Exchange of nuclei between dikaryons in *Coprinus lagopus*

K. M. ŚWIEŻYŃSKI

The research of Pontecorvo and his school (1959) has focussed attention on the importance of parasexual processes in fungi which bring about exchange of genetic material without the normal sexual cycle. One of the simplest ways of exchanging genetic material in dikaryotic mycelia of *Basidiomycetes* would be an exchange of nuclei between two dikaryons leading to the formation of a recombinant dikaryon.

As far as the author is aware, there is only one published example of such an exchange in *Holobasidiomycetes*. Kimura (1958) fruited jointly pairs of dikaryotic mycelia of *Coprinus macrorhizus* and found that most fruitbodies were formed by the components, but one fruitbody apparently originated from a recombinant mycelium. Several examples of exchange of nuclei in rusts were described (Ellingboe 1961, review: Day 1960b). In this group of fungi however the dikaryotic mycelium appears to be less stable and multinucleate hyphae were observed several times (Allen 1926, Nelson a.a., 1955).

The present study was undertaken to find out, whether recombinant dikaryons may be formed in *Coprinus lagopus*.

MATERIALS AND METHODS

Coprinus strains, used in this study, come from wild-type and mutant strains kindly supplied by Dr. P. R. Day from the John Innes Institute, England. Their origin is described in previous papers (Świeżyński and Day 1960a,b).

Three alleles of the *A* mating type locus i.e. A_2 , A_5 and A_6 , three alleles of the *B* mating type locus i.e. B_1 , B_5 and B_6 , and three auxotrophic mutants. $a = ad$ 8, $m = me$ 5 and $c = chol$ 1 with nutrient requirement of adenine, methionine and choline respectively, were used in this study. These loci fall into two linkage groups. The loci a and m are linked with A , the distance $A - a$ being 1.27 units and $A - m$ being 23 units. In the second linkage group B is linked with c at a distance calculated from different crosses to be 5.94 to 15.4 units (Day 1958, 1960a).

Recombinants were obtained from the above material by making single basidiospore isolations from suitable matings.

All the experiments, unless otherwise stated, were performed at 30° C in Petri dishes 80 mm. in diameter. Three types of nutrient media were used:

a) A simplified Fries (1953) minimal medium. In 1 litre it contains 20 g. glucose, 2 g. asparagine, 0.5 g. ammonium tartrate, 1 g. KH_2PO_4 , 2.25 g. Na_2HPO_4 , 0.29 g. Na_2SO_4 , 0.04 mg thiamin and 20 g. agar.

b) Minimal medium supplemented with adenine (0.01 g/litre), methionine (0.01 g/litre) or choline (0.2 mg/litre), singly or doubly, and termed respectively: a, m, c, am, ac and mc. For instance mc is a minimal medium supplemented with methionine and choline.

c) A modified Day (1959) complete medium. It contains all the components of the minimal medium and moreover to each litre are added: 1.35 g. yeast extract, 0.75 g. peptone and 1.25 ml. hydrolysed yeast nucleic acid.

Fruitbodies were produced in bottles of 500 ml volume on sterilised horse manure at about 26° C.

To test the possibility of exchange of nuclei between dikaryons, blocks of 1 mm. side of 2 different dikaryotic mycelia were placed on Petri dishes side by side and allowed to grow jointly. Usually 3 inocula (or pairs of inocula) were placed in the centre of the Petri dish (in an equilateral triangle, 15 mm. side). The extent of growth was established by measuring the largest distance from the inoculum to the margin of the colony (the radius). In each dikaryon both component nuclei were double mutants and had one nutrient requirement in common. The paired dikaryons had one nucleus in common, but each dikaryon of the pair was homozygous for a different nutrient requirement. The 3 dissimilar nuclei present in each pair of dikaryons were mutually compatible. To detect any influence of cytoplasm dikaryons were synthesised in both directions. Reciprocal matings were not always successful, because some strains were unable to become dikaryotised. This limited the variety of combinations. All the investigated combinations of dikaryons are presented in table 1. For instance in the combination of type I^a the component nuclei of the first dikaryon require methionine and choline (one nucleus) and adenine and methionine (the other nucleus). Both nuclei require methionine and therefore the mycelium with the first dikaryon will grow only on minimal medium supplemented with methionine. In a similar way the second dikaryotic mycelium will grow only on minimal medium supplemented with adenine. A recombinant dikaryon may be recognised if it is composed of the nucleus A_2B_1 mc from the first dikaryon, and the nucleus A_5B_5 ac from the second one. A mycelium with such a recombinant

Table 1
Investigated combinations of dikaryons

Type	Component dikaryons	Nutrient requirements of the dikaryons		
		first	second	recombinat
I	a	m*	a**	c***
	b			
II	a	a	c	m
	b			
III	a	c	m	a
	b			

Notice: Strain used as acceptor of nuclei is underlined

* methionine

** adenine

*** choline

dikaryon would require choline. Only cytoplasmic differences exist between type I^a and I^b. Types I, II and III differ in nutrient requirements of the component dikaryons and in mating types. Combinations of A and B alleles with auxotrophic mutants could not be chosen freely as not all possible combinations were available.

