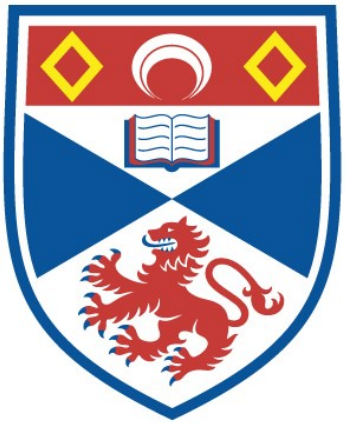
THE MATING SYSTEMS OF FUNGI, WITH SPECIAL REFERENCE TO POLYSTICTUS VERSICOLOR (L.) FR

Margaret Partington

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



1959

The Mating Systems of Fungi, with special reference to *Polystictus versicolor*(L.) Fr.

by

Margaret Partington B.Sc.

Thesis presented for the Degree of Doctor in Philosophy in the University of St. Andrews.

May, 1959.

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

ms 2115

Declaration

I hereby declare that the following thesis is based on the record of work done by me, that the thesis is my own composition and that it has not previously been presented for a higher degree.

The research was carried out in the Department of Botany at St Salvator's College of the University of St. Andrews under the direction of Prof. J.H.Burnett.

Certificate

I certify that Margaret Partington has spent nine terms of Research work under my direction, and that she has fulfilled the conditions of Ordinances No. 16 and 61. (St. Andrews) and that she is qualified to submit the accompanying Thesis in application for the degree of Doctor of Philosophy.

Career

I graduated with Honours in Botany at the University of Liverpool in June 1956. In October of that year I was admitted as a Research Student in the Department of Botany, in the University of St. Andrews, under Ordinances 16 and 61.

With the support of a grant from the Nuffield Foundation I have spent eleven terms of Research work.

Acknowledgements

I wish to acknowledge the continued help and encouragement of Prof. J.H. Burnett, who supervised this work, also to thank Miss. R. Aitcheson and the late Mr. A. Patrick for help in preparation of the photographs.

I also wish to acknowledge the support of a grant from the Nuffield Foundation.

CONTENTS

I. INTRODUCTION.

Much work has been done on the genetics of basidiomycetes since Kniep andbensaude established the basic features of bipolar and tetrapolar heterothallism in the early 1920's.

Since then, the breeding systems of over 200 species of Hymenomycetes have been recorded. In addition some observations have been made on the physiological and genetical control of these reactions.

The distribution of mating-type factors in fruit bodies collected over a wide area has been considered previously, and estimates that there are of the order of 100 alleles per locus have been made by Whitehouse(1949b.) More recent work by Raper et al. (1958). indicates that alleles at the mating type locus, at least in Schizophyllum commune are more numerous than was previously supposed. After sampling sporophores collected from different stations all over the world, estimates that there are some 300 alleles at the 'A' locus in Schizophyllum were obtained.

The fruit bodies of Polystictus versicolor studied here were collected over a very limited area, often from adjacent positions on the same tree, and their mating-type consitutions were analysed. It was thought that an intensive survey of the distributions of mating-type alleles within the mycelium in one infected tree would be useful and that such data would provide information on the genetical nature of the mycelium.

The physiology of the mating-type reactions in the tetrapolar Basidiomycetes is known to be highly complex Papazian(1950), Raper(1953). The reactions between haploids in compatible and incompatible pairings of Polystictus versicolor and Collybia velutipes have been studied.

Miles and Raper(1956), describe a method of splitting a dicaryotic mycelium into its component monocaryons by growing it on a medium containing Sodium taurocholate or cholic acid. The monocaryotic fragments are recovered after blending the mycelium. The presence of the bile salts is thought to increase the rate of breakdown of the dicaryon. This method has been used in studies designed to determine which nuclei migrate during diploidisation in an illegitimate dicaryon x monocaryon pairing eg. A_1B_2(A_1B_1 A_2B_2).

Another feature of the mating reaction of bipolar and tetrapolar fungi, is the control of nuclear migration and association. There is little evidence for the control of these reactions by factors other than the mating-type factors, Burnett (1956), there is however some evidence that direction of migration is controled by other factors (Kennedy and Burnett, unpublished.) In an attempt to discover whether nuclear migration in paired haploids of Polystictus versicolor is governed by the mating-type factors or by other closely linked genes, the speeds of the migrating nuclei in the two types of compatible pairing possible in a tetrapolar fungus were studied and compared.

Previous workers in this department have studied the breeding systems in Gasteromycetes, in particular Nidularia/

Nidularia denudata, Kennedy(1956), and the distribution and numbers of mating-type factors in both local and widespread populations of Polyporus betulinus, a bipolar fungus were studied by Saunders (1956).

The objects of the present investigation, therefore were as follows:

1) To obtain estimates of the numbers of alleles in local populations of Polystictus versicolor, a tetrapolar fungus, for comparison with those obtained for the bipolar fungus Polyporus betulinus, and with other previously recordd data.

2) To sample fruit bodies from a limited area, as all previous data, with the exception of that of Saunders, is based on samples taken over an extreemly wide area.

3) To investigate the genetical nature of the mycelium.

4) To investigate the effects of the mating -type factors on various physiological processes, such as hyphal growth and fusion, nuclear migration and clamp formation.

II. MATERIALS

The selection of materials was mainly dictated by the availability of various species in and around St. Andrews at particular times of the year.

Tetrapolar forms only were considered.

The following species were studied:-

Polystictus versicolor (L.) Fr.	Tetrapolar
Collybia velutipes (Curt.) Fr.	Tetrapolar or variable.
Crucibulum vulgare (Tul.)	Tetrapolar
Hypholoma fasciculare (Huds.)	Tetrapolar

A summary of the collections made is given in the following table.

Table I.

Species	Date of Collection	No. of Fruit Bodies sampled	Habit and location
P. versicolor	Oct. 1956	12	Closely agg. on stump. Ladebraes, St. Andrews.
P. versicolor	Oct. 1957	2	"
"	Nov. 1956	2	Felled wood. Dyersbrae, St.Andrews.
"	Nov. 1958	7	Closely agg. on two stumps. Tentsmuir, Fife.
C. velutipes	Nov. 1956	7	1 group terrestrial. University Botanic Garden, St.Andrews.
H. fasciculare	Sept. 1957	12 8	Scattered, some cluste terrestrial. U.B.G., St. Andrews.

The collections of Crucibulum vulgare were made available by Professor Burnett ex Freshfield, Lancs.

All collections of Polystictus versicolor were made from deciduous woodland, were locally restricted, and adjacent fruit bodies were collected. A diagram was drawn at the time of collection, showing the spatial relationships of the fruit bodies, and their positions on the stump.

This method of sampling and its implications will be discussed later.

III. METHODS

A. Media.

The medium used throughout for maintaining stock cultures, for single spore isolations, and for mating experiments, was 2% malt agar and 2% Difco agar at its natural pH 6.5. The incubation temperature was 25°C. except where otherwise stated.

The composition of the medium described by Raper and San Antonio (1954), and used in one particular series of pairing experiments, and in liquid form for blending experiments, is given in the appendix, as are the compositions of various media used in attempts to fruit the dicaryotic mycelia in culture.

B. Spore isolations.

Spore deposits were obtained by placing each fruit body collected, pore surface downwards in a sterile petri dish. From this spore print a spore suspension was made in sterile distilled water. It was not found necessary to make a standard spore dilution. The suspension was plated out on 2% malt extract agar, and after incubation at 25°C. for approximately 36 hours germination had proceeded far enough for isolation of the spores.

Under the high power of the binocular dissecting microscope (x 105) a small piece of the medium on which the spore was germinating was removed, with a previously sterilised needle, and transferred to a test-tube slope. Between fifteen and

twenty such isolations were made from the spores of each fruit body.

C. Blending Technique.

The material to be blended was grown in liquid culture in 60 ml. of Raper's standard medium, in 250 ml. Erlenmeyer flasks for varying periods of time up to three weeks. 0.15% sodium taurocholate was added to the medium before inoculation.

Before blending, the medium was decanted off, the mycelial pad washed in distilled water, and suspended in 40 ml. of distilled water. The "Atomix" Blendor was sterilised by autoclaving at 20 lbs. for 15 mins. The mycelium was blended for 1, 1½, 2, 3, and 4 mins. at about 8000 revs./ sec. After each period of blending, a 1 ml. aliquot was removed and suspended in 9 ml. of sterile distilled water; each of these suspensions was then diluted 1/100, so that a final dilution of 1/1000 was obtained. From the final dilution of each series 0.5 ml. was plated on 2% malt extract agar, and the plates incubated for 24 hours. During this time the water was absorbed by the agar. The fragments of mycelium were then isolated on to the plates marked in squares on the underside of the bottom of the petri dish; each dish took 30 to 35 isolates. 100 isolates were made from each of the combinations blended, and after 24 hours incubation were microscopically examined for presence or absence of clamps.

D. Slide matings and fusion experiments.

Plain agar was used in these experiments; firstly because it is relatively poor in nutrients, and therefore only sparse mycelial growth occurs, which facilitates observations, and secondly it is claimed that the low nutritional level stimulates hyphal fusions (Robak, 1942, Cabral, 1951, and Saunders, 1956).

A glass slide was sterilised by dipping it in 70% alcohol and flaming; it was then placed in a sterile petri dish containing moist filter paper. A drop of melted agar was put in the centre of the slide, and a sterile coverslip placed on top thus flattening the agar. 2 x $\frac{3}{4}$" No.0 coverslips were used, with 3" x 1" slides. After inoculation the slides were incubated at 25°C. and care was taken to maintain the filter paper in moist condition throughout the experiment.

IV. THE FUNGUS IN CULTURE

A. Vegetative growth.

On 2% malt extract agar at 25°C. in the dark the basidiospores of Polystictus versicolor germinate readily, and a good growth of mycelium occurs. After growth on this medium for a week or more the mycelium forms a closely felted mat, which is white or tinged with various shades of yellow. Malt extract agar on which Polystictus versicolor is growing always becomes bleached.

Dicaryotic hyphae have numerous well defined clamps, no conidia were recorded on either the dicaryotic or monocaryotic mycelia, a few chlamydospores were occasionally found. Vandendries (1933) however records conidia on the monocaryon. No conidia were found on the strains in culture here.

B. Fruiting of Polystictus versicolor in culture.

Fruit bodies of this fungus have been obtained only rarely in the laboratory. Lohwag (1952) reports having obtained normal fruit bodies and substantiates his report by a photograph. He cites two other workers, Bayliss (1908) and Cool (1912) as also having obtained normal fruit bodies in culture.

During the three years in which Polystictus versicolor has been in culture here, no fruit bodies have been obtained. Both naturally occurring and artificially produced dicaryons have been grown on 2% malt extract agar, 2% peptone agar and

a variety of other media (see appendix), but without any success.

Badcock (1943) records that he obtained sporophores of Polystictus versicolor after growing the mycelium on beech sawdust plus a variety of additional nutrients in the apparatus shown (Fig. 1) for seven weeks. The composition of the medium used is given in the appendix. Fruit bodies can be obtained more quickly but with greater risk of contamination, by growing the mycelium in boiling tubes containing the same medium as above and plugged with sterile cotton wool.

He gives the following conditions as necessary for the formation of fruit bodies:-

1) Provision of a medium containing readily available nutrients in addition to the wood substances.

2) Plentiful water.

3) An atmosphere not saturated, but of relatively high humidity.

4) Exposure to daylight of moderate intensity.

Although these conditions were maintained with great care over a considerable period of time in experiments carried out here, no sporophores were produced.

Papazian (1950) published a method by which he had obtained numerous fruit bodies of Schizophyllum commune. He grew the mycelium in test-tubes filled with sterilised vermiculite, saturated with 5% malt solution. The test-tubes had glass stoppers, and after inoculation, were maintained in an upright

Fig.1. Apparatus used by Badcock(1943) for fruiting wood-rotting Basidiomycetes in culture.

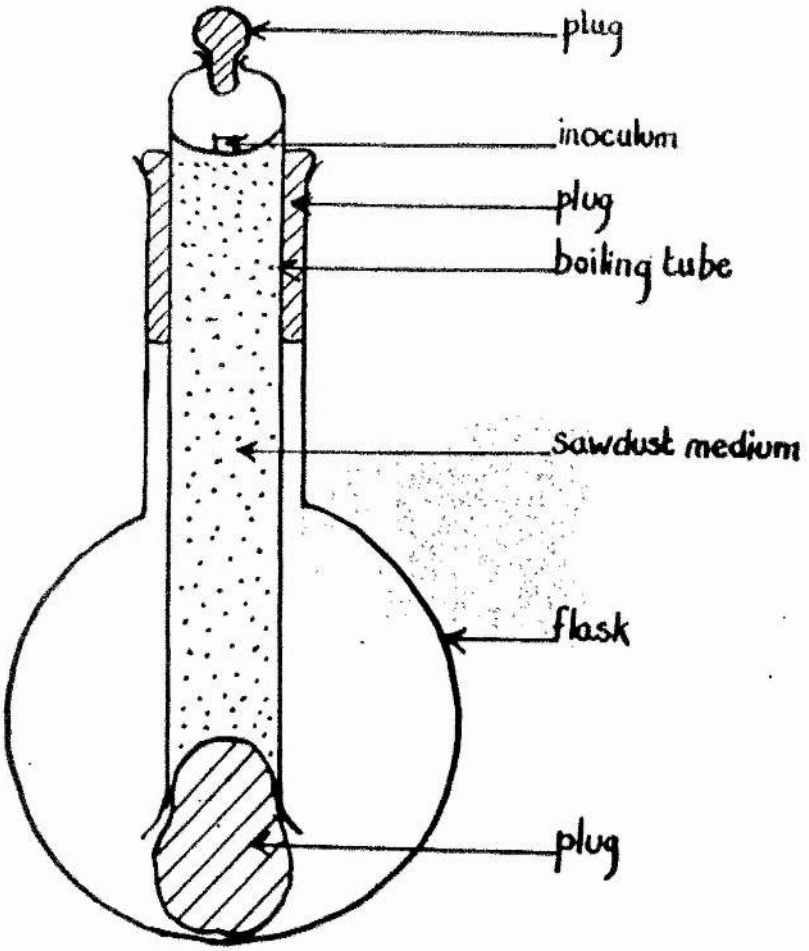


FIG 1.

position at room temperature until a good growth of mycelium had been obtained. They were then placed inside hollow soft tiles and maintained in a moist condition, fruit bodies were rapidly produced.

This method was repeated here with Polystictus versicolor. A good growth of mycelium was obtained on the vermiculite, but no fruit bodies were produced.

What is claimed to be a simple technique for producing fruit bodies of wood destroying fungi, was published by Tamblyn & Da Costa (1958). The dicaryons were grown on an enriched sawdust medium in glass jars until a good growth of mycelium had been obtained. Blocks of readily destroyed wood cut to fit the mouths of the jars were then put in place and the mycelium allowed to grow through them. Under moist conditions fruit bodies were produced on the sides of the blocks.

The composition of the enriched sawdust medium is given in the appendix.

In this laboratory, readily available sawdusts, e.g. oak, ash and beech, and the same additional nutrients were used but no sporophores were obtained.

C. Pairing technique.

Pairings between monocaryotic isolates were made in small

petri dishes of 5 cm. diameter containing malt extract agar medium. The two inocula were placed about 1 cm. apart near the centre of the dish; the mycelia had usually grown together and could be inspected for presence or absence of clamps after incubation at 25°C. for four to five days. This was done by lifting out a small piece of mycelium from the line of junction of the two mycelia, and examining it microscopically. In cases where no clamps were observed in the first examination plates were set aside, and re-examined a few days later.

V. EXPERIMENTAL RESULTS

A. Mating Reactions.

A routine check on the tetrapolarity of Polystictus versicolor was made by taking fifteen monocaryotic isolates and pairing them in all combinations. The isolates fell into four groups with regard to mating type.

The four groups of isolates from fruit body P.V.2 arranged according to mating types were:-

$A_1 B_1$	1, 3.
$A_2 B_2$	2, 8, 11.
$A_2 B_1$	4, 5, 9, 10, 14.
$A_1 B_2$	6, 7, 12, 13, 15.

These groups will be referred to later.

Papazian (1950) and later Raper (1953) record the following specific reactions as occurring in each of the possible combinations between monosporous mycelia of a tetrapolar fungus.

In a single stock A_1, A_2, B_1, B_2 the following reactions occurred:-

1. Unlike factors at A and B loci, i.e. A_1, B_1 x A_2, B_2 dicaryotisation of both mycelia.

2. Unlike factors at A locus, like factors at B, i.e. $A_1 B_1$ x $A_2 B_1$ or $A_1 B_2$ x $A_2 B_2$, gives a limited heterocaryon along the line of contact of the two mycelia. This is probably the "barrage" described by Vandendries.

3. Like factors at A locus, unlike factors at B, i.e. $A_1 B_1$ x $A_1 B_2$ or $A_2 B_1$ x $A_2 B_2$ yield a reciprocally constituted

dicaryon throughout both participating mycelia. This heterokaryon was named "flat" by Papazian. It is usually completely stable, and can be subcultured without apparent change. No clamps are formed.

4. Like factors at A and B loci, i.e. $A_1 B_1 \times A_1 B_1$ or $A_2 B_2 \times A_2 B_2$ usually show no reaction, but fusions between hyphae of the two strains have been recorded.

Raper states that these reactions are not specific to Schizophyllum commune and have been recorded in Lenzites betulina and Collybia velutipes.

There are few instances recorded of interaction between mating-types in the Polyporaceae. Interactions between two monosporous cultures of identical mating type in the bipolar fungus Polyporus betulinus have been recorded by Saunders and Burnett (unpub.), "there may be considerable branching and piling up of hyphae to give a well defined mycelial ridge or barrage, or a somewhat less well defined zone of aversion may develop". Also Mounce (1929) studying Fomes pinicola records that aversion always develops associated with abnormal hyphal proliferation and pigment production in a zone between two incompatible mycelia.

Raper suggests that one of the reasons why they have not been widely reported is that too rich a medium has been used in mating experiments, and that under these conditions cultural differences tend to disappear.

A specific search was made for such reactions in Polystictus versicolor using the basic medium used by Raper and San Antonio (1954). The following pairings were made using isolates of known mating-type:-

TABLE II

Common A.	Common B.
1 x 6	1 x 4
1 x 7	1 x 5
1 x 12	1 x 9
1 x 13	1 x 10
1 x 15	1 x 14

Although in some cases "barrages" did occur, they bore no relationship to the mating-type factors involved. No "flat" heterokaryons were recorded.

To ascertain the mating-type factors of the various monocaryotic isolates used in the above experiment, fifteen were paired in all combinations on malt agar, and their reactions were recorded. These are given in Table II.

B. Establishment and extent of Dicaryon and calculations on the rate of nuclear migration by various methods.

The speed and direction of migration of nuclei in compatible pairings of a Basidiomycete fungus was first studied by Buller (1931). He observed that whether an inoculum was

		A_1B_1		A_2B_1					A_2B_2			A_1B_2				
		1	3	4	5	9	10	14	2	8	11	6	7	12	13	15
A_2B_2	2	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
	8	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
	11	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
A_1B_2	6	−	−	+	+	+	+	+	−	−	−	−	−	−	−	−
	7	−	−	+	+	+	+	+	−	−	−	−	−	−	−	−
	12	−	−	+	+	+	+	+	−	−	−	−	−	−	−	−
	13	−	−	+	+	+	+	+	−	−	−	−	−	−	−	−
	15	−	−	+	+	+	+	+	−	−	−	−	−	−	−	−
A_1B_1	1	−	−	−	−	−	−	−	+	+	+	−	−	−	−	−
	3	−	−	−	−	−	−	−	+	+	+	−	−	−	−	−
A_2B_1	4	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+
	5	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+
	9	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+
	10	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+
	14	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+

TABLE II

haploid or diploid migration of nuclei occurred at a speed of approximately 1.5 mm./hr. in a legitimate pairing, e.g. $A_1B_1 \times A_2B_2$ or $A_1B_1(A_1B_1 + A_2B_2)$. These estimations were based on the length of time which elapsed between the inoculation of the small mycelium and appearance of clamps on the periphery of the large colony, and the distance between the small inoculum and several points on the periphery of the large one.

Dowding and Bakerspeigal (1954) and Kimura (1954) used a radial method of sampling colonies undergoing diploidisation. Samples were taken from the colonies under consideration by means of a sterilised aluminium tube, or by removing tufts of mycelium transferring to another petri dish and after further incubation from 20 to 70 hours ~~were~~ examining for presence or absence of clamps.

Dowding and Bakerspeigal (1954) studying the Ascomycete Gelasinospora tetrasperma obtained a nuclear migration rate of 10.5 mm./ hr. by this method.

Kimura (1954) studying a number of tetrapolar fungi including Coprinus macrorhizus f. microsporas Rea and Lentinus tigrinus Bull. Fr. obtained nuclear migration speeds varying between 0.58 mm./ hr. and 3.2 mm./ hr. in legitimate combinations.

An extensive series of experiments on nuclear migration rates in compatible mon. x mon. pairings of Polystictus versicolor was carried out, using a variety of methods.

During the first series of experiments in which a small inoculum was inoculated on the periphery of a large one and the large inoculum sampled by a "Grid" method, as described below, rather high figures for nuclear migration rates were obtained and an irregular pattern of dicaryotisation recorded. To account for the above phenomena, it was postulated that some hyphae in each colony have a higher growth rate than those of the rest of the colony. If such hyphae are present, then records of the time of contact between two monocaryotic mycelia growing towards each other will be subject to error, as will nuclear migration rates calculated without taking their presence into account.

It is generally acknowledged that nuclei migrate along vegetative hyphae. Girbart (1955) working in Germany and using phase contrast microscopy, investigated nuclear migration rates in Polystictus versicolor, and Dowding (1958) using similar methods, obtained data on the migration rates of nuclei in the Ascomycete Gelasinospora tetrasperma. Thus nuclei have been seen to migrate along normal vegetative hyphae, therefore there seems to be no reason to suppose that they do not migrate along faster growing hyphae if present.

A preliminary experiment was made in which monocaryotic strains were grown in slide culture as has been described. After five days growth at 25°C. in the dark the slides were examined under a binocular microscope and measurements were taken of the difference in length between the longest leading hyphae and the hyphae forming the rest of the colony. Six

strains were studied and there was found strong evidence that there are in fact some hyphae with a growth rate higher than that of the hyphae forming the major part of the colony.

After recording the presence of the long hyphae, a further series of experiments on nuclear migration rates was made taking into account their presence, and using a "radial" method of sampling.

Although nuclear migration rates obtained by the methods described above, taking into consideration the presence of "long" hyphae, are lower than the first estimates obtained for Polystictus versicolor, they are still more than ten times greater than the actual growth rate of this fungus.

Snider and Raper (1958) published data on migration rates of nuclei in Schizophyllum commune. "Biochemical mutants were used as markers, and the values obtained are based upon the time required for the advancing front of a population of nuclei to travel from point to point in a mycelium in which the hyphae between points rarely lie exactly in a straight line". They record 1.3 mm./hr. as a minimum estimate, and 2.5 mm./hr. as a maximum.

Estimates obtained for Polystictus versicolor in plate culture are approximately twice those obtained by Raper and Snider. This could be due

a) to variation between individual fungi with different growth rates and different nutritional requirements.

b) to the fact that in these experiments on Polystictus versicolor in plate culture it was only possible to record nuclear migration rates of one cm. or over.

c) to the fact that these measurements on Polystictus versicolor were of the "effective" migration rate, i.e. nuclei were moving through a multi-branched system of hyphae.

In order to obtain what was hoped would be a more accurate estimate of nuclear migration speeds, pairings were done on thin films of agar on slides.

a) The Grid Method.

