	choline	leaky
cho-c	IV	34/63	choline	leaky
cho-d	IV	30/61	choline	leaky
ino1	VI	31/62	inositol	

Table 2 continued

Name	Linkage Group	Segregation	Requirement	Description
ino1-b	VI	30/59	inositol	
ino1-c	VI	32/61	inositol	
ino2	?	29/62	inositol	very leaky
leu	III	30/62	leucine	responds less well to isoleucine
lys	?	6/123	lysine	low germling viability
ni1-b	IV	32/63	niacin	
ni2-b	III	35/64	niacin	
ni2-c	III	34/63	niacin	
ni2-d	III	34/64	niacin	
ni5	III	29/61	niacin	adaptive
ni5-b	III	30/60	niacin	adaptive
pan	VI	32/63	pantothenate	
pdx	III	30/59	pyridoxine	
pdx-b	III	31/62	pyridoxine	
pur	?	28/61	purine	responds best to adenine
rib	IV	29/63	riboflavin	
s	III	31/64	cysteine or methionine	
thy	III	32/60	thymine	responds less well to cytosine, slow growth
u1-b	III	30/57	uracil	leaky
u3	_	29/59	uracil	poor response
com	VII	32/62	none	compact, highly branched
com-b	VII	31/64	none	compact, highly branched
min*	V	32/62	none	very compact, highly branched (dome?)

Table 2 continued

Name	Linkage Group	Segregation	Requirement	Description
min-b	V	28/61	none	very compact, highly branched (dome?)
spi**	V	22/45	none	viewed from top forms cw spiral
a1	-	29/62	?	responds to casein hydrolysate
n1	-	35/63	?	responds slightly to any nucleic acid base
x30	-	31/59	?	
x31	-	32/63	?	responds somewhat to pyrimidines
x32	-	28/64	?	
x33	-	34/63	?	
x34	-	31/64	?	
x35	-	27/59	?	
x36	-	31/63	?	
x37	-	32/60	?	
x38	-	30/60	?	
x39	-	31/63	?	
x40	-	34/63	?	
x41	-	33/64	?	
x42	-	30/63	?	
x43	-	32/61	?	responds slightly to casein hydrolysate
x44	-	28/62	?	
x45	-	24/63	?	leaky
xL1	-	2/24	?	low germling viability

* mutations induced previously by others
** spontaneous mutation found in stock

be impossible. Strains carrying x43 will grow somewhat without vitamins and bases, but not at all without amino acids. None of the amino acid pool plates, however, increased its growth significantly. Virtually every mutant grows as well on migration complete as on yeast medium.

Mutations for the same requirement at the same locus were considered alleles if they came from different incubation flasks but separate isolates of the very same mutation if they came from the same flask. The latter situation did not arise in this study.

Allelism among the mutations: For most of the mutations listed in Table 2, complementation in heterokaryotic allelic tests was satisfactory as a demonstration of allelism. However, in the cases of ad7 and ni5, the former slow growing and the latter leaky, growth response was somewhat ambiguous. The dikaryons were allowed to fruit and the progeny were tested before any conclusions were reached. The two isolates of the morphological mutations min and com would not fruit when mated. Allelism was assumed on the basis of colony morphology and mapping to the same position.

Segregation of the mutations: The segregation frequencies presented in Table 2 represent, in all but one case, the sums of two separate first backcrosses to wild type. The exception is that of lys, in which the total of 123 represents the sum of the two original and three additional generations of backcrosses to wild type.

The segretation ratios approximate 1:1 for all but two mutations, lys and xL1. Germling viability is only about 50% with both of these and the survivors are almost all wild type. Chromosomal abnormalities may be associated with these mutations.

Linkage of the mutations: Linkage tests were done only with those mutations listed in Table 2 deemed useful for this study. Of these, all but four mapped to six of the seven linkage groups previously described (Ellingboe and Raper 1962) (Middleton 1964). The four mutations (ad6, ad7, ad8 and ad10) which showed no linkage to any gene also showed no linkage with each other.

Meiotic distances between loci, separated into values from 2-point and 3- or multi-point crosses, are presented in Table 3. Two-point data are not available for most of the gene pairs, but were included in an effort to find some order in the erratic behaviour of a few mutations - specifically pdx, ul, and min. The first two both show different cross-over frequencies with the same marker in different crosses, with ul (and ul-b) always appearing in excess of their wild type allele in multi-point crosses (264 progeny among the 359 from such crosses were uracil auxotrophs). min appeared to be linked to ni3 (67 of 207 = 32 units) but not to aro, which is linked to ni3. Nor did ad4 or ad9, flanking markers of min, appear to be linked to ni3.

The order of the markers shown in Figure 1 is based entirely on 3-point analysis of 3- or multi-point crosses. These analyses indicated unambiguous orders for almost all sets of three markers. One exception occurred in a cross of nil to ad1 arg2 in which nil appeared to be to the left of arg2. In a sample of 100 progeny, if nil were to the left of arg2, there were 39 crossovers between ad1 and nil, 2 between arg2 and nil, and no double crossovers. If nil were to the right of arg2, there would have been 2 double crossovers and there would have been no single crossovers between nil and arg2. The other exception was in a cross of pdx-b ul-b with thy ni2 ad3 in which progeny genotypes could not be

Table 3. Distances between meiotically linked genes.

	2-point crosses		3- & multi-point crosses	
genes	sample size	distance (centimorgans)	sample size	distance (centimorgans)
ad11, pab	79	23	141	31±2
ni5, u1-b	77	39	412	43±11
ni5-b, u1-b			111	41
ni5, s			210	47±5
ni5, ad3	39	31	131	34±2
ni5-b, ad3	44	43	222	38±1
ni5, thy			196	66±2
ni5, pdx-b			609	48±11
ni5-b, pdx-b			197	47±4
u1-b, s			210	22
u1-b, ad3			216	20±5
u1-b, ad3-c			47	10
u1-b, ni2	78	46	94	31±1
u1-b, pdx-b			252	27
s, ad3	135	6	97	12
s, ad3-b			111	12
s, ni2			410	23±6
s, thy			209	20±7
s, leu			202	31±8
s, pdx-b			384	25±4
ad3, ni2			281	19±4
ad3, ni2-c	48	13		
ad3-b, ni2			158	25±3

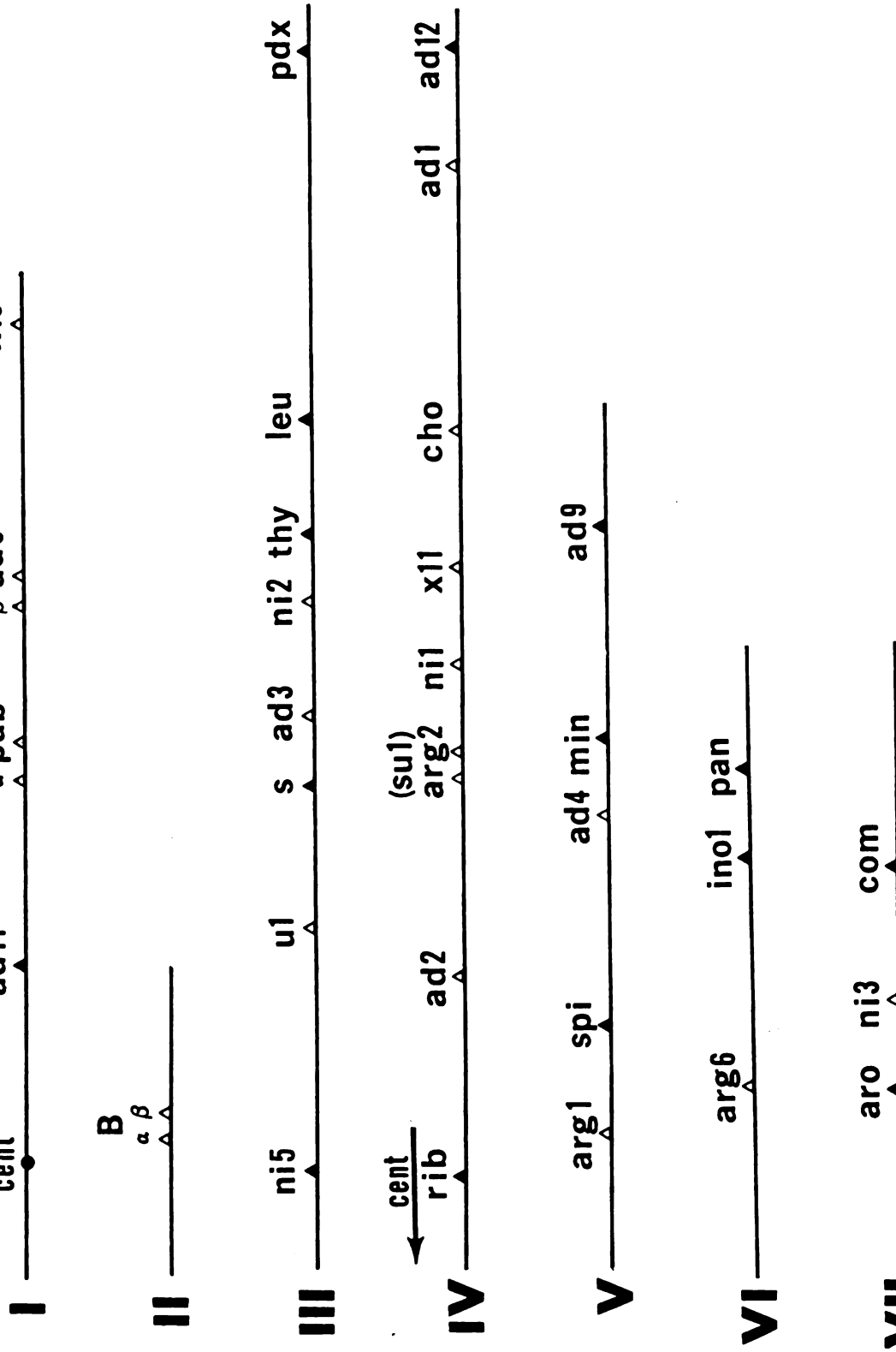
Table 3 continued

genes	2-point crosses sample size	2-point crosses distance (centimorgans)	3- & multi-point crosses sample size	3- & multi-point crosses distance (centimorgans)
ad3, thy			244	26±4
ad3, leu	46	26	111	24
ad3, pdx-b	22	27	511	41±4
ni2, thy			369	7±2
ni2, leu			105	16
ni2, pdx-b			308	26±4
thy, leu			105	17
thy, pdx-b	74	29	321	19±5
leu, pdx-b			303	38±7
rib, ad2			58	14
rib, arg2			414	22±5
rib, su1			96	23
rib, ad1			125	45±5
ad2, arg2			97	21
ad2, su1			59	29
ad2, ni1			97	20
arg2, ni1			197	2±1
arg2, ad1			225	36±4
arg2, ad12			83	41
ni1, ad1	106	37	100	43
ni1, ad12			185	42±11
cho, ad12	45	41	185	49±9

Table 3 continued

genes	2-point crosses sample size	2-point crosses distance (centimorgans)	3- & multi-point crosses sample size	3- & multi-point crosses distance (centimorgans)
arg1, spi			174	12±7
arg1, ad4			316	11±8
arg1, ad4-b	58	32	78	33
arg1, min	82	22	217	9±2
arg1, ad9			108	22
spi, ad4			157	19±3
spi, ad4-b			78	37
ad4, min			317	12±4
ad4, ad9	109	37		
min, ad9			108	16
arg6, ino1			120	18±13
arg6, pan			412	21±15
ino1, pan			160	5±1
aro, ni3	45	32	164	24±10
aro, com			104	34
ni3, com	92	8		

Figure 1. Linkage map of Schizophyllum commune. The seven meiotic linkage groups correspond to seven independent mitotically segregating chromosomes. Solid arrows indicate genes discovered in this study, while outlined arrows represent previously studied genes. Spacing between adjacent markers is approximately to scale and based on meiotic linkage data from this study (Table 3) and previous publications (Ellingboe and Raper 1962), (Middleton 1964).


A
B
I
II
III
IV
V
VI
VII
cent
ad11
α pab
β ad5
x15
α β
ni5
u1
s
ad3
ni2
thy
leu
pdx
cent
rib
ad2
(su1)
arg2
ni1
x11
cho
ad1
ad12
arg1
spi
ad4
min
ad9
arg6
ino1
pan
aro
ni3
com

interpreted to indicate any single gene order, but rather several different inconsistant orders, depending upon which three markers were being considered. The problem in this case seems to have been due to the erratic behaviour of ul-b; results from a cross identical but for the omission of ul-b indicated a consistant gene order.