Samples of mycelia or single basidiospore isolates were tested for nutrient requirement and mating type as follows:

To test nutrient requirement, unless otherwise stated, samples of mycelium were inoculated to 3 doubly supplemented media: am, ac and mc respectively. Prototrophic mycelia were able to grow on each of them, and auxotrophic mycelia requiring all the compounds were unable to grow on any of them. Auxotrophic mycelia with single requirements were unable to grow on one of the media, and doubly auxotrophic mycelia were unable to grow on two of them.

To determine the mating type 4 wild type tester stocks were used, each compatible for one mating type locus with all the strains. For instance if spores originating from a dikaryon: $A_5B_5 \times A_6B_6$ were analysed, the following strains were used as tester stocks: A_5B_1 , A_6B_1 , A_2B_5 and A_2B_6 .

The determination of nutrient requirement was usually made first to establish the expected mating type alleles.

PRELIMINARY EXPERIMENTS

Each of the 10 dikaryotic mycelia used in the combinations listed in table 1 was tested on minimal and supplemented media. All had the expected growth requirements. They were also allowed to fruit in dung bottles. Each bottle was inoculated with a different dikaryotic mycelium. Two of them, used twice in combinations listed on table 1 (because reciprocal combinations could not be obtained) were inoculated to two dung bottles each. All bottles with methionine requiring mycelia fruited, producing 1—4 fruitbodies in the bottle. Only 2 fruitbodies were obtained from choline requiring mycelia and one of them with great delay (after 20 days). No fruitbodies were obtained from mycelia requiring adenine (table 2). Spore analysis was made from 2 fruitbodies developed from methionine requiring mycelia and 1 fruitbody developed from a choline requiring mycelium. The spores were found to have the expected growth requirements and mating type alleles.

Each dikaryotic mycelium was also grown on complete medium in Petri dishes. Their growth was more or less normal. The margin of the colonies was even, but usually the margin of the submerged growth extended further than the margin of the surface growth. The average

Table 2

Growth, fruiting ability and hyphal tip characteristic of the component dikaryons

Dikaryon	Growth mm*	Number of		Dikaryons	Number of hyphae tip isolates				lost	total
		bottles	fruitbodies		Monokaryons with requirement of:					
					ac	am	mc			
$A_2B_1\ mc \times A_6B_6\ am$	23	1	4	16	—	—	—	1	1	18
$A_2B_1\ mc \times A_6B_6\ am$	24	1	1	18	—	—	—	—	—	18
$A_2B_6\ mc \times A_5B_1\ am$	22	2	3	13**	—	3	—	1	1	18
$A_6B_6\ am \times A_5B_5\ ac$	22	1	—	8	4	3	—	—	3	18
$A_6B_6\ am \times A_5B_5\ ac$	24	1	—	13	2	1	—	—	2	18
$A_6B_1\ am \times A_5B_6\ ac$	25	1	—	15	2	1	—	—	—	18
$A_6B_1\ am \times A_5B_6\ ac$	27	1	—	18	—	—	—	—	—	18
$A_5B_6\ ac \times A_2B_5\ mc$	30	2	1	15	1	—	—	1	1	18
$A_6B_5\ ac \times A_2B_6\ mc$	29	1	1	16	1	—	—	1	—	18
$A_6B_5\ ac \times A_2B_6\ mc$	23	1	—	17	—	—	—	1	—	18
Total		12	10	149	10	8	—	5	8	180

* Average radius of the 3 colonies growing on complete medium in Petri dishes, measured after 6 days of growth.

** Ten of the dikaryons required both adenine and methionine.

radius of the colonies after 6 days of growth ranged from 22—30 mm. (table 2).