The following experiments were designed primarily to obtain estimates of the rate of nuclear migration in compatible matings; the method of scoring employed does, however, provide information on the pattern of distribution of the dicaryon in such matings.

A large "acceptor" mycelium was inoculated onto a malt-extract agar plate and allowed to grow for 72 hours. The small "donor" was then inoculated 1.5 cm. away from the periphery of the"acceptor". The two mycelia were allowed to grow until hyphae from one inoculum were just in contact with those from the other. This was taken as zero time.

Sampling. A series of 1 mm. squares was marked on the under side of the bottom of each petri dish before sampling. The squares were numbered 1 - 25. The samples were plugs of agar taken from each square by means of a No.1 cork borer previously

Fig. 2. Positions of samples taken from each agar plate in "Grid Experiments".

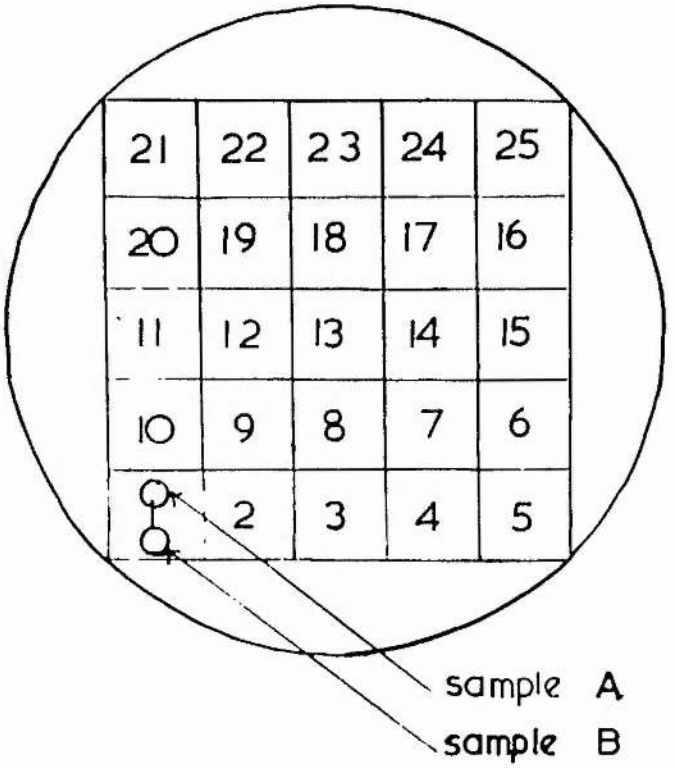

21
22
23
24
25
20
19
18
17
16
11
12
13
14
15
10
9
8
7
6
2
3
4
5
sample A
sample B

Fig 2

sterilised by dipping in alcohol and flaming. Each sample was then transferred to a clean agar plate marked in the same way, and placed in a corresponding square.

It was possible to take two complete series of samples from each plate, so by sampling each of the three replicates twice, six hourly samples could be taken.

Samples were incubated for 24 hours before examining for presence or absence of clamps.

The diameter of the cork borer used was 3.5 mm. Fig. 2 shows the grid and the positions of the samples within each grid square. Full details of the scoring are given in appendix.

A list of the combinations examined in this way is given in Table III.

Table III.

Using isolates from Polystictus versicolor 2.

$A_1B_2 \times A_2B_1$	$A_1B_1 \times A_2B_2$
4 x 6)	1 x 2)
4 x 15)	1 x 8)
4 x 14)	1 x 11)
6 x 4)	2 x 1)
15 x 4)	8 x 1)
14 x 4)	11 x 1)

The large "acceptor" inoculum is recorded first.

Two replicates were made for each combination.

Results. Fig. 3 shows the extent of dicaryotisation after six hours contact between the two monocaryotic isolates.

The following conventions were used:-

1. Samples were numbered 0, 1, 2, 3, 4, 5.

2. If clamps appeared in one particular square during the scoring they are marked in the corresponding square on the diagram by the number of the sample in which they occurred. Thus, if clamps were recorded in square 6 in sample taken at zero time a "0" is put in square 6 in the diagram.

By studying Fig. 3 it can be seen that dicaryotisation occurred in an uneven fashion, nuclear associations often being formed some distance away from the zone of contact between the two mycelia, before they were recorded in the contact zone.

Dicaryotisation patterns and migration rates in $A_1B_2 \times A_2B_1$ pairings were compared with those in $A_2B_2 \times A_1B_1$ pairings; there were significant differences.

Calculations on rate of nuclear migration. Table IV gives the maximum nuclear migration rates in various pairings as extracted from the diagrams

By calculation from this the average nuclear migration rate can be obtained.

Average maximum nuclear migration rate of Polystictus versicolor = 16.36 mm./ hr.

Fig. 3. Extent of dicaryon as determined by the "Grid Experiments".

* Indicates the position of the "donor" inoculum.

4 X 15

2	2	2		3	
2	3	3	1	2	1
5			4	3	2

*

15 X 4

3	5	5	5		
	4	4	3	3	
1	2	1	1		
3	4			1	1
4	3	2	0	1	

*

4 X 14

				4	3
2	2	2		3	2
1			1	1	1
1	1	3	2	2	

*

14 X 4

	3	2	1	1	
	1	1	1	2	3
1	3	1		0	0
	1	2	0	0	

*

4 X 6

		2			
		2			3
3	2	1	3		2
0	1	0			1

*

6 X 4

			2		
	1	4			2
2	2	3	4	4	1
2	3	1	3	2	1

*

FIG 3.

Table IV.

Pairing	Maximum migration rate
4 x 15	15 mm./ hr.
15 x 4	16.6 mm./ hr.
4 x 14	13.3 mm./ hr.
14 x 4	13.3 mm./ hr.
4 x 6	20.0 mm./ hr.
6 x 4	20.0 mm./ hr.

The growth rates of monocaryotic mycelia of Polystictus versicolor are recorded in Table V. Nuclear migration estimates in this case appear to be some twenty times greater than the growth rates.

Table V.

Growth rates of monocaryotic and dicaryotic mycelia P. versicolor. Measurements are increase in diameter of colony (mm./hr.).

Monocaryons		Dicaryons	
Strain	Growth Rate	Strain	Growth rate
4	0.5 mm./ hr.		
14	0.497 mm./ hr.	4 x 14	0.458 mm./hr.
6	0.458 mm./ hr.	4 x 6	0.467 mm./hr.
19	0.521 mm./ hr.		
3	0.560 mm./hr.	19 x 3	0.558 mm./hr.
9	0.540 mm./hr.	19 x 9	0.625 mm./hr.
13	0.437 mm./hr.	19 x13	0.485 mm./hr.

The results obtained here correspond with those obtained by Mittwoch (1954) for Coprinus lagopus in that the growth rate of the dicaryon is intermediate between the growth rates of its two monocaryotic components. There is one exception, the dicaryon formed between strains 9 and 19, in this case the growth rate of the dicaryon is higher than that of either of its component monocaryons. (See Fig. 4).

b) The "Long Hypha" Experiments.

Monocaryotic isolates from various strains of Polystictus versicolor were inoculated and grown on thin films of agar on slides, prepared as previously described (see Section on Methods.) The slides were kept on moistened filter paper in petri dishes and after inoculation were incubated at 25°C. for periods of up to five days.

Measurements of difference in length between normal hyphae and "Long" hyphae were taken after two, four and five days growth at 25°C. The lengths recorded are the average of measurements of 30 "long" hyphae, 10 from each plate.

Table VI./

Fig.4. Graphs to show the growth rates of the dicaryon and the monocaryon at 25°C.

——— Dicaryon.

- - - - - Monocaryon.

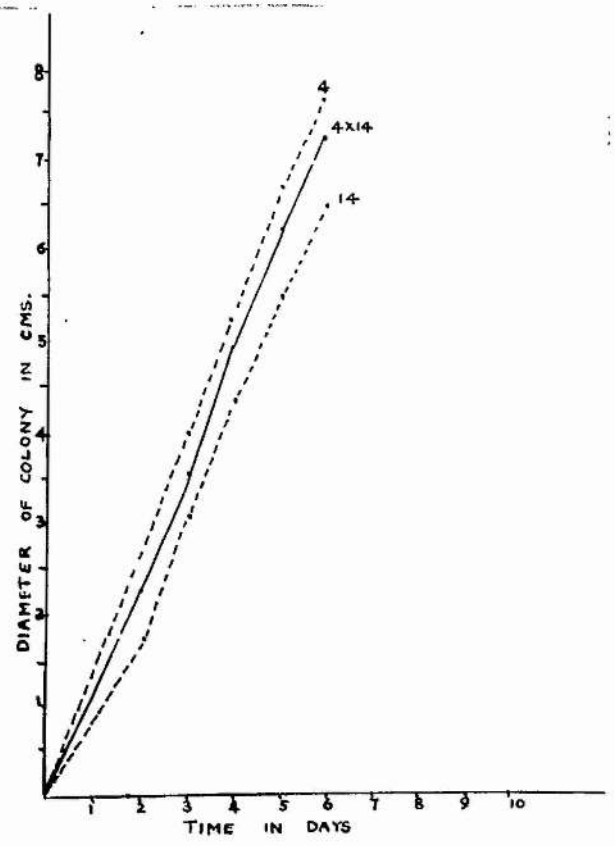

4
4X14
14
DIAMETER OF COLONY IN CMS.
1 2 3 4 5 6 7 8 9 10
TIME IN DAYS

Fig 4.

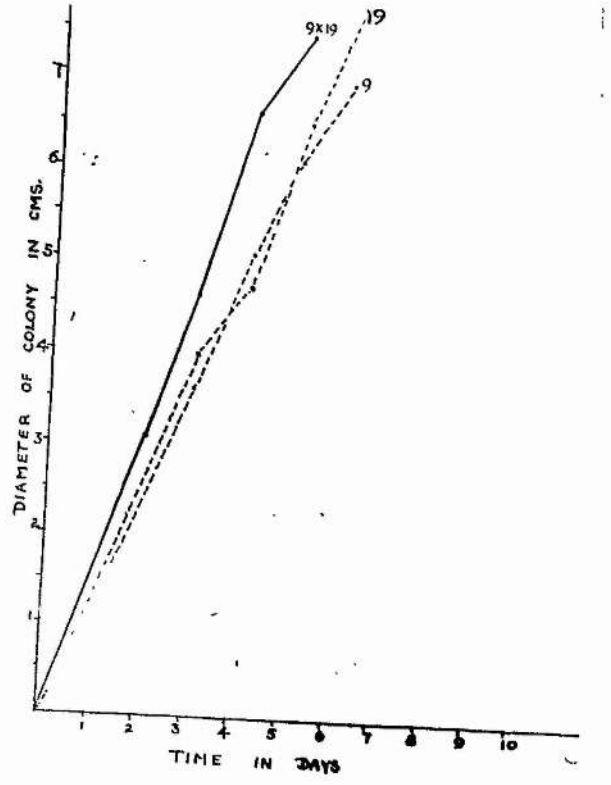

9X19
19
9
DIAMETER OF COLONY IN CMS.
1 2 3 4 5 6 7 8 9 10
TIME IN DAYS

Table VI.

Strain used	Length after 2 days	Length after 4 days	Length after 5 days
4	1.378 mm.	3.270 mm.	3.670 mm.
6	1.242 mm.	3.400 mm.	3.610 mm.
14	1.541 mm.	10.910 mm.	10.980 mm.
19	1.624 mm.	9.060 mm.	9.860 mm.
3	1.604 mm.	11.250 mm.	10.040 mm.
9	1.197 mm.	9.808 mm.	9.962 mm.
10	1.343 mm.	7.961 mm.	8.240 mm.
13	1.468 mm.	8.543 mm.	8.724 mm.

The results from these experiments indicate that there are some hyphae which do in fact have a growth rate which is higher than the growth rate of the majority of the hyphae, and that they continue to grow at this higher rate for at least five days.

This difference does not, however, appear to be large enough to account for the very rapid penetration of nuclei through the "acceptor" mycelium that was recorded in the previous experiment. After recording the presence of "long hyphae" a further series of experiments on nuclear migration rates in plate culture was made.

c) The Radial Method.

In these experiments two inocula of equal size were used and a "radial" method of sampling, adapted from the methods described by Dowding and Bakerspigal (1954) and Kimura (1954). A summary of Kimura's results compared with those obtained by other works in the field and those obtained here during the past two years is given in Table VII.

Two compatible strains were inoculated on malt agar plates 3 cms. apart; they were then incubated at 25°C. for two days. After this initial period the matings were examined under the dissecting microscope at frequent intervals so as to determine as accurately as possible the time when the two strains made contact. After the first contact had been recorded the mycelia were allowed to grow for a further four hours before sampling.

Samples were taken by means of a No. 1 cork borer; plugs of agar were taken from both inocula. The positions of the samples taken are shown in Fig. 5; the actual number of samples depends on the size of the inoculum at the time. Plugs of agar were transferred to clean malt agar plates marked out in the same way as the originals. They were incubated at 25°C. for 24 hours before scoring for presence or absence of clamps.

Table VII/

Table VII.

Estimates on the rates of nuclear migration.

Author	Species studied	Rate of Migration (mm./hr.)	Pairing studied	Growth rate compared with migration rate.	Method of Measurement.
Buller, 1933.	Coprinus lagopus	1.5	di. x mon.	10 x	Time taken for clamps to appear on periphery of large inoculum divided by distance from small inoculum to periphery of large inoculum.
Dowding & Bakerspigal, 1954.	Gelasinospora tetrasperma	10.5	mon. x mon.	———	Tufts of hyphae sampled along radii of large inoculum then transferred to fresh agar and examined after 24 hours for presence or absence of clamps.
Kimura, 1954.	Coprinus macrorhizus	3.2	di. x mon. mon. x mon.	10.7 x 21.3 x	Plugs of agar sampled along radii of inoculum, transferred to fresh agar plate, incubated 24 hours then examined for presence or absence of clamps.
	Pleurotus ostreatus	1.12	di. x mon. mon. x mon.	5.1 x 8.0 x	
	Lentinus tigrinus	0.72	di. x mon.	2.4 x	
	Trametes vittata	0.58	di. x mon. mon. x mon.	2.1 x 2.1 x	
Girbardt, 1955.	Polystictus versicolor	0.25	single dicaryon	approximately equal	Direct observation by phase contrast microscopy.
Dowding, 1958.	Gelasinospora tetrasperma	40	mon. x mon.	———	Direct observation by phase contrast microscopy.
Raper & Snider, 1958.	Schizophyllum commune	1.3 - 2.5	mon. x mon. biochemical mutants.	10 x	Radial sampling by removal of plugs of agar and transferring to fresh agar plate.

Fig. 5. Positions of samples taken in the "radial experiments"

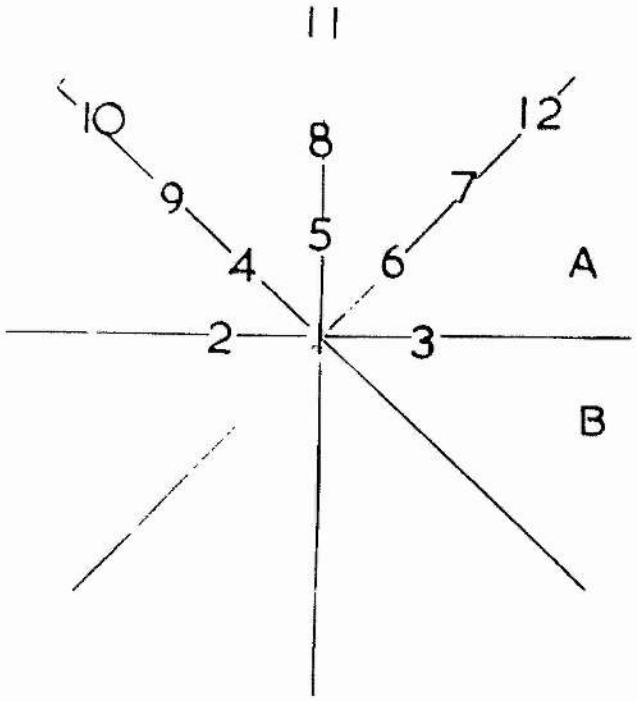
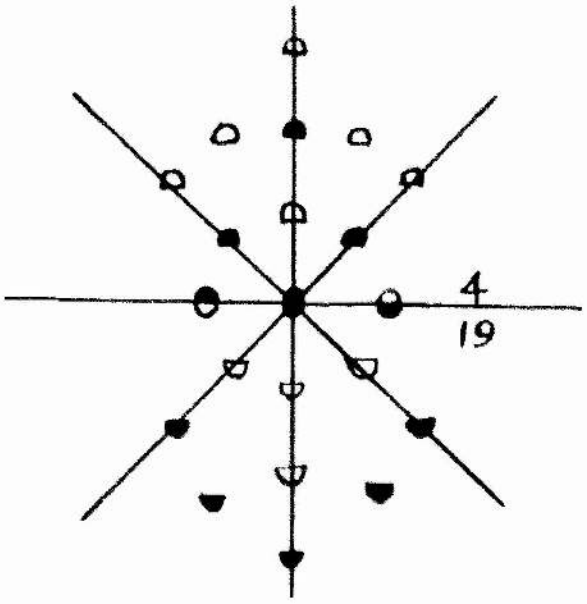


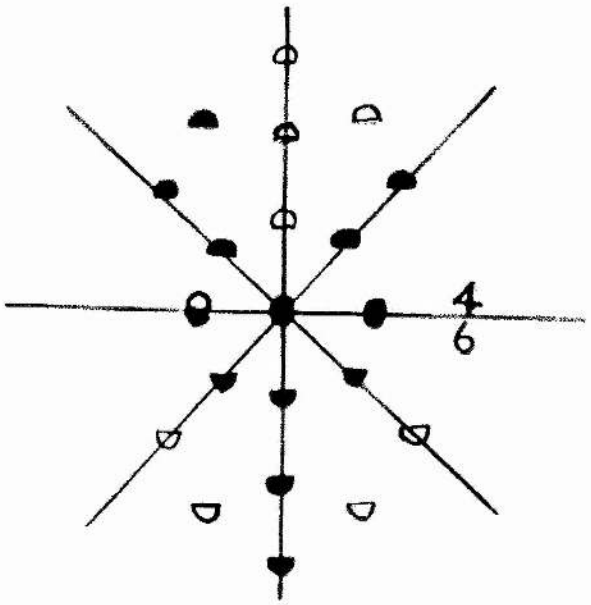
FIG. 5.

Fig. 6. Extent of the dicaryon as determined by the "Radial Experiments".

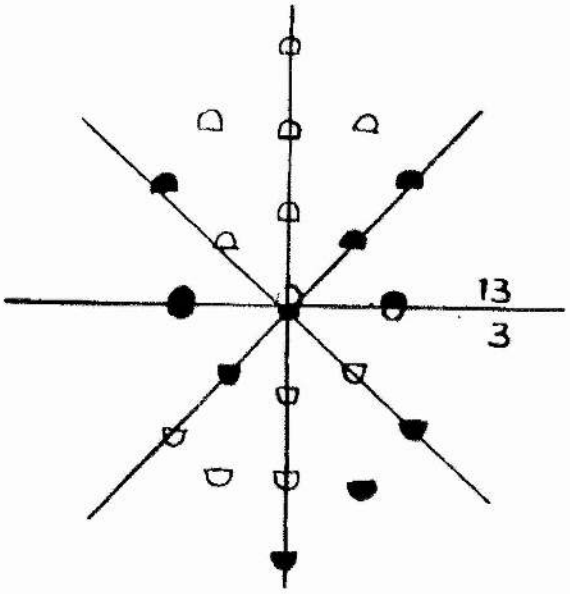
● indicates presence of clamps
○ indicates absence of clamps.



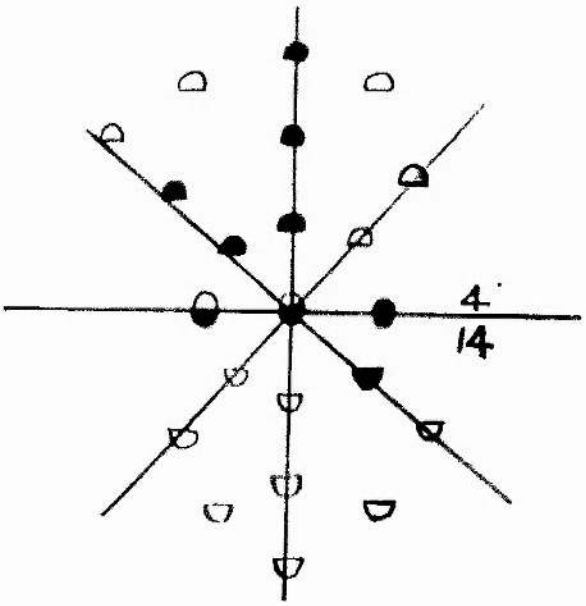
4X19



4X6



3X13



4X14

Fig. 6.

Table VIII gives a list of the combinations studied and the nuclear migration rates recorded for P.V. 14.

Fig. 6 shows the distribution of the dicaryon in the combinations studied.

Details of scoring are given in the appendix.

Table VIII.

Nuclear migration rates.

Pairing	Max. migration rate	Pairing	Max. migration rate
$A_1B_2 \times A_2B_1$		$A_1B_1 \times A_2B_2$	
$4 \rightarrow 6$	7.5 mm./hr.	$3 \rightarrow 13$	5.0 mm./hr.
$6 \rightarrow 4$	5.0 mm./hr.	$13 \rightarrow 3$	7.5 mm./hr.
$4 \rightarrow 14$	2.5 mm./hr.	$9 \rightarrow 13$	2.5 mm./hr.
$14 \rightarrow 4$	7.5 mm./hr.	$13 \rightarrow 9$	5.0 mm./hr.
$4 \rightarrow 19$	7.5 mm./hr.	$10 \rightarrow 13$	5.0 mm./hr.
$19 \rightarrow 4$	5.0 mm./hr.	$13 \rightarrow 10$	7.5 mm./hr.

The arrow indicates the direction in which migration was being recorded.

Results and observations. Again there was no recognisable difference in speed of nuclear migration between matings of $A_1B_2 \times A_2B_1$ and $A_2B_2 \times A_1B_1$. Dicaryotisation patterns in both types of pairing were equally irregular, and nuclear migration rates in both were found to vary between 2.5 mm./hr. and 7.5 mm./hr.

The following calculation was made:-

Mean rate of travel of nuclei in A_1B_2 x A_2B_1 pairing = 5.82 mm./hr. ± 1.634

Mean rate of travel of nuclei in A_2B_2 x A_1B_1 pairing = 5.4 mm./hr. ± 1.717

Statistical comparison of nuclear migration rates in pairing A_1B_1 x A_2B_2 and A_2B_1 x A_1B_2 follows. The difference between the mean rates of nuclear migration was not found to be significant.

Mean rate of nuclear migration A_1B_2 x A_2B_1 = 5.82 mm./hr.
" " " " " A_2B_2 x A_1B_1 = 5.4 mm./hr.

Standard deviation = $\sqrt{\frac{s(x-\bar{x})^2}{N}}$

$sx_1 = 35.0$ $\qquad$ $sx_2 = 32.5$

$N_1 = 6$ $\qquad$ $N_2 = 6$

$\bar{x}_1 = 5.82$ $\qquad$ $\bar{x}_2 = 5.41$

$s(x-\bar{x})^2 = 16.06$ $\qquad$ $s(x-x)^2 = 17.7$

$\sigma_1 = \sqrt{\frac{16.06}{6}}$ $\qquad$ $\sigma_2 = \sqrt{\frac{17.7}{6}}$

$= \sqrt{2.67}$ $\qquad$ $= \sqrt{2.95}$

$= 1.634$ $\qquad$ $= 1.717$

Using t test for significance for small samples.

