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Analysis of an incompatible di-mon mating in *Coprinus lagopus*

Analiza krzyżówki dikarion-monokarion (di-mon) u Coprinus lagopus

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INTRODUCTION

In the tetrapolar *Basidiomycetes*, to which group *Coprinus lagopus* belongs, mating type is determined by two independently inherited series of alleles: A and B. Two monokaryotic mycelia are able to initiate jointly a dikaryon only if their nuclei contain different alleles at both the A and B loci.

If a small inoculum of a dikaryotic mycelium is added to the margin of a monokaryotic colony, the nuclei of the dikaryon usually migrate into the monokaryotic colony to establish a new dikaryon (Buller 1930). This is called a di-mon mating. When both the nuclei of the dikaryon are compatible with the monokaryon, $(A_xB_x + A_yB_y) \times A_zB_z$ the mating is doubly compatible. When only one nucleus of the dikaryon is compatible, $(A_xB_x + A_yB_y) \times A_xB_x$* the mating is singly compatible and when neither nucleus is compatible, $(A_xB_x \times A_yB_y) \times A_xB_y$ the mating is incompatible. In the incompatible di-mon mating the nucleus of the monokaryon has a common A allele with the first nucleus of the dikaryon and has a common B allele with the other nucleus.

The problem of migration has been discussed in detail in previous papers (Świeżyński and Day 1960b, Świeżyński 1961a).

Occasionally di-mon matings give rise to new dikaryons with recombinant nuclei. These were found in doubly compatible di-mon matings by Crowe (1960) and Papazian (1950) in singly compatible matings by Kimura (1958) and in incompatible matings by Kimura (1958), Quintanilha (1939) and Papazian (1950, 1954). Crowe claimed to have demostrated recombination of linked genes in di-mon

* Instead of the A_xB_x nucleus sometimes A_xB_z is used (Kimura 1958).

matings. In each example the recombinants originated from exchange of genes between two compatible nuclei. Recombinants were obtained easily without selective pressure, which indicates, as C r o w e has pointed out, that recombinant nuclei must occur fairly frequently. Nevertheless recombinant dikaryons are less frequent in doubly and singly compatible di-mon matings than dikaryons which arise by migration of both nuclei of the parent dikaryon (C r o w e 1960, K i m u r a 1958).

Parag and R a p e r (1960), using selective pressure, obtained common B heterokaryons in compatible di-mon matings. A common B heterokaryon was also obtained in a compatible di-mon mating by K i m u r a (1958).

The conditions favouring formation of recombinant nuclei and the mechanism of recombination remain obscure. The present investigation was undertaken to collect information relating to this problem through the study of changes in nuclear composition of a monokarytic mycelium invaded by nuclei of an incompatible dikaryon.

MATERIALS AND METHODS

Three strains of *C. lagopus* were used in the combination: (A_5 *m*. B_5 *c* + A_6 *a*. B_6 *c*) × A_5. B_6. It was found from preliminary experiments that in such di-mon matings dikaryotic mycelium is easily formed at the periphery of the monokaryotic mycelium.

The subscripts at the letters A and B indicate the alleles of the mating type loci. The letters *a*, *c* and *m* represent recessive genes involving requirement of adenine (*a* = *ad—8*), choline (*c* = *chol 1*) and methionine (*m* = *meth—5*) respectively. Loci *a* and *m* are linked with A, the distance A — *a* being 1,30 units and A — *m* being 25 units. B is linked with *c* at a distance calculated from different crosses to be 2.2 to 15.1 (D a y and A n d e r s o n 1961).

Chromosomes carrying mating type loci originating from nuclei of the three strains may be identified by mating type and growth requirement. The three chromosomes containing the A mating type locus are of the composition: A_5 *m*, A_6 *a* and A_5 respectively. The three chromosomes, containing the B mating type locus are: B_5 *c*, B_6 *c* and B_6. In this way the origin of chromosomes taking part in the formation of recombinant nuclei can be determined.