The stability of the dikaryons was checked by making hyphal tip isolates. After 5 days of growth on complete medium, 16 samples, each containing a few hyphae, were taken from the margin of the mycelium of each plate. Of these 8 samples contained only hyphal tips growing under the surface of the agar, and 8 samples contained hyphae growing both on and under the surface. The samples were transferred to complete medium. After 24 hours some hyphae easy to isolate individually grew from the inocula. From samples of each dikaryotic mycelium 18 single hyphal tips were isolated (9 from the submerged and 9 from the mixed parts). After 48 hours the isolates developed into colonies. They were first tested for ability to grow on minimal medium supplemented with the compound required by the original dikaryon. If they were unable to grow, their nutrient requirement was determined and they were checked for the presence of clamps. Out of 180 isolates made, 8 did not grow, 23 appeared to be monokaryotic and the remainder were dikaryotic. Both types of monokaryotic mycelia could be isolated sometimes from hyphal tips of a given dikaryotic mycelium. An interesting case was observed in one dikaryotic mycelium requiring methionine. Out of 13 isolates with clamps (apparently dikaryotic), 10 required both adenine and methionine for growth (table 2).

Of the 23 monokaryotic hyphal tip isolates, 15 originated from samples taken from submerged hyphae.

GROWTH OF PAIRS OF DIKARYOTIC MYCELIA ON COMPLETE MEDIUM

The six pairs of dikaryotic mycelia listed on table 1 were inoculated to plates of complete medium. Each pair was inoculated to a separate plate. The mycelia were allowed to grow for 6 days and this group of plates is called transfer 1. On the 6th day from each plate blocks of ca. 3 mm. side of dense mycelium, developing in the area where both mycelia met, were used to inoculate new plates. Again 6 plates were inoculated and grown for 6 days. This group of plates is called transfer 2. In the same way mycelia of transfer 3 were grown from inocula taken from plates of transfer 2.

After 6 days each plate was covered or nearly covered with mycelium. The transfer 1 mycelia tended to form separate sectors, between which areas of dense mycelium of various size and shape could be found (Plate I, fig. 1 and 2). In later transfers the growth was more intermixed, but the dense areas could still be found, sometimes of considerable size (Plate I, fig. 3 and 4). No such areas of dense mycelium were found when single dikaryotic mycelia were grown on complete medium.

Plate I

Plate I

Fig. 1. — Pairs of dikaryotic mycelia inoculated to complete medium after 6 days of growth. Transfer 1, combination of type II. Different growth of both dikaryotic mycelia and dense growth in the meeting area visible

Fig. 2. — As fig. 1, but transfer 1, type I. Both dikaryotic mycelia growing vigorously, dense areas as less pronounced.

Fig. 3. — As fig. 1, but transfer 3, type I. Dense areas surround the inocula and spread in stripes

Fig. 4. — As fig. 1, but transfer 3, type II. Uneven growth of mycelia is visible. Dense areas are less pronounced

Fig. 5. — Recombinant dikaryotic mycelium auxotrophic for adenine, after 6 days of growth on minimal medium supplemented with adenine

Fig. 6. — Pairs of dikaryotic mycelia inoculated to minimal medium after 6 days of growth. Transfer 1, type I. Sectors with vigorous growth and sectors with faint growth clearly visible

Fig. 7—9. — The same series as fig. 6, but various kinds of growth in transfer 3: Fig. 7 presents mycelia in combination of type I, fig 8 — type II and fig. 9 — type III. Further explanations in the text.

Pictures reproduced in fig. 1—9 made by S. Kowalski

Table 3

Growth on minimal medium of samples isolated from complete medium

Type*		Samples from second transfer		Samples from third transfer	
		isolated	growing on minimal medium	isolated	growing on minimal medium
I	a	3	3	3	3
	b	3	3	3	1
II	a	3	2	3	2
	b	3	3	3	2
III	a	3	3	3	1
	b	3	1	3	3
Total		18	15	18	12

* Cf. table 1

After 6 days of growth, blocks of 1 mm. side were taken from dense areas of the second and the third transfer to inoculate minimal plates. The results are presented on table 3. Most isolates were able to grow on minimal medium. This indicates that in dense growing areas nuclei of both original dikaryons are usually present.

FRUITING ABILITY OF DIKARYOTIC MYCELIA GROWING JOINTLY ON COMPLETE MEDIUM

After 6 days of growth, samples of dense mycelium of each of the 3 transfers were inoculated to dung bottles, to test their fruiting ability. From each plate 1 bottle was inoculated, samples being taken from all the 3 colonies growing on each plate. The results are presented in table 4. Mycelia in each bottle fruited, frequently producing several fruitbodies. The fruiting was completed within 12 days from inoculation.

Single basidiospore isolates from nine fruitbodies (3 from each of the three types listed in table 1) were analysed, their growth requirements and mating type being determined.