$N_1 = 6$ $\qquad$ $N_2 = 6$

$s(x_1) = 35$ $\qquad$ $s(x_2) = 32.5$

$s(x_1^2) = 224.9$ $\qquad$ $s(x_2^2) = 193.7$

$\bar{x}_1 = 5.82$ $\qquad$ $\bar{x}_2 = 5.4$

$t = /$

$$t = \frac{(\bar{x}_1 - \bar{x}_2)\sqrt{\frac{1}{N^1}}}{\sqrt{\left\{ s(x_1^2) - \frac{[s(x_1)]^2}{N_1} + s(x_2^2) - \frac{[s(x_2)]^2}{N_2} \right\}}}$$

$\bar{x}_2 - \bar{x}_1 = .42$ $\qquad \frac{N_1 + N_2}{N_1 . N_2 (N_1 + N_2 - 2)} = N^1$

i.e. $\frac{12}{36(10)} = N^1$

$\therefore \sqrt{30} = \sqrt{\frac{1}{N^1}}$

$\therefore \sqrt{\frac{1}{N^1}} = 5.47.$

Also $s(x_1^2) - \frac{[s(x_1)]^2}{N_1} + s(x_2^2) - \frac{[s(x_2)]^2}{N_2}$

$= 224.9 - \frac{(35)^2}{6} + 193.75 - \frac{(32.5)^2}{6}$

$= 224.9 - \frac{1225}{6} + 193.75 - \frac{1056.2}{6}$

$= 38.55$

From formula $t = \frac{.42 \times 5.47}{\sqrt{38.55}}$

$= \frac{2.287}{6.2}$

$= .36$

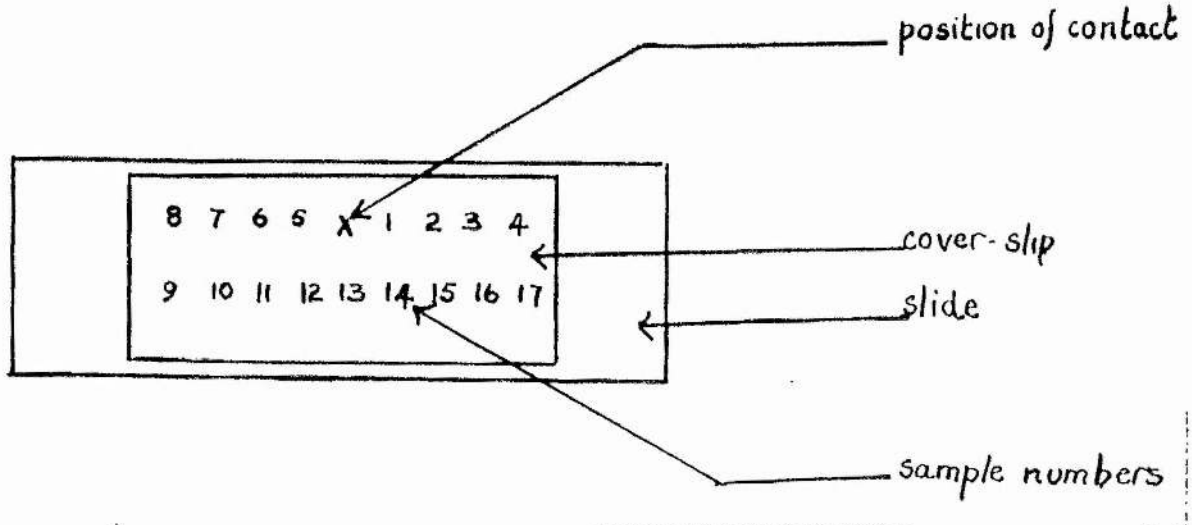
Value for t obtained here is much smaller than value in table 2.228. Fisher and Yates (1953)

$\therefore$ difference is not significant.

d) Slide Matings.

The slides were prepared as has been previously described, and inocula were placed at either end of the cover slip. Slides were incubated at 25°C. and examined daily until hyphae from the two inocula grew together. Three replicates of each combination were used. Growth of hyphae under these conditions was slow, and it was usually ten or twelve days before contact was recorded.

Sampling. The slides were carefully examined under the microscope until fusions between the two inocula were seen when a spot of Indian Ink was placed on the underside of the slide to mark their exact position. Pairings were then incubated for further periods of 4, 8 or 10 hours, as required. After this period the cover-slip was carefully removed leaving as much of the agar film on the slide as possible. Samples were taken on either side of the contact zone at 0.5 cm. intervals.



This was done by scraping away a small amount of agar with a sharply pointed flattened sterile needle, transferring the agar to a test tube slope, and incubating for 48 hours before examining for presence or absence of clamps.

Full details of the scoring are given in the appendix. The average rate of nuclear migration at 25°C. calculated by this method is 0.71 mm./hr.

No evidence has been obtained to suggest that incompatibility factors affect the nuclear migration rate.

The actual rates of nuclear migration recorded in various pairings are given in Table IX.

Table IX.

Total distance of nuclear migration in various pairings.

At 25°C.			At 35°C.		
Pairing	Time after (t_o)	Max. dist. travelled (mm.)	Pairing	Time after (t_o)	Max. dist. travelled (mm.)
4 x 14 4 x 6 4 x 26 10 x 13	4 hrs.	0	4 x 14 4 x 6 4 x 26 10 x 13	4 hrs.	5.00 5.00 5.00
			Ave. distance travelled per hour = 0.92 mm.		
4 x 14 4 x 6 4 x 26 10 x 13	8 hrs.	10.0 5.0 10.0 5.0	4 x 14 4 x 6 4 x 26 10 x 13	8 hrs.	10.0 10.0 15.0 10.0
Average distance travelled per hour = 0.93 mm.			Average distance travelled per hour = 1.4mm.		

Table IX (cont.)

At 25°C.			At 35°C.		
Pairing	Time after (t_o)	Max.dist. travelled (mm.)	Pairing	Time after (t_o)	Max.dist travelle (mm.)
4 x 14	10 hrs.	10.0	4 x 14	10 hrs.	15.0
4 x 6		10.0	4 x 6		10.0
4 x 26		10.0	4 x 26		10.0
10 x 13		10.0	10 x 13		15.0
Average distance travelled per hour = 1.0 mm.			Average distance travelled per hour = 1.22 mm.		

Q_{10} after 8 hours = 1.50

Q_{10} after 10 hours = 1.22

Raper and Snider recorded a Q_{10} of 1.7 for nuclear migration in Schizophyllum commune for the interval 22°C. to 32°C. and from this result draw the conclusion that a chemical rather than a physical process is limiting.

To check this result, and obtain a Q_{10} value for Polystictus versicolor a further series of pairings were incubated at 35°C., and sampled as described previously.

A mean nuclear migration rate at 35°C. of 1.18 ± 0.448 mm./hr. was recorded.

The Q_{10} of nuclear migration for the interval 25°C. to 35°C. was then calculated as follows:-

Mean distance travelled per hour at 35°C. = 1.18 mm. ∓ 0.488

Mean distance travelled per hour at 25°C. = 0.643 mm. ∓ 0.188

$$Q_{10} = \frac{1.18}{0.643}$$

$$= 1.835$$

The monocaryons used in the above experiments were grown in plate culture at 25°C. and 35°C. and their growth rates compared. (See Figs. 8, 9 and 10). The average growth rates for various monocaryotic strains and the Q_{10} of growth is given in Table X.

Table X.

Comparison of growth rates of Polystictus versicolor monocaryons at 25°C. and 35°C.

Strains considered.	Growth rate mm./hr. 25°C.	Growth rate mm./hr. 35°C.	Q_{10} Growth Rate
4	0.5	0.65	1.3
14	0.479	0.50	1.06
6	0.45	0.29	0.64
3	0.56	0.30	0.53
9	0.54	0.52	0.962
10	0.58	0.45	0.776

At 25°C. nuclear migration rate was 0.643 ± 0.188 mm./hr., 1.38 times the growth rate at that temperature.

At 35°C. migration was at the rate of 1.18 ± 0.448 mm./hr., which is 2.62 times larger than the growth rate.

It will be seen from Table X that Q_{10}'s for growth rate are in the majority of cases lower than 1, indicating a physical process is rate limiting.

Figs. 8, 9, 10. Graphs to show the growth rates of monocaryons, measured as increase in diameter of the co lony in cms.

- - - - Growth rate at 25°C.

——— Growth rate at 35 C.

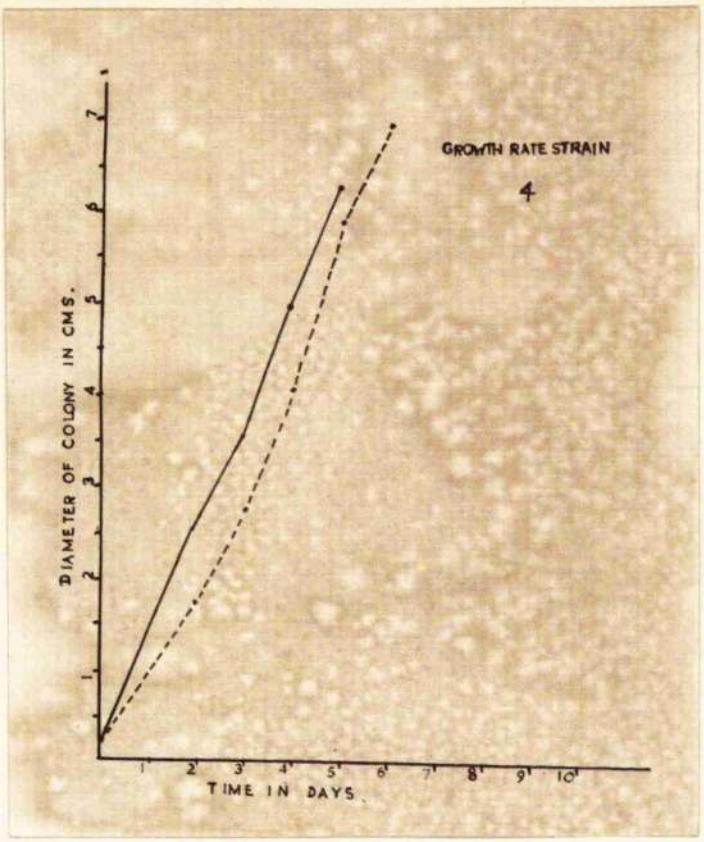
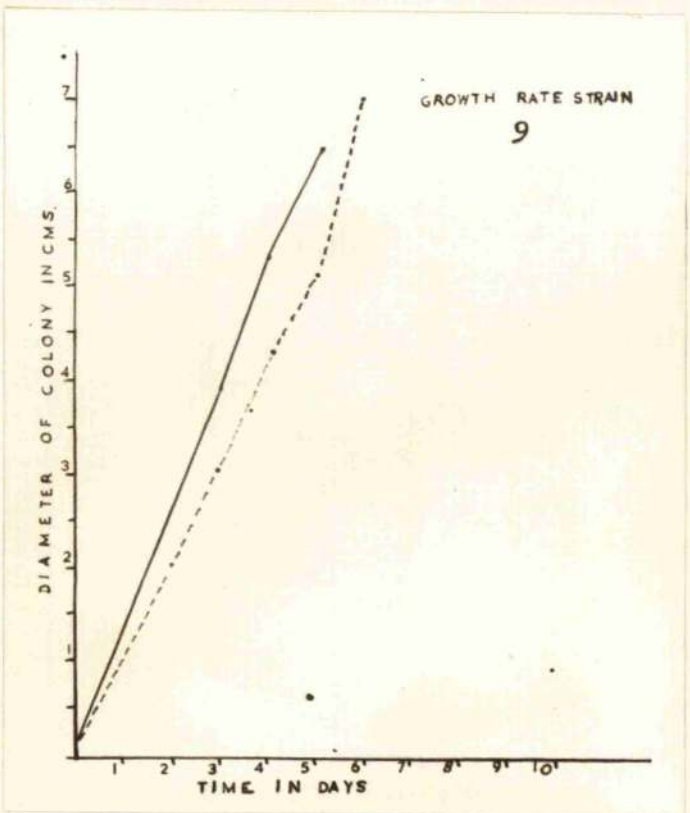

GROWTH RATE STRAIN
4
DIAMETER OF COLONY IN CMS.
TIME IN DAYS

FIG. 8.


GROWTH RATE STRAIN
9
DIAMETER OF COLONY IN CMS.
TIME IN DAYS

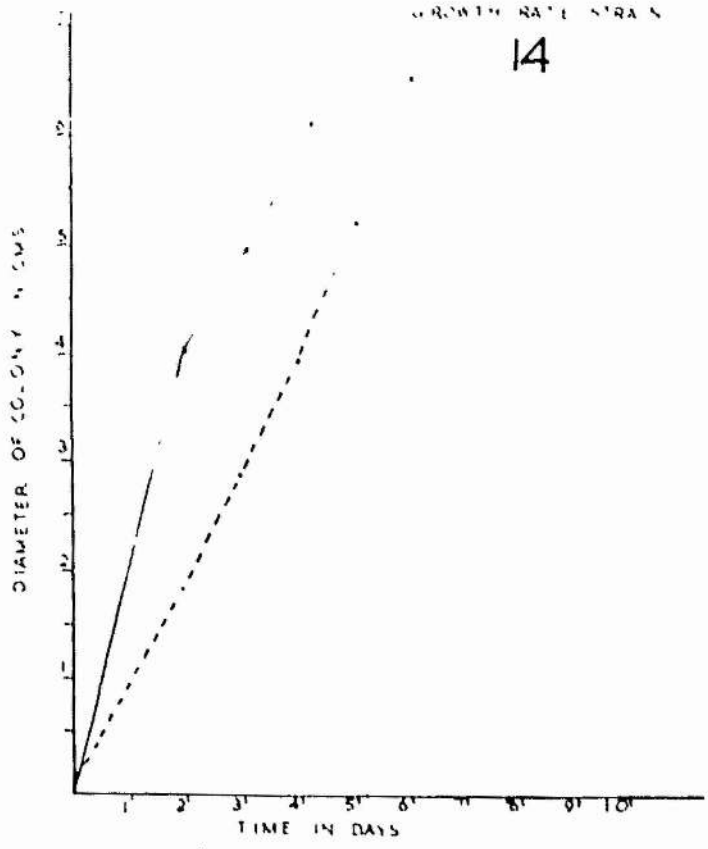
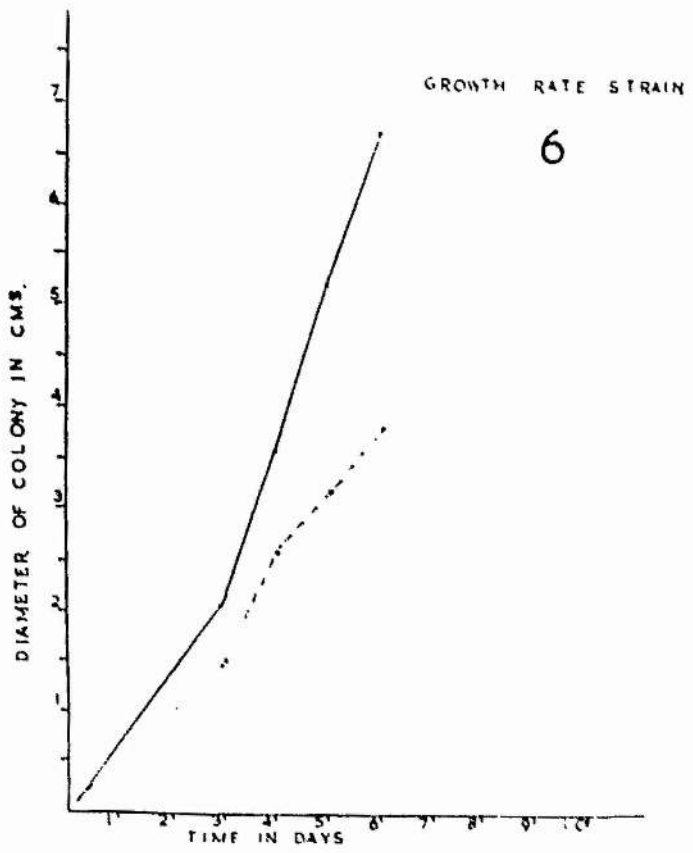

GROWTH RATE STRAIN
14
DIAMETER OF COLONY IN CMS
TIME IN DAYS

FIG. 9.


GROWTH RATE STRAIN
6
DIAMETER OF COLONY IN CMS.
TIME IN DAYS

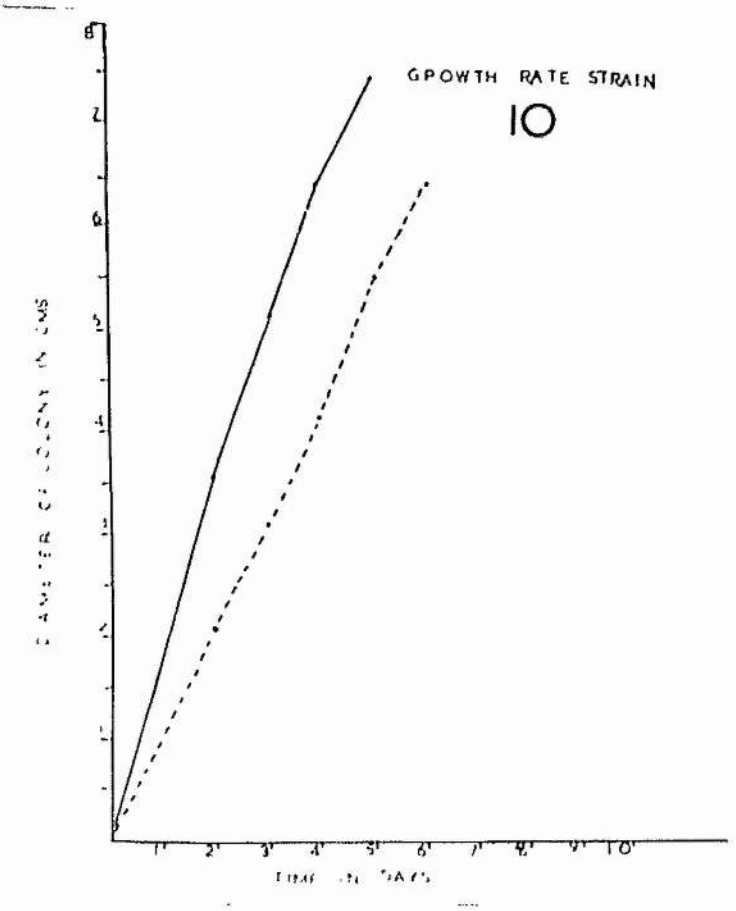

GROWTH RATE STRAIN
10
TIME IN DAYS

FIG. 10.

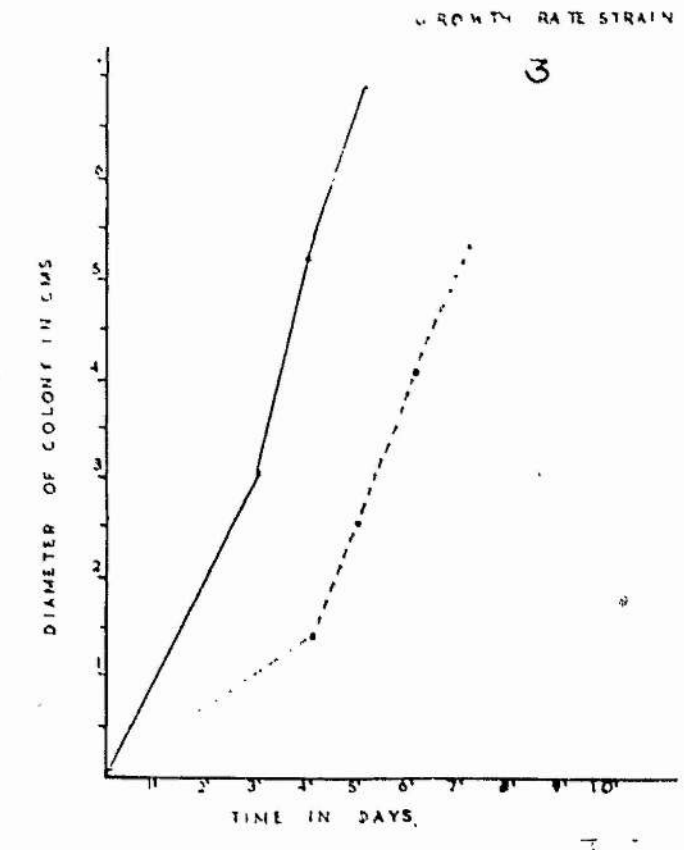

3
DIAMETER OF COLONY IN CMS
TIME IN DAYS

Table XI.

Rates of nuclear migration obtained by different methods.

Polystictus versicolor.

Method	Rate of migration. mm./hr.	Matings studied	Comparison with growth rate.
Large v. small inoculum, plate culture, grid samples.	10	mon. x mon.	16 x
Equal inocula, radial samples I.	5.82	" "	8 x
Equal inocula, radial samples II.	1.75	" "	2 x
Slide matings	25°C. 0.643 35°C. 1.18	" " " "	1.3 x 2.6 x

C. Membrane experiments.

A series of experiments was designed in which various combinations of monocaryons were grown in malt agar, one on either side of a cellophane membrane. The aim of the experiment was to ascertain whether there is any stimulation or inhibition of the growth rate of one monocaryon by the presence of another of

(a) opposite and compatible mating type

(b) incompatible mating type

(c) identical mating type.

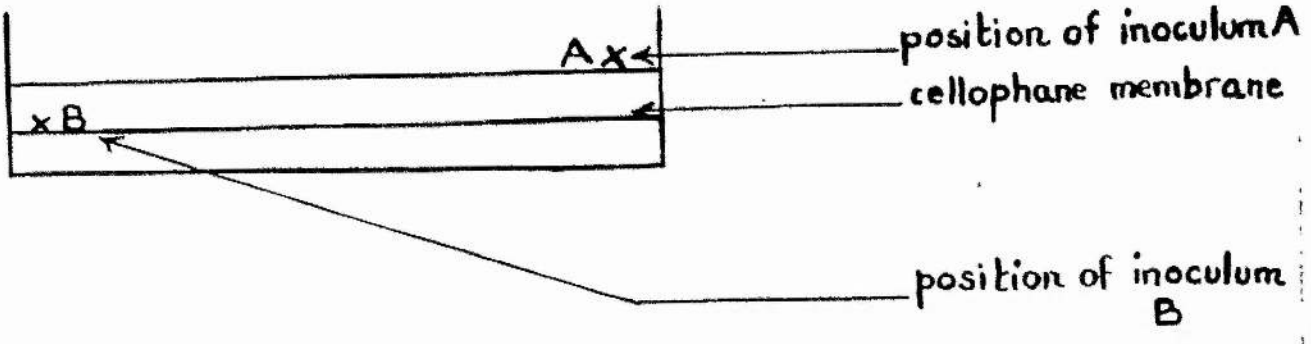
Any chemical substance produced by either monocaryon could diffuse through the permeable cellophane membrane. Physical

stimulus by contact between leading hyphae of the two strains was prevented by the membrane and, therefore, if stimulation or inhibition occurs it must be of a chemical nature.

Using 8 cm. petri dishes a thin layer of agar was poured and allowed to cool. From this layer a small plug was removed with a sterile cork borer, and one of the mycelia was inoculated into the hole. A cellophane disc of the same diameter as the petri dish was placed over the lower layer, and another layer of agar poured over it. The second mycelium was then inoculated into the agar surface.

The growth rates of the two inocula were recorded daily. Particular care was taken when recording growth rates of submerged inocula, and it was found necessary to examine the plate under the dissecting microscope (x 105), and to mark the daily growth increments with wax pencil. Two measurements at right angles to each other were used and their mean taken as the diameter of the colony.