Meiotic crossing-over frequencies in S. commune are highly variable and it is not surprising that, even with standard deviations taken into account, distances are rarely additive. There are several instances of markers recombining more frequently with adjacent than with more distal markers. Thus it is cautioned that while the gene order of Figure 1 is very likely correct, the distances listed in Table 3 are only approximations.

Determination of the adenine synthetic pathway steps affected by the adenine mutations: There are nine steps leading from ribose-5-phosphate to 5'-phosphoribosyl-5-aminoimidazole-4-carboxamide, two more to inosine-5'-phosphate, which marks the branch point for synthesis of adenosine-5'-phosphate and guanosine-5'-phosphate, and two more to adenosine-5'-phosphate. Xanthosine-5'-phosphate is the intermediate between inosine-5'-phosphate and guanosine-5'-phosphate.

The adenine mutations were tested for growth response on adenine, inosine, xanthine, and 5-aminoimidazole-4-carboxamide (AIC). It was assumed that the mutants were enzymatically capable of adding the phosphate and ribose moieties to these intermediates. Xanthine was included as a check for the leakiness of the pathway - i.e. the organisms' ability to convert a similar substance to adenine indirectly by other biochemical means. The mutants did not respond well to AIC, even applied at 10^{-3}M. But the data do provide a basis for the classification of some of the

mutations with a high degree of certainty and an indication for some of the others as to which part of the pathway they operate upon.

None of the mutants responded positively to xanthine. Both ad4 and ad9 responded only to adenine (Table 4) and are "pink" mutations, therefore known to code for the two polypeptides of adenylosuccinase, the enzyme for both the last synthetic step and the conversion of 5'-phosphoribosyl-5-aminoimidazole-4-(N-succino) carboxamide to 5'-phosphoribosyl-AIC. This dual role explains the partial restoration of growth achieved by ad4 and ad9 strains with inosine. The only other mutation which did not respond fully to inosine was ad12, ad12 must code for adenylosuccinate synthetase, the enzyme mediating the penultimate step. ad1, ad2, ad5, and ad10 definitely responded to AIC and can be assigned to the first eight steps of the pathway. ad3 showed some response to AIC and may possibly also be in this group. Growth of ad11 and ad7 strains was apparently supported only by inosine and adenine, so these can be each assigned one of the two steps between 5'-phosphoribosyl-AIC and inosine-5'-phosphate. The responses of ad6 and ad8 to AIC were uncertain.

Synthesis of common-AB diploids: Nineteen different common-AB heterokaryons were set up in an attempt to obtain variously marked diploids (Table 5). Only four produced rapidly growing sectors (Figure 2), and these four did so with different facility. Each was later found to be diploid. A diploid of heterokaryon A was isolated nine times from eighty plates. One diploid was isolated from three hundred plates of heterokaryon C, and two diploids were isolated from 160 plates of heterokaryon K. Heterokaryon P produced many sectors (about thirty on eighty plates) but only two were analysed. Both proved to be diploid. Those heterokaryons from which no diploids arose were grown on 150 to 800

Table 4. Response of *ad* mutations to intermediates of the purine synthetic pathway.

	Growth response to intermediates			
mutation	5-aminoimidazole-4-carboxamide	inosine	xanthine	adenine
ad1	+*	+	-	+
ad2	+	+	-	+
ad3	±	+	-	+
ad4	-	±	-	+
ad5-b	+	+	-	+
ad6	?	+	-	+
ad7	-	±	-	±
ad8	?	+	-	+
ad9	-	±	-	+
ad10	+	+	-	+
ad11	-	+	-	+
ad12	-	-	-	+

* + = growth, - = no growth

Table 5. Genotypes of forced common-AB heterokaryons and diploids.

Name	Genotype of heterokaryon	Diploid obtained
A	$\frac{A41}{A41}\ \frac{B41}{B41}\ \frac{s\ +}{+\ ni2}\ \frac{+\ +\ arg2}{ad2\ su1\ arg2}$	yes
B	$\frac{A42}{A42}\ \frac{B42}{B42}\ \frac{s\ +}{+\ ni2}\ \frac{+\ +\ arg2}{ad2\ su1\ arg2}$	no
C	$\frac{A41}{A41}\ \frac{B42}{B42}\ \frac{+\ +}{ad2\ su1\ arg2}\ \frac{arg6\ ino1\ +}{+\ +\ pan}\ \frac{ni3}{+}$	yes
D	$\frac{pab\ A41\ +}{+\ A41\ ad5\text{-}b}\ \frac{B41}{B41}\ \frac{+\ arg2}{su1\ arg2}\ \frac{+}{ni3}$	no
E	$\frac{pab\ A41\ +}{+\ A41\ ad5\text{-}b}\ \frac{B42}{B42}\ \frac{+\ arg2}{su1\ arg2}\ \frac{+}{ni3}$	no
F	$\frac{A41}{A41}\ \frac{B42}{B42}\ \frac{ni2\ +\ +}{+\ leu\ pdx\text{-}b}\ \frac{ad2\ su1\ arg2}{+\ +\ arg2}$	no
G	$\frac{A41\ ad5\text{-}b}{A41\ +}\ \frac{B42}{B42}\ \frac{+}{s}\ \frac{su1\ arg2}{+\ arg2}\ \frac{+}{pan}\ \frac{ni3}{+}\ \frac{+}{ad6}$	no
H	$\frac{A41\ ad5\text{-}b}{A41\ +}\ \frac{B42}{B42}\ \frac{+}{s}\ \frac{su1\ arg2}{+\ arg2}\ \frac{+}{pan}\ \frac{ni3}{+}\ \frac{+}{ad8}$	no
I1	$\frac{A41\ ad5\text{-}b}{A41\ +}\ \frac{B42}{B42}\ \frac{+\ arg2}{su1\ arg2}\ \frac{+\ +}{ni3\ aro}$	no
I2	$\frac{A41\ ad5\text{-}b}{A41\ +}\ \frac{B41}{B41}\ \frac{+\ arg2}{su1\ arg2}\ \frac{+\ +}{ni3\ aro}$	no
J	$\frac{pab\ A41}{+\ A41}\ \frac{B41}{B41}\ \frac{+}{ni2}\ \frac{+\ +\ arg2}{rib\ su1\ arg2}$	no
K	$\frac{A41}{A41}\ \frac{B41}{B41}\ \frac{ad4\ +}{+\ ad9}\ \frac{arg6\ +}{+\ pan}\ \frac{ni3}{+}$	yes
L	$\frac{A41}{A41}\ \frac{B41}{B41}\ \frac{ni2}{+}\ \frac{+\ arg2}{su1\ arg2}\ \frac{ad4}{+}\ \frac{+\ +}{ni3\ aro}$	no
M	$\frac{ad11\ A42}{+\ A42}\ \frac{B41}{B41}\ \frac{+\ +}{s\ ni2}\ \frac{+\ arg2}{su1\ arg2}$	no
N	$\frac{pab\ A41}{+\ A41}\ \frac{B42}{B42}\ \frac{+\ arg2}{su1\ arg2}\ \frac{+\ +}{ni3\ aro}$	no

Table 5 continued

Name	Genotype of heterokaryon	Diploid obtained
O	$\frac{A41}{A41}\ \frac{ad5\text{-}b}{+}\ \frac{B42}{B42}\ \frac{+}{s}\ \frac{su1\ arg2}{+\ \ arg2}\ \frac{ni3}{+}\ \frac{+}{ad10}$	no
P	$\frac{A41}{A41}\ \frac{B47}{B47}\ \frac{s\ pdx\text{-}b}{+\ \ +}\ \frac{+}{rib}\ \frac{+\ \ ad9}{ad4\ \ +}\ \frac{+\ \ pan}{arg6\ \ +}\ \frac{+}{ni3}$	yes
Q	$\frac{A41}{A41}\ \frac{B42}{B42}\ \frac{s}{+}\ \frac{+\ \ arg2}{su1\ \ arg2}\ \frac{pan}{+}\ \frac{+\ \ +}{ni3\ aro}\ \frac{ad6}{+}$	no
R	$\frac{A41}{A41}\ \frac{B42}{B42}\ \frac{s}{+}\ \frac{+\ \ arg2}{su1\ \ arg2}\ \frac{pan}{+}\ \frac{+\ \ +}{ni3\ aro}\ \frac{ad8}{+}$	no

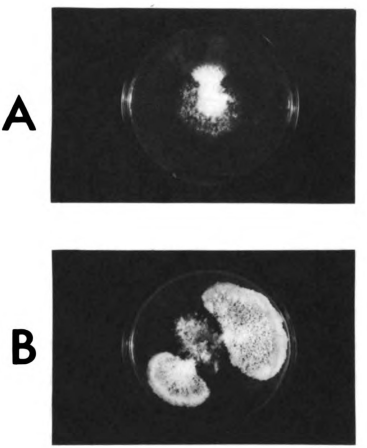


Figure 2. Diploids of Schizophyllum commune. A. Sector arising on twelve-day-old plate of common-AB heterokaryon A on minimal medium plus arginine. B. Sectors arising on fourteen-day-old plate of common-AB heterokaryon P on minimal medium.

plates each. The proclivity to diploidize is apparently a highly variable property among strains.

Verification of diploidy by mating to wild type haploid: Tables 6 thru 9 show the frequency of recovery of auxotrophic markers and of mating type factors in crosses of the four common-AB diploids with wild type haploids. Two different expected marker frequencies are listed in these tables. The second columns list the marker frequencies predicted on the basis of trisomic segregation of chromosomes and no subsequent loss of any disomic chromosomes from the progeny. The third columns list predicted frequencies based in trisomic segregation followed by total haploidization of all progeny. The fourth and fifth columns contain marker frequencies observed originally upon colony maturation, and subsequently after total haploidization of the aneuploid progeny, respectively. Only those progeny with irregular growth pattern were considered probable aneuploids. These progeny were isolated and allowed to sector. Hyphal tips of a single sector were isolated and allowed to sector again. The process was continued until stable growth was obtained, at which point the progeny were considered haploid.

The data from Tables 6 thru 9 show that the original observed frequencies of each marker were almost always between the values expected from no loss of chromosomes and those expected from total haploidization - usually closer to the latter. This indicates either poor germination of aneuploids or instability and extensive loss of disomic chromosomes among the aneuploid progeny. The former is suggested by spore germination percentages of about 75% from diploid x haploid matings. The latter is suggested by the tremendous initial variation in growth rate of the germlings, a difference which tended to disappear with time.

Table 6. Recovery of markers in 216 progeny of a cross of Diploid A-5 $\left(\frac{A41}{A41}\ \frac{B41}{B41}\ \frac{s\ +}{+\ ni2}\ \frac{+\ +\ arg2}{ad2\ su1\ arg2}\right)$ to wild type A42 B42 haploid.

	Marker Frequency			
	expected with trisomic segregation and		observed	
Marker	no haploidization	total haploidization	originally	after haploidization
A41	99	132	109	140
A41 A42*	66	-	48	
A42	33	66	41	57
Ax			18	19
B41	108	144	123	141
B41 B42	72	-	50	
B42	36	72	43	75
ad2	36	72	41	69
arg2**	72	72	38	56
ni2	36	72	54	98
s	36	72	34	67

* either A41 or A42 may occasionally be replaced by Ax

** those progeny bearing arg2 coupled with su1 are here scored as $arg2^+$

Table 7. Recovery of markers in 174 progeny of a cross of Diploid A-8 $\left(\frac{A41}{A41}\ \frac{B41}{B41}\ \frac{s\ +}{+\ ni2}\ \frac{+\ +}{ad2\ su1}\ \frac{+\ arg2}{arg2}\right)$ to wild type A42 B42 haploid.