Abnormalities were found in 1 fruitbody of type I and 1 fruitbody of type III. Therefore from these two types new samples, 5 fruit bodies from each, were tested. This makes a total of 19 analysed fruitbodies.

In the abnormal fruitbody of type I apparently all 3 nuclei recombined. A thorough inspection of the gills from which the sample of basidiospores was taken and an analysis of a larger number of single spore isolates (72 were tested) indicated that probably a mixed sample

Table 4

Fruiting ability of pairs of dikaryotic mycelia

Type*		Number of fruitbodies in bottles from transfer:			Total number of	
		first	second	third	bottles	fruitbodies
I	a	7	2	3	3	12
	b	4	7	4	3	15
II	a	1	6	3	3	10
	b	2	5	1	3	8
III	a	5	4	3	3	12
	b	1	2	2	3	5
Total		20	26	16	18	62

* Cf table 1

of gills originating from 2 different fruitbodies was taken. In this way the total of analysed fruitbodies increased to 20 (table 5).

Table 5

Analysis of fruitbodies

Type*	Nuclei taking part in the formation of the fruitbody						Total number of fruitbodies
	two nuclei originating from one component dikaryon with requirement of:			two nuclei originating each from a different dikaryon with requirement of:			
	adenine	methionine	choline	adenine	methionine	choline	
I		8	×	×	×	1**	9
II	1	×		×	2	×	3
III	×	8***			×	×	8
Total	1	16			2	1	20

* Cf. table 1.

** Fruitbody from the mixed sample of gills.

*** All isolates from one of the fruitbodies required both adenine and methionine for growth.

In the abnormal fruitbody of type III over 90% of spores germinated, but out of 64 basidiospore isolates tested all required both adenine and methionine for growth. Moreover some of them required choline. The mating type was determined in 8 isolates. The alleles: $A_2 A_5 B_1 B_6$ were found in the isolates, the allele A_5 appearing only once. The requirement of choline was linked with B_6 . It follows from these data that in 7 out of 8 examples tested, strains requiring adenine contained the A_2 allele, although — basing on the characteristic of the original dikaryons — it was expected that A_5 will be closely linked with adenine requirement.

In the remaining 7 fruitbodies from combinations of type III the abnormal reaction did not appear.

Summarised results of the analysis of fruitbodies are presented in table 5. From these results the following conclusions may be drawn:

1. Fruitbodies are most easily formed by dikaryotic mycelia requiring for growth methionine.

2. In mixed cultures fruitbodies may be obtained from dikaryotic mycelia requiring adenine for growth.

3. Fruitbodies may be formed from recombinant dikaryotic mycelia. Such fruitbodies were obtained from recombinant mycelia requiring methionine (2 cases) and requiring choline (1 case).

FORMATION OF RECOMBINANT DIKARYONS ON SUPPLEMENTED MEDIA

Blocks of ca 3 mm. side were taken from dense areas of plates from transfer 1 and transfer 3, grown on complete medium, and inoculated to supplemented media. Media were selected in such a way, that only recombinant dikaryotic mycelia would be able to grow on them singly. The plates were incubated for 6 days and then blocks of ca 1 mm side were taken from the marginal parts of the colonies to check their growth requirements. If the testing demonstrated, that no or very few recombinant dikaryons were produced, new plates were inoculated and grown for 6 days (transfer 2). In the same way transfer 3 was made, if necessary. The results are presented in table 6. Recombinant dikaryons requiring adenine for growth were formed very quickly and already from transfer 1 only recombinant mycelia were obtained. Recombinant dikaryons requiring methionine were obtained in 7 out of 12 examples in transfer 1 and in all but one example in transfer 2. No choline requiring recombinants were obtained in 3 successive transfers.

Two recombinant dikaryons requiring methionine and two requiring adenine were taken for a more detailed analysis. Each was grown on a separate plate supplemented with methionine and adenine respectively and shown to be able to cover the plate with a more or less normal mycelium (Plate I, fig. 5). They were also inoculated to dung bottles. The methionine requiring recombinants fruited and spore analysis demonstrated the expected recombinations of growth requirements and mating type loci. The adenine requiring recombinants were unable to fruit. They were therefore crossed with two wild type strains compatible respectively with one and the other of the expected component nuclei of the recombinant dikaryons. Fruitbodies were obtained from these crosses and spore analysis demonstrated in each case the expected recombinations of growth requirements and mating type loci.