The following Fig. 11 shows the positions of the inocula A and B.



The combinations examined are given in the following table:-

Table XII.

Incompatible Combinations	Compatible Combinations	Identical
4 x 13 A_1B_2 x A_2B_2	7 x 6 A_1B_2 x A_2B_1	4 x 4
6 x 13 A_2B_1 x A_2B_2	7 x 14 A_1B_2 x A_2B_1	6 x 6
14 x 13 A_2B_1 x A_2B_2	3 x 13 A_1B_1 x A_2B_2	13 x 13 3 x 3

Growth rates are shown graphically in Figs. 12 and 13.

The growth rates of the submerged mycelia were in general somewhat lower than those in mycelia grown above the collophane membrane, this is, however, probably due to a decrease in oxygen tension. No stimulatory or inhibitory effect could be discerned which could be attributed to the presence of another mycelium.

Table XIII.

Average growth rates of isolate "6" under various conditions.

Position of Strain 6.	Position of other strain.	Mating-type of other strain (with respect to 6).	Growth rate of 6. (mm./hr.)
Below cellophane disc.	Above cell.disc.	Compatible	0.30 ± 0.10
Above " "	Below " "	Compatible	0.51 ± 0.14
" " "	Below " "	Incompatible	0.46 ± 0.12
Below " "	Above " "	Incompatible	0.32 ± 0.18
Above " "	Below " "	Strain 6	0.49 ± 0.13
Below " "	Above " "	" "	0.33 ± 0.16
Growth rate of 6 in normal plate culture at 25°C.			0.46 ± 0.02

Figs. 12, 13. Graph to show growth rates of monocaryons in "Membrane Experiments".

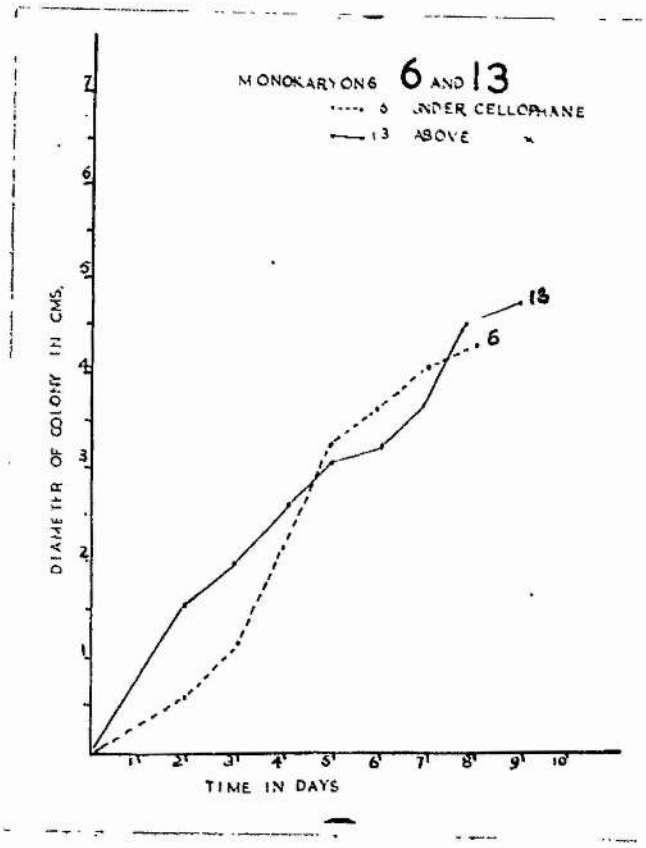
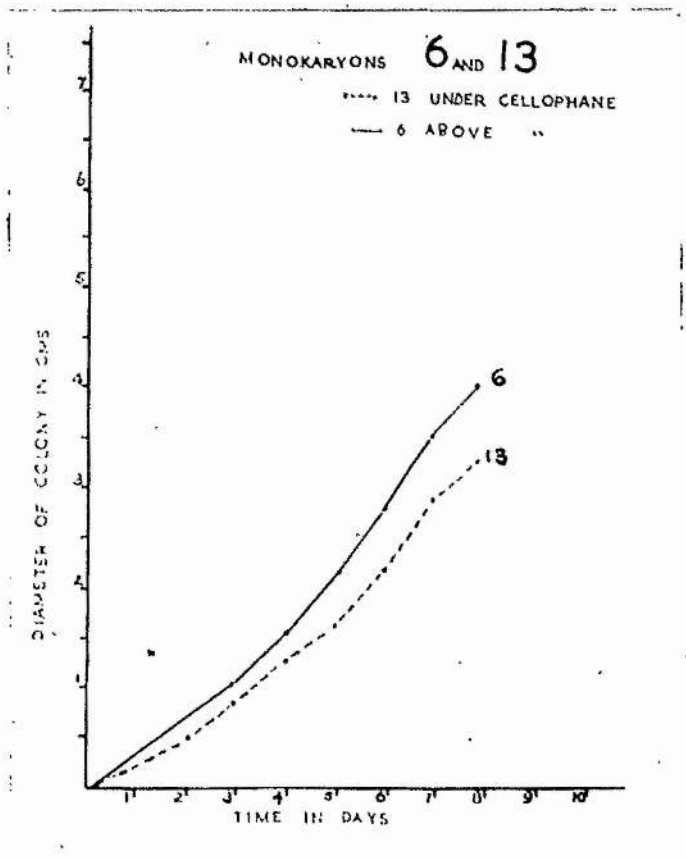

MONOKARYONS 6 AND 13
..... 6 UNDER CELLOPHANE
— 13 ABOVE "
DIAMETER OF COLONY IN CMS.
TIME IN DAYS
13
6

FIG. 12.


MONOKARYONS 6 AND 13
..... 13 UNDER CELLOPHANE
— 6 ABOVE "
DIAMETER OF COLONY IN CMS
TIME IN DAYS
6
13

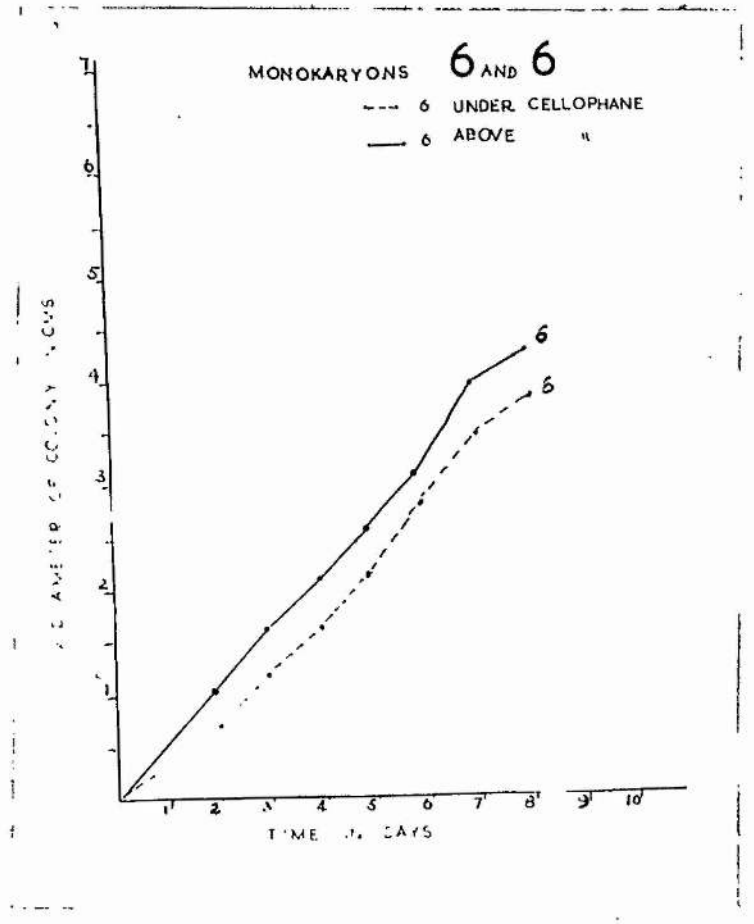
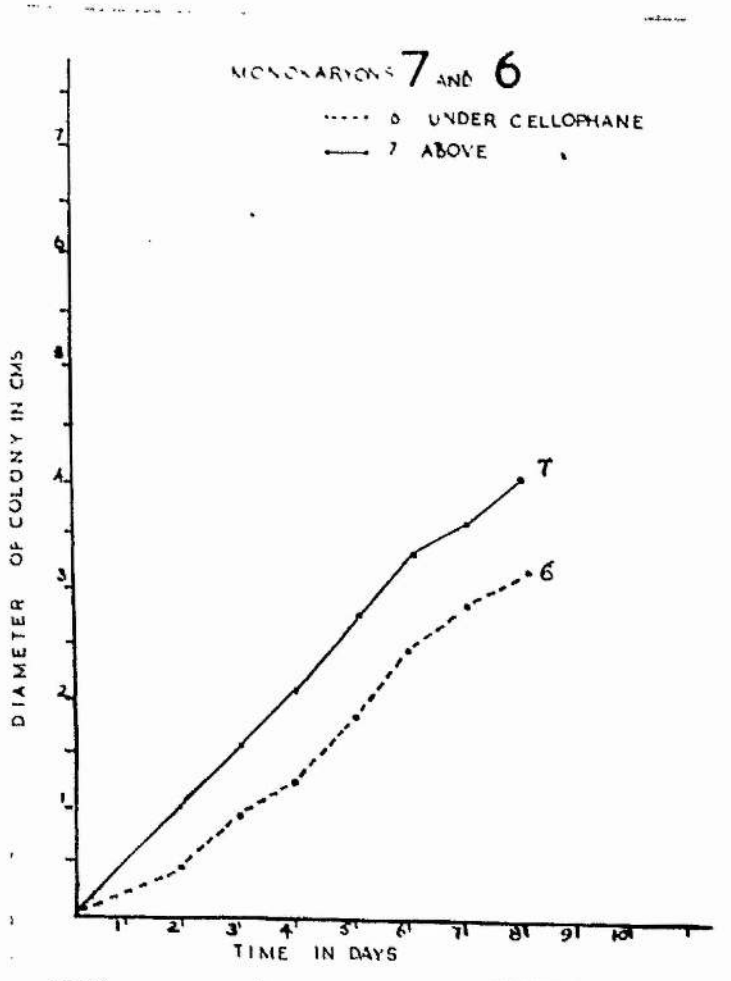

MONOKARYONS 6 AND 6
--- 6 UNDER CELLOPHANE
— 6 ABOVE "
6
6
TIME IN DAYS

FIG. 13.


MONOKARYONS 7 AND 6
6 UNDER CELLOPHANE
7 ABOVE
DIAMETER OF COLONY IN CMS
7
6
TIME IN DAYS

D. Interaction of Incompatibility Factors.

Little data is at present available on the mode of action of the incompatibility factors. It is known that in tetrapolar species when two mycelia having incompatibility factors at "A" and "B" loci meet, a heterocaryotic mycelium the dicaryon is formed.

Stable dicaryons are formed in tetrapolar species between nuclei of the following types:-

a) $A_xB_n + A_yB_m \equiv A_1B_1 + A_2B_2$

b) $A_xB_m * A_yB_n \equiv A_2B_1 + A_1B_2$

In the majority of cases this dicaryon has two nuclei in each cell and forms clamp connections.

There are no readily detectable differences between dicaryon of these two constitutions. Both are equally stable, are apparently produced equally readily, both bear clamp connections and both are capable of producing fruit bodies under suitable conditions.

When examining a series of pairings under the microscope for presence or absence of clamp connections and therefore for presence or absence of the dicaryon no differentiation is made between dicaryons of the two kinds. However, if the number of clamps in $A_xB_nA_yB_m$ matings are counted and compared with the number of clamps formed in $A_xB_mA_yB_n$ pairings distinct differences may be noticed.

In the fungi examined the differences are pronounced in Polystictus versicolor, present in Crucibulum vulgare, but absent in Hypholoma fasciculare.

The data recorded in experiments designed to detect this phenomenon are given below (

The differences in average clamp numbers have been proved to be significant by statistical methods. (see appendix)

Pairings were made in large petri dishes 8 cm. in diameter. Inocula were placed approximately 3 cm. apart, and pairings were incubated at 25°C. for three to four days. By this time the mycelia had grown together and were ready for scoring.

Scoring: The positions in which the samples were taken are shown in Fig. 14. Five samples were taken from each plate, and scored and recorded in the order shown. Each sample was examined under the microscope, and the number of clamps present in each of twenty fields was counted and recorded. Monocaryotic strains used in these experiments were first examined for the occurrence of "false clamps"; none was recorded.

Table XIV gives the combinations of Polystictus versicolor examined, both inter and intra-fruit bodies, e.g. P.V. 14 x 14, and PV 2 x 14, were studied in this way.

The average clamp numbers recorded in counts made on various fruit-bodies are given in Table XV. The Standard Deviations are included for comparison. The histogram Fig. 15 illustrates clearly the persistant occurrence of high average

Fig. 14. Positions of samples as taken in "Clamp Number Experiments."

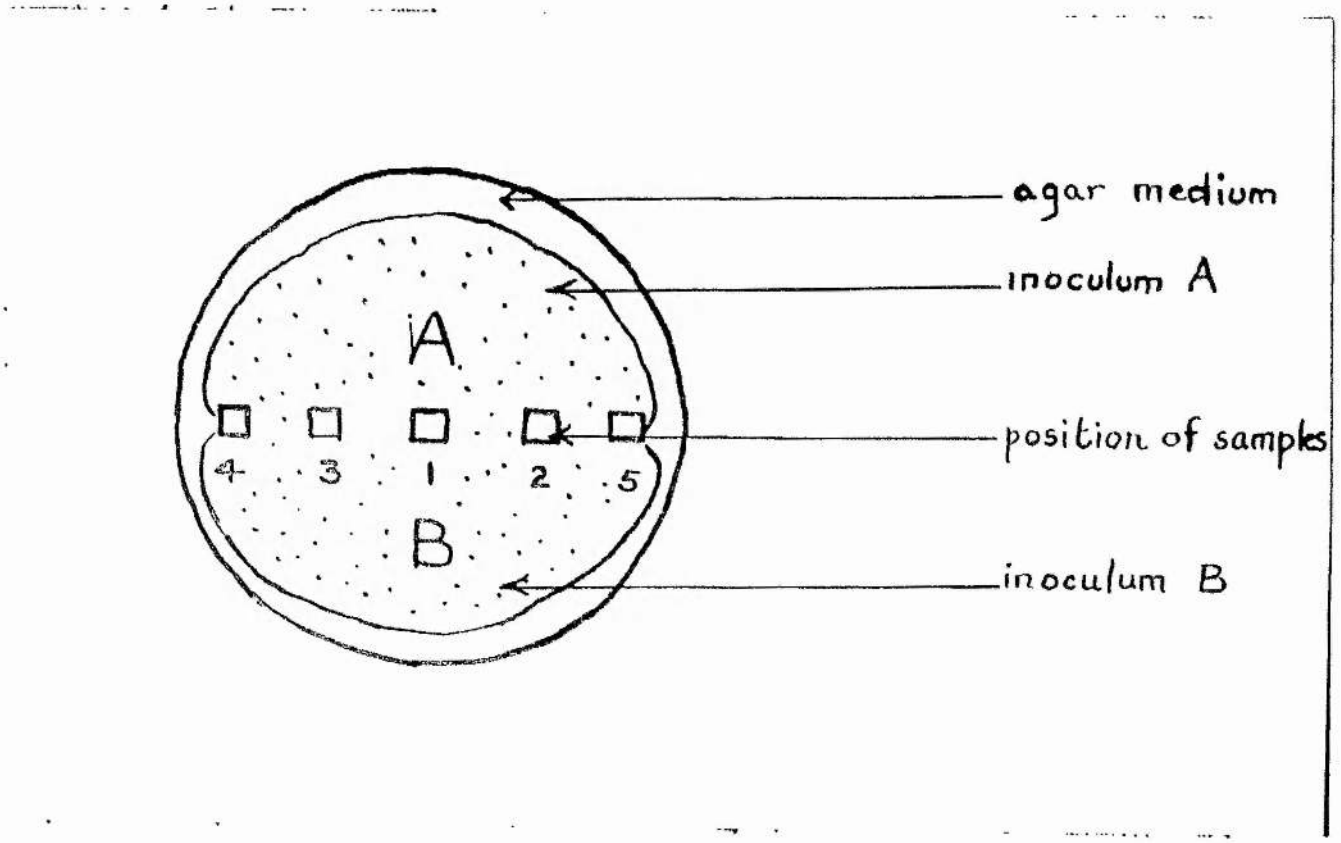

agar medium
inoculum A
A
position of samples
4
3
1
2
5
B
inoculum B

FIG. 14.

Fig. 15. Histogram to show the difference in numbers of clamps recorded in $A_1B_1 \times A_2B_2$ pairings in various fruit bodies as compared with those recorded in $A_2B_1 \times A_1B_2$ pairings.

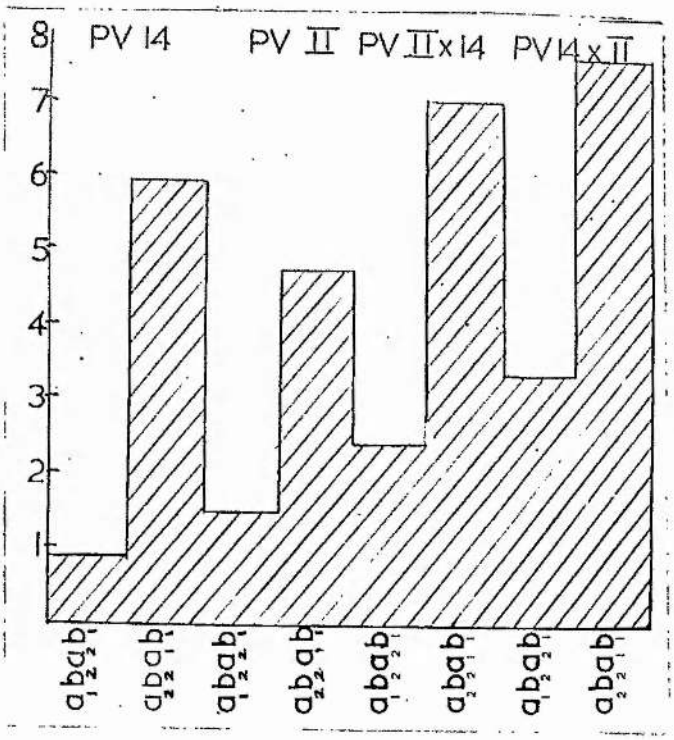


FIG. 15.

clamp numbers in $A_xB_n + A_yB_m$ pairings and the comparatively low numbers recorded in $A_xB_m + A_yB_n$ pairings.

The full figures obtained are given in the appendix, also a statistical treatment of the same.

Hypholoma fasciculare and Crucibulum vulgare were studied in the same way as a comparison.

Table XIV.

Fruit bodies are denoted by the initials of the species and a number; thus P.V. 2 Mating-type factors are denoted A_1: A_2: B_1: B_2.

Polystictus versicolor						*Crucibulum vulgare*		*Hypholoma fasciculare*	
P.V. 14	P.V.14	P.V. 2	P.V.2	P.V.2 x 14	P.V.2 x 14				
$A_1B_2xA_2B_1$	$A_2B_2xA_1B_1$	$A_1B_2xA_2B_1$	$A_2B_2xA_1B_1$	$A_2B_2xA_2B_1$	$A_1B_2xA_2B_1$	$A_1B_1xA_2B_2$	$A_2B_1xA_1B_2$	$A_1B_1xA_2B_2$	$A_2B_1xA_1B_2$
4 x 5	1 x 3	7 x 4	1 x 2	2 x 3	7 x 5	1 x 2	3 x 5	1 x 9	7 x 2
4 x 6	1 x 9	7 x 5	1 x 8	2 x 9	7 x 6	1 x 4	3 x 10	1 x 12	7 x 3
4 x 14	1 x 10	7 x 9	1 x 11	2 x 10	7 x 14	1 x 18	3 x 15	1 x 13	7 x 4
4 x 15	1 x 24	7 x 10		2 x 24	7 x 15	2 x 7	3 x 17	1 x 15	7 x 11
4 x 22	1 x 25	7 x 14		2 x 25	7 x 19	2 x 9			
4 x 26						2 x 16			

Table XV.

Average clamp numbers, Standard Deviation and Significance level.

Fruit Bodies	Combination	Average Clamp Number.	Standard Deviation	Significance level.
P.V. 2	A_1B_1x A_2B_2	5.17	21.76	5% and 1%
P.V. 2	A_1B_2x A_2B_1	1.278	18.98	
P.V. 14	A_1B_1x A_2B_2	5.9	29.05	5% and 1%
P.V. 14	A_1B_2x A_2B_1	0.446	6.92	
P.V.2 x 14	A_1B_1x A_2B_2	7.662	22.6	5% and 1%
P.V.2 x 14	A_1B_2x A_2B_1	3.228	15.36	
P.V.14 x 2	A_1B_1 xA_2B_2	8.185	7.81	5% and 1%
P.V.14 x 2	A_1B_2x A_2B_1	1.49	5.718	

E. Nature of the Mycelium.

a) Wood Sampling.

When fruit bodies were collected from Tentsmuir a piece of the wood on which they were growing was also brought into the laboratory. Small pieces of wood were taken from the places marked in Fig. 18 and by means of a sterile needle were transferred to sterile agar plates. The mycelium was allowed to grow out from the wood samples and after three days was examined under the microscope for presence or absence of clamps. All samples

were found to have produced dicaryotic mycelia.

b) Fusion Experiments.

Several fruit bodies were collected from the same tree and when analysed they were found to carry different combinations of a limited number of mating type factors. This suggested that mycelial infections in the tree, originally distinct from each other, might, by hyphal fusions, become combined to form a single physiological unit.

Fusions of this nature have been described by Miss Saunders as occurring between dicaryons and monocaryons, between two dicaryons and between two monocaryons in plate cultures of Polyporus betulinus. The successful fusions are listed in Table XVI. Fusions between genetically identical and genetically dissimilar mycelia were obtained.

Table XVI.

Combinations in which fusions were obtained.

a) Saunders (1956). Bipolar Polyporus betulinus

Dicaryons	Monocaryons	Dicaryons & monocaryons
1.2 x 1.3	1 x 3 x 4	1.4 x 3
1.2 x 2.3		1.4 x 1
1.2 x 3.4		1.4 x 4
1.2 x 1.2		

b) Partington (1958). Tetrapolar Polystictus versicolor

1.2:1.2x4.6:4.6	2.2x1.1	1.1(1.1 + 2.2)
1.2:1.2x5.6:5.6	1.2x1.2	1.2(1.2 + 2.1)
1.2:1.2x1.6:1.6		2.2(1.1 + 2.2)
1.1:2.2x3.4:3.4		6.6(1.1 + 2.2)
5.6:5.6x 1.6:1.6		
6.6:6.6x3.4:3.4		
5.6:5.6x4.6:4.6		

Fusions between dicaryons, in plate culture of other Polyporaceae including Polystictus versicolor have been observed by Robak (1942) and Cabral (1951), but without knowledge of the genetic constitution the mycelia involved.

In the work described below fusions between dicaryons and monocaryons of Polystictus versicolor of known mating-type were studied.

Both Robak and Cabral used plain agar as a medium in their studies and it was used in this work for reasons previously given. The method of preparing the slides for inoculation has been described in the section on "Methods". Inocula were placed at either end of the cover slip (2 x $\frac{7}{8}$") and the slides incubated for four to five days: after this period the two inocula had grown together and a microscopical examination was then made to determine whether or not fusions had occurred.