	Marker Frequency			
	expected with trisomic segregation and		observed	
Marker	no haploidization	total haploidization	originally	after haploidization
A41	77.5	103.3	90	102
A41 A42*	51.7	-	32	
A42	25.8	51.7	33	53
Ax			19	19
B41	87	116	95	121
B41 B42	58	-	43	
B42	29	58	36	53
ad2	29.	58	41	55
arg2**	58	58	42	48
ni2	29	58	48	67
s	29	58	35	51

* either A41 or A42 may occasionally be replaced by Ax

** those progeny bearing arg2 coupled with su1 are here scored as arg2+

Table 8. Recovery of markers in 125 progeny of a cross of Diploid C: $\left(\frac{A41\ B42}{A41\ B42}\ \frac{+\ +}{ad2\ su1}\ \frac{arg2\ arg6}{arg2\ +}\ \frac{ino1\ +}{+\ pan}\ \frac{ni3}{+}\right)$ to wild type A42 B41 haploid.

Marker	Marker Frequency: expected with trisomic segregation and no haploidization	expected with trisomic segregation and total haploidization	observed originally	observed after haploidization
A41	56	74.7	67	72
A41 A42*	37.3	-	18	
A42	18.7	37.3	27	38
Ax			13	15
B42	62.5	83.3	71	87
B42 B41	41.7	-	30	
B41	20.8	41.7	24	38
ad2	20.8	41.7	32	38
su1**		41.7		36
arg2**	55.5	41.7	34	32
arg6**		69.4		
		41.7		22
ino1	20.8	41.7	16	21
ni3	20.8	41.7	42	51
pan	20.8	41.7	33	44

* either A41 or A42 may occasionally be replaced by Ax

** these markers cannot be counted unless the progeny are totally haploidized. Thus values offset between lines in the second, third, and fourth columns represent total number of arginine auxotrophs

Table 9. Recovery of markers in 79 progeny of a cross of Diploid P-1: $\left(\frac{A41}{A41}\ \frac{B47}{B47}\ \frac{s\ pdx\text{-}b}{+\quad +}\ \frac{+}{rib}\ \frac{+\quad ad9}{ad4\ +}\ \frac{+\quad pan}{arg6\ +}\ \frac{+}{ni3}\right)$ to wild type A42 B41 haploid.

		Marker Frequency			
		Expected with trisomic segregation and		observed	
Marker		no haploidization	total haploidization	originally	after haploidization
	A41	34	45.3	40	46
A41	A42*	22.7	-	10	
	A42	11.3	22.7	18	22
	Ax			11	11
	B47	39.5	52.7	43	55
B47	B41	26.3	-	17	
	B41	13.2	26.3	19	24
	ad4**	26.3	26.3	45	28
	ad9**		52.7		25
	arg6	13.2	26.3	9	13
	ni3	13.2	26.3	22	36
	pan	13.2	26.3	25	44
	pdx-b	13.2	26.3	18	27
	rib	13.2	26.3	20	29
	s	13.2	26.3	19	23

* either A41 or A42 may occasionally be replaced by Ax

** these markers cannot be counted unless the progeny are totally haploidized. Thus values offset between lines in the second, third, and fourth columns represent total number of adenine auxotrophs

The observed marker frequencies upon total haploidization usually matched the predicted values well, although there appears to be selection against progeny carrying arg6 or inol (Tables 8 and 9).

It was not possible to predict the frequency of recombinant A mating type factors. Thus their numbers were merely subtracted from the total and the predicted numbers of the parental A factors were based upon this reduced total. Progeny which were compatible with both parental and recombinant A factors were scored as disomic and containing the two parental A factors. However, such progeny, having completely haploidized, occasionally proved to have a recombinant A factor (Tables 6 and 8). They were thus originally disomic with one of the A factors being recombinant.

It was considered undesirable to perform heterokaryotic allelic tests for aneuploid, and therefore unstable, progeny. It should therefore be noted that sul, arg2, and arg6 in Table 8 and ad4 and ad9 in Table 9 were not distinguished and scored except in haploidized progeny. arg2 and arg6 tester strains were used in heterokaryotic allelic tests on the progeny of Diploid C x haploid (Table 8). Arginine prototrophic progeny were tested at the same time with the arg2 tester to distinguish those truly wild type from those bearing sul with arg2.

Crossing-over in matings of diploids x wild type haploids: The frequency of crossing-over in suitably marked regions in the diploid x haploid crosses provided support for two assumptions made about these matings, namely, that the three homologous chromosomes participate equally, and that intimate pairing over any chromosome region involves any two of the three at random. Expected numbers of crossover genotypes were generated when these assumptions were considered along with normal

meiotic distance between two genes and the fraction of crossovers between these genes which would actually be detectable in fully haploidized progeny (Tables 10 thru 13). Only two-thirds of crossovers between A factors and between markers in coupling were detectable since one-third of the crossovers would be between chromatids with identical A factors or identical wild type alleles of the coupled markers. Only one-sixth of crossovers between markers in repulsion were detectable since only the doubly auxotrophic crossover product is distinguishable from the three parental genotypes. Crossing-over between ad2 and sul could only be detected one-sixth of the time in Diploids A-5 and A-8, but two-thirds of the time in Diploid C in which sul with arg2 was distinguished from wild type. There was a fairly good match between expected and observed frequencies in all but one instance, and the assumptions therefore seem reasonable.

Crosses to produce compatible, common-A, and common-B diploids: Crosses were made between each of the common-AB diploids and appropriate haploids with most of the same markers in an attempt to synthesize a series of common-A, common-B, and compatible diploids with the same, or similar, arrangements of the mutations as were present in the common-AB diploids. The strains which were crossed to make the haploids for these crosses were either the very same ones that were incorporated into the common-AB diploids or else siblings marked identically but for mating type. The series of diploids should then provide a basis for determining the effect of the incompatibility factors in the diploid on the mechanism(s) of somatic recombination.

The crosses and the expected frequency of the desired diploids among the progeny are given in Tables 14, 15 and 16. The expected frequency of

Table 10. Expected and observed numbers of crossovers among 216 progeny of a cross of Diploid A-5 to wild type haploid.

Marked region	Meiotic length	Fraction of crossovers detectable in haploids	Number of crossovers	
			expected	observed
Aα - Aβ	16*	2/3	23	19
ad2 - su1	21	1/6	8	10
ni2 - s	23±6	1/6	8±2	14

Table 11. Expected and observed numbers of crossovers among 174 progeny of a cross of Diploid A-8 to wild type haploid.

Marked region	Meiotic length	Fraction of crossovers detectable in haploids	Number of crossovers	
			expected	observed
Aα - Aβ	16*	2/3	19	19
ad2 - su1	21	1/6	6	7
ni2 - s	23±6	1/6	7±2	11

* highly variable

Table 12. Expected and observed numbers of crossovers among 125 progeny of a cross of Diploid C to wild type haploid.

Marked region	Meiotic length	Fraction of crossovers detectable in haploids	Number of crossovers	
			expected	observed
Aα - Aβ	16*	2/3	13	15
ad2 - su1	21	2/3	17	13
arg6 - ino1	18±13	2/3	15±11	17
ino1 - pan	5±1	1/6	1	4
arg6 - pan	21±15	1/6	4±3	8

Table 13. Expected and observed numbers of crossovers among 79 progeny of a cross of Diploid P-1 to wild type haploid.

Marked region	Meiotic length	Fraction of crossovers detectable in haploids	Number of crossovers	
			expected	observed
Aα - Aβ	16*	2/3	8	11
s - pdx-b	25±4	2/3	13±2	28
ad4 - ad9	37	1/6	5	6
arg6 - pan	21±15	1/6	3±2	3

* highly variable

Table 14. Cross of common-AB Diploid A-5 x haploid, desired diploid progeny and their expected frequency, and expected frequency of progeny viable on selective medium.

	Linkage Group							Fraction of total progeny
	I	II	III	IV	V	VI	VII	
Cross	$\frac{A41}{A41}$	$\frac{B41}{B41}$	$\frac{s\ +}{+\ ni2}$	$\frac{ad2\ su1\ arg2}{+\ +\ arg2}$	$\frac{+}{+}$	$\frac{+}{+}$	$\frac{+}{+}$	
				X				
	$\frac{A42}{}$	$\frac{B42}{}$	$\frac{s\ ni2}{}$	$\frac{ad2\ su1\ arg2}{}$	$\frac{+}{}$	$\frac{+}{}$	$\frac{+}{}$	
Desired compatible diploid	$\frac{A41}{A42}$	$\frac{B41}{B42}$	$\frac{s\ +}{+\ ni2}$	$\frac{ad2\ su1\ arg2}{+\ +\ arg2}$	$\frac{+}{+}$	$\frac{+}{+}$	$\frac{+}{+}$	
Fraction of chromosome configurations suitable for a compatible diploid	$\frac{1}{3}$	$\frac{1}{3}$	$\frac{1}{6}$	$\frac{1}{6}$	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2592}$
Desired common-A diploid	$\frac{A41}{A41}$	$\frac{B41}{B42}$	$\frac{s\ +}{+\ ni2}$	$\frac{ad2\ su1\ arg2}{+\ +\ arg2}$	$\frac{+}{+}$	$\frac{+}{+}$	$\frac{+}{+}$	
Fraction of chromosome configurations suitable for a common-A diploid	$\frac{1}{6}$	$\frac{1}{3}$	$\frac{1}{6}$	$\frac{1}{6}$	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{5184}$
Desired common-B diploid	$\frac{A41}{A42}$	$\frac{B41}{B41}$	$\frac{s\ +}{+\ ni2}$	$\frac{ad2\ su1\ arg2}{+\ +\ arg2}$	$\frac{+}{+}$	$\frac{+}{+}$	$\frac{+}{+}$	
Fraction of chromosome configurations suitable for a common-B diploid	$\frac{1}{3}$	$\frac{1}{6}$	$\frac{1}{6}$	$\frac{1}{6}$	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{5184}$
Fraction of chromosome configurations permitting growth on minimal + arginine	1	1	$\frac{1}{6}$	$\frac{1}{2}$	1	1	1	$\frac{1}{12}$

Table 15. Cross of common-AB Diploid C x haploid, desired diploid progeny and their expected frequency, and expected frequency of progeny viable on selective medium.

	Linkage Group							Fraction of total progeny
	I	II	III	IV	V	VI	VII	
Cross	$\frac{A41}{A41}$	$\frac{B42}{B42}$	$\frac{+}{+}$	$\frac{ad2\ su1\ arg2}{+\ +\ arg2}$	$\frac{+}{+}$	$\frac{+\ +\ pan}{arg6\ ino1\ +}$	$\frac{+}{ni3}$	
				X				
	A42	B41	+	ad2 + +	+	arg6 ino1 +	ni3	
Desired compatible diploid	$\frac{A41}{A42}$	$\frac{B42}{B41}$	$\frac{+}{+}$	$\frac{+\ +\ arg2}{ad2\ su1\ arg2}$	$\frac{+}{+}$	$\frac{+\ +\ pan}{arg6\ ino1\ +}$	$\frac{+}{ni3}$	
Fraction of chromosome configurations suitable for a compatible diploid	$\frac{1}{3}$	$\frac{1}{3}$	$\frac{1}{2}$	$\frac{1}{6}$	$\frac{1}{2}$	$\frac{1}{3}$	$\frac{1}{3}$	$\frac{1}{1944}$
Desired common-A diploid	$\frac{A41}{A41}$	$\frac{B42}{B41}$	$\frac{+}{+}$	$\frac{+\ +\ arg2}{ad2\ su1\ arg2}$	$\frac{+}{+}$	$\frac{+\ +\ pan}{arg6\ ino1\ +}$	$\frac{+}{ni3}$	
Fraction of chromosome configurations suitable for a common-A diploid	$\frac{1}{6}$	$\frac{1}{3}$	$\frac{1}{2}$	$\frac{1}{6}$	$\frac{1}{2}$	$\frac{1}{3}$	$\frac{1}{3}$	$\frac{1}{3888}$
Desired common-B diploid	$\frac{A41}{A42}$	$\frac{B42}{B42}$	$\frac{+}{+}$	$\frac{+\ +\ arg2}{ad2\ su1\ arg2}$	$\frac{+}{+}$	$\frac{+\ +\ pan}{arg6\ ino1\ +}$	$\frac{+}{ni3}$	
Fraction of chromosome configurations suitable for a common-B diploid	$\frac{1}{3}$	$\frac{1}{6}$	$\frac{1}{2}$	$\frac{1}{6}$	$\frac{1}{2}$	$\frac{1}{3}$	$\frac{1}{3}$	$\frac{1}{3888}$
Fraction of chromosome configurations permitting growth on minimal + arginine	1	1	1	$\frac{1}{2}$	1	$\frac{1}{3}$	$\frac{1}{2}$	$\frac{1}{12}$

Table 16. Cross of common-AB Diploid P-1 x haploid, desired diploid progeny and their expected frequency, and expected frequency of progeny viable on selective medium.