Table 6
Growth requirements of pairs of dikaryotic mycelia grown on supplemented media

Type*	Source transfer	Medium supplemented with	Transfer 1				Transfer 2				Transfer 3			
			number of isolates with requirement of											
			a	m	c	+	a	m	c	+	a	m	c	+
I	a	c	3	—	—	—	2	—	—	1	2	—	—	1
	3	c	3	—	—	—	—	—	—	3	2	—	—	1
	1	c	3	—	—	—	2	—	—	1	3	—	—	—
	3	c	3	—	—	—	3	—	—	—	3	—	—	—
II	a	m	—	—	—	3	1	2	—	—	×	×	×	×
	3	m	—	2	—	1	—	3	—	—	×	×	×	×
	1	m	—	3	—	—	—	3	—	—	×	×	×	×
	3	m	—	2	—	1	—	3	—	—	×	×	×	×
III	a	a	3	—	—	—	×	×	×	×	×	×	×	×
	3	a	3	—	—	—	×	×	×	×	×	×	×	×
	1	a	3	—	—	—	×	×	×	×	×	×	×	×
	3	a	3	—	—	—	×	×	×	×	×	×	×	×

* Cf. table I.
 ** Able to grow on minimal medium.
 × the transfer was not made.

GROWTH OF PAIRS OF DIKARYOTIC MYCELIA ON MINIMAL MEDIUM

Paralell to inoculations to complete medium, described previously inoculations were made in the same way to plates of minimal medium. As before three successive transfers were made, each growing for 6 days. The growth of mycelium was usually irregular, but quite considerable. Examples of such mycelia, after 6 days of growth on minimal medium are presented in plate I, fig. 6—9.

Table 7

Growth requirements of fainter growing mycelium

Type*		Number of isolates requiring			
		a	m	c	***
I	a	9	—	—	—
	b	8	—	—	1
II	a	9	—	—	—
	b	5	1	—	3
III	a	4	1	1	3
	b	2	—	—	7
Total		37	2	1	14
among them transfer:	first	13	1	1	3
	second	13	—	—	5
	third	11	1	—	6

* Cf. = table 1.

** Able to grow on minimal medium.

Inocula taken from dense parts of mycelium, used for making successive transfers, always produced growth on minimal medium. In most colonies areas with fainter growth of mycelium could also be detected. The results of the checking growth requirements of the fainter mycelium are presented in table 7. Isolates requiring adenine largely predominate, also in combinations of type III, where such mycelia are expected to contain recombinant dikaryons. Occasionally mycelia with other requirements are present, in one case also a mycelium with choline requirement.

Minimal plates from transfer 3 were kept in the incubator for a prolonged period of time. In one of the plates a rudimentary fruitbody developed (Plate II, fig. 1) indicating that a joint growth of dikaryotic auxotrophic mycelia is able to produce conditions necessary for fruiting, even on minimal medium.

Plate II

Fig. 1. — A rudimentary fruitbody developed on minimal medium in a plate inoculated with a pair of complementary auxotrophic dikaryotic mycelia

Fig. 2. — Network of aerial hyphae, a few millimeters beyond the margin of a 6 days old mixed dikaryotic colony. Many pairs and groups of hyphae sticking together visible. The area enclosed by the rectangle magnified on fig. 3

Fig. 3. — Two hyphae sticking together in aerial dikaryotic mycelium. Morphological changes and the line along which the hyphae appear to fuse is clearly visible. Arrows indicate places where clamps are present

Fig. 4. — Network of hyphae from the same mycelium as Fig. 2. Places with many hyphae sticking together indicated by arrows

Fig. 5. — Network of hyphae from the same mycelium as fig. 2. Morphology of the thickened part of mycelium visible. The area enclosed by the rectangle magnified on fig. 6

Fig. 6. — Network of hyphae, shown on fig. 5, magnified. Several pairs of hyphae sticking together visible

All pictures taken from living mycelium, growing in Petri dishes.

Picture reproduced on fig. 1 made by S. Kowalski

Pictures reproduced on fig. 2—6 made by Z. Klawender

Plate II

SINGLE HYPHA ANALYSIS OF MYCELIA GROWING ON MINIMAL MEDIUM

From each of the 3 transfers growing on minimal medium, after 6 days of growth, 18 samples containing a few marginal hyphae were isolated from each plate. They were taken from areas with most vigorous, either submerged or surface growth. The samples were transferred to minimal medium. After 24 hours new growth started from the inocula from which single hyphae could be isolated, sometimes branched and up to 2 mm. long. After the single hyphae were isolated, the inocula were allowed to grow for 4 days, and the number of colonies able to develop was scored. The results are presented in table 8. It appears that in all combinations of pairs of mycelia isolates containing only a few hyphae may grow sometimes on minimal medium.