The combinations studied are listed in Table XVI. Mating-type factors carried by the mycelia considered are recorded thus: e.g. $A_{1.2}B_{1.2}$

c) Blending Experiments.

Rawitscher (1933) in his review suggested that diploidisation in a combination/

A_1B_2 (A_1B_1 x A_2B_2) is effected by both the nuclei A_1B_1 and A_2B_2 moving from the diploid mycelium into the haploid mycelium, and there taking possession of the cytoplasm. This explanation has been favoured by **Buller** (1931), Chow (1934), Dickson (1934) and Oikawa (1939).

Quintanilla (1939), however, disagrees with this explanation and postulates that in a di. x mon. mating such as A_1B_2(A_1B_1 x A_2B_2) conjugate nuclei for the A_1B_2 nucleus are formed by chromosome exchange between the A_1B_1 and A_2B_2 nuclei. This produces a nucleus of the constitution A_2B_1 which can then form conjugate pairs with the A_1B_2 nucleus originally present and diploidise the mycelium.

Kimura (1958) in an extensive survey of the reactions occurring between mycelia in illegitimate diploidisations in Coprinus macrorhizus f. microsporus found that both nuclei of the diploid mycelium migrated into the haploid mycelium, with one exception in which a recombinant nucleus was recorded.

Papazian (1950), working on Schizophyllum commune found from thirteen different illegitimate combinations, six instances supporting Rawitscher's hypothesis and two in favour of Quintanilla's hypothesis.

There is no available data on diploidisation in di. x mon. pairings in Polystictus versicolor; in order to obtain such data the following series of experiments was carried out.

When a di. x mon. pairing, e.g. $A_1B_1(A_1B_2 \times A_2B_1)$ is allowed to fruit and the basidiospores thus formed are analysed with regard to their mating-type factors, all four spore types are recoverable in the progeny (see Fig.16). If, however, the mycelium is not allowed to fruit, and after diploidisation has taken place, the resultant mycelium is split into its component monocaryons by means of a "blending" technique, recombination cannot occur. The monocaryons recovered after blending will be the same as those present in the original pairing and if somatic crossing over has taken place it will be detected by the presence of the fourth type of nucleus, in this case A_2B_2.

Miles and Raper (1956), in studies on the "splitting" of dicaryons in fungi which do not produce oidia, grew them on media containing sodium taurocholate or cholic acid which, they state, has a pronounced "splitting" effect, and hence nuclei of both homocaryotic types can be recovered easily.

This technique was used as follows to "split" the diploidised mycelium in illegitimate di. x mon. matings.

The cultures for blending were grown on liquid medium the composition of which is given in the appendix (Raper & San Antonio, 1954). Control flasks contained the basic medium; sodium taurocholate up to 0.15% was added to a second series of flasks. The blending technique used is described in the section on "Methods". The material for blending was prepared as follows.

Fig. 16. The mating-type constitutions of monocaryons isolated by a) Fruiting
b) Blending
a di. x mon. pairing of the constitution
A_1B_1 (A_2B_1 x A_1B_2)

A monocaryon of known mating type was inoculated onto a malt agar plate and allowed to grow for four days. A small dicaryotic mycelium, also of known mating type, was then inoculated in position B, and the plates incubated until dicaryotisation had occurred. A sample of the dicaryotised mycelium was then taken from position C and inoculated onto liquid medium in 250 cc. Erlenmeyer flasks. This mycelium was then allowed to grow for periods of up to three weeks.

The combinations treated in this way are given in Table XVII.

Table XVII.

Using monocaryons from P.V. 2.

Combination.	Mating type.
(4 x 12) 3	(A_1B_2 x A_2B_1) A_1B_1
(4 x 13) 3	" " "
(4 x 15) 3	" " "
(4 x 12) 1	" " "
(4 x 13) 1	" " "
(4 x 15) 1	" " "

The results of blending these combinations after

a) Growth on a standard or basic medium

b) Growth on the basic medium with the addition of 0.15% sodium taurocholate,

are given in Table XVIII.

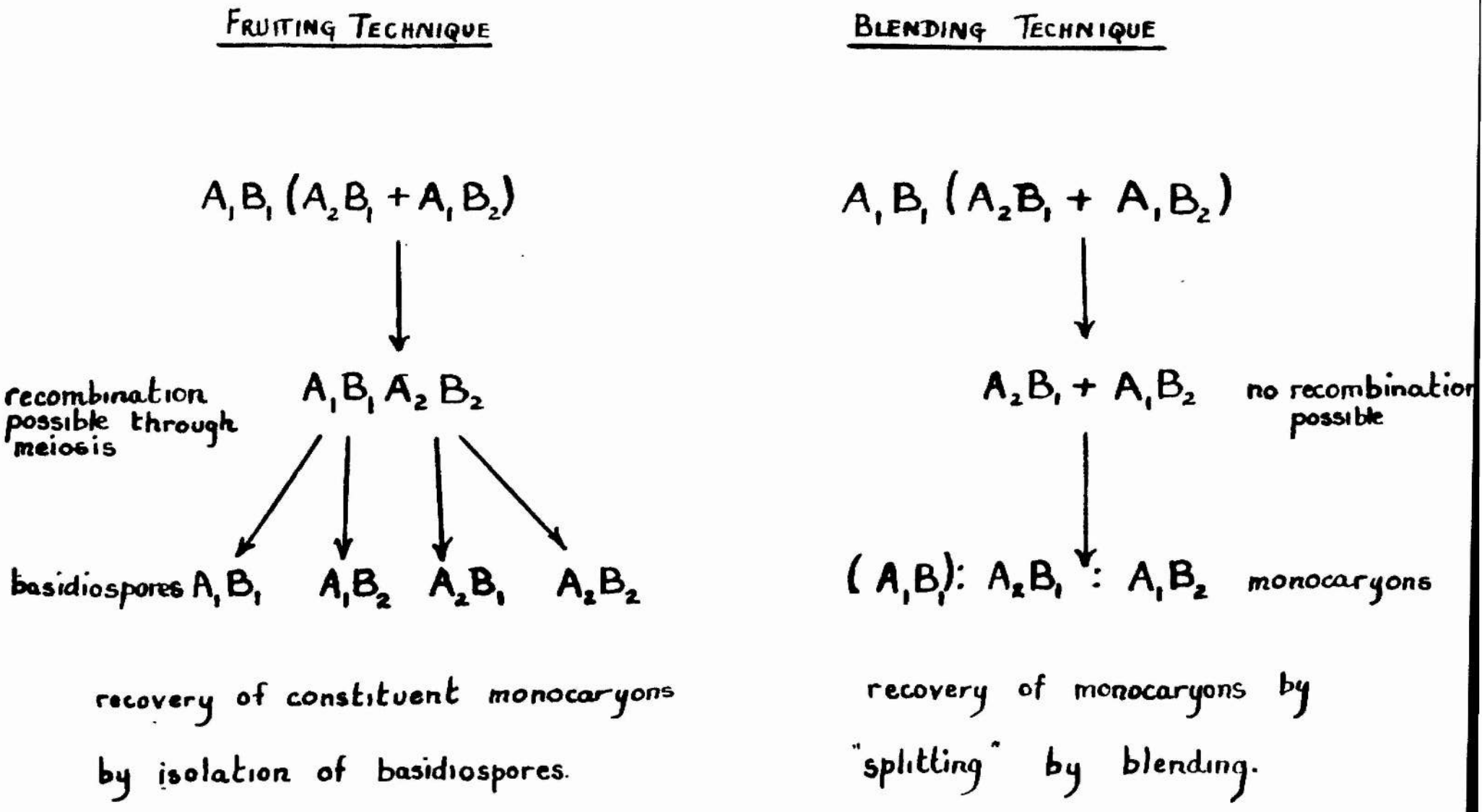

FRUITING TECHNIQUE
BLENDING TECHNIQUE
A1B1 (A2B1 + A1B2)
A1B1 (A2B1 + A1B2)
recombination possible through meiosis
A1B1 A2B2
A2B1 + A1B2
no recombination possible
basidiospores A1B1 A1B2 A2B1 A2B2
(A1B1): A2B1 : A1B2 monocaryons
recovery of constituent monocaryons by isolation of basidiospores.
recovery of monocaryons by "splitting" by blending.

FIG. 16.

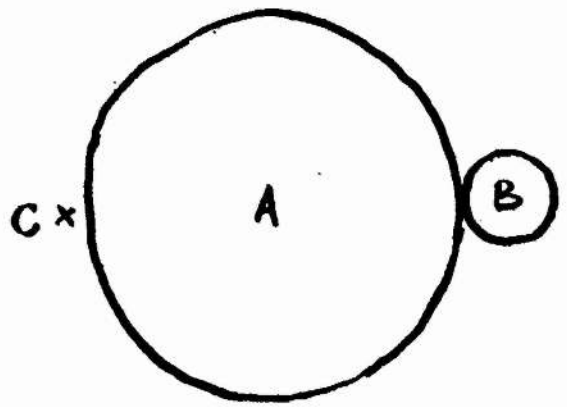


Fig. 16a. Position from which hyphae were taken for growth in liquid culture for Blending experiments.

A - large haploid inoculum
B- small diploid inoculum
C - position from which hyphae were taken

Table XVIII.

Combination Blended	No. of mono./ 100 Basic media	No. of mono./ 100 Basic media + Na taurocholate	Mating-types recovered Basic		Basic + Na tauro.	
			A_1B_2	A_2B_1	A_1B_2	A_2B_1
(4 x 12) 1	7	24	11	13	0	7
(4 x 13) 1	7	28	12	16	3	4
(4 x 15) 1	10	20	8	12	8	2
(4 x 13) 3	6	22	20	2	5	1

The mating types recovered in the blending experiments were identified in the following way. Each monocaryotic isolate was paired with monocaryons from the parent fruit body carrying each of the four possible combinations of the mating-type factors. An example of these pairings is given in Table XIX.

Table XIX.

Haploids	A_1B_1	A_2B_2	A_2B_1	A_1B_2
1	-	-	+	-
2	-	-	+	-
3	-	-	+	-
4	-	-	-	+
5	-	-	-	+
6	-	-	+	-
7	-	-	+	-
8	-	-	-	+
9	-	-	-	+
10	-	-	+	-

Calculation of the dicaryon splitting coefficient.

$$\text{Dicaryon Stability Coefficient} = \frac{\text{No. of dicaryons}}{\text{No. of isolates.}}$$

$$\text{"splitting coefficient"} = 100 \left(1 - \frac{\text{Dicaryon stab. coef. Na taur}}{\text{Dicaryon stab. coef.}} \right)$$

$$= 100 \left(1 - \frac{24}{100} \times \frac{100}{93} \right)$$

$$= 100 \left(1 - \frac{24}{93} \right)$$

$$= 100 \times \frac{69}{93}$$

$$= \underline{75.2}$$

The figure obtained in the previous calculation is of the same order as that obtained by Raper for Polystictus versicolor. He records a splitting coefficient of 89. "Splitting" coefficients given for other fungi treated with sodium tauro-cholate vary from 83.5 recorded for Pleurotus ostreatus to 100 for Polyporus betulinus.

In these experiments only monocaryotic fragments of the constitutions A_1B_2 and A_2B_1 were isolated from the blended material. The absence of fragments bearing the mating-type factors A_1B_1 and A_2B_2 points in favour of Rawitscher's (1933) hypothesis i.e. that both nuclei of the diploid mycelium migrate into the haploid mycelium. The evidence presented here cannot, however, be considered as conclusive.

F. Spatial Distribution of the Mating-Type Factors.

In this series of experiments the following notation was employed.

Each mating-type factor as identified was assigned a number and a letter. The constitution of the sporophore of a tetrapolar species can be generally represented as $A_{n:m}$ $B_{x:y}$ (mating-factors at two loci, A and B). The pair of compatible monocaryons from fruit body B_4 would be given the notation $B_{4/1}$ and $B_{4/4}$.

a) Intra population studies in Polystictus versicolor.

The number of fruit bodies sampled, their habitat and location have been recorded previously. The data will be given again here for convenience.

Table XX.

Date of Collection	No. of fruit bodies sampled	Location.
October, 1956.	12 f.b.	Ladebraes
November, 1956	2	Dyersbrae
October, 1957	2	Ladebraes
November, 1958.	7 (2 stumps)	Tentsmuir.

Total number of fruit bodies collected = 23.

The sites from which the Tentsmuir and Ladebraes samples were taken, and the distribution of the mating-type factors within the samples are illustrated in Figs. 17 and 18.

Fig. 17. Spatial distibution of mating-type factors in fruit bodies of Polystictus versicolor(L). sampled from Ladebraes, St. Andrews.

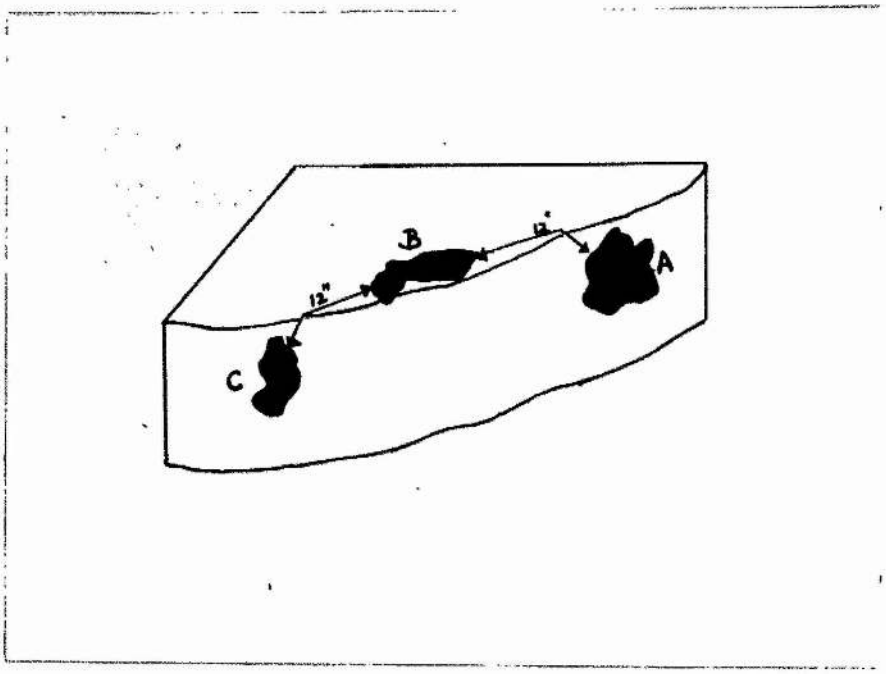

B
12"
12"
A
C

FIG. 17.

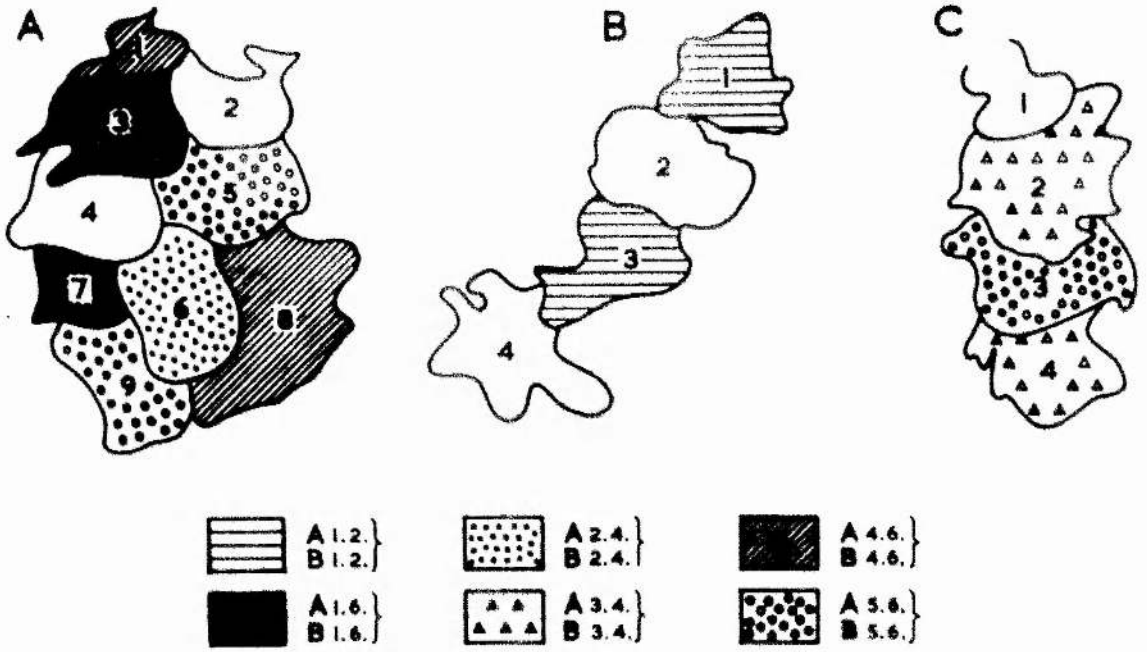

A
1
2
3
4
5
6
7
8
9
B
1
2
3
4
C
1
2
3
4
A 1.2.
B 1.2.
A 2.4.
B 2.4.
A 4.6.
B 4.6.
A 1.6.
B 1.6.
A 3.4.
B 3.4.
A 5.6.
B 5.6.

Fig. 18. Spatial distribution of mating-type factors in fruit bodie s of Polystictus versicolor(L.) sampled from Tentsmuir, Fife.

* indicates position from which Wood samples were taken.

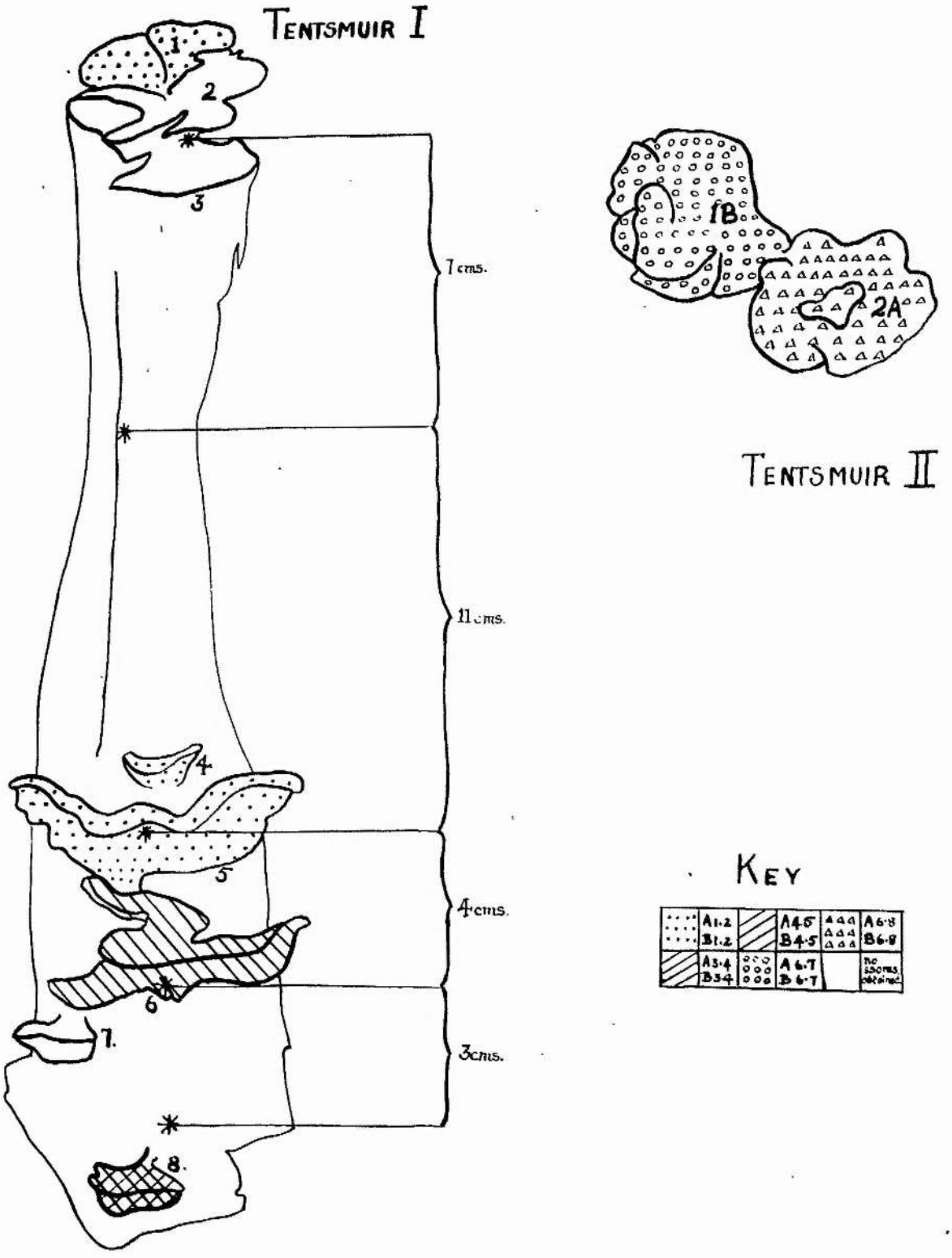

TENTSMUIR I
1
2
3
7 cms.
11 cms.
4
5
4 cms.
6
7
3 cms.
8
1B
2A
TENTSMUIR II
KEY
A1-2
B1-2
A4-5
B4-5
A6-8
B6-8
A3-4
B3-4
A6-7
B6-7
No scores obtained

FIG. 18.

To obtain these results, one pair of compatible monocaryons was isolated from one fruit body in the sample, and used as testers. These were paired with testers derived from each of the other fruit bodies in the same way. Thus the mating-type factors in each of the fruit bodies were identified.

Spores from fourteen fruit bodies were sampled from the Ladebraes population, in two collections 1956 and 1957. They were found to carry eight different alleles at each locus and were numbered 1 to 8. In the Tentsmuir population eight different alleles were recorded from the seven fruit bodies sampled. In the two fruit bodies from Dyersbrae four alleles at each locus were recorded.

In collecting Polystictus versicolor adjacent fruit bodies were sampled, and after studying the distribution of mating-types within these samples the following points emerge:-

(a) It is a common occurrence for fruit bodies from the same tree to carry different mating type factors.

(b) Adjacent fruit bodies often carry different mating-type factors.

(c) Several fruit bodies on the same tree carry the same mating-type, but in different combinations, which suggests that there is a limited number of allelomorphs present in the mycelium within the stump.

(d) The groups of mating-types appear to be different in each stump.

Ladebraes Isolates	Tentsmuir Isolates									
	A4/1	A4/3	A6/1	A6/6	1B/1	1B/9	2A/1	2A/6	A5/8	A5/1
C2/2	+	+	+	+	+	+	+	+	+	+
C2/7	+	+	+	+	+	+	+	+	+	+
A5/1	+	+	+	+	+	+	+	+	+	+
A5/6	+	+	+	+	+	+	+	+	+	+
A7/1	+	+	+	+	+	+	+	+	+	+
A7/7	+	+	+	+	+	+	+	+	+	+
A1/1	+	+	+	+	+	+	+	+	+	+
A1/3	+	+	+	+	+	+	+	+	+	+
B4/1	+	+	+	+	+	+	+	+	+	+
B4/6	+	+	+	+	+	+	+	+	+	+

TABLE XXI

b) Inter population studies.

Tester mycelia containing each of the eight alleles recorded in the Ladebraes population were paired in all combinations with monocaryons carrying the eight factors from the Tentsmuir population. No common alleles were recorded. The four alleles identified in the Dyersbrae population were found to be different from those in either the Tentsmuir or the Ladebraes samples. The total number of mating-types identified in the three areas sampled was twenty out of a possible total of forty-six from twenty-three fruit bodies.