Cross	Linkage Group I	II	III	IV	V	VI	VII	Fraction of total progeny
	$\frac{A41}{A41}$	$\frac{B47}{B47}$	$\frac{s\ pdx\text{-}b}{+\quad +}$	$\frac{+}{rib}$	$\frac{+\ ad9}{ad4\ +}$	$\frac{+\ pan}{arg6\ +}$	$\frac{+}{ni3}$	
				X				
	A42	B41	s pdx-b	rib	ad4 +	arg6 pan	ni3	
Desired compatible diploid	$\frac{A41}{A42}$	$\frac{B47}{B41}$	$\frac{s\ pdx\text{-}b}{+\quad +}$	$\frac{+}{rib}$	$\frac{+\ ad9}{ad4\ +}$	$\frac{+\ pan}{arg6\ +}$	$\frac{+}{ni3}$	
Fraction of chromosome configurations suitable for a compatible diploid	$\frac{1}{3}$	$\frac{1}{3}$	$\frac{1}{3}$	$\frac{1}{3}$	$\frac{1}{3}$	$\frac{1}{6}$	$\frac{1}{3}$	$\frac{1}{4374}$
Desired common-A diploid	$\frac{A41}{A41}$	$\frac{B47}{B41}$	$\frac{s\ pdx\text{-}b}{+\quad +}$	$\frac{+}{rib}$	$\frac{+\ ad9}{ad4\ +}$	$\frac{+\ pan}{arg6\ +}$	$\frac{+}{ni3}$	
Fraction of chromosome configurations suitable for a common-A diploid	$\frac{1}{6}$	$\frac{1}{3}$	$\frac{1}{3}$	$\frac{1}{3}$	$\frac{1}{3}$	$\frac{1}{6}$	$\frac{1}{3}$	$\frac{1}{8748}$
Desired common-B diploid	$\frac{A41}{A42}$	$\frac{B47}{B47}$	$\frac{s\ pdx\text{-}b}{+\quad +}$	$\frac{+}{rib}$	$\frac{+\ ad9}{ad4\ +}$	$\frac{+\ pan}{arg6\ +}$	$\frac{+}{ni3}$	
Fraction of chromosome configurations suitable for a common-B diploid	$\frac{1}{3}$	$\frac{1}{6}$	$\frac{1}{3}$	$\frac{1}{3}$	$\frac{1}{3}$	$\frac{1}{6}$	$\frac{1}{3}$	$\frac{1}{8748}$
Fraction of chromosome configurations permitting growth on minimal medium	1	1	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{3}$	$\frac{1}{6}$	$\frac{1}{2}$	$\frac{1}{144}$

of the desired diploid was calculated as the product of the expected frequency of appropriate marker configurations for each linkage group without selection. These figures were then used to determine the number of spores to be plated to have a realistic chance of isolating the desired diploid progeny. The expected frequency of progeny able to grow on a particular selective medium was calculated in the same way. The predicted and observed numbers of both progeny able to grow on their selective medium and those among them which were the desired diploids are summarized in Table 17. The lower than "expected" numbers of progeny able to grow on their selective medium is likely due in large part to a lower than "expected" incidence of disomy, as suggested by the crosses of these diploids to wild type haploids.

Progeny of A-5 and those of C that grew on minimal medium plus arginine were screened for inability to grow on minimal medium. All the progeny were observed for stable growth, acceptable morphology, and were tested for mating type. This lowered the number of potential desired diploids to 6 from A-5, 4 from C, and 7 from P-1. All these were mated to wild type haploids or, if compatible, allowed to fruit. These tests provided a basis for elimination of most of the presumptive diploids. The recovery of desired strains is listed in Table 17. The one possible common-A progeny diploid from A-5 would not cross with haploids and exhibited deteriorating morphology.

Characterization of daughter diploids: Spontaneous fruiting of the diploids was encouraged or matings were made to wild type haploids, and the progeny were screened for markers for which the diploid should have been heterozygous. Nuclei were stained and counted to distinguish compatible diploids from "accidental" dikaryons. The results of the

Table 17. Expected and observed frequencies of common-A, common-B and compatible diploids from crosses of common-AB diploids x haploids.

Diploid mated	Total spores plated	Progeny able to grow on selective medium			Common-A or common-B diploids			Compatible diploids		
		expected		obs.	expected		obs.	expected		obs.
		fraction	number	number	fraction	number	number	fraction	number	number
A-5	7000	$\frac{1}{12}$	600	104	$\frac{1}{5184}$	1	1?	$\frac{1}{2592}$	2 - 3	0
C	5000	$\frac{1}{12}$	400	144	$\frac{1}{3888}$	1	0	$\frac{1}{1944}$	2 - 3	2
P-1	10,000	$\frac{1}{144}$	70	48	$\frac{1}{8748}$	1	0	$\frac{1}{4374}$	2	3

latter test correlated with the morphology of the strain. Compatible diploids have a rather flat, uneven appearance on migration complete or completely supplemented minimal media, with fruiting bodies scattered singly or in small groups (Figure 3), whereas dikaryons have a characteristic knotty appearance and large groups of fruiting bodies. On old plates the difference disappears.

The parental configurations of linked genes in the cultures derived from the diploid x haploid crosses were determined from the segregation patterns in selfing or crosses of the cultures with wild type haploids. From the data presented in Tables 19, 21, 23, and 25, it can be argued that there is approximately the same frequency of recombination in fruiting of compatible diploids as in compatible dikaryons.

Diploid C-34: C-34 was derived from a cross of Diploid C with a homokaryon (Table 15). No cells with two nuclei were visible under the microscope. The colony morphology was typical of a compatible diploid. It fruited spontaneously. Eighty progeny were obtained and the recovery of markers was determined (Table 18). One of the A factors was recombinant and thus had one subunit in common with A41. All auxotrophic mutations present in Diploid C were recovered. Three - ad2, sul, and ni3 - were recovered in approximately 1:1 segregation with the wild type alleles. The arrangements of linked markers and frequency of crossing-over between them in C-34 are given in Table 19. There appeared to be extreme selection against arg6 and inol, two genes that were present in coupling. Selection against these markers - arg6 in particular - was observed repeatedly in this study and is the probable cause of the great excess of pan, a gene linked to arg6 and inol but in repulsion to them.

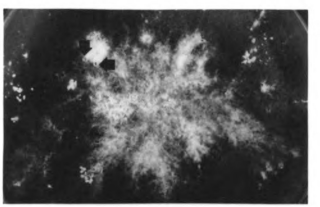


Figure 3. Compatible diploid C-34 on minimal medium plus arginine. Arrows indicate fruiting bodies.

Table 18. Recovery of segregating markers among 80 progeny from spontaneous fruiting of compatible Diploid C-34.

Marker	Number of progeny
A41	37
Aα1β5	43
B41	41
B42	39
ad2	48
su1	45
arg6	4
ino1	1
pan	76
ni3	35

Table 19. Crossover frequency among 80 progeny from spontaneous fruiting of compatible Diploid C-34.

Marked region	Meiotic length	Parental arrangement		Crossovers	
		cis	trans	expected	observed
ad2,su1	21		X	17	19
arg6,ino1	18±13	X		14±10	3
arg6,pan	21±15		X	17±12	6
ino1,pan	5±1		X	4±1	3

Diploid C-44: C-44 was derived from a cross of Diploid C with a homokaryon (Table 15). The colony morphology was typical of a compatible diploid. Only about 2% of the cells with a visible nucleus had two nuclei. Seventy-eight progeny were obtained from spontaneous fruiting of C-44. The marker frequency among the progeny indicated a 1:1 segregation with the wild type allele in each case (Table 20). Interestingly, however, twenty-eight of the progeny were initially slow growing, requiring two extra days for the germlings to become large enough to transfer. All twenty-eight bore arg6 and inol. This showed that selection was operating against these alleles in C-44 as well as in C-34. The arrangements and recombination frequencies of linked markers in C-44 are presented in Table 21.

Diploid P-3: P-3 was derived from a cross of Diploid P-1 with a homokaryon (Table 16). The colony morphology was typical of a compatible diploid and only a few percent of the cells appeared to have two nuclei. It fruited spontaneously. Ninety progeny were obtained and tested for marker frequency (Table 22). One of the A factors was recombinant, having a subunit in common with A41. All markers present in Diploid P-1 were recovered and segregated approximately 1:1 with their wild type alleles, except ni3 for which there was a marked excess of $ni3^{+}$. The arrangements and recombination frequencies of linked markers in P-3 are presented in Table 23.

Culture P-21: P-21 was derived from a cross of Diploid P-1 with a homokaryon (Table 16). The colony morphology had the knotty texture typical of a dikaryon. It had two nuclei in about 40% of those cells in which nuclei were stained. Only a small percent of the cells took up stain, indicating that the 40% figure might be due to unstained second

Table 20. Recovery of segregating markers among 78 progeny from spontaneous fruiting of compatible Diploid C-44.

Marker	Number of progeny
A41	34
A42	38
Ax	6
B41	39
B42	39
ad2	36
su1	35
arg6	38
ino1	34
pan	42
ni3	45

Table 21. Crossover frequency among 78 progeny from spontaneous fruiting of compatible Diploid C-44.

Marked region	Meiotic length	Parental arrangement		Crossovers	
		cis	trans	expected	observed
Aα,Aβ	16*			12	6
ad2,su1	21		X	16	19
arg6,ino1	18±13	X		14±10	12
arg6,pan	21±15		X	16±11	12
ino1,pan	5±1		X	4±1	2

* highly variable

Table 22. Recovery of segregating markers among 90 progeny from spontaneous fruiting of compatible Diploid P-3.

Marker	Number of progeny
A41	45
Aα1β5	45
B41	48
B47	42
s	49
pdx-b	43
rib	35
ad4	42
ad9	39
arg6	43
pan	52
ni3	15

Table 23. Crossover frequency among 90 progeny from spontaneous fruiting of compatible Diploid P-3.

Marked region	Meiotic length	Parental arrangement		Crossovers	
		cis	trans	expected	observed
s,pdx-b	25±4		X	23±4	30
ad4,ad9	37		X	33	25
arg6,pan	21±15		X	19±14	11

nuclei. The 40% is still much higher than the percentages obtained for C-34, C-44, and P-3, and too high to be due to cells in the process of division or random cellular mistakes. The culture fruited and progeny were tested for segregation of markers (Table 24). All markers present in P-1 were recovered and all segregated approximately 1:1 with their wild type alleles. The arrangements and recombination frequencies of linked markers in P-21 are presented in Table 25.

Culture P-19: P-19 was derived from a cross of Diploid P-1 with a homokaryon (Table 16). It was originally tested against stocks of mating types A47 B47, A1 B41, A41 B1, and A42 B1. It dikaryotized all but the first and was thus scored as common-B (B47). However, haploid sectors from P-19 all proved to contain B41, although this factor had not been thought to be present in P-19. Several tests were therefore initiated to determine the nature and mating behaviour of the P-19 culture.

P-19 had highly irregular morphology and was never observed to fruit spontaneously. Microscopic investigation revealed many clamp connections and at least equal numbers of pseudo-clamps, usually septate, and many with nuclei trapped within the pseudo-clamps. Many cells contained two nuclei. This description is similar to that of a common-B heterokaryon (Raper 1966).

The observed inability of P-19 to dikaryotize an A47 B47 haploid was reconfirmed in matings with another strain of the same mating type. P-19 also proved unable to dikarytize an A41 B47 strain. It did, however, successfully dikaryotize A47 B1 and A41 B42 haploids, the former with no difficulty, the latter rather more slowly (it required six weeks to fruit).

From all these observations it was concluded that P-19 was partially dikaryotic but had some irregularity in the function of its B mating type

Table 24. Recovery of segregating markers among 89 progeny from spontaneous fruiting of compatible Culture P-21.

Marker	Number of progeny
A41	38
A42	40
Ax	11
B47	46
B41	43
s	42
pdx-b	48
rib	45
ad4	46
ad9	43
arg6	43
pan	44
ni3	47

Table 25. Crossover frequency among 89 progeny from spontaneous fruiting of compatible Culture P-21.