All experiments were performed in Petri dishes at 30°C. The origin of the *Coprinus* strains, the media used and the methods of determination of nutrient requirement and mating type are described in a previous paper (Ś w i e ż y ń s k i, 1961b).

The di-mon matings were prepared as follows. Four identical Petri dishes, 100 mm diameter, containing complete medium were inoculated in the centre with a sample of the dikaryotic mycelium:

(A_5 *m*. B_5 *c* + A_6 *a*. B_6 *c*). At the same time 8 inocula of the monokaryotic mycelium: A_5B_6 were placed round the dikaryotic one in a circle, 10 mm in diameter (Fig. 1). The dikaryotic colony developing in the centre is a rich source of nuclei which migrate easily through

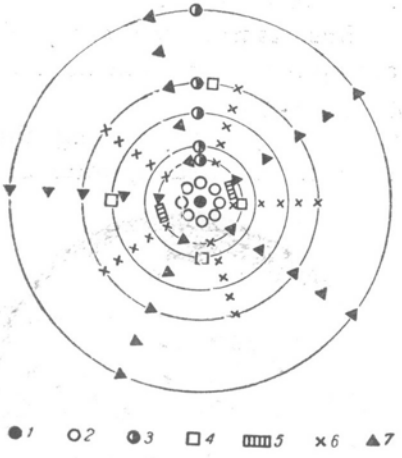

Fig. 1 — Sampling places
1 — inoculation of the dikaryotic mycelium; *2* — inoculation of the monokaryotic mycelia; *3* — colony margin after 2, 3, 4, 5 and 8 days respectively; *4* — hyphal tip isolation to complete medium after 2, 3, 4 and 5 days respectively; *5* — isolation of cubes 1 mm side to complete medium after 2, 3, 4 and 5 days respectively; *6* — isolation of cubes 5 mm side to minimal medium after 5 days; *7* — isolation of cubes 5 mm side to minimal medium after 8 days

the surrounding young monokaryotic mycelium. The monokaryotic strain developed so quickly that there was no danger of dikaryotic mycelium growing over it and reaching the margin of the colony.

Within 24 hours the dikaryotic mycelium met the surrounding monokaryotic one. Within 2 days the average total diameter of the colonies on the 4 Petri dishes reached 22 mm. After 3 days the margins of the colonies became fainter like a common A heterokaryon (Świeżyński and Day 1960a) and their average diameter reached 29 mm. Within 8 days the whole surface of the plates was covered with mycelium.

From each plate samples were taken (4 replicates) after 2, 3, 4, 5 and 8 days from inoculation. They were taken in 3 ways:

1. Discs about 1 cm^2 were cut out from the margin of the colonies and transferred to glass slides. Under a dissecting microscope 4 hyphal tips were isolated from each disc and transferred to complete medium. One sample per plate was taken after 2, 3, 4 and 5 days from inoculation.

2. Cubes of 1 mm side were taken at the position of the mycelium margin after 2 days of growth and transferred to complete medium. They were taken after 2, 3, 4 and 5 days from inoculation. Each time the samples were taken from adjacent places.

3. Discs about 0,25 cm^2 were taken from the whole surface of the colonies and inoculated to minimal medium. They were taken after 5 and 8 days from inoculation. This method of sampling favored isolation of recombinant dikaryons, as dikaryotic mycelia containing both migrant unchanged nuclei were auxotrophic.

The places, where samples were taken are presented on fig 1 and a picture of one of the plates, after all the samples were taken from it, is shown on Fig. 2.

Each sample was analysed for the presence of clamps, mating type and growth requirement. Moreover some were fruited and their progeny analysed.