Cross matings between Ladebraes and Tentsmuir isolates are given in Table XXI.

c) Other fungi sampled.

Two other basidiomycete fungi were studied in the same way. They were Hypholoma fasciculare and Collybia velutipes.

A group of seven fruit bodies of Collybia velutipes, all apparently arising from the same stock was sampled. The fruit bodies were all found to carry the same mating-type alleles.

Two collections of Hypholoma fasciculare were made. All fruit bodies were collected from the same area, the first sample being taken on September 18th, the second some four days later. Mating-type factors proved to be identical in each of the fruit bodies sampled within the two groups, and also between the two samples taken on different days. The two crops of fruit bodies were probably produced by fructification of the same mycelium. A diagram showing the spatial distribution of the two collections is given in Fig. 19.

Fig. 19. Spatial distribution of mating-type factors in fruit bodies of Hypholoma fasciculare sampled from the University Botanic Gardens St, Andrews.

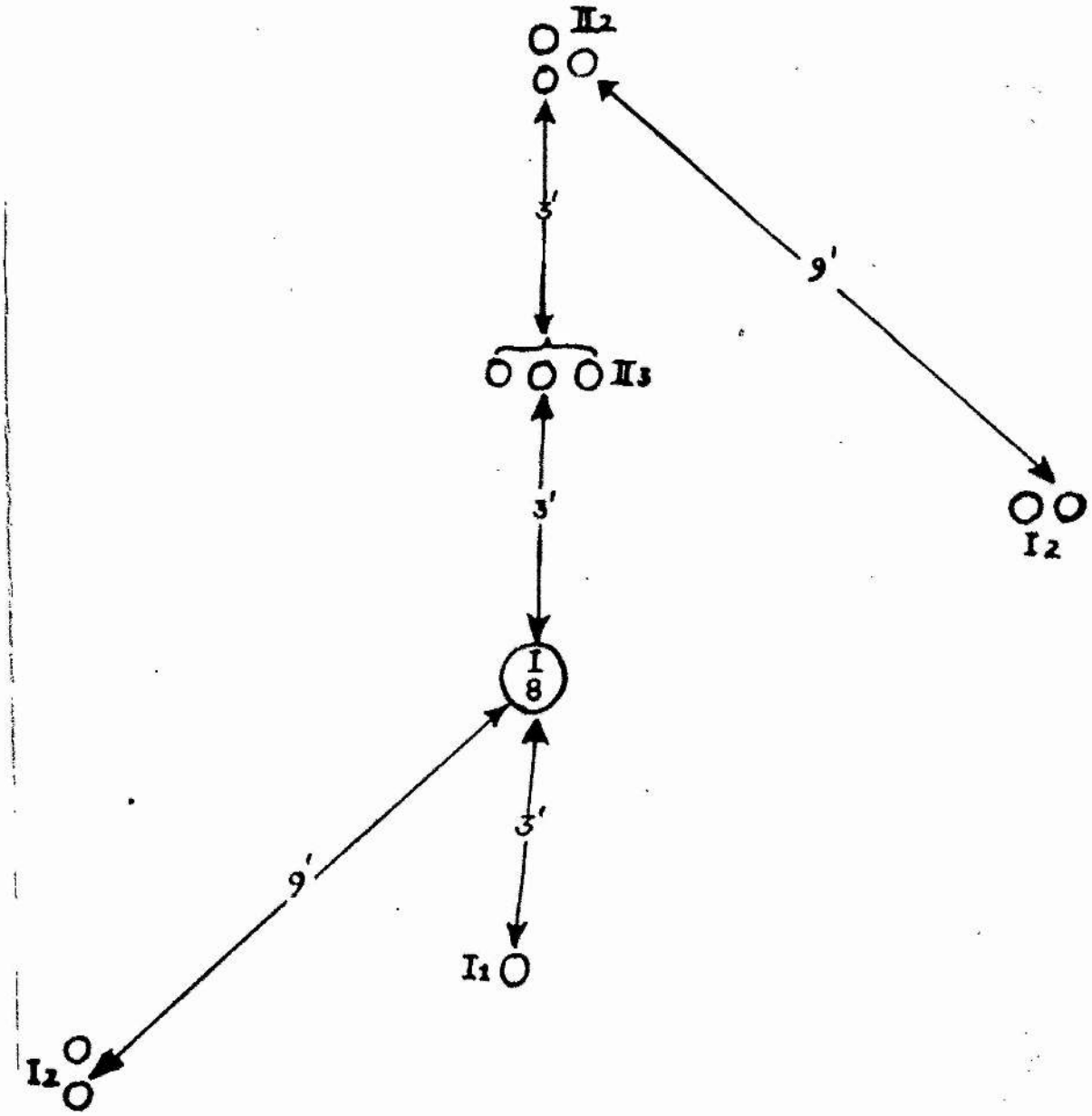


FIG. 19.

Table XXII.

Summary of the numbers of mating type factors recorded in various species of Hymenomycetes.

Author	Species	No. of fruit bodies sampled	Max. no. factors possible.		No. of factors recorded	
Brunswick, 1924	Coprinus comatus	5½	11		9	
Mounce & Macrae, 1937	Fomes roseus	5	10		9	
Mounce & Macrae, 1937	Fomes subroseus	10	20		20	
Barnett, 1937	Auricularia auricula judae	5	10		10	
Saunders, 1956.	Polyporus betulinus	57½	115		26	
Brunswick, 1924	Coprinus fimentarious	13½	27	27	27	27
Hanna	Coprinus lagopus	7	14	14	14	14
Fries & Jonassen, 1941	Polyporus abietinus	14	28	28	28	28
Roschal, 1950	Schizophyllum commune	12	24	24	24	24
Kimura, 1952	Coprinus macrorhizus f. microsporus	10	20	20	19	19
		5	10	10	9	9
Eggertson, 1953.	Polyporus obtusus	24	48	48	39	39
Partington, 1958.	Polystictus versicolor	23	46	46	20	20
Raper et al. 1958.	Schizophyllum commune	114	228	228	96	56
By calculation total possible factors in natural population					339	64
					within 5% limits	

VI. DISCUSSION

A. The number and Distribution of Mating-Type Factors.

Sexual reproduction in the Higher Basidiomycetes was first reported by Bensaude (1917) and later by Kniep (1920, 1922). It was shown that some species of Hymenomycetes were heterothallic and that in these compatibility was controlled by a series of multiple alleles, either at one locus (bipolar forms) or at two loci (tetrapolar forms); other species were shown by Kniep to be homothallic. Since that time the mating systems in more than 200 species of Basidiomycetes have been investigated, these are mostly listed by Whitehouse (1949b) and by Quintanhilha and Pinto-Lopes (1950).

The significance of heterothallism as an out-breeding mechanism was first pointed out by Mather (1942) and later elaborated by Whitehouse (1949b). The latter estimated the efficiency of bipolar and tetrapolar heterothallism as out-breeding mechanisms by calculations based on the total possible number of alleles in the population, 'N' and the number 'n' recorded in a sample of 'r' fruit-bodies, on the presumption that all alleles occur in the population with equal frequency.

Whitehouse also summarised the data available on the number of mating-type factors present at the A and B loci. The numbers of fruit-bodies sampled, on which Whitehouse

based his calculations varied from 5 - 14, and in all cases the number of alleles recorded was very close to the maximum possible number. Whitehouse concluded that "the number of allelomorphs at the loci for heterothallism in natural populations of Hymenomycetes and Gasteromycetes is of the order of 100 per locus". All collections made previous to 1949, are of small numbers of fruit-bodies from a wide area.

Since then reports have been published on larger samples but results can be most conveniently reviewed on a basis of the size of area sampled. The data then fall into two groups.

a) Those in which fruit-bodies were collected over a wide area.

b) Those in which local populations of varying size were sampled.

a) Samples from a wide area

Brunswick (1924) collected fruit bodies of Coprinus fimentarius from places as far apart as Berlin, Hamburg and Vienna, which are separated by hundreds of miles. He sampled 13½ fruit bodies and recorded 27 allelomorphs at each of the A and B loci, the maximum possible number. Likewise, Hanna's (1925) collection of Coprinus lagopus, Fr. Bull. included Canadian fruit bodies from hundreds of miles apart as well as one from England. Again, the maximum possible number of alleles were recorded.

The specimens of Fomes roseus and Fomes subroseus studied by Mounce and Macrae (1937) were collected from Quebec, Ontario, New York, British Columbia, Oregon, and New Brunswick, the distances between these places ranging from about three hundred to over one thousand miles. Fries and Jonasson (1941) sampled fourteen fruit bodies of Polyporus abietinius; the two groups analysed came from areas separated by some two hundred miles although each group was collected within a relatively restricted area. Fourteen fruit bodies were sampled, which is the largest number examined by any of the workers considered by Whitehouse. Out of a possible total of 28 alleles at the A and B loci, 23 and 26 were recorded respectively.

The most recent evidence on numbers of mating-type factors per locus has been given by Raper et al.(1958). In this study fruit-bodies collected from stations all over the world were analysed with regard to their mating-type factor constitution. "114 homokaryotic strains were analysed and the sample contained 96 distinct interfertile A factors and 56 different B factors. Assuming random distribution and equal frequency of factors of both series, these data indicate

399 with 5% limits of 562 and 216 A factors

and 64 with 5% limits of 79 and 53 B factors, in the natural population."

b) Samples from localised areas.

Eggertson (1953) working on an isolated population of the tetrapolar fungus Polyporus obtusus, identified 39 factors at each of the A and B loci, out of a possible total of 48. He does not give any indication of the total size of the population from which the samples were taken, but it may be concluded that it was confined to a fairly restricted area since he described the infection as a local occurrence.

Roschal (1950) in an examination of twelve fruit bodies of Schizophyllum commune all collected within a 10 acre tract of forest at Lake Geneva, Wisconsin, found 23 A and 21 B factors out of the possible 24 at each locus.

In a survey of the number of mating type factors in the bipolar fungus Polyporus betulinus in the British Isles (Saunders, 1956) three collections were made.

1. Thirty-three fruit bodies were sampled from a local population at Freshfield, Lancs. and nineteen different alleles were identified.

2. Twenty-nine fruit-bodies were sampled from Stravithie, Fife, from which sixteen alleles were isolated.

3. Eighteen fruit-bodies were collected from various parts of the country in a survey collection and from these, five new alleles were identified. The number of fruit bodies sampled in the total collection was 81, but 57½ is the

number quoted. This is because certain trees either had more than one fruit-body carrying identical mating-types, or two or more fruit-bodies contained one mating-type factor in common. To allow for the possibility that such fruit-bodies had not come from independent mycelia, Saunders calculated the number of fruit-bodies per tree as half the total number of mating-type factors found in that tree. Thus from 57½ fruit-bodies sampled, only 26 different mating-type factors were identified, and almost half of these were found to be common to all three collections, i.e. 12 out of 26. If, however, Miss Saunders' assumptions were incorrect, then there would be 26 alleles recorded out of a possible total of 160.

The samples of Polyporus betulinus should be sufficient to demonstrate the number of mating-type factors per locus throughout the range of this fungus, if one accepts the premise of Whitehouse that fruit-bodies sampled from a small area may be expected to carry as many mating-type factors as the same number sampled from a large area. In this case the postulate that there are of the order of 100 alleles per locus appears to be invalid. Application of Whitehouse's (1949b) formula indicates that there are about 26 alleles in the natural population of Polyporus betulinus, i.e; N = 26

c) Results of sampling local populations of Polystictus versicolor.(L.)

In the present investigation four local populations of Polystictus versicolor were sampled. Spores from fourteen fruit-bodies from Ladebraes in two collections 1956 and 1957, from seven fruit-bodies from Tentsmuir 1958, and from two fruit-bodies from Dyersbrae 1956 were analysed. The total number of alleles identified was twenty at each of the A and B loci out of a possible forty-six from twenty-three fruit bodies, and from the Whitehouse (1949b) formula the total number of alleles in the natural population can be calculated to be of the order of 22.

The results obtained for Polystictus versicolor appear to differ widely from previously published data for tetrapolar forms. The following possible explanations are offered.

1. Small numbers of alleles may be related to ecological specialisation. Burnett (1956) suggested that mating-type factors originated through selection from genes having various different functions, and one might expect a greater number of such factors if the fungus were adapted to a wide range of habitats. Species in which distribution is restricted might in this case have fewer mating-type factors. Polyporus betulinus has been recorded as fruiting only on birch trees, and in the analysis made by Saunders (1956) 25 - 30 alleles

were recorded in the total population in the British Isles. This fungus, known to be ecologically restricted, has relatively low numbers of alleles. Fomes roseus and Fomes subroseus, two bipolar forms studied by Mounce and Macrae (1937) are restricted to Coniferous woods; ten fruit bodies were analysed, having been collected over a wide area, and 100% of the expected number of alleles was recorded. A comparable analysis was attempted by Saunders (1956) in which one fruit body from each of the twelve areas was taken at random and the number of mating-type factors present was analysed. A total of 17 out of a possible twenty-four was recorded, just over 70% of the possible number. Thus Polyporus betulinus is more ecologically specialised than Fomes roseus and Fomes subroseus and lower numbers of alleles were recorded. Eggertson (1953), in studies on the tetrapolar fuhgus Polyporus obtusus which is generally restricted to branches of Quercus though it has been recorded on other deciduous trees, recorded 39 alleles at each of the A and B loci out of a possible total of 48; this is 81% of the possible total and by calculation from Whitehouse's formula an estimate that there are 154 alleles in the total population can be obtained. Data recorded in the present study indicate low numbers of alleles in Polystictus versicolor which may infect dead wood of a variety of deciduous trees and cannot therefore be considered as being ecologically specialised.

From the evidence presented above, ecological specialisation or otherwise does not appear to be correlated with numbers of mating-type factors per locus in tetrapolar forms studied, although in the two bipolar forms discussed there is a suggestion of some correlation. But this hypothesis cannot be accepted on the small amount of evidence available.

2. The second possibility, is that no generalisation can be made with regard to the numbers of mating-type factors in the Hymenomycetes, because the number may vary widely from species to species. Similar numbers may exist in species which are closely related taxonomically or show similarities of habitat. Variability in number of mating-type factors is indicated by the data presented by Whitehouse and that summarised in Table XXII.

Studies made here on Hypholoma fasciculare and Collybia velutipes, when twenty and seven fruit-bodies were analysed respectively and in which all were found to carry identical mating-type factors, and on Polystictus versicolor in which twenty alleles were isolated out of a possible forty-six, seem to bear out this suggestion.

3. Methods of sampling appear to have a direct correlation with the number of alleles recorded. When investigating local populations of Polystictus versicolor, adjacent fruit bodies were sampled. All fruit bodies in the Ladebraes collection were taken from the same stump; in the Tentsmuir collections two stumps were sampled, these were within a hundred yards

of each other and again adjacent fruit bodies were sampled. Low estimates for numbers of alleles at the mating-type locus were obtained. Saunders (1956), when studying the numbers of mating-type alleles in Polyporus betulinus also sampled fruit-bodies from the same tree and obtained low estimates for numbers of alleles in the population.

If one fruit body were taken at random from each of the local populations of P. versicolor studied, e.g. one from Ladebraes 1956 and 1957, one from Dyersbrae and one from each of the stumps sampled at Tentsmuir, it is possible that, from the five fruit-bodies, ten alleles would be identified. This in fact was done by Saunders in an analysis of data from the "survey" collections of Polyporus betulinus. One fruit body was taken from each of the twelve areas sampled and a total of seventeen out of a possible twenty-four mating-type factors were recorded. A somewhat higher estimate than was obtained by considering the total number of fruit-bodies sampled i.e.45 as compared with 25.

Estimates made by Raper (1958) in which he sampled fruit-bodies of Schizophyllum commune from all over the world and the earlier estimates by Hanna (1925) on Coprinus lagopus, and Mounce and Macrae (1937) on Fomes roseus and Fomes subroseus were obtained by the latter method of sampling.

d) Genetical Structure of local populations of Polystictus versicolor and their potential outbreeding efficiency.

Since several fruit-bodies of P. versicolor sampled from the same tree carry the same mating-type factors, there must be a limited number of alleles present in one stump. On comparing the alleles in one stump with those in another (see Table XXI) no common alleles were recorded. Thus, although the data at present available are not sufficient to allow any definite conclusions to be drawn, one is led to suspect that each stump carries a group of alleles which is genetically distinct from the group of alleles carried by any other stump. When fruiting occurs and large numbers of sporophores are produced, because of the limited number of alleles available, some sporophores must carry the same two alleles.

These local concentrations of alleles may form discreet populations, gene exchange taking place only within the populations. Potential out-breeding efficiency would be maintained by the occasional addition of new factors when a basidiospore carrying different alleles became established on the stump and this may well be demonstrated by further work.

Mating-type factors in the vegetative mycelium are generally considered to be extremely stable. Kniep (1929)

noted the stability of these factors during vegetative growth; attempts to induce mutations by means of X-rays in Schizophyllum commune - Papazian (1958), in Polyporus betulinus and Vorraria granulata - Burnett and Saunders (unpubl.) proved abortive. Further experiments with Coprinus lagopus using mixtures of oidia and short hyphae and treating with acriflavine, camphor and epichlorhydrin also gave negative results - Papazian (1958).

Brunswick (1924), Zattler (1924) and others, however, have noted the frequent occurrence of unexpected factors on mycelia arising from basidiospores, these factors can now probably be regarded as arising by crossing over and other complex phenomena - Papazian (1951) and Raper (1958).

Tetrapolar heterothallism permits a minimum of 50% cross compatibility, and with multiple allelic control the amount rises with the number of alleles (Mather, 1942). He calculated that with two loci each having three allelomorphs and all alleles being equally frequent in the population, sister mating occurs in $\frac{1}{4}$ of the cases and non sister mating in 4/9, i.e. potential outbreeding is 44.4%. If the number of alleles at each of the two loci is increased to eight, potential outbreeding will be 73.2%, with ten alleles 87.4%, and with twelve alleles 90.1%

But further increases in numbers of allelomorphs at the mating-type loci will increase the potential outbreeding efficiency of the fungus by smaller and smaller amounts, e.g. with twenty alleles the potential outbreeding efficiency it increased only to 95%.

Thus the local populations of P. versicolor at Tentsmuir and Ladebraes which have been examined in this study will be effectively outbreeding, with eight alleles at the A and B mating-type loci. Occasional addition of new alleles may occur as described above.

B. The Nature of the Mycelium.

When sporophores of P. versicolor from the same tree were analysed with regard to their mating-type factors, it was found that adjacent fruit-bodies often carried different mating-type factors. When sporophores of Hypholoma fasciculare were collected (Fig. 19) and examined, however, all were found to carry the same mating-type factors, as were the sporophores of Collybia velutipes examined.

Parker Rhodes (1951) has postulated that "sporophores of practicolous species which are separated by a distance greater than five yards are derived from different mycelia". There already exist published data which bear on the extent of a single mycelium, and, therefore, raise the question as to how a fungus individual may be defined.

Sporophores of Panaeolus campanulatus, three metres apart possessed different mating-type factors, (Vandendries, 1932) and this was also true for two sporophores of Coprinus lagopus from the same sample of dung (Hanna, 1925). The results reported above and studies made here lead one to doubt the universal applicability of Parker Rhodes' assumption.

More evidence is required, but consideration of the data presented for Hypholoma fasciculare suggest that the extent of the mycelium in some species at least is far greater than was previously supposed. The question now arises as to what

is the genetical nature of the mycelium in Hypholoma fasciculare and how does it differ from that in species such as Polyporus betulinus and Polystictus versicolor. The data for Hypholoma fasciculare may be interpreted in two ways.

a) There might be a single extensive mycelium which produces scattered clumps of and individual sporophores over a long period of time.

b) There might be a numb r of genetically identical mycelia with overlapping distributions, each producing single or clumps of sporophores.

The distribution patterns of mating-type factors in Polystictus versicolor are capable of similar alternative explanations but there are important differences:-

There may exist

a) a large number of physically distinct monocaryotic mycelia

b) a large number of physically distinct, dicaryotic mycelia, each derived from the fusion of two distinct monocaryons.

c) an extensive physiologically unitary mycelium derived by the fusion of genetically different monocaryons and/or dicaryons to give a single genetically heterogenous mycelium.

The interpretation that extensive mycelia occur in the substrate, sometimes genetically homogenous as in Hypholoma fasciculare and sometimes genetically heterogenous as in

Polystictus versicolor and P. betulinus is favoured for the following reasons.

Hyphal fusions have been shown to occur in plate cultures of Polyporaceae including Polystictus versicolor by Robak (1942) and Cabral (1951) but without knowledge of the genotypes involved. Saunders (1956) working with Polyporus betulinus achieved fusions in plate culture between two dicaryons, between monocaryons, and dicaryons and between two monocaryons of known mating-type. Fusions of the same three classes have been recorded in P. versicolor in this work (see page 42) between mycelia of known mating-type, of the same and different genetical constitutions.

Isolations of mycelia from decayed wood were made (see page 41.) these all proved to be dicaryotic and therefore leads one to believe that P. versicolor mycelium does in fact exist in the wood as a physiologically unitary and genetically heterogenous mycelium. The only evidence which leads one to doubt this hypothesis is that published by Nobles (1958). Monocaryotic hyphae are of various species of Polyporaceae are calimed to have been isolated frequently from decays in trees, but details are not given nor have they been mentioned in earlier publications.

Little is known of the control of sporophore production in the Basidiomycetes (Hawker 1956). The simultaneous and

synchronised development of closely aggregated sporophores must be due to either a common external stimulus, or a common internal one if there is but a single mycelium. In culture, different dicaryons of P. betulinus form hymenial pores at characteristically different times (Saunders, 1956) and this is said to be the case in other fungi. Simultaneous production of sporophores by different dicaryons is therefore somewhat more likely if they are all developed from a single physiologically unitary mycelium. The simultaneous fusion of a large number of monocaryons followed by fruiting seems unlikely.

It has not yet proved possible to demonstrate in nature that hyphal fusions will produce a genetically diverse mycelium which forms a single physiological unit, although an approach to this has been made in plate culture.

The blending technique and use of sodium taurocholate described by Miles and Raper (1956) has wide applicability. It was shown, by Raper, and by the results obtained here, to be an efficient method for "splitting" the dicaryon into its component monocaryons. It eliminates the laborious micro-surgical operations on hook cells of hypha during clamp formation employed by Harder (1927) and more recently by Fries and Aschan (1952) and Papazian (1955), which until recently was the only method of recovering monocaryotic strains from the dicaryon if the dicaryon did not either bear monocaryotic oidia or produce fruit bodies readily in culture.

As Miles and Raper point out it is often desirable to know the nuclear constitution of a fruit body produced in nature, i.e. to recover the component monocaryons from a dicaryon of unknown constitution which will not produce fruit-bodies readily. The identification of wood-rotting basidiomycetes in cases where it has not been found possible to obtain single spore isolates is also facilitated. The "splitting" technique could be used to provide a solution to the problem of particular interest here, in splitting a dicaryon obtained from an infected tree into its component monocaryons. This method appears to be the only way of determining whether a physiologically homogenous yet genetically heterogenous mycelium exists in P. versicolor. Such studies, however, have not yet been attempted.

The splitting technique was used here in experiments on illegitimate di. x mon. pairings, e.g. $A_1B_1(A_2B_1 + A_1B_2)$ for which no data for P. versicolor is available.

Di. x mon. pairings were first described by Buller (1931) (the Buller Phenomenon), and three kinds were distinguished.

