STUDIES ON SOMATIC RECOMBINATION IN DIKARYONS OF *SCHIZOPHYLLUM COMMUNE* *

YAIR PARAG

Biological Laboratories, Harvard University, Cambridge, Mass.
Division of Biology, California Institute of Technology, Pasadena, Calif.†

Received 27.x.61

1. INTRODUCTION

Schizophyllum commune is a heterothallic, higher Basidiomycetous fungus, in which the pattern of mating is genetically controlled by two incompatibility factors, *A* and *B*, each with a series of alternative states (A^1 , A^2 , A^3 , etc., and B^1 , B^2 , B^3 , etc.). Diploid basidia $A^1A^2B^1B^2$ produce four classes of basidiospores; A^1B^1 , A^1B^2 , A^2B^1 and A^2B^2 . A mycelium of mating type A^1B^1 is incompatible with A^1B^1 , A^1B^2 and A^2B^1 , but it is compatible with A^2B^2 as well as A^2B^3 , A^3B^3 , etc., with which it establishes dikaryotic mycelia, e.g. ($A^1B^1 + A^2B^2$). When a dikaryon is mated with a homokaryon which is genetically compatible with at least one of the dikaryotic components (compatible di-mon matings) the homokaryon is converted into a dikaryotic mycelium: ($A^1B^1 + A^2B^2$) $\times$ $A^2B^3 \rightarrow (A^1B^1 + A^2B^3)$ ("Buller phenomenon"—Buller, 1931; Quintanilha, 1933; Raper, 1953). When a dikaryon is mated with a homokaryon that is incompatible with both of the dikaryotic components (incompatible di-mon matings), the homokaryon is not uniformly dikaryotised. Only occasionally are dikaryotic sectors formed in the previously homokaryotic mycelium: ($A^1B^1 + A^2B^2$) $\times$ $A^1B^2 \rightarrow$ occasional dikaryotic sectors ("Illegitimate Buller phenomenon"—Buller, 1931; Quintanilha, 1933; Raper, 1953).

Two hypotheses were early advanced to explain the phenomenon: (a) migration of both nuclei from the original dikaryon into and through the homokaryon to re-establish the original dikaryon at the periphery of the homokaryon, e.g. ($A^1B^1 + A^2B^2$) $\times$ $A^1B^2 \rightarrow (A^1B^1 + A^2B^2)$ (Rawitscher, 1933), and (b) interference by the nucleus of the homokaryon with conjugate division of the pair of nuclei of the dikaryon following the establishment of anastomoses, with chromosomal exchange to yield a nucleus now genetically compatible with the homokaryon, e.g. ($A^1B^1 + A^2B^2$) $\times$ $A^1B^2 \rightarrow^2 (A^2B^1 + A^1B^2)$ (Quintanilha, 1933). Results as predicted by both hypotheses have been repeatedly obtained (Quintanilha, 1939; Papazian, 1950; Gans and Prud'homme, 1958; Kimura, 1958; Ellingboe and Raper, 1962 and personal communication); the mechanisms and concepts relating to incompatible

* Work presented to the Department of Biology, Harvard University as a portion of a dissertation in partial fulfilment of the requirements for the degree of Ph.D. The work was supported in part by a grant from the National Institutes of Health, U.S. Public Health Service (C-2221) to Prof. John R. Raper. The manuscript was prepared for publication while in residence at the Division of Biology, California Institute of Technology.

† Present address: Address following April 1962: Botany Department, Hebrew University, Jerusalem, Israel.

di-mon matings are recently reviewed in detail by Ellingboe and Raper (1962).

The discovery that somatic recombination is quite common among fungi (Pontecorvo, 1956) suggests that somatic recombination as it may be involved in the "Buller phenomenon" is not an anomaly peculiar to the higher Basidiomycetes. Consequently, since somatic recombination occurs also in numerous organisms without relation to any process comparable to the "Buller phenomenon", it is evident that a re-evaluation is needed of Quintanilha's idea about chromosomal exchange induced by the nucleus of the incompatible homokaryotic mate. In order to test this, it was necessary to seek evidence for somatic recombination in a dikaryotic mycelium that had not been exposed to an incompatible homokaryotic tester.