Marked region	Meiotic length	Parental arrangement		Crossovers	
		cis	trans	expected	observed
Aα,Aβ	16*			14	11
s,pdx-b	25±4	X		22±4	21
ad4,ad9	37		X	33	22
arg6,pan	21±15		X	15±11	17

* highly variable

factors. The B41 factor was apparently unable to participate in matings when in the presence of the B47 factor, but it functioned normally when alone in the haploid sectors from P-19. The B47 factor was never found in the haploid sectors.

Mating type tests of the progeny of P-19 crossed with either an A47 B1 or an A41 B42 haploid showed that those progeny, and only those progeny, which had inherited the B47 factor of P-19 had also inherited anomalous mating behaviour. Specifically, they could dikaryotize A1 B41, A2 B2, and (for those progeny A41) A47 B1 and one A47 B41 testers. With another particular A47 B41 strain, it gave the "barrage" reaction typical of common-B interactions. This latter A47 B41 strain was a great-great-grandparent of P-19, but had contributed none of P-19's mating type factors.

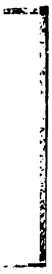
The A42 B41 haploid parent of P-19, which had contributed its B41 factor to P-19, was mated to both A41 B47 haploids which had gone into P-1, the diploid parent of P-19. One of these matings was normal. The other was neither normal nor "barrage", but produced instead a weak, slow growing dikaryon. Substitution of control strains with the same mating type for either ancestor of P-19 in this mating restored normal dikaryotization.

The other data relevant to understanding of P-19 are the marker frequencies among the progeny from crosses of P-19 with A47 B1, A41 B42, and A42 B1. The last of these was a mating type test cross originally done on P-19 which produced a fruiting body. In the cross with A47 B1 (Table 26) the A41 factor from P-19 and the A47 factor from the haploid segregated approximately 1:1, but the A42 factor of P-19 was absent from the progeny. Recombinant A factors, which would have been predicted as a

Table 26. Recovery of segregating markers among 80 progeny from a mating of compatible Culture P-19 to wild type A47 B1 haploid.

Marker	Number of progeny
A41	35
A47	45
A42	0
Ax	0
B47	60*
B1	37*
B41	0
s	8
pdx-b	6
rib	41
ad4	2
ad9	43
pan	41
ni3	0

* 17 progeny were disomic for the B chromosome and possessed both B47 and B1.



result of crossing-over between A41 and A47 or A42 were also absent. There was an excess of B47 (from P-19) relative to B1 (from the haploid). Three markers - rib, ad9, and pan - were recovered at 1:1 ratios with their wild type alleles while the other markers - ad4 (in repulsion with ad9), s and pdx-b (in coupling) and ni3 - were absent or rare.

In the cross of P-19 with A41 B42 (Table 27) most biochemical markers were recovered at near 1:1 ratios with their wild type alleles. The sole exception was ad4 which was totally absent from the progeny. The A42 factor from P-19 segregated 1:1 with A41 even though there were supposedly two doses of A41 entering the cross.

Progeny of the cross of P-19 with A42 B1 were not fully analysed (no mating types, no distinction between ad4 and ad9). Nineteen of the progeny scored as auxotrophic for all markers, even for arg6 which, by its absence from the progeny of the other P-19 crosses and haploid sectors, was thought not to be present in P-19. Probably these progeny were just not viable on sub-optimal media of any kind. They have been excluded from the data of Table 28. Two markers - ni3 and s - were rare in this sample. The latter marker is particularly interesting as s is linked to pdx-b which was not rare. This would indicate that s and pdx-b were present in repulsion. Data from the other P-19 crosses and the haploid sectors indicated that s and pdx-b were in coupling.

None of the progeny from these three crosses involving P-19 ever sectored, except seventeen progeny of the cross to A47 B1. This group proved to be disomic for only the chromosome containing the B factor.

The varying frequencies of the various markers in the progeny of the three crosses of P-19 make determination of crossover frequency difficult. Therefore the arrangements of linked markers in P-19 have been determined by analysis of its haploid sectors.

Table 27. Recovery of segregating markers among 122 progeny from a mating of compatible Culture P-19 to wild type A41 B42 haploid.

Marker	Number of progeny
A41	58
A42	52
Ax	12
B47	51
B42	71
B41	0
s	55
pdx-b	59
rib	69
ad4	0
ad9	62
pan	53
ni3	53

Table 28. Recovery of segregating markers among 73 partially analysed progeny from a mating of compatible Culture P-19 to wild type A42 B1 haploid.

Marker	Number of progeny
s	1
pdx-b	16
rib	16
ad4 and/or ad9	37
pan	8
ni3	1

Somatic recombination: Type A Diploids: Arginine prototrophs were isolated from common-AB diploids A-5 and A-8 as rapidly growing sectors. (See Table 5 for genotype of Diploid A.) Haploid sectors which arise by mitotic haploidization should show a requirement for either ni2 or s since these heterozygous linked markers were present in repulsion. Sectors which arose as a result of mitotic crossing-over between sul and its centromere, followed by reassortment to give homozygosity for sul, should be prototrophic for ni2 and s. Crossovers between ad2 and the centromere would lead to homozygosity for ad2 also. Crossovers between ad2 and sul would not lead to homozygosity for ad2.

About one plate in four eventually produced a sector. Tables 29 and 30 show that, of 123 sectors purified from these two diploids, only 4 from A-5 had ni2 or s and were therefore considered to be haploid. These data also show that the frequency of crossovers in the region centromere - ad2 relative to the frequency in region ad2 - sul was greater in A-5 than in A-8. No sectors showed any instability which would have suggested aneuploidy, and none of the four haploids were both ni2 and s.

Somatic recombination: Type C Diploids: Common-AB Diploid C (see Table 5 for genotype) produced arginine prototrophic sectors more rarely than did the type A diploids - about one sector per 25 plates (Figure 4A,B). There was, as in A-5 and A-8, a high ratio of diploid to haploid recombinants (51:14, see Table 31), as judged by the recovery of non-selective markers heterozygous and in repulsion. Haploids bearing arg6 and inol were recovered only once. The one that was isolated was recovered from a sector of very weak growth in the less sparse background. No crossovers between arg6 and inol or inol and pan were recovered, nor was there any instability that could have been associated with aneuploidy.

Table 29. Recombinant sectors isolated from common-AB Diploid A-5.

	Genotype			
Number	ad2	arg2	ni2	s
30	+	+	+	+
36	ad2	+	+	+
1	ad2	+	ni2	+
3	ad2	+	+	s

Table 30. Recombinant sectors isolated from common-AB Diploid A-8.

	Genotype			
Number	ad2	arg2	ni2	s
7	+	+	+	+
46	ad2	+	+	+

Figure 4. Sectors arising from diploid and partially dikaryotic strains of Schizophyllum commune. A. Rapid-growing sector from nine-day-old growth of Diploid C on arginineless medium. B. Rapid-growing sector from twenty-day-old growth of Diploid C on arginineless medium. C-D. Thin morphological sectors from twenty-two-day-old growth of Diploid P-1 on completely supplemented minimal medium. E-F. Thick morphological sectors from fourteen-day-old growth of Culture P-19 on completely supplemented minimal medium.

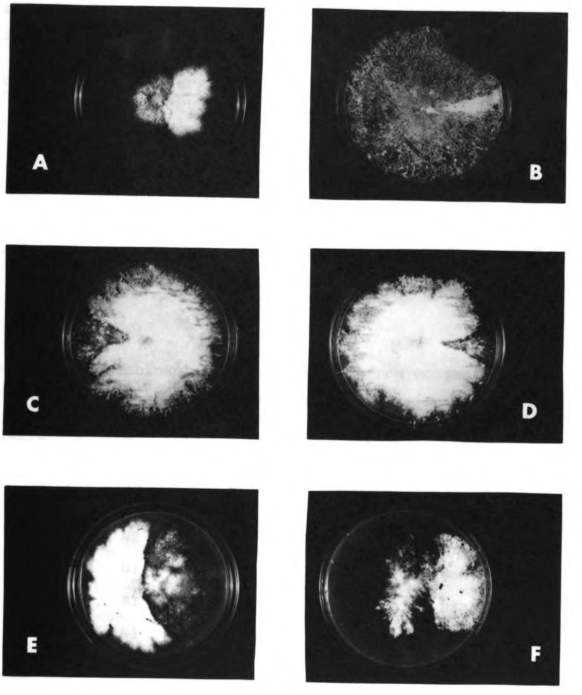

A
B
C
D
E
F

Table 31. Recombinant sectors isolated from common-AB Diploid C.

	Genotype					
Number	ad2	arg2	arg6	ino1	pan	ni3
8	+	+	+	+	+	+
43	ad2	+	+	+	+	+
9	ad2	+	+	+	pan	+
4	ad2	+	+	+	pan	ni3
1	+	arg2	arg6	ino1	+	+

Table 32. Recombinant sectors isolated from compatible Diploid C-34.

			Genotype					
Number	A	B	ad2	arg2	arg6	ino1	pan	ni3
7	41 1-5	41 42	ad2	+	+	+	+	+
5	41 1-5	41 42	+	+	+	+	+	+
1	1-5	41	ad2	+	+	+	pan	+
2	1-5	41	ad2	+	+	+	pan	ni3
1	41	41	ad2	+	+	+	pan	ni3
2	41	42	ad2	+	+	+	pan	+

The compatible diploids C-34 and C-44 had an uneven type of morphology (Figure 3). Detection of rapidly growing sectors was very difficult on any but young plates. The twenty-four isolated (Tables 32 and 33) included only six haploids, (a percentage similar to that in Diploid C), all from C-34. Two of these haploids were secondary sectors from sectors that were presumably aneuploid. No crossovers in the arg6 inol pan linkage group were in evidence.

Somatic recombination: Type P Diploids: It was originally supposed that haploid sectors from these diploids (see Table 5 for genotype) should be detectable as pink sectors due to presence of ad4 or ad9 (present in repulsion). However no such sectors ever arose from P-1 when plated on yeast or migration complete medium. When grown on completely supplemented minimal medium, the diploid had denser morphology with a more clearly defined growing edge. A few pink sectors were found on supplemented minimal medium. More importantly, many sectors appeared which were of different morphology, i.e. thinner, uneven, and, if not actually growing more rapidly, at least expanding laterally at a faster rate (Figure 4C,D). When isolated, these usually proved to be aneuploid or haploid. Almost all the unstable and, therefore, presumably aneuploid sectors eventually produced stable colonies which were considered haploid. In some cases two or more different haploids from the same original aneuploid were isolated and these "haploid families", sometimes of three sector generations, were numbered to indicate the order in which they arose. It was found that all ad4 and ad9 strains eventually turned pink on any medium, but at highly different rates, which accounted for the early difficulty in identifying pink sectors. Every allele in every type P

Table 33. Recombinant sectors isolated from compatible Diploid C-44.

			Genotype					
Number	A	B	ad2	arg2	arg6	ino1	pan	ni3
2	41 42	42 41	ad2	+	+	+	+	+
4	41 42	42 41	+	+	+	+	+	+

diploid or culture was recovered (though often at frequencies far different from 50%), except the B47 factor from P-19.

Forty sectors isolated from common-AB diploid P-1 were haploid or ultimately produced haploids or haploid families. These are listed in Tables 34 and 35. Thirty-two of the sectors produced only one stable haploid (Table 34). Eight eventually produced families of stable subcultures including two or more genotypes (Table 35). Crossing-over had occurred in only one of the forty, and that crossover was between arg6 and pan. (There is the possibility that the sector is stably disomic for the chromosome on which arg6 and pan are located.)

Compatible Diploid P-3 sectored less often than its parent P-1. Twenty-six sectors were isolated which, upon subculturing, eventually produced stable haploid colonies (Tables 36 and 37). Some aneuploids were fairly stable. Total haploidization often took five or six weeks of continuous growth. Twenty-two of the sectors produced only one stable genotype (Table 36). Four sectors eventually produced stable subcultures of two genotypes (Table 37). There were no crossover types.

Culture P-21 sectored rarely compared to P-1 and P-3. Nineteen sectors eventually yielded haploids (Tables 38 and 39). Fifteen of the sectors produced only one stable genotype (Table 38). Four sectors eventually produced stable subcultures of two genotypes (Table 39). Four were the result of crossing-over. Two crossovers were between pan and arg6 (also possibly stably disomic for this chromosome), one was between s and pdx-b, and one was between ad4 and ad9.