1. Compatible di. x mon. pairings e.g. $A_1B_1(A_2B_2 + A_3B_3)$
2. Hemi-compatible pairings e.g. $A_1B_1(A_1B_1 + A_2B_2)$
3. Non-compatible or illegitimate pairings e.g. $A_1B_1(A_2B_1 + A_1B_2)$

The mechanism involved in diploidisation, particularly in non-compatible or illegitimate pairings, has been the subject of much speculation. Rawitscher (1933) suggests that during diploidisation in such a pairing, both nuclei of the dicaryon migrate into the monocaryon and there take possession of the cytoplasm, and thus diploidise it. Quintanhilha (1939), however, disagreed with this explanation and postulated that conjugate mates for the incompatible nucleus are formed by chromosome exchange between the two nuclei of the dicaryon, thus pairing can occur and the mycelium can be diploidised.

In both Schizophyllum and Coprinus lagopus it has been demonstrated that a dicaryon e.g. ($A_1B2 + A_2B_1$) can in an incompatible pairing produce a nucleus of the constitution A_1B_1 or A_2B_2 (Papazian, 1954). In some cases the new dicaryon has the constitution $A_2B_1 + A_1B_2$, but in others the constitution $A_2B_2 + A1B_1$. Distinction between these two dicaryons is difficult since both produce identical projeny. They can, however, be distinguished by the blending technique (Fig. 16, and p.**45**) without the prolonged series of di. x mon. matings with the unknown dicaryon described by Papazian (1954).

More recently, Kimura (1958) working on illegitimate di. x mon. pairings in Coprinus macrorhizus f. microsporus, found that both nuclei of the dicaryotic mycelium migrated into the monocaryotic mycelium and thus diploidised it, in the majority of cases studied. Occasional instances of

supposed recombination were recorded. Kimura postulates that there is an extensive series of alleles, independent of the incompatibility factors which modify the conjugation affinity of the nuclei in legitimate di. x mon. pairings, and the greater the difference between the two alleles governing two compatible nuclei, the greater the combination affinity between them.

In studies on P. versicolor made here, using the blending technique, in all cases the monocaryons recovered, after blending the di. x mon. mycelium, had the same mating-type factor constitution as the original dicaryon (Page 47 and Table XVIII).

The evidence obtained suggests that both nuclei of the dicaryon migrate into the monocaryotic mycelium and there take possession of the cytoplasm. The fate of the A_1B_1 nuclei originally present in the dicaryon is unknown, but since none were isolated in the mycelial fragments after blending, one can only presume that they were either

a) destroyed or

b) that the numbers of A_1B_1 nuclei were so small that they were undetectable, or

c) nuclear fusion followed by Mitotic recombination at one locus occurred and A_1B_2 or A_2B_1 nuclei were produced.

The methods by which a genetically heterogenous yet physiologically unitary mycelium could be established have

been demonstrated in the laboratory. They are

(1) Hyphal fusions between dicaryons and between two monocaryons, and

(2) Diploidisation of monocaryotic mycelia by dicaryotic mycelia, of the three kinds first described by Buller.

The factors controlling the establishment of the dicaryon are discussed in the next section.

C. The Establishment of the Dicaryon.

a) Hyphal Fusions.

Hyphal fusions between two hyphae of a basidiomycete fungus were first described by Buller (1931) and are of two kinds; (1) hypha to peg fusions and (2) peg to peg fusions. In all cases fusion is tip to tip. These fusions occur between hyphae of the same mycelium thus forming a three-dimensional network and between hyphae of different strains as a preliminary to nuclear migration, and diploidisation in compatible pairings. Fusions between hyphae of two incompatible strains may also occur as has been demonstrated in Polyporus betulinus by Saunders (1956) and in Polystictus versicolor in the present study. Fusions also occur between dicaryotic and monocaryotic hyphae and between hyphae of two dicaryons (see Page 42). The significance of these fusions was first realised by Buller (1931) - "hyphal anastomoses make possible sexual co-operation, facilitate the passage of nuclei through a haploid mycelium whilst it is being diploidised, aid the flow of food materials to fruiting bodies whilst these are developing, diminish the deleterious effects of small wounds in a mycelium, and in any one species make possible the co-operation of numerous monosporous mycelia in the formation of one or more fruit-bodies." Thus hyphal fusions have been established as a necessary preliminary to nuclear migration, clamp formation and the establishment of the dicaryon.

younger part than through the older part of the mycelium.

Similar observations to those made by Buller were recorded by Oikiawa (1939) in Galerea tenera.

The above estimations, as is pointed out by Kimura (1954) are rough approximations, they are based on the length of time elapsed from the inoculation of the small mycelium, to the first appearance of clamps on the periphery of the large inoculum, and the distance between the small inoculum and several points on the periphery of the large inoculum.

Kimura (1954) designed a series of experiments to overcome these difficulties. Large and small inocula were again used, but instead of waiting until clamp bearing mycelia appeared on the periphery of the large inoculum, they were sampled whilst diploidisation was still in progress. Large numbers of samples were taken by means of a sterile aluminium tube, transferred in sequence to fresh agar in other petri dishes, and after incubation were examined for presence or absence of clamps.

Estimates of speeds of nuclear migration obtained, together with estimates made by other authors, are given in Table VII (Page 26).

Kimura records that nuclei derived from the small inoculum when advancing through the large mycelium which they are diploidising, may travel through any part of the mycelium but progress more rapidly through the older part

than through the younger part. This is exactly the opposite to the situation recorded by Buller (1931).

More direct methods of estimating nuclear migration rates have been used recently. By phase contrast microscopy Dowding and Bakerspigal (1954) saw nuclei of the Ascomycete Gelasinospora tetrasperma travel slowly through septal pores from one cell to another. Girbardt (1955) studying Polystictus versicolor, saw conjugate pairs of nuclei migrate at the time clamp connections were being formed. The speed of nuclear migration in Polystictus versicolor was recorded as being approximately equal to that of hyphal elongation - 0.25 mm. per hour. Dowding (1958) working on Gelasinospora tetrasperma observed nuclear migration in mated monocaryons. The material was grown on slides on yeast enriched malt-agar. Under these conditions cytoplasm streamed rapidly, and nuclei passed along the mycelium at a speed equal to that of the cytoplasm which carried them. Nuclei were timed to travel through the hyphae at a rate of 40 mm. per hour. No indication of the growth rates of the strains studied is given.

Nuclear migration speeds in mated biochemical mutants of Schizophyllum commune have been studied by Raper and Snider (1958), and were recorded as being as much as ten times greater than the growth rates of hyphal tips at the same temperature. After studying migration at 22°C. and 32°C. a Q_{10} of 1.7 for nuclear migration was obtained. This result

suggests that a chemical reaction is rate limiting.

Because of the inconsistency of the results obtained previously, experiments on nuclear migration rates were made here. A variety of methods were used which gave a wide range of results (see Table XI).

The pairing done on slides are thought to give the most accurate estimates for the following reasons:

1) Observation of the actual time of fusion of the two mycelia is greatly facilitated, because of the reduction in numbers of hyphae.

2) Because of the thinness of the agar film, hyphae are forced to grow more nearly in two dimensions only, not three as is the case in plate cultures.

3) Movements of the nuclei over smaller distances can be detected.

Nuclear migration rates obtained by the slide technique were approximately 1.5 times the growth rates of the hyphae.

A temperature effect similar to that reported by Raper and Snider was recorded here. A Q_{10} of 1.54 was obtained, again suggesting that a chemical process is rate limiting.

Raper and Snider record 1.3 mm.per hour as a minimum and 2.5 mm. per hour as a maximum estimate of the speed of nuclear migration in Schizophyllum commune. The figure 0.71 mm. per hour obtained for Polystictus versicolor is

approximately half this minimum estimate.

It can hardly be expected that nuclear migration rates in different fungi having different growth rates and different nutritional requirements will be the same; they can, however, be expected to be of the same order. The only previously recorded figure for Polystictus versicolor is that given by Girbardt (1955). This figure, 0.25 mm. per hour, was obtained by direct observation of moving nuclei of a single dikaryotic strain under the phase contrast microscope. Movement of nuclei in paired compatible haploids was not considered. The reason for the discrepancy between the results obtained by Girbardt and those obtained here is not known.

Nuclear migration rates do not appear to be controlled by the mating-type alleles; they may be governed by other genes closely linked to the mating-type loci, or by genes which, control cytoplasmic factors preventing the successful establishment of nuclei bearing incompatible mating-type factors in the cytoplasm. Evidence supporting this premise has been obtained here by comparing estimates obtained for nuclear migration rates in $A_1B_1 \times A_2B_2$ and $A_1B_2 \times A_2B_1$ pairings, obtained by the radial method. No significant differences in the average rates were recorded (see Pages 27-29)

That the genes governing nuclear migration are closely linked to the mating-type loci is borne out by the fact that only in compatible pairings i.e. those with unlike factors at both loci, does normal nuclear migration and subsequent establishment of the dicaryon take place. Although nuclear migration does occur in pairings with similar 'A' and 'B' factors, only in Schizophyllum commune have stable heterokaryons been recorded e.g. "flat" common A heterokaryon recorded by Papazian (1950) and Raper (1953).

Buller (1933) working on Coprinus lagopus, Dowding and Bakerspigal (1954) and Dowding (1958) working on the Ascomycete Gelasinospora tetrasperma, report the presence of pores in the septa through which the nuclei pass, carried along by streaming of the cytoplasm. Girbardt (1958), however, has published electron microscope photographs in which the septum in Polystictus versicolor is shown to be without pores. It is difficult to imagine how nuclei pass along a multi-septate hypha in the absence of septal pores, unless each septa breaks down before and is reorganised after a nucleus passes through. This process would occupy a considerable amount of time, and judging from the high speeds for nuclear migration recorded in some cases (see Table VII) seems completely impossible. It is possible that electron microscope pictures do not show a physico-chemical pore.

The path of nuclear migration has been open to question. Buller (1933) records that the migrating nuclei pass preferentially through the younger parts of the mycelium, i.e. the peripheral hyphae and Kimura reports that in general nuclei pass first through the older parts of the mycelium, but may travel through any part. Detailed studies made here indicate that in Polystictus versicolor there is an irregular pattern of dicaryotisation, no particular preference for either the younger or the older parts of the mycelium is shown and the path taken by the nuclei appears to be different in different pairings (see Figs. 3 and 6).

c) The Control of Clamp Connection Formation.

Clamp connections on the hyphae of Basidiomycetes were first observed by Hoffman (1856) and soon came to be considered as a mycelial characteristic of this group. Kniep (1915) and Bensaude (1918) described modes of formation of clamp connections cytologically and revealed the relationship between this process and that of conjugate division. The true significance of the dicaryon and its associated clamp connections was appreciated shortly afterwards when Kniep and Bensaude described heterothallism in the Basidiomycetes. In many species clamp connections are to be found associated with conjugate division in the dicaryons and are absent in

the monocaryon. Clamp connections may be found on mycelia derived from a single spore in homothallic species, e.g. Stereum hirsutum (Kniep, 1918) and Coprinus narcoticus (Brunswick 1924), and the dicaryotic mycelia of many fungi e.g. Corticum bombycinum and Calocera viscosa (Kniep, 1918) do not bear clamp connections.

Heterokaryons are formed from incompatible matings in some species the best known being the common A "flat" heterokaryon in Schizophyllum commune described by Papazian (1950 c). Pseudo-clamps and, it is claimed, even rarely true clamps are found at some septa. A common B heterokaryon has also been described in Schizophyllum commune, it differs from the common A in that it is of more limited extent and in that clamp connections have not been recorded.

Illegitimate pairings between monocaryons have been studied in some detail in various species of Coprinus by Brunswick (1924) and Vandendries (1923 a and b). Brunswick termed such pairings "durchsbrechungskopulations", and found that such matings occurred in Coprinus spp. only in the presence of common B factors. "Pseudoschnallen", i.e. false clamps were recorded occasionally in the common B heterokaryons. Vandendries and Brodie (1933) recorded "Pseudoschnallen" in common B heterokaryons of Polystictus versicolor.

From this evidence certain conclusions can be drawn. They are:

1) Mating type factors interact in some way.

2) Different mating type factors at both loci are necessary for normal clamp formation to occur.

3) Like factors at either loci appear to prevent the formation of normal clamps, but false clamps may be formed.

This interaction between the mating type factors in illegitimate pairings has been studied frequently, Vandendries and Brodie (1933), Papazian (1950 c) and others, but little attention has been given to the interactions between mycelia carrying compatible mating type alleles.

The results obtained in experiments described earlier (Page 37-41) indicate that in Polystictus versicolor an interaction affecting clamp connection occurs between mycelia of compatible mating-types. A significant difference has been recorded between the number of clamps formed in a pairing $A_1B_1 \times A_2B_2$ and the number formed in a pairing $A_1B_2 \times A_2B_1$, in intra fruit body pairings. The same differences were recorded in inter-fruit body pairings, more clamps were always recorded in $A_1B_1 \times A_2B_2$ pairings than in $A_1B_2 \times A_2B_1$ pairings. The fact that high clamp numbers have always been recorded in pairings which happen to have been given the notation $A_1B_1 \times A_2B_2$ is due to chance, but the fact there is a significant difference in the numbers of clamps formed by the two differently constituted dicaryons has been clearly demonstrated.

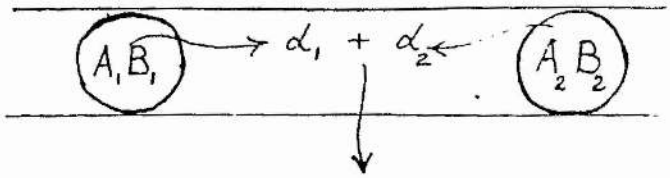
Further proof that there is interaction between nuclei within the cytoplasm is given by Papazian (1958). He grew the two components of the "flat" heterokaryon of Schizophyllum commune on either side of a cellophane membrane and obtained no interaction. He therefore concludes that contact and plasmogeny are necessary, but a cellophane membrane might also prevent the diffusion of large molecules.

It was thought that the presence of compatible mating-type factors in paired hyphae might stimulate the growth rates of these hyphae. The experiments in which compatible, non-compatible and common A and common B strains were grown on either side of a cellophane membrane did not produce any positive results. (Tables XII and XIII). The growth rates of the strains studied remained unchanged, and one can only assume that there is not any chemical stimulation of the growth rates. Physical stimulation also appears to be absent since no increase in the growth rate of any particular strain was recorded when it was grown in the presence of another compatible strain in the absence of a cellophane membrane.

Different mating type factors at both loci are necessary for the establishment of a dicaryon, in this case the the nucleus of a compatible type is readily accepted into the cytoplasm of the acceptor mycelium. The nucleo-cytoplasmic

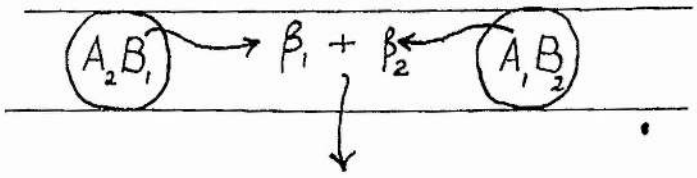
Fig. 20 Diagramatic representation of mechanism proposed as acting in the stimulation of clamp connection formation, in compatible pairings of *Polystictus versicolor* (L).

COMPATIBLE PAIRING (I).



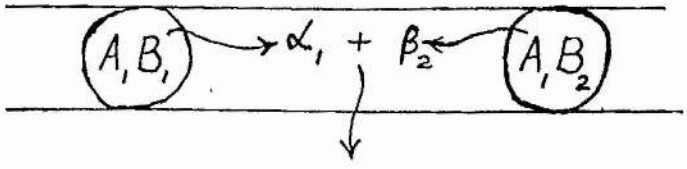
CLAMPS

COMPATIBLE PAIRING (II).



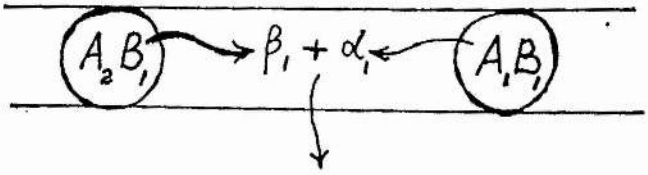
DIFFERENT NUMBER OF CLAMPS

COMMON "A" FACTORS.



NO CLAMPS OR "FALSE" CLAMPS

COMMON 'B' FACTORS.



NO CLAMPS OR "FALSE" CLAMPS

FIG. 20.

ratio is adjusted from 1:1 to 2:1 and conjugate division and clamp formation can then occur. It is thought that some chemical substance is produced by interaction between the two mating-type factors within the nucleus; this is then secreted into the cytoplasm surrounding the nucleus. When a nucleus of one strain migrates via another compatible strain, the substance produced by the invading nucleus will react with that produced by the resident nucleus. The reaction regulates the nucleo-cytoplasmic balance, facilitating the change from 1:1 to 2:1, then stimulates conjugate division and the formation of clamp connections. This hypothesis necessitates the existence of two complementary pairs of substances, each capable of reacting with the other to produce a distinct physiological effect i.e. both pairs of substances on reacting together will stimulate the formation of clamp connections; one combination, however, stimulates clamp formation to a greater extent than the other.

The mechanism postulated is represented diagramatically in Fig. 20.

The substances produced by interaction of A_1 with B_1 and by A_2 with B_2 are given the notation α_1, and α_2; those produced by A_1B_2 and A_2B_1 are given the notation β_1 and β_2 respectively. The combinations $(\alpha_1 + \alpha_2)$ and $(\beta_1 + \beta_2)$ will

stimulate the formation of clamp connections on reacting together ($\alpha_1 + \alpha_2$) having a greater effect than ($\beta_1 + \beta_2$). The combinations ($\alpha_2 + \beta_1$) and ($\alpha_1 + \beta_2$) produced by interaction of nuclei with common A factors do not stimulate clamp connection formation, neigher do the combinations ($\alpha_2 + \beta_2$) and ($\alpha_1 + \beta_1$) given by interaction of nuclei with common B factors.

The exact physiological mechanism which promotes the formation of clamp connections is still unknown. Substances stimulating their formation in the two types of compatible pairings possible in a tetrapolar fungus have been postulated, the existence of these substances has not however been proved. The following events are necessary preliminaries to the initiation and formation of normal clamp connections and the establishment of the dicaryon.

a) The apposition of two strains carrying different mating-type factors at both loci.

b) The establishment of hyphal fusions between the hyphae of the two strains.

c) Migration of nuclei of one strain into the hyphae of the other compatible strain.

d) Interaction between the two unlinked alleles governing the mating-type of the fungus, with the production of some substance which is secreted into the cytoplasm, followed by interaction between the "hormones" produced by the two compatible nuclei when in the same cytoplasm with then stimulates clamp formation.

VII CONCLUSIONS

The numbers and distribution of mating-type factors in Polystictus versicolor have been studied. Local populations have been sampled and in many cases the mating-type factors of fruit-bodies from adjacent positions have been analysed. It is thought that local populations of Polystictus versicolor exist as discrete units, interbreeding occasionally taking place between them. The number of alleles at the mating-type loci in these local populations is not thought to provide an accurate estimate of the number of alleles in the population as a whole as has been postulated (Whitehouse, 1949 b). The evidence presented here, however, is insufficient for any definite conclusions to be drawn.

The genetical and physiological nature of the mycelium in Polystictus versicolor has been studied and compared with that in other fungi. It is thought that the mycelium of P. versicolor exists as a genetically heterogenous yet physiologically homogenous unit. It has not proved possible to demonstrate that such a situation exists in nature, though a method of doing so has been suggested. Studies in the laboratory on hyphal fusions and diploidisation phenomena have elucidated a mechanism by which a genetically heterogenous mycelium could be established.

The reactions between paired compatible haploids have been studied and the existence of substances stimulating and

stimulate the formation of clamp connections on reacting together ($\alpha_1 + \alpha_2$) having a greater effect than ($\beta_1 + \beta_2$). The combinations ($\alpha_2 + \beta_1$) and ($\alpha_1 + \beta_2$) produced by interaction of nuclei with common A factors do not stimulate clamp connection formation, neither do the combinations ($\alpha_2 + \beta_2$) and ($\alpha_1 + \beta_1$) given by interaction of nuclei with common B factors.

The exact physiological mechanism which promotes the formation of clamp connections is still unknown. Substances stimulating their formation in the two types of compatible pairings possible in a tetrapolar fungus have been postulated, the existence of these substances has not however been proved. The following events are necessary preliminaries to the initiation and formation of normal clamp connections and the establishment of the dicaryon.

a) The apposition of two strains carrying different mating-type factors at both loci.

b) The establishment of hyphal fusions between the hyphae of the two strains.

c) Migration of nuclei of one strain into the hyphae of the other compatible strain.

d) Interaction between the two unlinked alleles governing the mating-type of the fungus, with the production of some substance which is secreted into the cytoplasm, followed by interaction between the "hormones" produced by the two compatible nuclei when in the same cytoplasm which then stimulates clamp formation.

controlling the formation of clamp connections has been postulated.

The rates of nuclear migration in compatible pairings of Polystictus versicolor have been studied. Nuclear migration does not appear to be controlled by the mating-type factors. Rates recorded here were found to be somewhat lower than expected by comparison with the results obtained by previous authors, this is thought to be due to a refinement of technique.

VIII SUMMARY.

1. The numbers and distribution of mating-type factors in local populations of Polystictus versicolor have been studied, and the data available on the number and distribution of mating-type alleles in other fungi have been reviewed. Possible explanations for the low numbers recorded in Polystictus versicolor are given, and the potential out-breeding capacity of the local populations is discussed.

2. Growth rates of dicaryotic and monocaryotic strains of P. versicolor at 25°C. and 35°C. have been studied.

3. Studies have been made on the genetical and physiological nature of the mycelium in P. versicolor and it is thought that the mycelium exists in nature as a physiologically homogenous and genetically heterogenous unit. The mechanisms by which such a mycelium could be established have been studied. They are (a) hyphal fusions and (b) di. x mon. pairings.

4. The effects of different mating-type factors in compatible pairings on (a) hyphal fusion; (b) nuclear migration; (c) clamp connection formation; have been investigated, and a suggestion made concerning a model for gene action in connection with the formation of clamps.

BIBLIOGRAPHY

ARNOLD, J.D. (1935) A comparative study of certain species of Marasmius and Collybia in culture. Mycologia 27, 388-417.

BADCOCK, E.C. (1943) Methods for obtaining fructifications of wood-rotting fungi in culture. Trans.Brit. mycol.Soc. 26, 127-132.

BAYLISS, J.S. (1908) The Biology of Polystictus versicolor. J. econ. Bot. 3, 1-24.

BENSAUDE, M. (1919) Sur la sexualité chez les Champignons Basidiomycètes. C.R. Acad. Sci. Paris 165, 286-289.

BRODIE, H.J. (1948) Tetrapolarity and unilateral diploidisation in the Bird's Nest Fungus Cyathus stercoreus. Amer.J.Bot. 35, 312-320.

BRODIE, H.J. (1936) The occurrence and function of oidia in the Hymenomycetes. Amer.J.Bot. 23, 309-327.

BULLER, A.H.R. (1931) Researches on Fungi. Vol.II. London.

(1931) Researches on Fungi. Vol.IV. London.

(1933) Researches on Fungi. Vol.V. London.

(1941) The diploid cell and the diploidisation process in plants and animals with special reference to the higher fungi. Bot.Rev. 7, 335-431

BURNETT, J.H. (1956) Mating systems of Fungi I. New Phytol. 55, 50-90.

BURNETT, J.H. & PARTINGTON, M. (1957). Spatial distribution of fungal mating-type factors. Proc. R. phys. Soc. Edinb. 26, 61-68.

BRUNSWICK, H. (1924) Untersuchungen über die Geschlects und Kernverhältnisse bie der Hymenomyzetengattung, Conrinus. Bot. Abh. K. Goebel. 5, 152 pp.

CABRAL, R.V. de G. (1951) Anastomoses micelias sur valor no diagnostico dos Poliporoses. Bull.Soc. Brot. 15, 291-362.