An earlier possible approach to this problem was the use of compatible di-mon matings (Papazian, 1950; Crowe, 1960); many of the new components of the dikaryotic mycelia derived from these matings, up to 15 per cent., could be explained only by exchanges between the nuclei of the original dikaryon. Crowe (1960) used morphological markers in addition to the incompatibility factors, and the frequencies of intra- and inter-chromosomal exchanges, as well as the occurrence of double exchanges, were consistent with expectation from a meiotic process (Pontecorvo, 1958; Parag, 1960). There remained the question, however, whether the observed frequency and indicated mechanism accurately reflected only processes that occurred in the original dikaryon, or whether the observed results were also influenced by the homokaryotic tester. Such influence of the tester in the compatible matings might arise either (a) from the induction of genetic exchange, as postulated by Quintanilha (1933) for incompatible matings, or (b) through the selection of certain classes of nuclei.

The objectives of the present work were three: (a) to determine whether somatic recombination occurs regularly in the vegetative dikaryon before it is exposed to the possible inductive or selective effects of a homokaryotic tester; (b) to develop, by the use of appropriately marked genotypes, techniques for the selective recovery of somatic recombinants; and (c) to attempt a precise analysis of the process of somatic recombination in *S. commune*.

2. MATERIAL AND METHODS

Some of the strains used in this study came from the collection of Prof. J. R. Raper; others were derived from these during this study, either by mutations or by recombinations; still others were generated by back-crossing mutant strains for 6-11 generations to the standard strain 699 by Dr A. H. Ellingboe. Most of the mutations were either described before (Raper and Miles, 1958) or were U.V.-induced mutants obtained by Mr A. S. Flexer: *st* (streak, a morphological mutant); *arg-1*, *arg-2* (two unlinked arginine-requiring mutants), *ad-1*, *ad-4* (two unlinked adenine-requiring mutants), *ura-1*, *nic-2*, *cho*, *pab* (requiring uracil, nicotinic acid, choline and *p*-amino benzoic acid, respectively). Two new mutations (see below

have been used: *pol^r* (a recessive polymyxin resistant) (Parag, 1961), and *su-arg-2* (a suppressor of *arg-2*). Wild alleles in crosses or dikaryons are written only when relevant. A tentative linkage map of these markers and the *A* and *B* factors is given in fig. 1; this map is based on data of Papazian (1951), Raper and Miles (1958), Raper, Baxter and Ellingboe (1960), Parag (1960), Ellingboe and Raper (1962). The basic media routinely employed were complete medium, minimal medium, and minimal medium with appropriate supplements (Raper and Krongelb, 1958; Raper and Miles, 1958; Snider and Raper, 1958). A basic temperature of 22° C. was commonly used, but 32° C. was used upon occasion, especially to prevent fruiting of dikaryons in tests and in di-mon matings and also sometimes to accelerate the mating reactions. Dikaryons were grown for long periods for successive tests, and, in order to keep them in active vegetative growth,

FIG. 1.—A tentative genetic map of the loci relevant to this study.
(? - - linkage not certain).

they were transferred successively to new plates. The inoculum for each transfer was a long peripheral strip of mycelium containing a large number of hyphal tips and representing a large fraction of the hyphal systems of the dikaryons.

Shake cultures were made in 50 ml. medium in 250 ml. Erlenmeyer flasks and were shaken continuously for adequate aeration. The mycelia for pour-plates were macerated in the Waring Blender (two minutes) and washed in water by centrifugation. The concentration of the suspension was roughly estimated by measuring the optical density with a Klett-Somerson colorimeter; more exact determinations of viable fragments were obtained by counting the emergent mycelia upon complete medium following pour-plating.

The tests for mating types of homokaryons and of the components of dikaryons were performed as suggested by Papazian (1950). The biochemical deficiencies were tested by the procedure described by Raper and Miles (1958). The test for biochemical deficiencies carried by the components of the dikaryon was based on the separate selection from the dikaryon of the two nuclear types of di-mon matings. The tester strains carried appropriate deficiencies, and from the growth or lack of growth of the derived dikaryons on a series of test media, the genotype of the original dikaryon could be deduced. Details of these tests will be given below with the experiments in which they were required.

The terminology concerning the incompatibility factors, the types of matings and the mating reactions follows that of Papazian (1950), Raper (1953), and Raper, Baxter and Middleton (1958). The superscripts denote the factor-specificities, while the subscripts denote the specific alleles of the two subunits, α and β , which are separable by crossing over (thus, $A_{1-1}^{41} = \alpha 1 \beta 1$). Letter superscripts denote *A*-factor recombinants (e.g. $A_{1-1}^{41} \times A_{3-4}^{47} \xrightarrow{\text{crossing over}} A_{1-4}^d$ and A_{3-1}^e). The sub-unit constitution of the incompatibility factors is described only when it is relevant. In the di-mon mating, the dikaryon of the mating is termed the *original* or *tested dikaryon*, the homokaryon is termed *homokaryotic tester*, and the resulting dikaryon is termed *derived dikaryon*. A dikaryon prior to such di-mon matings is termed *unmated dikaryon*. The terms *homozygous* and *heterozygous* describe the relation of the two alleles at a given locus in the dikaryon or in the heterokaryon in the same way these terms are used for diploid organisms.