In the progeny testing the basidiospore suspension was spread in Petri dishes. Usually after 24 hours single colonies were transferred to

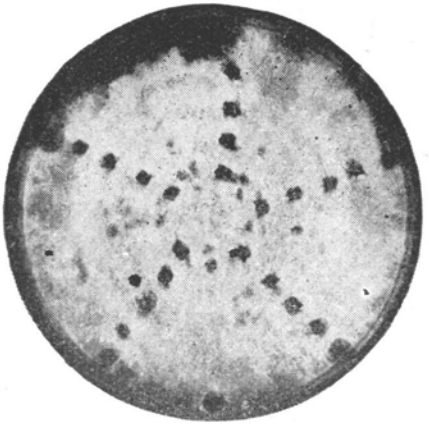

Fig. 2 — One of the plates on which the incompatible di-mon mating was performed. The picture has been made after all the samples were taken

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complete medium and at the same time the germination percentage was determined from counts of 100 — 500 basidiospores. After 48 hours the growth of each isolated colony was scored and they were tested for growth requirements and mating type.

RESULTS

Analysis of hyphal tips isolated to complete medium

Not every hyphal tip developed into a colony. Therefore of the 4 hyphal tips isolated from each plate only 2 were analysed from each sampling date.

If the isolates were unable to grow on minimal medium but grew on minimal supplemented with choline and if clamps were present it was assumed that the isolates contained the dikaryon with both migrant nuclei. If the isolate grew on minimal medium, produced no

clamps and was compatible with the $A_6 B_5$ tester stock it was assumed that the isolate contained the resident nucleus: $A_5 B_6$.

If the isolate was able to grow on minimal medium and produced clamps it was assumed that a recombinant dikaryon is present. One isolate of this kind developed weaker and was incompatible with the tester stock $A_1 B_6$. It was concluded that this isolate was a common B heterokaryon (A_5 *a*. B_6 *c* + A_5. B_6).

Table 1

Types of nuclei present in hyphal tip isolates

Sampling date (days)	Number of isolates with				Total
	the resident nucle (monokaryon)	both migrant nuclei (dikaryon)	recombinant nuclei (dikaryon)	common B nuclei (heterokaryon)	
2	8				8
3	2	3	2	1	8
4		8			8
5	1	7			8

Some isolates containing recombinant dikaryons and the common B heterokaryotic mycelium were allowed to fruit and the diagnosis was checked in progeny analyses described in one of the following sections.

The composition of hyphal tip isolates is shown in table 1. In samples taken after 2 days, when the mycelia hardly met, only nuclei of the original monokaryon were present. After 3 days (2 days after the colonies met) samples with both migrant nuclei and samples with recombinant nuclei were found. In samples taken after 4 and 5 days both migrant nuclei were present in nearly every case.

Analysis of cubes, 1 mm side, isolated to complete medium

The analysis was performed in the same way as the analysis of the hyphal tips. The results are presented in table 2. Most isolates contained both migrant nuclei. Two points are worth mentioning:

1. After 2 days (1 day after the colonies met) the two migrant nuclei were found, which indicate that migration of both nuclei starts nearly as soon as the colonies meet.

2. Almost every sample isolated contained both migrant nuclei which indicates together with results presented in table 1 that the migrants may colonise the monokaryotic mycelium rather densly.

Table 2

Types of nuclei present in cubes 1 mm side isolated to complete medium

Sampling date (days)	Number of isolates with			Total
	the resident nuclei (mono-karyon)	both migrant nuclei (dikaryon)	recombinant nuclei (dikaryon)	
2	1	6	1	8
3		8		8
4		8		8
5		8		8

Analysis of samples isolated to minimal medium

One hundred samples were taken after 5 days and one hundred after 8 days. The analysis was performed in a similar way to the analysis of samples isolated to complete medium, but more care was taken to purify the isolates i.e. all apparently recombinant dikaryotic mycelia were transferred twice on complete medium and then tested again on minimal medium to check, whether ability to grow on minimal medium and presence of clamps are not due to mixed growth of monokaryotic prototrophic and dikaryotic auxotrophic mycelium. It is apparent from progeny tests (Table 5) that the precautions were not sufficient to eliminate every mixed growth, although in the majority of cases the progeny test confirmed conclusions based on the analysis of the mycelium. No attempt was made to isolate more than 1 component from mixed growth.