Table 8

Ability to grow on minimal medium of samples containing a few hyphae

Type*		Number of samples	
		isolated	growing on minimal medium
I	a	54	1
	b	54	2
II	a	54	3
	b	54	18
III	a	54	5
	b	54	6
Total		324	35
Among them transfer:	first	108	7
	second	108	17
	third	108	11

* Cf. table 1.

The single hyphal isolates were transferred to minimal plates and incubated for 4—6 days. Altogether 108 single hyphal tips were isolated, 41 originating from dikaryon combinations of type I, 36 from type II and 31 from type III. None of them developed into colonies able to grow on minimal medium.

Another 54 hyphal tips were isolated from samples of dense mycelia from colonies grown on complete medium. None grew on minimal medium.

The single hyphal isolates did not show normal growth, but they developed a faint mycelium, typical for auxotrophic mutant strains. A sample of such colonies was taken to check the growth requirement and the presence of clamps. The results are presented in table 9. All the isolates required either adenine or adenine and choline. In all colonies

requiring only adenine clamps were present. All colonies requiring both adenine and choline had no clamps.

The mating types of the colonies requiring both adenine and choline were determined. All colonies originating from combinations of type I had the mating type $A_5 B_5$, all colonies from type II had the mating type

Table 9
Growth requirements of single hyphal isolates

Type*	Number of isolates requiring:		
	a	ac	total
I	a	10	1
	b	14	1
II	a	11	1
	b	8	—
III	a	7	2
	b	6	5
Total	56	10	66

* Cf. Table 1.

$A_5 B_6$ and all colonies from type III had the mating type $A_6 B_5$. This result was expected on the assumption that these colonies are monokaryotic and the nuclei present in them are unchanged component nuclei of one of the original dikaryons (cf. table 1).

MICROSCOPIC OBSERVATIONS ON JOINTLY GROWING DIKARYOTIC MYCELIA

To get some idea, how cytoplasmic connections between dikaryotic mycelia might be established, pairs of such mycelia, growing on plates with complete medium, were observed microscopically. On the agar surface in the periphery of the colonies only single hyphae could be seen, but in aerial mycelium, reaching frequently the colony margin, pairs or groups of hyphae sticking together could be seen (Plate II fig. 2—6). Morphological changes in hyphae sticking together and — partial at least — disappearance of the walls separating them indicates that longitudinal hyphae fusions take place. This picture was observed in monokaryotic and in dikaryotic mycelia, singly or in combinations. Sometimes such aerial hyphae, fused or not, grow beyond the hyphae developping on agar surface, and falling down to the agar, start a marginal surface growth.

DISCUSSION

Three types of combinations of dikaryons were analysed (table 1). In two of them, in which recombinant dikaryotic mycelia required methionine or adenine, the recombinants were obtained several times (tables 5

and 6). Recombinant dikaryotic mycelia with adenine requirement were formed very easily without any selective pressure applied or even against it (tables: 6, 7 and 9). There are indications that recombinant dikaryons may be formed also from choline requiring nuclei (table 5). They were not obtained frequently, because such dikaryotic mycelia are apparently eliminated by mycelia requiring adenine (table 6). It appears from these results that recombinant dikaryons are formed without difficulty. The frequency of their formation largely depends on the genetical constitution of the strains. The cytoplasm did not influence their formation to any large extent in the analysed material.

Two simple ways of forming recombinant dikaryons may be suggested:

1. Fusion of hyphae containing different dikaryons and rearrangement of nuclei within the cell. A network of hyphae was observed in dikaryotic mycelia (Plate II, fig. 2—6) in which many hyphal fusions must have taken place and there is no reason to expect that such fusions would be less frequent between hyphae originating from different mycelia. Polykaryotic hyphae are apparently not stable as in over 160 isolated hyphal tips none appeared to contain 3 kinds of nuclei. It must be expected therefore, that if such fused hyphae produce new growth, the number of nuclei per cell must be reduced to one pair, and this pair may be recombinant.

2. Dikaryotisation of monokaryotic (dedikaryotised) hyphae. It has been shown that single hyphae taken either from the original dikaryotic mycelia (table 2) or from their joint growth (table 9) may contain nuclei of one type only. If such monokaryotic cells exist in the colony and if they are dikaryotised by a nucleus of a foreign dikaryon (Buller phenomenon), a recombinant dikaryon will be formed.