Culture P-19 grew very irregularly. Many thick clumps or well defined feathery arms were common. Even small morphological or pink sectors were easily detectable at the edge of such growth (Figure 4E,F).

Table 34. Recombinant sectors isolated from common-AB Diploid P-1.

	Genotype							
Number	s	pdx-b	rib	ad4	ad9	arg6	pan	ni3
5	+	+	+	+	ad9	+	pan	ni3
4	+	+	rib	+	ad9	+	pan	ni3
3	+	+	+	ad4	+	+	pan	ni3
2	+	+	rib	+	ad9	+	pan	+
2	+	+	+	ad4	+	arg6	+	ni3
1	+	+	+	+	ad9	arg6	+	ni3
4	s	pdx-b	+	ad4	+	+	pan	ni3
2	s	pdx-b	rib	+	ad9	+	pan	ni3
2	+	+	+	+	ad9	+	pan	+
*1	+	+	+	+	ad9	+	+	ni3
2	s	pdx-b	+	+	ad9	+	pan	ni3
1	+	+	rib	ad4	+	arg6	+	ni3
1	s	pdx-b	+	+	ad9	arg6	+	+
2	s	pdx-b	+	+	ad9	+	pan	+
32	Total mutant at each locus (of 32)							
	11	11	9	10	22	5	26	25

* crossover genotype

Table 35. Haploid families from recombinant sectors of common-AB Diploid P-1.

	Genotype							
Order	s	pdx-b	rib	ad4	ad9	arg6	pan	ni3
1	s	pdx-b	rib	+	ad9	+	pan	+
2	+	+	+	+	ad9	+	pan	+
1	+	+	rib	ad4	+	+	pan	ni3
2	+	+	+	+	ad9	+	pan	ni3
1a	+	+	rib	ad4	+	+	pan	ni3
1b	+	+	+	ad4	+	+	pan	ni3
2a	+	+	rib	+	ad9	+	pan	ni3
2b	+	+	+	+	ad9	+	pan	ni3
1	+	+	rib	ad4	+	arg6	+	ni3
2	+	+	rib	ad4	+	+	pan	ni3
1	s	pdx-b	+	+	ad9	+	pan	ni3
2a	s	pdx-b	+	ad4	+	+	pan	ni3
2b	+	+	rib	ad4	+	+	pan	ni3
2c	+	+	+	ad4	+	+	pan	ni3
1	s	pdx-b	+	+	ad9	+	pan	ni3
2	s	pdx-b	rib	+	ad9	+	pan	ni3
1	+	+	rib	+	ad9	+	pan	ni3
2	+	+	+	+	ad9	+	pan	ni3
3	s	pdx-b	rib	+	ad9	+	pan	ni3
1	+	+	+	ad4	+	+	pan	ni3
2	s	pdx-b	+	+	ad9	+	pan	ni3
	Total mutant at each locus							
	5	5	9	5	7	1	8	7
Of:	12	12	14	12	12	9	9	8

Table 36. Haploid sectors isolated from compatible Diploid P-3.

			Genotype							
Number	A	B	s	pdx-b	rib	ad4	ad9	arg6	pan	ni3
2	1-5	47	s	+	rib	ad4	+	+	pan	ni3
1	42	41	s	+	+	ad4	+	+	pan	+
1	1-5	41	+	pdx-b	rib	+	ad9	+	pan	+
1	1-5	47	+	pdx-b	+	ad4	+	arg6	+	+
1	42	41	s	+	+	+	ad9	arg6	+	+
1	1-5	41	s	+	rib	+	ad9	+	pan	+
1	42	47	+	pdx-b	+	ad4	+	+	pan	+
1	1-5	47	s	+	+	+	ad9	arg6	+	+
1	1-5	47	+	pdx-b	+	+	ad9	arg6	+	+
1	1-5	41	+	pdx-b	+	ad4	+	arg6	+	ni3
1	1-5	41	s	+	+	+	ad9	arg6	+	+
1	42	47	+	pdx-b	+	ad4	+	+	pan	ni3
1	1-5	47	+	pdx-b	rib	+	ad9	+	pan	+
1	1-5	41	s	+	+	ad4	+	arg6	+	+
1	42	47	+	pdx-b	rib	+	ad9	+	pan	ni3
1	42	41	s	+	+	ad4	+	arg6	+	+
1	1-5	41	+	pdx-b	rib	ad4	+	+	pan	+
1	1-5	47	s	+	+	+	ad9	+	pan	+
1	42	41	+	pdx-b	rib	+	ad9	arg6	+	+
1	42	41	+	pdx-b	+	+	ad9	+	pan	+
1	1-5	47	s	+	+	ad4	+	+	pan	+
22			Total mutant at each locus (of 22)							
	1-5:14 42:8	41:11 47:11	11	11	8	11	11	9	13	5

Table 37. Haploid families from sectors of compatible Diploid P-3.

			Genotype							
Order	**A**	B	s	pdx-b	rib	ad4	ad9	arg6	pan	ni3
1	42	41	s	+	rib	ad4	+	+	pan	+
2	42	41	s	+	+	ad4	+	+	pan	ni3
1	42	47	s	+	+	+	ad9	+	pan	ni3
2	42	47	+	pdx-b	+	+	ad9	+	pan	ni3
1	1-5	41	s	+	+	+	ad9	+	pan	+
2	1-5	47	+	pdx-b	+	+	ad9	+	pan	+
1	1-5	41	+	pdx-b	+	ad4	+	+	pan	ni3
2	1-5	41	s	+	+	ad4	+	+	pan	ni3
			Total mutant at each locus							
	1-5:2 42:2	41:3 47:2	4	3	1	2	2	0	4	3
Of:	4	5	7	7	5	4	4	4	4	5

Table 38. Haploid sectors isolated from compatible Culture P-21.

			Genotype							
Number	A	B	s	pdx-b	rib	ad4	ad9	arg6	pan	ni3
2	41	47	s	pdx-b	+	ad4	+	+	pan	+
1	41	47	s	pdx-b	+	+	ad9	arg6	+	+
*1	41	47	+	+	+	+	ad9	+	+	+
1	41	41	+	+	rib	ad4	+	arg6	+	+
2	42	47	s	pdx-b	+	+	ad9	+	pan	+
1	42	47	+	+	+	ad4	+	+	pan	+
1	42	41	+	+	+	ad4	+	arg6	+	ni3
1	41	41	+	+	rib	ad4	+	+	pan	ni3
1	41	47	s	pdx-b	rib	ad4	+	arg6	+	+
1	42	47	s	pdx-b	rib	+	ad9	+	pan	+
1	41	41	+	+	+	ad4	+	+	pan	ni3
1	42	47	+	+	rib	ad4	+	+	pan	ni3
1	42	41	+	+	rib	ad4	+	+	pan	+
15			Total mutant at each locus (of 15)							
	41:8 42:7	41:5 47:10	7	7	6	10	5	4	11	4

* crossover genotype

Table 39. Haploid families from sectors of compatible Culture P-21.

			Genotype							
Order	A	B	s	pdx-b	rib	ad4	ad9	arg6	pan	ni3
1	42	47	s	pdx-b	rib	+	ad9	+	pan	+
*2	42	47	+	pdx-b	rib	+	ad9	+	pan	+
1	41	47	+	+	+	ad4	+	arg6	+	ni3
2	42	47	s	pdx-b	rib	ad4	+	arg6	+	+
**1	42	41	s	pdx-b	rib	ad4	ad9	+	+	ni3
*2	42	41	s	pdx-b	rib	ad4	ad9	+	pan	+
1	41	41	+	+	+	ad4	+	arg6	+	ni3
2	41	47	+	+	+	ad4	+	arg6	+	ni3
			Total mutant at each locus							
	41:2 42:3	41:2 47:3	3	3	3	3	2	2	2	3
Of:	5	5	6	6	5	4	4	5	5	6

* crossover genotype

** two-crossover genotype

Forty-six sectors were recovered, thirty of which produced one stable haploid genotype (Table 40) and sixteen of which produced two or more stable haploid genotypes (Table 41 and Figure 5). Fifteen crossovers were evident - thirteen between s and pdx-b, and two between ad4 and ad9.

Frequency of somatic crossing-over: The C diploids produced few haploids, none the result of crossing-over. This made comparisons of crossover frequency impossible. The P diploids and cultures are compared in Table 42. There were four regions, Aα - Aβ, s - pdx-b, ad4 - ad9, and arg6 - pan, in which crossovers were detectable, but each diploid or culture did not have all of these regions marked. Crossover frequencies were compared assuming that each suitably marked region common to the two strains compared represented an independent opportunity for crossover. Numbers of crossovers per common opportunity were then compared for each strain in every possible pair-wise combination. The significance of their crossover frequency differences were calculated by Contingency Chi^2. Significant differences are apparent between all combinations except P-1 and P-3, and also P-21 and P-19.

Mitotic mapping: Since it is apparent that crossovers are very rare in the common-AB and compatible diploids, these strains were used to identify independently assorting chromosomes. The compatible diploid was particularly valuable as all seven meiotic linkage groups were marked.

Linked genes were used in calculations as single units, with the one crossover type from P-1 omitted. Each gene or unit was compared with every other gene or unit, in pairs, for frequency of recombination. The pairs were arranged in their two possible reciprocal allele combinations and the number of each combination was counted. The minority combinations were considered recombinant types. Recombination frequencies

Table 40. Haploid sectors isolated from compatible Culture P-19.

			Genotype						
Number	A	B	s	pdx-b	rib	ad4	ad9	pan	ni3
1	41	41	s	pdx-b	rib	+	ad9	+	+
1	42	41	s	pdx-b	rib	ad4	+	+	+
1	41	41	+	+	rib	ad4	+	+	+
1	41	41	s	pdx-b	rib	ad4	+	pan	ni3
2	41	41	+	+	rib	ad4	+	pan	ni3
*1	42	41	+	pdx-b	+	+	ad9	+	+
*1	41	41	+	+	rib	ad4	ad9	+	ni3
1	42	41	+	+	+	ad4	+	pan	+
1	42	41	+	+	rib	+	ad9	pan	+
*1	42	41	+	+	+	ad4	ad9	+	+
*1	41	41	+	pdx-b	rib	ad4	+	+	ni3
*1	42	41	+	pdx-b	rib	ad4	+	+	+
1	42	41	s	pdx-b	rib	+	ad9	+	+
1	42	41	s	pdx-b	rib	ad4	+	+	ni3
2	41	41	+	+	rib	ad4	+	pan	+
2	42	41	+	+	rib	ad4	+	+	+
5	41	41	+	+	rib	ad4	+	+	ni3
*1	42	41	+	pdx-b	rib	+	ad9	pan	+
*1	41	41	+	pdx-b	rib	ad4	+	+	+
1	41	41	s	pdx-b	rib	ad4	+	+	+

Table 40 continued

Number	A	B	Genotype s	pdx-b	rib	ad4	ad9	pan	ni3
1	42	41	s	pdx-b	+	ad4	+	+	ni3
1	42	41	+	+	rib	ad4	+	pan	ni3
1	42	41	+	+	+	ad4	+	+	+
30			Total mutant at each locus (of 30)						
	41:19 42:11	41:30 47:0	7	12	25	25	7	9	13

* crossover genotype

Table 41. Haploid families from sectors of compatible Culture P-19.