Most samples isolated from central parts of the colonies after 5 and 8 days grew much weaker than samples isolated from the periphery (Fig. 3 and 4). The difference appears not to be linked with ability to grow on minimal medium, as those growing very poorly at the first transfer in further transfers differentiated into various classes some able and others unable to grow on minimal medium.

The results of the analysis of the samples are presented in tables 3 and 4. The rarity of both migrant nuclei, which were present in great majority in samples isolated to complete medium is obviously due to selective pressure (isolation to minimal medium). A new class of mycelium appears which is monokaryotic, with $A_6\,a$. $B_6\,c$ nuclei evidently formed from only one of the migrant nuclei.

Samples taken after 8 days contained more recombinant nuclei than samples taken after 5 days (54 compared with 35) and fewer samples

were made up of the original monokaryon (25 compared with 50). Both differences are not statistically significant.

The number of recombinant dikaryotic mycelia isolated was greatest near the periphery of the colony and the number of isolates with the original monokaryon was greatest near the centre of the colony. Only the second gradient is statistically significant ($p < 0{,}001$). This indicates

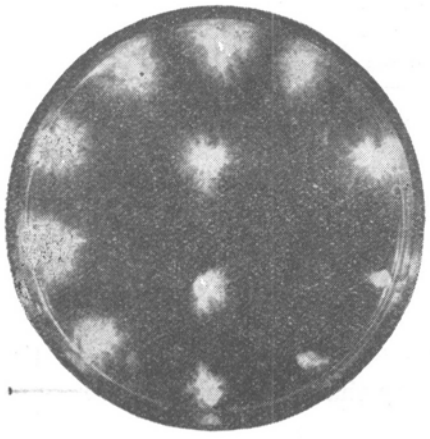

Fig. 3 — Plate with minimal medium containing 3 days old samples. The samples were taken from outer parts of the colony shown on Fig. 2. Most samples are able to grow

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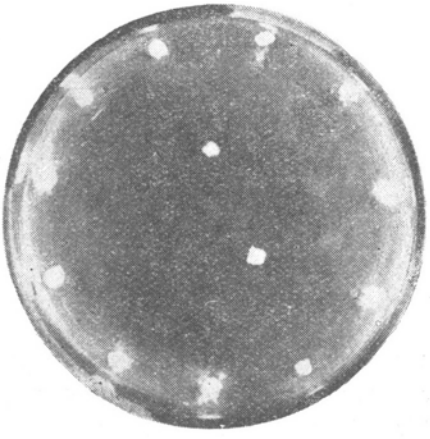

Fig. 4 — Plate with samples similar to those presented on Fig. 3 but isolated from central parts of the colony. Most samples are unable to develop visible growth, although in further transfers some of them will develop well on minimal medium

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Table 3

Types of nuclei present in cubes 5 mm side isolated after 5 days to minimal medium

Sampling place	Number of isolates containing				Total
	resident nuclei	both migrant nuclei	recombinant dikaryons	migrant nuclei $A_6a.B_6c$	
Periphery (I)	1		10	9	20
II	7	1	7	5	20
III	11	1	8		20
IV	14		6		20
Centre (V)	17		3		20
Total	50	2	34	14	100

Table 4

Types of nuclei present in cubes 5 mm side isolated after 8 days to minimal medium

Sampling place	Number of isolates containing				Total
	resident nuclei	both migrant nuclei	recombinant dikaryons	migrant nuclei $A_6a.B_6c$	
Periphery (I)	3	2	14	1	20
II	2		15	3	20
III	5	1	8	6	20
IV	3		9	8	20
Centre (V)	12		8		20
Total	25	3	54	18	100

that the resident nuclei are not eliminated from older parts of the mycelium but their increase may be slower than the increase of recombinant nuclei in the new growth.