The results obtained do not allow the rejection of either of the alternatives, nevertheless the first appears to be the more likely one. Hyphal fusions are formed in large numbers in aerial mycelium and this mycelium frequently reaches the periphery of the colony, making the marginal growth of recombinants possible. On the other hand monokaryotic hyphal tips appear to be formed most frequently (or exclusively?) under the surface of the agar. Hyphal fusions appear to be less likely there, even for mechanical reasons. Moreover monokaryotic hyphal tips appear to have lost the ability to accept compatible nuclei found in the same hypha (Świeżyński 1961). It may be expected that the acceptance of nuclei from foreign hyphae may also be difficult.

Published data concerning *Coprinus* and other *Basidiomycetes* indicate that both mechanisms may operate in bringing about exchange of nuclei between dikaryons. Hyphae fuse in fungi very frequently (Buller 1933) and Hein (1930) observed in *Psalliotia campestris* also longitudinal

hyphal fusions. Studies of Nelson (1956) and Nelson et al. (1954, 1955) indicate that in rusts a polykaryotic mycelium is an intermediate stage in formation of recombinant dikaryons. On the other hand the splitting of dikaryons bringing about the formation of monokaryotic mycelia has also been observed in many fungi. It was found in connection with periodic changes of mycelium, formation of oidia, differentiation of hyphae in fruitbodies and germination of basidiospores of "homothallic" species (Papazian 1950, 1958, Ashan 1952, Terra 1955, older literature in reviews of Buller 1941 and Raper 1953).

There are several indications that in compatible and incompatible di — mon matings exchange of genetic material between nuclei may occur (Crowe 1960, Kimura 1958, review of older data, Papazian 1958). If recombination of this kind had occurred in the material analysed, it would be expected to bring about two kinds of changes:

1. The formation of dikaryotic mycelia able to grow on minimal medium. For instance in the first dikaryon of type I (Table 1) a recombinant nucleus of the constitution $A_2 B_6 m$ might be formed. Such a nucleus would be compatible with the nucleus $A_5 B_5 ac$ of the other dikaryon and in this way a recombinant dikaryon might be formed, able to grow singly on minimal medium. Hyphal tip isolates with such dikaryons would also be able to grow on minimal medium. No such dikaryons were ever observed, although the cultures have been kept for three successive transfers on minimal and complete media and then some were kept for prolonged periods on supplemented media.

2. Formation of recombinant abnormal nuclei. In the analysed material only one case of abnormal reaction was observed i.e. the appearance of additional requirement of adenine. This was found in some hyphal tip isolates originating from one of the dikaryotic mycelia (table 2) and in the progeny of one fruitbody formed by a mixed growth of mycelia, the same dikaryotic mycelium being one of the components (Table 5). This phenomenon does not appear to be due to exchange of genetic material between nuclei. However no satisfactory explanation of this result could be found and a separate analysis of the case is intended.

These results suggest that in dikaryons of *C. lagopus* singly or in joint growth the tendency to somatic exchange of genetic material between nuclei does not exist or is very weak. This may be an indication, that such somatic recombinations are linked with migration of nuclei, which took place in all described cases of somatic recombination in *Holobasidiomycetes*. In our experiments, performed only with dikaryotic mycelia, there was no opportunity for nuclear migration.

In the course of experiments two interesting phenomena appeared:

1. Crossfeeding between dikaryotic mycelia. Single, auxotrophic

ones were unable to grow on minimal medium, but pairs of them with different growth requirements were able to form large colonies. The crossfeeding was also apparent in fruitbody formation. Single auxotrophic dikaryotic mycelia were unable to fruit or fruited poorly (Table 2). On the contrary pairs of such mycelia with complementary growth requirements fruited profusely (Table 4). In single cultures dikaryotic mycelia requiring adenine for growth were never observed to fruit, but such a fruitbody was obtained in joint growth of mycelia (Table 5). The cross-feeding may also be important in fruiting of wild type mycelia. Difficulties in obtaining fruitbodies from single dikaryotic mycelia (Terra 1953) may be due to lack of such crossfeeding.

2. Combinations of nuclei advantageous for dikaryotic growth are not necessarily the same as those most favorable for fruitbody formation. Throughout the whole course of these experiments it was found, that dikaryons composed of nuclei with adenine requirement are most stable (irrespective of nutrient medium used). On the contrary the best fruiting was observed regularly in dikaryons auxotrophic for methionine.

It appears likely that the exchange of nuclei has an evolutionary significance, making possible the formation, multiplication and fruiting of the best adapted dikaryotic mycelia. It may be of special importance in parasites in which monokaryotic and dikaryotic mycelia develop on different hosts. In such parasites it is additionally significant that basidiospores formed on recombinants, and able to produce best combinations, are liberated jointly and have more chance to reach jointly the, frequently distant, alternative hosts.