			Genotype						
Order	A	B	s	pdx-b	rib	ad4	ad9	pan	ni3
1	42	41	+	+	rib	ad4	+	+	ni3
2a	41	41	+	+	+	ad4	+	+	ni3
2b	41	41	+	+	rib	ad4	+	+	ni3
1	41	41	+	+	rib	ad4	+	+	+
*2	41	41	+	pdx-b	rib	ad4	+	+	ni3
3	42	41	+	+	rib	ad4	+	+	ni3
1	42	41	+	+	rib	ad4	+	+	+
*2	42	41	+	pdx-b	rib	ad4	+	+	+
1a	42	41	+	+	+	ad4	+	+	ni3
1b	42	41	+	+	rib	+	ad9	pan	+
2a	42	41	+	+	rib	+	ad9	+	+
*2b	42	41	+	pdx-b	rib	+	ad9	+	+
*1	42	41	+	pdx-b	+	+	ad9	pan	ni3
*2	41	41	+	pdx-b	+	+	ad9	pan	+
*3	42	41	+	pdx-b	rib	+	ad9	pan	ni3
1a	42	41	s	pdx-b	rib	ad4	+	+	+
1b	42	41	s	pdx-b	rib	ad4	+	+	ni3
2	42	41	+	+	rib	ad4	+	+	+
1	42	41	+	+	rib	ad4	+	+	+
2	41	41	+	+	rib	ad4	+	+	+
1	41	41	+	+	rib	ad4	+	+	+
*2	41	41	+	pdx-b	rib	ad4	+	+	+
*1	41	41	+	pdx-b	rib	ad4	+	pan	+
*2	41	41	+	pdx-b	rib	ad4	+	pan	ni3
1	41	41	s	pdx-b	rib	ad4	+	+	ni3
2	41	41	+	+	rib	ad4	+	+	+
1	41	41	+	+	rib	ad4	+	+	ni3
2	41	41	+	+	rib	ad4	+	pan	+
1	41	41	s	pdx-b	rib	ad4	+	+	ni3
2	42	41	s	pdx-b	rib	ad4	+	+	ni3

Table 41 continued

Order	A	B	Genotype s	pdx-b	rib	ad4	ad9	pan	ni3
1	42	41	+	+	rib	ad4	+	pan	ni3
2	42	41	+	+	rib	ad4	+	+	ni3
1	41	41	s	pdx-b	rib	ad4	+	+	ni3
2	41	41	+	+	rib	ad4	+	pan	+
*1a	41	41	+	pdx-b	rib	ad4	+	+	+
1b	41	41	s	pdx-b	rib	ad4	+	+	+
2	41	41	s	pdx-b	rib	ad4	+	pan	+
*1	42	41	+	pdx-b	+	+	ad9	pan	ni3
2	42	41	+	+	+	+	ad9	pan	ni3
			Total mutant at each locus						
	41:11 42:10	41:16 47:0	5	13	15	14	3	8	12
Of:	21	16	24	24	19	17	17	21	24

* crossover genotype

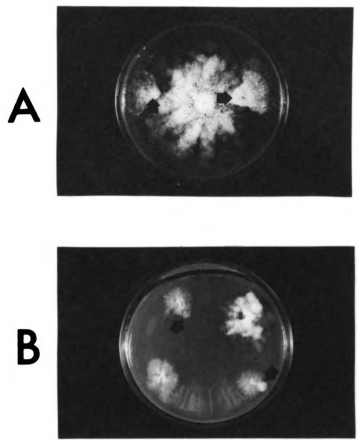


Figure 5. Secondary sectoring of aneuploid isolates of culture P-19. A. Two sectors from an aneuploid plug on yeast medium. B. Irregular growth and sectors from hyphal tip isolates of an aneuploid sector on completely supplemented minimal medium. Sectors are indicated by arrows.

Table 42. Pair-wise comparison of number of crossovers in strains P-1, P-3, P-21, and P-19.

Strains compared	Total haploids	Common regions	Crossovers in common regions	Chi^2	Significance
P-1 P-3	40 26	3	1 0	0.67	.3 - .5
P-1 P-21	40 19	3	1 4	5.44	.02
P-1 P-19	40 46	2	1 15	11.37	.001
P-3 P-21	26 19	3	0 4	5.05	.02
P-3 P-19	26 46	2	0 15	9.42	.01 - .001
P-21 P-19	19 46	3	2 15	2.79	.10

are listed in Table 43 together with their sample numbers. In haploid families where both "parental" and "recombinant" types were present, both were counted. For any pair of genes or gene units, a difference between "parental" and "recombinant" frequencies significantly different from that predicted by 1:1 segregation would have been considered evidence that those genes or units were actually syntenic and that recombination between them could only have occurred by crossing-over.

The recombination frequencies within P-1 and P-3 were considered together, and among them the lowest value was that between (arg6-pan) and ni3, and even this value showed no significant deviation from the 1:1 ratio that was predicted by independent assortment of whole chromosomes (Chi^2 = .10 - .05).

Thus it appears that the meiotic linkage groups correspond to independent chromosomes, and that there are, therefore, at least seven chromosomes in Schizophyllum commune. Figure 1 illustrates the seven chromosomes with markers separated approximately to scale according to their meiotic distances from their nearest neighbours.

Table 43. Somatic recombination frequencies between meiotic linkage groups in diploids P-1 and P-3. Sample totals are listed in parentheses.

Linkage Group markers	A	B	ad4,ad9	arg6,pan	ni3	s,pdx-b	rib
		Upper right: Diploid P-1					
A		-	-	-	-	-	-
B	.44 (27)		-	-	-	-	-
ad4,ad9	.50 (26)	.48 (27)		.32 (44)	.48 (44)	.50 (46)	.50 (48)
arg6,pan	.50 (26)	.37 (27)	.46 (26)		.30 (40)	.45 (44)	.41 (46)
ni3	.41 (27)	.39 (28)	.37 (27)	.44 (27)		.34 (44)	.41 (46)
s,pdx-b	.45 (29)	.43 (28)	.45 (29)	.48 (29)	.47 (30)		.43 (46)
rib	.48 (27)	.48 (27)	.44 (27)	.41 (27)	.42 (26)	.43 (30)	
	Lower left: Diploid P-3						

DISCUSSION

The mutations induced by NG were numerous and proved to be very useful. The exact mode of action of the mutagen is unknown, but it is capable of acting as an alkylating agent that can induce chromosome breaks and aberrations, and also as a very potent point mutagen (Hollaender 1971). The former occurs at alkaline pH, the latter optimally at about pH 6. NG was used at pH 7, so most of the mutations are likely point mutations. Chromosomal aberrations of some kind would probably account for the two mutations that have a high degree of lethality, and perhaps deletions over at least two cistrons could explain some of the auxotrophs with unknown requirement.

The total linkage data - mitotic and meiotic - are very satisfactory in that all linkage groups except II have at least three usable markers, and one in particular - group III - has a series of eight auxotrophic markers for seven different substances, only two of which present difficulties when used in multi-point crosses.

Despite statistical analysis of the mitotic data which indicate that all seven linkage group are on different chromosomes, it may be argued that some of the groups are syntenic but separated by distances long enough to make even mitotic crossing-over likely. It is much more probable, however, that meiotic data indicating such synteny are spurious. Spurious indications of loose linkage were found commonly during this study.

In largely restricting the mutagenesis project to a search for simple auxotrophs, rather than for genes that suppress or modify other genes, affect colony morphology, or confer resistance to drugs, in verifying the existence of seven chromosomes and indicating the direction of the centromere on chromosome IV, a map has been produced which should make S. commune more amenable to easy, standard genetic analysis.

The spontaneous production of diploids by common-AB heterokaryons proved to be a highly strain-specific phenomenon. While it is impossible to compare the total number of cells containing both nuclei of the heterokaryon among plates of different heterokaryons, it is likely safe to say that differences in diploidization frequencies such as those between heterokaryon P (30 apparent diploids from 80 plates) and heterokaryons G and H (no sectors from 800 plates) are real differences. This large variation was also noted by Casselton (1965) working with oidia from common-A heterokaryons of C. lagopus. It is unfortunate that among those heterokaryons which failed to yield diploids were those which contained the adenine mutations for which no linkage was apparent, and those with mutations bracketing the A mating-type factor which were necessary for studying Specific Factor Transfer.

The purpose of crossing the common-AB diploids to wild type haploids was to ascertain that they were, in fact, diploids containing all the markers originally put into the heterokaryons from which they arose. It was not expected that the alleles be recovered in frequencies predicted by simple trisomic segregation. Aneuploids are unstable and it is reasonable that many of the disomic chromosomes in progeny of these crosses will have been lost by the time the progeny have grown to testable size. This process would bring allele frequencies closer to

those expected from total haploidization. Indeed, a majority of the progeny did not sector at all after their initial five days of growth and were presumed haploid. Most of the allele frequencies fell between those predicted by trisomic segregation with no subsequent loss of chromosomes and those predicted by trisomic segregation followed by total haploidization. When allowed to haploidize, the progeny showed allele frequencies close to expected values. Those that deviated usually did so because of selection effects noted throughout this study - for niacin requirement or against arg6. Trisomic segregation assumes equal participation of the three homologous sets of chromosomes and close pairing of any two of these three, randomly, in any chromosome region. This mechanism generates a prediction of the percent of detectable crossover genotypes, compared to standard meiotic values, which should be found in the progeny. The four common-AB diploids, upon crossing, produced crossover-type progeny in close to the expected numbers in most cases. A glaring exception were crossovers in the s pdx-b region from the cross of P-1 with haploid. Whether this reflects selection peculiarities or preferential pairing or crossover "hot spots" cannot be determined from the data.

Initial efforts to detect daughter diploids with desired genotypes by testing entire samples of progeny from the appropriate diploid x haploid crosses were totally unsuccessful. Thus it became necessary to test much larger samples and also to use a selective system to pick out likely candidates. In calculating the number of spores from each cross to plate on selective medium it was assumed that trisomic pairing is random, that crossing-over could be ignored and that all progeny were equally viable and stable. The first assumption has been borne out by

crossover data. The second is only partially true; crossovers would not alter the number of progeny able to grow on selective medium but would lower the number marked in the desired way. The last assumption is certainly false, as aneuploids are unstable and tend toward haploidy. Haploidy would lower the number of viable progeny on the selective plates, as fewer auxotrophic markers would be "covered" by a wild type allele. The results showed that numbers of both selected progeny and desired diploids were lower than "predicted".

The absence of common-B and, with one possible exception, common-A diploids from the selected progeny was puzzling. There is no a priori reason to suspect that they might be unstable or inviable. Diploids have been obtained from both common-A heterokaryons of C. lagopus (Casselton 1965) and common-B heterokaryons of S. commune (Parag and Nachman 1966).

The compatible diploids and partially dikaryotic cultures that were recovered by this procedure present several interesting questions. Although there is no necessity to suppose that compatible diploids would be unstable, they do, by virtue of being compatible, contain the genetic machinery necessary for the maintenance of a dikaryon - of coordinated but separate nuclei. For a diploid to persist we must assume that the machinery to keep nuclei apart either is not operating or will not separate them if they have originally been "tricked" together. It is established, since recombination is known to occur between the nuclei of a dikaryon (Crowe 1960) (Parag 1962), that spontaneous diploid nuclei must arise in dikaryons. But they are transient. Thus the mere occurrence of diploidy does not guarantee its persistance, and the question remains as to what allows the diploid state to persist in the compatible diploids.

The dik gene (Koltin and Raper 1968) and perhaps others like it seem to be agents for maintaining a dikaryon. The diploids produced in these experiments cannot have been due to dik mutations since progeny and sectors from the diploids mate normally. But the ability of compatible diploids to persist may well be due to failure, for some reason, of dik^+ to act. Perhaps the product of dik^+, or other genes that it may control, require some time to build up to effective concentration in the cytoplasm. A germinating compatible diploid spore might establish a diploid mycelium before any of its cells become dikaryotic. This would predict a degree of instability in compatible diploids, especially in older cells. The compatible diploid samples examined microscopically in this study came from young regions close to the growing edge, yet still contained a small number of two-nucleus cells. Perhaps these were true dikaryotic cells and not just cells seen in the act of division.

The notion of cytoplasmic accumulation of substances which would cause a diploid nucleus to form two haploid nuclei is consistent with the observation that diploid nuclei of C. lagopus, stable when vegetative, become highly unstable in a dikaryon (Casselton 1965).

The nature and behaviour of the partially dikaryotic cultures used in this study can also be viewed in this perspective. Microscopic observations left no doubt that many cells of P-19 and P-21 were dikaryotic. While it was possible that the cells which appeared monokaryotic did so only because the second nucleus failed to pick up stain, it is also possible that there was a real and significant population of monokaryotic diploid cells.