CARTWRIGHT, K.St.J. & FINDLAY, W.P.K. (1946) The decay of timber and its prevention. H.M.S.O. London.

CHOW, C.H. (1934) Contribution a l'étude du développement des Coprins. Botaniste 26, 89-232.

COOL, C. (1912) Beiträge zur Kenntniss der Sporenkeimung und Reinkultura der höhern Pilzen. Meded. phytopath. Lab. Scholten 3, 1-38.

DICKSON, H. (1935) Studies on Coprinus sphaerosporus II. Ann.Bot., Lond. 49, 181-204.

DOWDING, E.S. & BAKERSPIGAL, A. (1954) The migrating nucleus. Canad. J. Microbiol. 4, 295-301.

EGGERTSON, E. (1953) An estimate of the number of alleles at the loci for heterothallism in a local concentration of Polyporus obtusus (Berk.) Canad. J. Bot. 31, 750-759.

FISHER, R.A. & YATES, F. (1953) Statistical tables for Biological, Agricultural and Medical Research. 4th edition. Oliver and Boyd, Edinburgh.

FRIES, N. & JONASSON, L. (1941) Über die Interfertilität verschiedener Stämme von Polyporus abietinus (Dicks) Fr. Svensk. bot. Tidskr. 35, 177-193.

FRIES, N. & ASCHAN, K. (1952) The physiological heterogeniety of the dikaryotic mycelium of Polyporus abietinus investigated with the aid of a microgical technique. Svensk bot. Tidskr. 46, 429-445.

FUNKE, G.L. (1924) Über die Isolierung von Basidiosporen mit dem Mikromanipulator nach Jansse undo Péterfi. Z. Bot. 16, 619-623.

GIRBARDT, M. (1955) Lebenbesbachtungen an Polystictus versicolor. Flora, 142, 540-563.

GIRBARDT, M. (1958) Über die Substruktur von Polystictus versicolor (L.). Arch. Mikrobiol. 28, 255-269.

HANNA, W.F. (1925). The problem of sex in Coprinus lagopus. Ann. Bot., Lond. 39, 431-457.

HARDER, R. (1927). Über Geschlechtsverlust bzw Verlust der Kopulations-fähigkeit bei Pholiota mutabilis. Ber. dtsch. bot. Ges. 45, 55-60.

HAWKER, L.E. (1956) Physiology of Reproduction in the Fungi. C.U. Press.

KENNEDY, E.M. (1956) Ph.D. Thesis, Liverpool.

KIMURA, K. (1954) Diploidisation in the Hymenomycetes. I. Preliminary Experiments. Biol.J. Okayama Univ. 1, 226-233.

KIMURA, K. (1958) Diploidisation in the Hymenomycetes. II. Nuclear behavior in the Buller Phenomenon. Biol.J. Okayama Univ. 4, 1-59.

KNIEP, H. (1915) Bietrage zur Kenntnis der Hymenomyzeten III. Über die Konjugierten Teilungen und die phylogenetische Bedeutung der Schnallenbildungen. Z. Bot. 7, 369-398.

(1920) Über morphologische und physiologische Geschlectdifferenzierung. Verh.phys.med.Ges. Würzburg. 46, 1-18.

(1922) Über Geschlechtsbestimmung und Reduktionsteilung. Verh. phys.med. Ges. Würzburg. 47, 1-28.

(1929) Vererbungserscheinungen bei Pilzen. Bibl. genet. 5, 371-478.

LOHMAG, K. (1952) Zur Fruchkörperbildung holzzerstörend-enhöherer Pilzer in Reinkultur. Svd. Ann. Mycol. II, 6, 5-7.

MATHER, K. (1942) Heterothally as an outbreeding mechanism in the fungi. Nature, Lond. 149, 54.

MILES, P.G. & RAPER, J.R. (1956) Recovery of component strains from dicaryotic mycelia. Mycologia, 48, 484-494.

MITTWOCH, U. (1954) The genetics of Conrinus lagopus II. J. Genet. 52, 551-554.

MOUNCE, I. (1929) Studies in forest pathology II. Bull. Dep. Agric. Canad. III.

MOUNCE, I. & MACRAE, R. (1937) The behavior of paired monosporus mycelia of Fomes roseus (Alb. & Schn.) Cooke. and Fomes subroseus (Wier) Overh. Canad.J. Res. 15, 154-161.

NOBLES, M.K. (1958) Cultural characters as a guide to the taxonomy and phylogeny of the Polyporaceae. Canad. J. Bot. 36, 883-926.

OIKAWA, K. (1939) Diploidisation and fruit body formation in Hymenomycetes. Sci. Rep. Tohoku Univ. 14, 245-260.

OORT, A.J.P. (1930) Die Sexualität von Coprinus fimentarius. Rec. Trav. bot. néeland. 27, 85-148.

PAPAZIAN, H.P. (1950) A convenient method of growing fruiting bodies of a bracket fungus. Bot. Gaz. 112, 138-139

(1950) Physiology of the incompatibility factors in Schizophyllum commune. Bot. Gaz. 112, 138-139.

(1950) The incompatibility factors and a related gene in Schizophyllum commune. Genetics, 36, 441-459.

(1954) Exchange of incompatibility factors between nuclei of a dicaryon. Science, 119, 691-693.

(1955) Sectoring variants in Schizophyllum. Amer. J. Bot. 42, 394-400.

(1958) The Genetics of Basidiomycetes. Adv. in Genetics. 9. 41-69.

PARKER, Rhodes, A.F. (1951) Basidiomycetes of Skokholm Island. VII. New Phytol. 50, 227-243.

QUINTANILHA, A. (1937) Contribution à l'étude génétique du phenomène de Buller. C.R. Acad. Sci., Paris. 205, 745-747.

QUINTANILHA, A. & PINTO LOPES, J. (1950) Apergu sur l'état actuel de nos connaissances concernant la "conduite sexuelle" des espèces d'Hymenomycètes. Bol. Soc. broteria. 24, 117-290.

RAPER, J.R. (1951) Sexual hormones in Achlya. Amer. Scientist, 39, 110-121.

RAPER, J.R. (1953) Tetrapolar sexuality. Quart. Rev. Biol. 28, 233-259.

RAPER, J.R. & SAN ANTONIO, J.P. (1953) Heterocaryotic mutagenesis in Hymenomycetes. I. Amer. J. Bot. 41, 69-86.

RAPER, J.R. et al. (1958) The genetic structue of incompatibility factors in Schizophyllum commune. Proc. N.A.S. America. 449, 890-900.

RAPER, J.R., KRONGELB, G.S., & BAXTER, M.G. (1958) The number and distribution of incompatibility factors in Schizophyllum. Amer. Nat. 92, 221-234.

RAWITSCHER, F. (1933) Besprechungen. Z.Bot. 26, 134-186.

ROBAK, H. (1942) Cultural studies on some Norwegian wood-destroying fungi. Medd. Vestland. Forstl. Forsksst. 25, 248 pp.

ROSHAL, J.Y. (1950) Incompatibility factors in a population of Schizophyllum commune. Thesis, University of Chicago.

SAUNDERS, M. (1956) M.Sc. Thesis, Liverpool.

SNIDER, P.J. & RAPER, J.R. (1958) Nuclear migration in the Basidiomycete Schizophyllum commune. Amer. J. Bot. 45, 538-547.

TAMELYN, N. & Da COSTA, E.W.B. (1958) A simple technique for producing fruit bodies of Basidiomycetes. Nature, Lond. 181, 597-597.

VANDENDRIES, R. (1923 a) Recherches sur la déterminism sexuel Basidiomycètes. Mem.Acad.R.Cl.Sci. 5, 1-98.

(1923 b) Nouvellos recherches sur la sexualité des Basidiomycètes. Bull.Soc.Bot. Belg. 56, 73-97.

(1932) La tetrapolarité sexuelle de Pleurotus columbinus. La Cellule 41, 267-278.

VANDENDRIES, R. & BRODIE, H.J. (1933) La tétrapolarité et l'étude expérimentale des barrages sexuels chez les Basidiomycètes. Bull. Acad. R. Belg. (1).

VANDENDRIES, R. & BRODIE, H.J. (1933) Nouvelles investigations dans le domaine de la sexualité des Basidiomycètes. La Cellule. 42, (2). 165-209.

VANDENDRIES, R. (1934) Le cycle conidien haploïde et diploïde chez les Basidiomycètes. C.R. Acad.Sci. Paris. 198, 842.

WHITEHOUSE, H.K.L. (1949 a) Heterothallism and sex in fungi. Biol. Rev. 24, 411-447.

(1949 b) Multiple allelomorph heterothallism in the fungi. New Phytol. 48, 212-244.

ZATTLER, F. (1924) Vererbungstudien an Hutpilzen (Basidiomyzeten) Z. Bot. 16, 433-499.

Appendix.

Composition of basic medium Raper and San Antinio(1954).

1 litre distilled water.

20 g. Glucose

2 g. Bacto Peptone

1 g. K_2HPO_4

2 g. Yeast

2 g. Malt extract

.5g. $MgSO_4$

.46g. KH_2PO_4

plus .15 g. sodium taurocholate as required.

Media used in Fruiting Experiments.

I . Badcock(1943).

Beech sawdust		
Maize meal	50	pts. by weight.
Bone meal	30	pts. by weight.
Potato starch	16	pts. by weight.
Sucrose	2	pts. by weight.
Wood ash	1	pt. by weight.

II. Medium used by Tamblyn and Da Costa. (1958).

The following tropical sawdusts were used in the original experiment in the proportions given below. Since these were unobtainable in this country, readily available sawdusts, e.g. oak, ash, beech, were used in the same proportions.

Medium given by Tamblyn and Da Costa.(1958).

Pinus radiata D. Don. (sapwood).	25 pts.
Eucalyptus obliqua L.Herit. (sapwood)	25 pts.
E. diversicolor F.v M.	10 pts.
Acacia dealbata Link.	10 pts.
Rhizophora sp.	10 pts.
Nothofagus cuninghamii Oerst.	10 pts.
Ceratopetalum apetalum D. Don.	10 pts.

The mixture can be varied to reflect the particular origins of any one culture. In experiments on the fruiting of Polystictus versicolor Oak, Ash, and Beech sawdusts were used in equal quantities .

The following nutrients were added /100gms. air dry sawdust.

2.5g.	Maize meal
1.5g.	Bone meal
.75g.	Potato starch
.2g.	Dried yeast.
.175g.	Casein hydrolysate
.0033g.	Thiamine hydrochloride

The firstm four are mixed in powder form with the sawdust, the remainder dissolved in water and used for moistening it.

Method of scoring in "Grid Experiments".

Numbers 1-5 correspond with sample numbers.

Numbers 1-24 correspond with numbers of Grid squares.

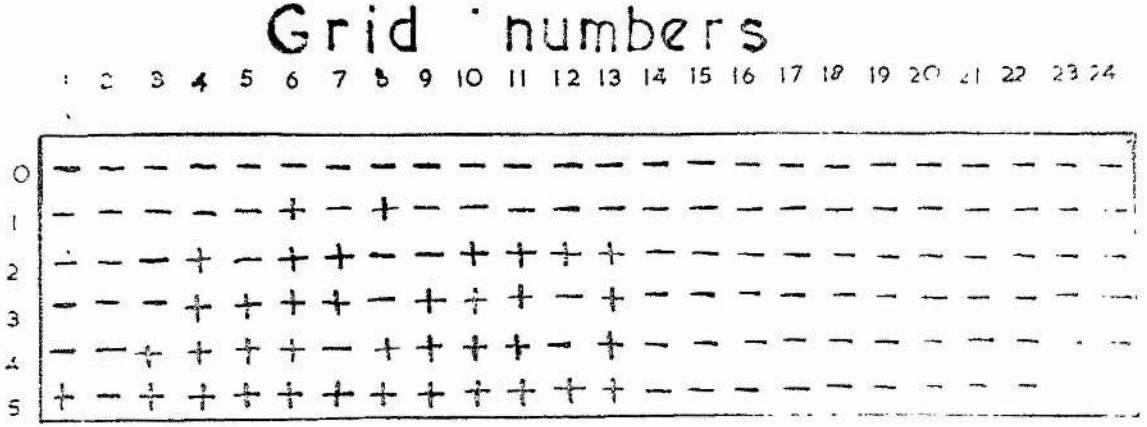
+ or − indicates presence or absence of clamps.

Appendix II

samples

pairing

4 x 5



Method of scoring in "Radial Experiments".

Numbers correspond to positions of samples,

+ or − indicates presence or absence of clamps.

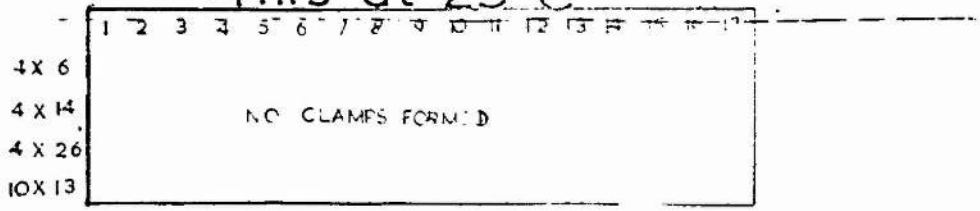
APPENDIX III

Strain nos.	Sample numbers. 1	2	3	4	5	6	7	8	9	10	11	12
13	−	+	+	−	−	+	+	−	+	−	−	−
13X3 $a_2b_2 \times a_1b_1$	−	+	−	−	+	−	+	−	+	−	−	−
	+	−	+	−	−	+	−	−	−	−	−	−
3	+	+	+	+	−	−	+	−	−	−	+	−
	+	+	−	+	−	−	+	−	−	−	+	−
	+	−	+	+	−	−	−	−	−	−	+	−

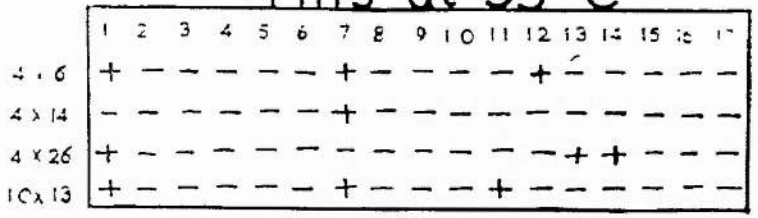
Table to show method of scoring in "Slide Matings". Numbers correspond to positions of samples, a + — indicates presence or absence of clamps.

APPENDIX IV

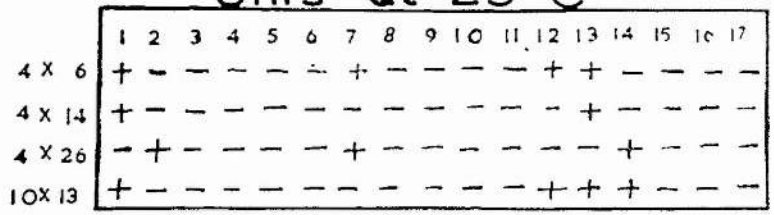
4hrs at 25°C



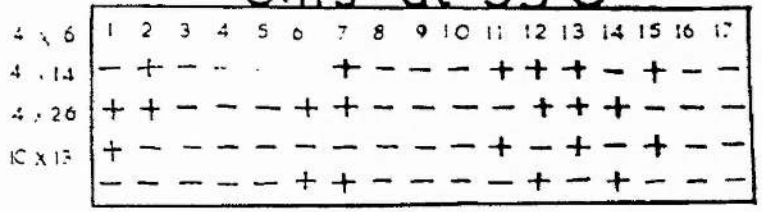
4hrs at 35° C



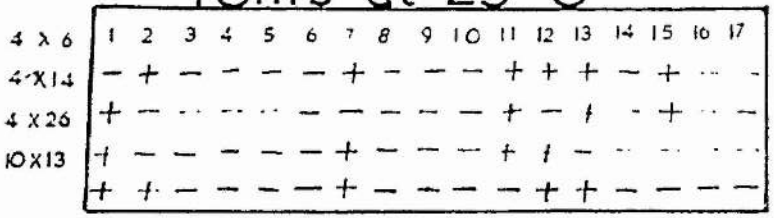
8hrs at 25°C



8hrs at 35°C



10hrs at 25°C



10hrs at 35°C

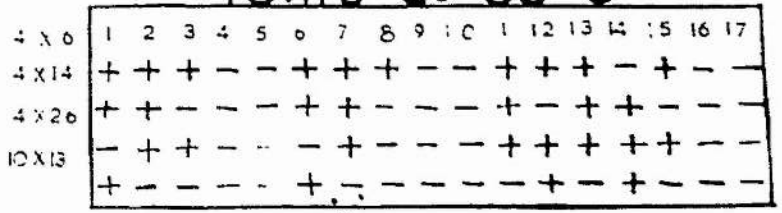


Table of clamp numbers.

Numbers in longitudinal columnsare numbers of clamps recorded/field in each of the 5 samples taken from one pairing.

Numbers in horizontal columns are clamp numbers recorded in the four replicates of each pairing.

Numbers in large type are the sum of the numbers of clamps in each of 20 fields.

PV 14 x PV 14

$a_2 b_2$	$a_1 b_1$	nos. of clamps.					total/sample				
1 x	3	11 9 8 7 15	4 5 5 5 7	8 8 6 11 9	3 0 2 0 0	1 4 4 2 3	136	103	123	34	67
		7 4 3 5 8	9 5 2 0 6	12 5 2 3 7	1 0 0 0 2	4 2 8 5 0					
		7 3 4 8 5	6 13 4 5 8	2 6 5 9 2	3 3 3 1 1	4 3 3 7 7					
		10 8 5 6 4	3 4 4 5 3	9 6 5 4 4	0 3 6 2 2	2 7 0 0 1					
x	9	6 4 5 5 6	6 9 5 3 5	3 3 6 7 6	2 6 5 7 7	1 8 11 9 1	118	111	129	125	123
		5 4 2 4 6	6 8 7 6 4	11 6 8 11 6	7 7 5 5 13	3 6 4 5 7					
		10 11 10 4 6	6 4 6 4 8	6 9 3 3 9	8 4 5 8 5	3 3 14 2 3					
		3 5 9 9 4	9 3 9 5 8	3 5 7 3 14	9 5 5 10 3	6 3 7 6 7					
x	10	2 7 6 13 8	9 4 9 6 4	10 8 10 4 2	10 10 7 6 7	10 11 12 7 7	127	152	176	138	144
		7 1 4 7 20	7 8 13 8 11	12 0 14 6 4	7 4 3 8 4	10 2 5 5 3					
		2 3 5 11 9	5 4 8 12 5	10 5 11 11 10	2 8 7 8 6	4 8 4 7 4					
		10 8 4 2 1	7 6 14 6 6	12 10 13 7 12	9 9 9 4 9	7 7 9 11 11					
x	24	7 14 6 14 8	5 10 9 11 10	5 4 11 4 5	5 10 6 8 11	7 10 5 11 3	168	120	105	112	145
		10 5 11 9 3	8 7 5 1 5	7 5 2 9 1	8 7 2 3 1	3 12 17 2 16					
		12 4 4 8 12	5 4 6 7 4	6 5 7 3 4	12 13 6 2 6	8 10 6 6 10					
		5 12 6 14 6	2 4 3 4 10	5 5 10 2 2	3 1 5 1 2	8 6 2 1 2					
x	25	2 4 5 4 3	5 4 8 12 7	6 3 4 4 6	0 2 3 4 5	2 3 7 7 7	114	106	93	102	112
		5 5 6 5 6	6 6 5 2 5	5 6 5 5 4	8 7 9 5 4	3 3 5 7 8					
		6 8 8 6 9	8 5 3 7 3	5 6 5 5 1	4 3 6 6 6	8 6 5 8 5					
		5 4 5 5 3	4 3 3 6 4	5 3 8 3 4	4 5 6 4 7	8 6 3 4 5					

Table of clamp numbers recorded in Hypholoma fasiculare and Crucibulum vulgare.

Each figure represents the sum of the numbers of clamps recorded in 20 fields.

Hypholoma fasiculare

	A_1B_1	A_2B_2			
1x9	35	28	18	23	14
1x12	36	25	19	20	15
1x15	35	22	20	17	16

	A_2B_1	A_1B_2			
7x2	28	22	17	22	18
7x3	32	24	26	17	-
7x4	22	21	18	-	19
7x11	36	27	18	-	-

Crucibulum vulgare

	A_1B_1	A_1B_2			
1x2	73	63	53	41	48
1x4	69	68	48	68	49
1x18	78	93	77	76	88

	A_1B_2	A_2B_1			
3x5	51	55	73	54	53
3x10	58	64	61	65	74
3x15	30	34	41	29	33
3x17	48	53	52	55	58

Table of clamp numbers recorded in Polystictus versicolor

Each figure represents the sum of numbers of clamps recorded in 20 fields.

PV. 2	A_1B_1		A_2B_2	
1x2	74	81	116	67	76
1x8	109	102	112	83	100
1x11	122	126	140	114	129

PV. 2	A_2B_1		A_1B_2		
7x4	7	19	17	7	8
7x5	48	35	28	23	15
7x9	11	13	14	19	16
7x10	33	14	19	10	12
7x14	93	65	61	37	15

PV. 14.	A_1B_1		A_2B_2	
1x3	136	103	123	34	67
1x9	118	111	129	125	123
1x10	127	152	176	138	144
1x24	168	120	105	112	145
1x25	114	106	93	102	112

PV. 14	A_2B_1		A_1B_2		
4x5	15	10	12	8	-
4x8	10	9	10	-	-
4x14	17	16	-	-	5
4x22	9	8	5	5	-
4x16	29	13	23	16	15
4x26	13	9	-	11	-

PV. 2x14.	A_1B_1		A_2B_2	
2x3	146	147	163	128	139
2x9	154	149	130	125	135
2x24	191	184	152	142	130
2x10	141	133	143	140	143
2x25	176	201	186	201	151

PV. 2x14.	A_2B_1		A_1B_2		
7x5	65	43	97	62	83
7x6	76	87	49	63	70
7x8	67	50	38	37	35
7x14	54	55	67	51	53
7x15	67	76	71	56	52

PV. 14x2.	A_1B_1		A_2B_2	
1x1	174	161	167	160	155
1x3	176	172	162	154	156

PV. 14x2.	A_2B_1		A_1B_2		
4x4	48	28	33	30	27
4x5	28	31	38	27	23
4x9	34	28	35	31	31

Statistical Treatment of Data from Clamp Number Experiments

Data from groups 3&4.

$SX_3 = 2992$

$n_3 = 30$

$SX_3^2 = 377806.$

$\frac{SX_3}{n_3} = 99.73$

$\sigma_3 = \sqrt{\frac{377806 - (99.7)^2}{30.}}$

$= \sqrt{2972.5}$

$SX_4 = 268$

$n_4 = 30$

$SX_4^2 = 3970.$

$\frac{SX_4}{n_4} = 8.9$

$\sigma_4 = \sqrt{\frac{3970 - (8.9)^2}{30}}$

$= \sqrt{53.09}$

using the "t" test for significance.

$n_3 = 30$

$m_3 = 99.73$

$s_3 = \sqrt{2972.5}$

$n_4 = 30$

$m_4 = 8.9$

$s_4 = \sqrt{53.09}$

$$t = \frac{m_3 - m_4}{\sqrt{\frac{n_3 s_3^2 + n_4 s_4^2}{n_3 + n_4 - 2}\left(\frac{1}{n_3} + \frac{1}{n_4}\right)}} = \frac{90.83}{\sqrt{\frac{90750}{58 \times 15}}}$$

$$= \frac{90.83}{\sqrt{104.3}}$$

$$t = \underline{8.9}$$

Significant at 1% and 5% levels.

Fisher and Yates Statistical Tables.