Aknowledgement

The author wishes to express his gratitude to Dr. P. R. Day, John Innes Institute, England, who has kindly checked the manuscript and made many valuable suggestions.

SUMMARY

1. From two jointly growing dikaryotic mycelia with mutually compatible nuclei a new dikaryotic mycelium may arise. The component nuclei of the new dikaryon are each derived from a different dikaryon. The frequency of such recombination largely depends on the genetical composition of the nuclei. Its mechanism and possible evolutionary significance are discussed.

2. Jointly growing auxotrophic dikaryons are able to crossfeed. By this means fruitbodies may be obtained from mycelia which are unable to fruit alone.

3. In jointly growing dikaryotic mycelia recombinant nuclei due to internuclear exchange of genetical material were not observed, although such mycelia were grown for prolonged periods of time.

REFERENCES

- Allen R. F., 1926, A cytological study of *Puccinia triticina* physiological form 11 on Little Club wheat, J. Agr. Res. 33: 201—222.
- Aschan K., 1952, Studies on dediploidisation mycelia of the basidiomycete *Collybia velutipes*, Svensk. Bot. Tidskr. 46: 366—92.
- Buller A. H. R., 1933, Researches on Fungi vol. V, repr. 1958, Hafner New York.
- Buller A. H. R., 1941, The diploid cell and the diploidisation process in plants and animals with special reference to higher fungi, Bot. Rev. 7: 335—431.
- Crowe L. K. 1960, The exchange of genes between nuclei of a dikaryon. Heredity 15: 397—405.
- Day P. R., 1958, John Innes Hor. Inst. Annual Report.
- Day P. R., 1959, A cytoplasmically controlled abnormality of the tetrads of *Coprinus lagopus*, Heredity 13: 81—87.
- Day P. R., 1960a, The structure of the A mating type locus in *Coprinus lagopus*, Genetics 45: 641—50.
- Day P. R., 1960b, Variation in Phytopathogenic Fungi, Ann. Rev. Microb., 14: 1—16.
- Ellingboe A. H., 1961, Somatic recombination in *Puccinia graminis* var. *Tritici*, Phytopathology 51: 13—15.
- Fries L., 1953, Factors promoting growth of *Coprinus fimetarius* under high temperature conditions, Physiol. Plant 6: 551—63.
- Hein J., 1930, Studies in the mycelium of *Psalliota campestris*, Amer. J. Bot. 17: 197—211.
- Kimura K., 1958, Diploidisation in the *Hymenomycetes*, II Nuclear behaviour in Buller phenomenon, Biol. J. Okayama Univ. 4: 1—59.
- Nelson R. R., 1956, Transmission of factors for urediospore colour in *Puccinia graminis* var. *tritici* by means of nuclear exchange between vegetative hyphae, Phytopathology 46: 538—40.
- Nelson R. R. and Wilcoxson R. D., 1954, Heterocaryosis as a mechanism of variation in rust fungi, Phytopathology 44: 400 (Abstr.).
- Nelson R. R., Wilcoxson R. D. and Christensen I. I., 1955, Heterokaryosis as a basis for variation in *Puccinia graminis* var. *tritici*, Phytopathology 45: 639—43.
- Papazian H. P., 1950, Physiology of incompatibility factors in *Schizophyllum commune*, Bot. Gaz. 112: 143—63.
- Papazian H. P., 1958, The Genetics of *Basidiomycetes*, Adv. Genet. 9: 41—69.
- Pontecorvo G., 1959, Trends in genetic analysis, Oxford Univ. Press.
- Raper J. R., 1953, Tetrapolar sexuality, Quart. Rev. Biol. 28: 233—59.
- Swieżyński K. M., 1961, Migration of nuclei in tetrapolar *Basidiomycetes*, Acta Soc. Bot. Pol. 30: 529—534.
- Swieżyński K. M., and Day P. R., 1960a, Heterokaryon formation in *Coprinus lagopus*, Genet. Res. 1: 114—128.
- Swieżyński K. M., and Day P. R., 1960b, Migration of nuclei in *Coprinus lagopus*, Genet. Res. 1: 129—139.
- Terra P., 1953, Détermination de la polarité sexuelle en trente espèces de Basidiomycètes saprophytes, Compt. Rend. Acad. Sci. 236: 115—170.
- Terra P., 1955, Amphithallie et hétérocaryose chez un basidiomycète bipolaire, *Stropharia umbonatescens*, Compt. Rend. Acad. Sci. 241: 1334—36.