3. EXPERIMENTAL PROCEDURES AND RESULTS

Two different experimental approaches have been used in the work described below: (a) *total isolation*, the analysis of random isolates from macerated dikaryon, and (b) *selection*, the examination of samples selected by means of nutritional competence or resistance to drugs.

(i) *Total isolation*

Only the incompatibility factors and their subunits were used as markers in the studies based on total isolation. Three different dikaryons were each tested at different ages:

- I. ($A_{1-1}^{41}B^{41} + A_{3-4}^{47}B^{42}$) (The subunit constitution of the *B*-
- II. ($A_{1-1}^{41}B^{42} + A_{2-2}^{51}B^{41}$) factor is not relevant here and has
- III. ($A_{1-1}^{41}B^{41} + A_{4-6}^{42}B^{42}$) been omitted)

The test for recombinants is the one described in detail by Raper (1961). Sizable portions of the dikaryons were macerated and spread upon the surface of complete medium. After 48 hours, well isolated mycelia, from micromycelia previously observed to be clearly derived from single hyphal fragments, were each divided into five pieces under the dissection microscope. Four pieces of each isolate were mated with testers of four mating types in order to detect recombinants (step 1, table 1); a fifth piece was saved for a second-step test in which the two products of intra-*A* recombination could be differentiated (step 2, table 1).

From seven samples of about 100 isolates each from the three dikaryons, seven and three recombinants were obtained from two samples of dikaryon I (table 2). All seven recombinants from one sample were homokaryotic and were analysed in detail (table 1). Four, three $A_{1-1}^{41}B^{42}$ and one $A_{3-4}^{47}B^{41}$ (classes *c* and *d*), can most easily be explained as resulting from independent assortment of whole chromosomes; the other three, $A_{3-1}^eB^{42}$, $A_{1-4}^dB^{42}$ and $A^{d/e}B^{41}$ (classes *e*, *f* and *g* respectively), may be explained as resulting from intra-*A* recombination; the first by crossing over only, the second by crossing

over and independent assortment (the third was not completely characterised). In the same sample, three parental type, homokaryotic isolates were found; no homokaryons were detected in the other samples.

TABLE 1
The pattern of interaction of isolates from a 40 day old dikaryon I
($A_{1-1}^{41}B^{41} + A_{3-4}^{47}B^{42}$)

Class	Frequency	Testers						Geno- types	Inter- pretation *
		First step				Second step			
		$A_{1-1}^{41}B^{41}$	$A_{1-1}^{41}B^{42}$	$A_{3-4}^{47}B^{41}$	$A_{3-4}^{47}B^{42}$	$A_{1-4}^d B^{47}$	$A_{3-1}^e B^{47}$		
<i>a</i>	98	+	F	F	+	/	/		P-dikaryon
<i>b</i>	3	+	—	F	—	/	/		P-homokaryon
<i>c</i>	3	F	—	+	—	/	/	$A_{1-1}^{41}B^{42}$	I.A.
<i>d</i>	1	—	+	—	F	/	/	$A_{1-1}^{47}B^{41}$	I.A.
<i>e</i>	1	+	—	+	—	F	+	$A_{1-4}^d B^{42}$	C.O.
<i>f</i>	1	+	—	+	—	+	F	$A_{3-1}^e B^{42}$	C.O. & I.A.
<i>g</i>	1	—	+	—	+	/	/	$A_{3-1}^d B^{41}$	C.O. (&I.A.?)

* P, parental ; I.A. independent assortment ; C.O. crossing over.

TABLE 2
Recombination in samples by total isolation

Dikaryon no.	Sample no.	Days after mating	Frequency of recombinants	
			Number	Per cent.
I	1	20	3/101	3
	2	40	7/108	6.5
	3	122	0/109	0
	4	157	0/107	0
II	1	21	0/110	0
	2	67	0/110	0
III	1	40	0/110	0
Total	7		10/755	1.3

(ii) Selection

Two types of experiments based on selection for recombinants, were performed: 1. Recombinants from a dikaryon heterozygous in repulsion for two biochemical deficiencies and homozygous for a third deficiency were selected by a homokaryotic tester, which carried

the two deficiencies for which the dikaryon was heterozygous. 2. Recombinants from a dikaryon heterozygous for a recessive mutation were selected via homozygosis for this mutation: The latter employed either a recessive suppressor for a biochemical deficiency or a recessive mutation for resistance to an antibiotic, in this case polymyxin.