Transient diploidy in dikaryons is a widely accepted reality, but the fact that the "dikaryons" haploidized via stages of aneuploidy

indicates that diploidy, in these "dikaryons" at least, is quite persistant. Haploidization is understood to be a phenomenon of diploid nuclei in which a non-disjunctional event creates a 2n+1 and 2n-1 pair of daughter cells. The 2n+1 cell loses one of its trisomic chromosomes at random and the 2n-1 cell becomes unstable and proceeds toward haploidy. Any analogous process in a dikaryon would necessitate either a control mechanism to keep the two nuclei from losing homologous chromosomes, and then have them unite to become a haploid, or else loss of one of the two nuclei. The former is highly improbable and the latter would create basically two classes of haploid products, each reflecting the constitution of one of the two nuclei. The data of Tables 38 thru 41, by contrast, show almost as many classes of haploid products as there are haploid products. It is more likely that haploidization in "dikaryons" actually occurs in some diploid cells. The frequency of haploidization and the persistence of the aneuploid stages indicate that these diploid cells are neither especially rare nor short-lived.

The origin of these dikaryons also merits consideration. It was realized that the selective plating technique allowed the possibility of occasional undetected mating of young germlings. The probability that any two adjacent germlings would be of proper genotype to form these dikaryons is about half the probability of properly marked compatible diploids being formed at meiosis. In addition there is the necessity of the adjacent haploids being close enough to mate undetected, and being able to grow together sufficiently to mate despite bearing several auxotrophic mutations which cause them to be selected against on the medium.

In view of these considerations and the likelihood of considerable diploidy in the dikaryons and of some instability in compatible diploids, the possibility exists that the dikaryons are partial breakdown products of compatible diploids.

The only strain which exhibited behavioural peculiarities was P-19. Its morphology was irregular and the behaviour of its B factors highly anomalous. Although it contains compatible A and B factors it never fruited. Only B47 participated in matings and this cannot be attributed to nuclear selection since the selection was absolute; crosses of P-19 to B47 strains would not dikaryotize. Only B41 was found in haploid sectors. Progeny of P-19 bearing its B47 factor were unable to dikaryotize an A47 B41 strain (the common-B "barrage" reaction occurred instead) which, although a great-great-grandparent of P-19, had no mating-type factors in common with it. These same progeny successfully dikaryotized other A47 B41 strains. The haploid parent of P-19 could mate only with difficulty with a compatible strain that constituted one genome of the diploid parent of P-19. Progeny from the one partially analysed and two completely analysed crosses of P-19 to wild type haploids were almost never aneuploid. This indicates that only one nucleus from P-19 participated in what were therefore essentially haploid x haploid crosses. The marker frequency data from these crosses can best be explained by assuming that the participant nucleus from P-19 was different in each case. (No matter how the mating-type factors are arranged into nuclei, and in order for all three of these crosses to have occurred, one of the three haploids would have had to have mated with a recombinant nucleus from P-19.)

This anomalous mating behaviour seems to be due to P-19's B47 factor or at least the chromosome with B47. Since mutations of B regularly

abolish B's ability to confer incompatibility, the mutation, if such it is, would more likely be of a modifier gene than B47 itself. A cluster of modifiers has been mapped close to B (Raper 1973). B47 seems able to suppress certain B41's, both the B41 of P-19, which it rendered incapable of participating in mating, and that of its great-great-grandparent, with which it reacted as if the interaction were common-B. The B41 factor of P-19, on the other hand, behaves normally once isolated from P-19. The exclusive appearance of B41 in haploid products is best explained by a high tendency of the B47 chromosome to non-disjoin. If this were so, B47 would almost always be included in the 2n+1 mitotic non-disjunctional product rather than the 2n-1 product which ultimately becomes the haploid. Beyond this, an anomaly which can act as a dominant allele to certain B41's - perhaps, specifically, ones in its lineage - and also cause its chromosome to non-disjoin defies easy definition.

Recombination in common-AB diploids appears, as expected, to be mitotic as in the parasexual cycle. Only one of forty haploids from P-1 was a crossover type, and stages of aneuploidy were easily isolated; only one of the forty was originally isolated as a complete haploid. Data from A-5, A-8 and C are scant, but none of the 18 haploids they produced was a crossover type. The large percentage of sectors that were apparently still diploid was unexpected. A preponderance of haploids among sectors collected in this way had been reported earlier (Mills and Ellingboe 1971). These recombinant sectors cannot be used in calculating frequency of crossing-over in the diploids because they are selected for. It should be noted that some of the diploid prototrophs which were homozygous for ad2 in addition to sul, and therefore scored as products of crossing-over between ad2 and the centromere, may have been

"non-disjunction" diploids - 2n+1 products of non-disjunction of chromosome IV from which the $\underline{ad2}^{+}$ $\underline{arg2}$ homologue was subsequently lost. None of the haploids isolated were originally collected as aneuploids.

Recombination in compatible diploids C-34, C-44, and P-3 also appeared to be parasexual. Of the 32 haploids they gave rise to, 26 were originally isolated as aneuploids and none was of crossover genotype. Again the input from the C diploids was disappointing as sectors were difficult to detect and most of those isolated were recombinant diploids.

The mode of recombination in the "dikaryons" is not as clear. Sixty-five haploid sectors were collected. All but nine of them went through stages of aneuploidy during isolation, which is predicted by a parasexual mechanism. Yet nineteen had crossover genotypes. That is a very high figure for parasexuality and more in accord with a meiotic type of mechanism. Both cultures had significantly higher crossing-over frequency than did compatible or common-$\underline{AB}$ diploids. And perhaps surprisingly, despite the peculiarities of P-19, no significant difference in crossover frequency was indicated between P-19 and P-21. The crossover type haploid products of P-19 are, however, peculiar in that thirteen of the fifteen represent crossing-over between $\underline{s}$ and $\underline{pdx-b}$ and that all of these are $\underline{s}^{+}$ $\underline{pdx-b}$.

In an attempt to explain these results it is useful to return to the idea of a tension between diploid and dikaryotic states. Diploidy is only maintained in vegetative cells whereas the dikaryotic state marks a step in a cell's committment to meiosis and reproduction. Therefore, in a dikaryon, given a tendency for nuclei to occasionally unite, a high degree of meiotic competence could be achieved in other than basidial cells and "precocious meiosis" occur. Similarly, particularly low

levels of whatever gene products confer meiotic competence in other cells might lead to diploidization.

The fission yeast Schizosaccharomyces pombe, while not dikaryotic, is a haplontic organism in which the diploid is normally confined to a single cell, briefly, prior to meiosis. Several meiotic mutants have been identified in this organism (reviewed by Bresch et al, 1968). Four of these genes which are necessary for successful meiosis have been found to cause, when mutant, persistance of the diploid state (Egel 1973). Thus a tension between meiotic competence and diploidy is demonstrated.

For the purposes of this study, the terms diploid and dikaryon applied to whole organisms become relative terms, referring to a variable majority but probably not all of their cells. It is possible that the "dikaryons" used are less dikaryotic than most, if they formed originally by partial breakdown of diploid germlings.

A compatible diploid is then, of necessity, an organism in which, for whatever reason, the vegetative state is maintained in most cells. Somatic recombination in such cells would be parasexual. An organism very predominantly dikaryotic would probably undergo somatic recombination in the form of precocious meiosis. Techniques such as incompatible di-mon matings can be used to pick out recombinant nuclei. Organisms in an intermediate state such as that argued for P-19 and P-21 should be able to undergo parasexual reduction by stages of aneuploidy, but the genotype of some of the haploid nuclei produced might reveal an earlier dikaryotic history in which meiotic-like recombination occurred.

The fact that thirteen of the fifteen crossover type haploids from P-19 were of genotype s^+ pdx-b could be due to selection. However, no selection has been previously observed for these markers. A more likely

explanation would be that a $\underline{s}^+$ $\underline{pdx\text{-}b}$ recombinant nucleus arose early in P-19, proliferated, and eventually made up a substantial fraction of the mosaic P-19 genome. Accepting this idea would mean considering all thirteen $\underline{s}^+$ $\underline{pdx\text{-}b}$ recombinants as results of one event. This is an inherent difficulty in studying somatic recombination via analysis of mitotic haploidization. Any crossover types which are identical or reciprocals of each other may have arisen earlier from the same event, but they are scored as independent events. P-19 is only the most glaring case.

Despite this difficulty, the observations have been made that (1) cultures which are largely or completely diploid, whether compatible or common-$\underline{AB}$, haploidize via stages of aneuploidy and show almost no evidence of somatic crossing-over, and (2) cultures which are compatible and substantially dikaryotic also haploidize via stages of aneuploidy and show definite evidence of somatic crossing-over. The discussion above is presented as a reasonable way of viewing these two sets of observations which, on the surface, appear irreconcilable.

A final assumption which must be made is that whatever gene or genes may be defective in P-19 are not those which confer ability to perform crossing-over.

The role of incompatibility factors in determining modes of somatic recombination is then an indirect one. Compatibility itself will not cause a switch from a vegetative state, in which parasexual phenomena occur, to a premeiotic state. It will, however, activate many genes of the type of $\underline{dik}$, or the meiotic genes of $\underline{S.\ pombe}$, which can effect the change. In such an indirect system slippage can occur. The genes for dikaryosis and meiosis will not immediately and totally be derepressed

by compatible mating-type factors, and under extreme conditions they might become derepressed without compatibility (haploid fruiting).

Specific Factor Transfer, if it is a phenomenon of highly specific and frequent crossing-over, would only occur in a meiotically competent cell, not in compatible diploids or probably even metastable diploid-dikaryons. Common-A and common-B diploids would be expected to behave like any other diploid and show parasexual somatic recombination. There were no data from this study bearing on these predictions.

SUMMARY

Seventy-four mutant strains of Schizophyllum commune were induced by mutagenesis with N-methyl-N'-nitro-N-nitrosoguanidine. Twenty-two of these, two morphological and twenty auxotrophic, were ultimately characterized as "new" genes and fourteen mapped to existing linkage groups. Another twenty-five mutations proved to be alleles of the newly isolated or stock strains. Tests of growth response to intermediates of purine synthesis made it possible to assign most of the twelve different adenine genes to regions of the purine pathway. Three of these genes could be assigned specific enzymes.

Common-AB diploids were isolated as spontaneous sectors from nutritionally forced common-AB heterokaryons. Their genotypes were determined by analysis of progeny from crosses to wild-type haploid strains.

Two of these diploids were homozygous for arg2 and heterozygous for sul, and produced rapidly growing sectors on selective media via mitotic crossover leading to homozygosity of sul, or via haploidization with loss of one arg2. A majority of the sectors were still diploid, as determined by prototrophy for non-selective heterozygous markers. No aneuploid sectors were formed, but those that were haploid showed no evidence of crossing-over and thus likely arose via a mitotic mechanism.

Another diploid was heterozygous for ad4 and ad9, linked "pink" adenine mutations in repulsion. This diploid produced sectors of non-

diploid morphology and/or pink colour, almost all of which were aneuploid. When finally haploidized, these sectors showed only a single incidence of crossing-over and thus also appeared to have arisen by mitotic haploidization.

The common-AB diploids were crossed to haploids optimally marked for selection of progeny diploids heterozygous for all markers (save those homozygous for arg2 and heterozygous for sul). Five progeny proved, via analysis of progeny from crosses to wild type haploid, or from spontaneous fruiting, to be 2n and heterozygous for the desired markers. Staining and counting of nuclei was done to distinguish diploids from dikaryons.

Two of the daughters were compatible diploids of the sul - arg2 selective type. Three daughters were of the ad4 - ad9 selective type, one diploid and two largely dikaryotic. One of the dikaryons possessed a B mating type factor with anomalous behaviour. A series of tests indicated that the anomalous B factor repressed the expression of certain other B factors, including that of the other dikaryon nucleus, making the dikaryon pseudo-common-B. It also indicated a marked tendency to non-disjoin. No usable common-A or common-B progeny diploids were obtained.

Recombinant sectors were collected from the progeny diploids and dikaryons in the same ways they were collected from the diploid parents. Analysis of the sectors showed a common incidence of aneuploidy in all cases. Haploids from the compatible diploids showed no evidence of crossing-over, while those from the dikaryons showed a significant frequency of crossing-over. It appears that the somatic recombination process in diploids, common-AB and compatible, is classically mitotic. The dikaryons apparently contain a percentage of diploid cells which can

undergo mitotic haploidization but whose nuclei give evidence of a previous history of meiotic-like behaviour involving crossing-over. The tendency toward "precocious meiosis" is apparently a property of the dikaryotic state rather than of compatibility *per se*.

Analysis of segregation of markers on separate meiotic linkage groups during haploidization of the diploids indicated that each linkage group corresponds to a separate chromosome. There are at least seven chromosomes in *Schizophyllum commune*.

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