i. *Selection by three biochemical deficiencies—the triple deficiency technique.* A dikaryon, IV, constituted of doubly deficient strains ($A^{41}B^{41} ura-1 nic-2 + A^{15}B^{15} ura-1 arg-2$), was macerated when 12 days old and plated together with a macerate of a doubly deficient, homokaryotic tester, $A^2B^2 nic-2 arg-2$. The only dikaryon that should be able to grow upon minimal medium would contain a singly deficient nucleus, derived by way of recombination from the original dikaryon, in combination with the tester nucleus:

Platings of the unmated dikaryons and of each of the three homokaryons on minimal medium and on the appropriately supplemented media served as controls. On the latter media, the input of viable fragments of the tested dikaryon was estimated (an average of seven plates). All the controls behaved as expected, with no growth on the minimal medium. On the minimal-medium plates inoculated with the dikaryon (7770 viable fragments per plate) plus the homokaryotic tester, $42.1 (\pm 0.85)$ wild type mycelia per plate (average of 10 plates) were found. Two hundred of these were isolated and tested after two successive transfers, and their stable prototrophy was confirmed. In order to test whether these mycelia were prototrophic because they each contained a recombinant nucleus, and if so, to determine the mechanisms of recombination, a smaller sample was tested for non-selective markers—in this case the incompatibility factors. The results were disappointing; two successive tests of the same 108 isolates gave inconsistent mating reactions. These inconsistencies cast doubt on recombination as the cause for prototrophy, and because of this, as well as for another reason (see discussion), this technique was abandoned.

ii. *Selection based on homozygosity of a recessive suppressor.* A suppressor of *arg-2* (*su-arg-2*) was used to attempt the selection of recombinants in the same way that such recessive suppressors had previously been

used in *Aspergillus* (Pontecorvo and Käfer, 1958). An *arg-2* revertant strain, kindly supplied by Dr S. Dick, was tested and found to have a recessive suppressor: (a) the dikaryon constituted of the revertant strain and an arginine-dependent (*arg-2*) strain failed to grow on minimal medium. (b) when this revertant was outcrossed to wild type, a quarter of the segregants were arginine dependent, and when the other three-quarters were mated with *arg-2*, a third of the resulting dikaryons failed to grow on minimal medium. These results are interpreted as resulting from the cross *arg-2 su-arg-2* $\times$ *arg-2⁺su⁺-arg-2* with the expected segregation: $\frac{1}{4}$ *arg-2 su⁺-arg-2*, $\frac{1}{4}$ *arg-2 su-arg-2*, $\frac{1}{2}$ wild type ($\frac{1}{4}$ *su-arg-2 arg-2⁺*, $\frac{1}{4}$ *su⁺-arg-2 arg-2⁺*) (Parag, 1960).

Dikaryon V (*A²B¹ arg-2 su-arg-2* + *A¹⁵B¹⁵ arg-2 ura-1*) was phenotypically arginine dependent; somatic recombination would be expected to lead to homozygosity for the recessive suppressor, among other combinations, and this would result in arginine independence of the dikaryon. Such dikaryotic mycelia would therefore be expected to give sectors capable of normal growth on medium lacking arginine *:

Eighteen distinct sectors appeared on six colonies of this dikaryon, and the different sectors were most probably independent in their origins. Seven of the sectors were parental homokaryons, *A²B¹ arg-2 su-arg-2*. The others were dikaryons having the same mating reactions as the original dikaryon, and all were uracil independent. These preliminary findings suggested that no recombination for the non-selective markers had occurred. The dikaryons were further tested by mating them with *A¹⁵B² ura-1 arg-2*, and the derived dikaryons were found to be phenotypically wild type. It was concluded, therefore, that the dikaryotic sectors were all (*A²B¹ arg-2⁺ su-arg-2* + *A¹⁵B¹⁵ arg-2 su⁺-arg-2 ura-1*). This, of course, means that reversion of *arg-2* to *arg-2⁺*, rather than recombination, accounts for the prototrophy of the dikaryons. These frequent reversions negate the usefulness of this specific suppressor for the selection of recombinants.

iii. *Selection for polymyxin resistance.* Selection of dikaryons that were homozygous for polymyxin-resistance, basically the same device as that described above, was next attempted. In the early attempts

* — *ura-1* is omitted from the diagram.

to apply this selection, however, it was found that a dikaryon heterozygous for the recessive trait of polymyxin resistance, *pol^r*, breaks down on polymyxin-complete medium to liberate the resistant homokaryon by sectoring (Parag, 1961). A modified technique was therefore devised to use another selective marker, *ura-1*, so that on polymyxin medium lacking uracil, only homozygous dikaryons or homokaryons with nuclei resulting from recombination between *ura-1* and *pol^r* would grow:

Two dikaryons being studied in these tests:

VI, $(A^{41}B^2 \text{ ura-1 } nic-2 pol^r-1 + A^{42}B^{42} \text{ arg-2})$

VII, $(A^{41}B^2 \text{ ura-1 } nic-2 \text{ arg-2 } pol^r-1 + A^{42}B^{42} \text{ ade-1 } cho)$

All the media for these experiments included bacto-agar (Parag, 1960 and 1961); the *polymyxin-selective* medium was minimal medium, and contained 100-150 mg./l polymyxin and all necessary supplements, except uracil; the *polymyxin-complete* included uracil as well.

The dikaryons were each transferred to shake cultures to prevent fruiting: dikaryon VI was transferred 30 days after mating, dikaryon VII was transferred 3 days after mating. A reconstruction experiment was performed with mycelia derived from large numbers of basidiospores from fruit bodies of dikaryon VI. These mycelia were macerated and were plated together with a macerate of dikaryon VI in known proportions on the polymyxin-selective medium. Only a qualitative estimate was made (*i.e.* the efficiency of the expression was not considered), and it was found that when the reconstructed resistant mycelia were mixed with parental sensitives in the proportion 1 : 6000, the resistant mycelia could be expressed by selection on the selective medium.

Altogether, about 0.75×10^8 viable mycelial fragments were tested (table 3), and 26 polymyxin-resistant mycelia, all dikaryons and supposedly containing recombinants, were isolated. These showed, however, neither recombination for mating types nor homozygosity for the recessive non-selective markers. In order to check the genotypes of the components of the resistant dikaryons, the isolates from dikaryon VI were mated with the homokaryotic tester $A^{41}B^2 \text{ ura-1 } nic-2 \text{ arg-2 } pol^r$, and the isolates from dikaryon VII were mated with $A^{42}B^{41} \text{ ura-1}$, $A^{42}B^{41} \text{ ade-1 } cho$, and $A^{41}B^2 \text{ ura-1 } nic-2 \text{ arg-2 } pol^r-1$, and the derived

dikaryons were tested for mating types, resistance and nutritional requirements. These tests indicated that the resistant isolates of dikaryon VI were ($A^{41}B^2$ *ura-1*? *nic-2*? *arg-2*+ *pol^r-1* + $A^{42}B^{42}$ *ura-1*+ *nic-2*+ *arg-2* *pol^r-1*), and the isolates of dikaryon VII were ($A^{41}B^2$ *ura-1* *nic-2*? *arg-2* *cho*+ *ade-1*+ *pol^r-1* + $A^{42}B^{42}$ *ura-1*+ *nic-2*+ *arg-2*+ *cho*? *ade-1*? *pol^r-1*). Although the genotypes were not completely determined, the results that were obtained ruled out as improbable any recombination between non-selective markers. Homozygosity for polymyxin resistance can be best ascribed to spontaneous mutations.

TABLE 3

The frequencies of polymyxin resistant mycelia selected from sensitive dikaryons ($pol^r + pol^s$)

Dikaryon	Days after mating	Viable fragments plated on the selective medium ($\times 10^6$)	Resistant mycelia	
			no.	frequency ($\times 10^{-6}$)
VI	30	0.78	0	0
	60 <i>a</i>	2	2	1
	<i>b</i>	0.75	4	5
	<i>c</i>	1.35	5	4
	75 <i>a</i>	0.03	0	0
	<i>c</i>	1.1	0	0
	Total	6.01	11	1.8
VII	23	1.07	15	15

One hundred and forty thousand viable fragments of the homokaryon $A^{42}B^{42}$ *ade-1* *cho*, the sensitive component of dikaryon VI, were plated on polymyxin medium, and 26 resistant mycelia were found after 21 days. This indicates a frequency of 1.8×10^{-5} for spontaneous mutation, and this is very close to the frequency with which the homozygous resistant dikaryons were selected from dikaryons VI and VII.

In addition, one experiment was performed on polymyxin-complete medium with dikaryon VIII ($A_{1-7}^2B^2$ *ura-1* *nic-2* + $A_{1-1}^{41}B^{41}$ *pol^r*), two weeks after mating. The mycelia isolated on this medium were expected to be resistant-homozygous dikaryons or resistant homokaryons, the latter to be tested for recombination between all the markers. This experiment is actually a total isolation of one component of the dikaryon following its selection from the dikaryon on the polymyxin medium (Parag, 1960, and 1961). Among 216 apparently resistant mycelia, 30 were dikaryons that were later found to be sensitive, 185 were parental, resistant homokaryons, and one had the morphology of a common-*A* heterokaryon. The genetic constitution of the latter was found, by means of tests for mating type and of

tests of derived, and appropriately marked, dikaryons to be as follows:

$$(A_{1-1}^{41}B^{41}ura-1^+ nic-2^+ pol^r + A_{1-1}^{41}B^2ura-1^+ nic-2^+ pol^r)$$

The heterokaryon carried one nucleus showing recombination between the *B* factor and all other markers, and the result was homozygosity for all markers, except the *B* factor.

4. DISCUSSION

In this study, somatic recombination in the unmated dikaryon of *S. commune* has been studied in samples obtained both by total isolation and by selection. The methods that have been used represent a new approach to the study of somatic recombination in the higher Basidiomycetes, and, as far as the author is aware, the present study provides the first evidence for somatic recombination in the unmated dikaryon. This study also details the first attempts to use selective techniques based on biochemical mutations or mutations for resistance for the enrichment of somatic recombinants in the higher fungi.

The experiments based on total isolation gave clear evidence that recombination does occur in the dikaryon with the consequent production of recombinants having non-parental mating types. The results, however, gave no decisive answer to the problem of the mechanism of recombination. The relative frequencies of independent assortment of chromosomes and of cross-overs among the seven recombinants isolated from dikaryon I can best be explained by precocious diploidy followed by meiosis (Raper, 1961), but the data are too few and the markers are insufficient to provide any conclusive answers.

The results of Crowe (1960), who detected recombinants following compatible di-mon matings, were similar to our results with dikaryon I, and it may be argued that two independent experiments with different methods agree in supporting an hypothesis of meiotic recombination. It must be kept in mind, however, that, in Crowe's experiments, the recombinants were detected after nuclei from the dikaryon had migrated through the homokaryotic tester. This migration or the association with a third nuclear type might affect the frequencies of the various recombinants or it might induce recombination of the type suggested by Quintanilha (1933) for incompatible di-mon matings.

In a somewhat larger sample of somatic recombinants than either of the above and derived from incompatible di-mon matings, Ellingboe and Raper (1962) found a sizable class of recombinants that were indistinguishable from meiotic products in respect to reassortment and linkage.

All of the available evidence, although fragmentary, thus points to the occurrence of meiotic or meiosis-like recombination in vegetative mycelia. The present study demonstrates the presence of such recombinant nuclear types in the unmated dikaryon, and it is entirely

consistent that this is the site of similar recombinational events in the other cases.

The single recombinant isolated from dikaryon VIII, however, appears to be exceptional; in this case, only the *B* factors were exchanged, and no exchange occurred between 5 other markers. The probability of such a recombinant to occur via meiosis is about 0.06. This recombinant is essentially similar to a majority of those found in incompatible di-mon matings by Ellingboe and Raper (1962). These authors interpret such recombinants, characterised by the exchange of incompatibility factors without any reassortment of numerous other markers, as the result of genetic exchange involving only a portion of the genome. Such exchange could be accomplished by crossing over in a short-lived diploid cell followed by haploidisation via "genome segregation" (Stern, 1958) or via "reductional grouping" (Huskins and Cheng, 1950; Patau, 1950).

Most of the efforts in this study have been directed toward the development of selective techniques, which unfortunately, have not given the expected results. The triple-deficiency technique was found to be inadequate to this study, especially since residual growth on the minimal medium allowed di-mon matings to occur before the selection operated. The observed prototrophs accordingly might be due to mixtures of three nuclear types in the same mycelium, to mixtures of genotypically distinct mycelia, or to recombination during nuclear migration into the homokaryotic tester. Mycelial or nuclear mixtures could thus account for the unstable mating reactions of the selected prototrophs. The method, however, has been found to be efficient for the selection of prototrophs, and it may be useful as a selective technique for the study of processes that occur in di-mon matings.

The techniques involving selection via the recessive suppressor of *arg-2* and via the polymyxin resistance suffered from the mischief of high frequency of reverse and forward mutations, respectively. The failures, however, should not necessarily be ascribed only to these technical difficulties. The polymyxin technique, used more extensively than either of the other selective methods, failed to detect any recombination in more than half a million mycelial fragments, although a reconstruction experiment indicated that this technique would, in fact, select recombinants from a heterozygous, sensitive dikaryon if they were present among the nuclei of the dikaryon. If recombination actually occurs between *pol^r* and *ura-1* in the two dikaryons tested, the frequency of such recombination must have been lower than 2×10^{-6} , whereas the frequency of recombination between incompatibility factors in another dikaryon had been found to be as high as 7×10^{-2} . It is possible that wide variation in the frequency of recombination in different combinations of genomes could account for the apparent ten-thousand-fold disparity, but it also may be a result of the very nature of the mechanism of recombination. The

findings of Ellingboe and Raper mentioned above are compatible with the discrepancies observed here between positive results when the technique could resolve recombination between the incompatibility factors and negative results when the technique could only resolve recombination between other markers that were not associated with the incompatibility factors.

In spite of the failures, the antibiotic-resistance technique remains very promising, although it requires the introduction of certain necessary modifications. Until another mutation for antibiotic resistance—with a lower mutation rate—is available, *pol^r* could be associated with a linked marker to distinguish between a new resistance mutation and recombination; an appropriate marker, *ad-4* (ca. 20 c.o. units from *pol*, Schildkraut, personal communication), is now available. Also, since recombination was so far observed in relatively high frequency between the incompatibility factors, markers close to the *A* factor and its centromere are essential. A dikaryon (*A¹ pab + B¹ ad-4 pol^r + str A² B², other markers*) with selection on polymyxin medium lacking *p*-aminobenzoic acid, is suggested.

There is no *a priori* reason to exclude the possibility that two processes of recombination occur in different stages and conditions—*i.e.* one in the unmated dikaryon, the other during the migration of nuclei through the tester, when they may be exposed to interference or effect by a third nucleus. The two processes could be distinct, and, as such, they could be different in the means by which inter-nuclear exchanges are achieved. In fact, the work of Ellingboe and Raper, cited above, supports such a dual mechanism. Their results indicate that in incompatible di-mon matings, recombination can take place either in the dikaryon prior to contact with the tester or after intimate contact of the dikaryon with the homokaryotic tester. The two processes are very likely as different in their mechanisms as are the recombinants resulting from them. Genetic recombination in the unmated dikaryon may be comparable, in terms of its products, to the parasexual process in other fungi, but it probably differs from the latter in the mechanism by which recombination is achieved. In the present work and in the other works cited here, no evidence has been found for mitotic recombination as demonstrated mainly by Pontecorvo and his coworkers in numerous Ascomycetous and imperfect fungi (Pontecorvo, 1956) and in a lower Basidiomycete, *Ustilago maydis* (Holliday, 1961). Whether two mechanisms—a precocious meiosis and a genetic exchange involving only a portion of the genome—exist in the unmated dikaryon is still to be resolved.

The results of this study as compared with those of Crowe (1960) and Ellingboe and Raper (1962) emphasise the need for further study of the process of somatic recombination in the original unmated dikaryon for comparison with the process as reflected in derived dikaryons. The selective techniques as devised here, when further developed to exploit their inherent capacities, will provide a sound quantitative basis for the study of somatic recombination in the dikaryon as well as the means to resolve the exact mechanism underlying the phenomenon of somatic recombination in this fungus.

5. SUMMARY

A study has been made of somatic recombination in unmated dikaryons of *Schizophyllum commune*. Somatic recombination between incompatibility factors has been demonstrated in samples taken by total isolation, and the pattern of recombination indicates that it may be meiotic in nature in contrast to the parasexual process in some *Ascomycetes*. Another mechanism, recombination involving only a portion of the genome, is suggested by results of tests in which recombination between other markers was investigated. No evidence for parasexuality was found.

Three selective techniques were developed. A "triple-deficiency" selection was found inadequate for this study, but it is a potential tool for studies of processes involved in di-mon matings, including somatic recombination. Two other selective techniques, selection for homozygosity for a recessive suppressor and selection for polymyxin resistance, are similar to those used in *Aspergillus nidulans*. Although recombinants have not been detected through the use of these selective techniques, slight modifications can adapt them for critical quantitative work. The need is emphasised for further studies with unmated dikaryon for comparison with studies with di-mon matings.

Acknowledgments.—The author wishes to thank Professor J. R. Raper for his encouragement, advice and invaluable suggestions, without which this work could not have been accomplished. The author wishes to express his sincere appreciation to Professor D. G. Catcheside for suggestions following his critical reading of the manuscript; to Drs A. H. Ellingboe and S. Dick and Mr A. S. Flexer for certain strains used in this study and for many invaluable suggestions; to Mrs M. G. Stein and Mrs R. Gornish for certain strains and for helping in some of the experiments; and to Mrs M. Benson for her invaluable assistance during this study.

6. REFERENCES

- BULLER, A. H. R. 1931. *Researches on Fungi*, IV. Longmans Green & Co., London.
- CROWE, L. K. 1960. The exchange of genes between nuclei of a dikaryon. *Heredity*, 15, 397-405.
- ELLINGBOE, A. H., AND RAPER, J. R. 1962. Somatic recombination in *Schizophyllum commune*. *Genetics* 47, 85-98.
- GANS, M., AND PRUD'HOMME, N. 1958. Échanges nucléaires chez le Basidiomycète *Coprinus fimetarius* (Fr.) *Comp. Rend. Acad. Sci. (Paris)*, 247, 1895-1897.
- HOLLIDAY, R. 1961. Induced mitotic crossing-over in *Ustilago maydis*. *Gen. Res. Camb.*, 2, 231-248.
- HUSKINS, C. L., AND CHENG, K. C. 1950. Segregation and reduction in somatic tissues. IV. Reductional groupings induced in *Allium cepa* by low temperature. *J. Hered.*, 41, 13-18.
- KIMURA, K. 1958. Diploidisation in the Hymenomycetes. II. Nuclear behavior in the Buller phenomenon. *Biol. J. Okayama Univ.*, 4, 1-59.
- PAPAZIAN, H. P. 1950. Physiology of the incompatibility factors in *Schizophyllum commune*. *Bot. Gaz.*, 112, 143-163.
- PAPAZIAN, H. P. 1951. The incompatibility factors and a related gene in *Schizophyllum commune*. *Genetics*, 36, 441-459.
- PARAG, Y. 1960. Genetic studies on somatic recombination and common-B heterokaryosis in *Schizophyllum commune*. Ph.D. Thesis, Harvard University.

- PARAG, Y. 1961. Sensitivity of *Schizophyllum commune* to chemical toxicants. *Can. J. Microbiol.*, 7, 838-841.
- PATAU, K. 1950. A correlation between separation of the two chromosome groups in somatic reduction and their degree of homologous segregation. *Genetics*, 35, 128.
- PONTECORVO, G. 1956. The parasexual cycle in fungi. *Ann. Rev. Microbiol.*, 10, 393-400.
- PONTECORVO, G. 1958. *Trends in Genetic Analysis*. Columbia Univ. Press, New York.
- PONTECORVO, G., AND KÄFER, E. 1958. Genetic analysis by means of mitotic recombination. *Adv. Genet.*, 9, 71-104.
- QUINTANILHA, A. 1933. Le problème de la sexualité chez les champignons. *Bol. Soc. broteria.*, 8, 1-99.
- QUINTANILHA, A. 1939. Étude Génétique du phenomene de Buller. *Bol. Soc. Broteria.*, 13, 425-486.
- RAPER, J. R. 1953. Tetrapolar sexuality. *Quart. Rev. Biol.*, 28, 233-259.
- RAPER, J. R. 1961. Parasexual phenomena in Basidiomycetes. *Rec. Adv. Bot.*, pp. 379-383, University of Toronto Press.
- RAPER, J. R., BAXTER, M. G., AND MIDDLETON, R. B. 1958. The genetic structure of the incompatibility factors in *Schizophyllum commune*. *Proc. Nat. Acad. Sci. (U.S.)*, 44, 538-546.
- RAPER, J. R., BAXTER, M. G., AND ELLINGBOE, A. H. 1960. The genetic structure of the incompatibility factors of *Schizophyllum commune*: The A-factor. *Proc. Nat. Acad. Sci. (U.S.)*, 46, 833-842.
- RAPER, J. R., AND KRONGELB, G. S. 1958. Genetic and environmental aspects of fruiting in *Schizophyllum commune* Fr. *Mycologia*, 50, 707-740.
- RAPER, J. R., AND MILES, P. G. 1958. The genetics of *Schizophyllum commune*. *Genetics*, 43, 530-546.
- RAWITSCHER, E. 1933. Besprechungen. *Z. Bot.*, 26, 134-136.
- SNIDER, P. J., AND RAPER, J. R. 1958. Nuclear migration in the Basidiomycete *Schizophyllum commune*. *Amer. J. Bot.*, 45, 538-546.
- STERN, C. 1958. The nucleus and somatic cell variation. *J. Cell. Comp. Physiol., Suppl.* 1, 1-34.