Analysis of the progeny of recombinant dikaryotic mycelia

A sample of 11 apparently recombinant dikaryotic mycelia isolated on minimal medium together with 6 * apparent recombinants isolated on complete medium and 1 common B heterokaryotic mycelium isolated on complete medium were allowed to fruit. All fruited except 1 isolate from complete medium. In this way the progenies of 17 mycelia were tested. In 2 cases samples taken from different fruitbodies gave different results. Therefore alltogether 19 fruitbodies were analysed.

At first from each fruitbody a small sample of 16 spores was tested. Later some of them were analysed on a larger scale. The results are presented in table 5.

The germination of basidiospores was in general very poor (2—71% germinated). In similar experiments with normal fruitbodies, obtained from wild type and mutant strains usually 50—90% basidiospores germinated.

The growth of germinated basidiospores was also poor. In normal fruitbodies nearly every isolated germinated basidiospore develops into a normal colony. In these experiments out of 1486 colonies isolated, only 649, less than one half, developed in a normal way (table 5) and not all of them gave clear cut growth requirement reactions. This

* Data in tables 1—4 have been corrected from the results of progeny testing. Therefore in tables 1 and 2 only 4 recombinants are listed.

Table 5

Progeny analysis

No. of isolate	Source	% germination	Number of spores														
			isolated	growing	Tested									not growing	A_5B_5	A_5B_6	A_6B_5	A_6B_6
					growing	A_5m	A_6a*	A_5	others	B_5c	B_6c	B_6	others					
1[1]	Recombinants from complete medium	71	32	21	9		6	3		5	1	3		2	1			1
2[2]		6	64	58	58	23	25(9)	10		7	30	3	18-B_5	1				1
3[1]		31	135	95	81		22	58	1—A_6	1	8	71	1-B_5	2		1		1
4[1]		13	64	20	18		6	11	1—A_6	8	5	5		3	1	1		1
5[3]		27	32	30	12	3	6(3)	3		6	6							
6[4]	common B	40	32	30	18		1	17			12	7		3				3
7[1]	Recombinants from minimal medium	16	206	18	13		6	7		3	5	5		4	2		1	1
8a[1]		67	16	10	4		2	2		4		4		2			2	
8b[3]		2	32	31	11	4	3	4		4	6		1-B_5[6]					
9[1]		28	16	3	3		1	2		2		1		2		1	1	
10[5]		5	212	35	23	13	3	6	1-A_5a	8	8	6	1-B_5	10	1	4	1	4
11[5]		6	136	18	10	5		3	2-A_6a	3	3	3	1-B_5	7		3	1	3
12[3]		5	79	75	29	8	15(6)	6		15	14			2			1	1
13[1]		6	106	75	69		28	40	1-A_6a	32	2	30	5-B_5	2	1	1		
14[1]		23	16	12	4		2	2		2	1	1		1		1		
15[1]		9	16	15	8		4	4		2	2	4						
16[1]		2	128	85	78		36	37	2-A_6 3-A_5a	32	6	30	10-B_5	10	2	1	4	3
17a[1]		8	132	3	4		1	3			2	2		12	2	9	1	
17b[3]		1	32	15	8	3	3	2		3	5			3		2		1
	Total		1486	649	460									66	10	24	12	20

* In brackets A_6am

1 Recombination between compatible nuclei (11 cases).

2 Recombination of linked genes between common A nuclei (1 case).

3 Original dikaryon (4 cases).

4 Common B heterokaryon (1 case).

5 Recombination between common B nuclei (2 cases).

6 Probably contamination.

a— } different fruitbodies of the same isolate.
b— }

suggests that nuclei making up the recombinant dikaryons were frequently abnormal, with decreased viability.

In 11 fruitbodies analysed the pair of compatible nuclei (the migrant ones) had recombined. Among them from isolates 3, 13 and 16 larger samples of single basidiospores were tested (81, 69 and 78 respectively). In isolates 13 and 16 all types of recombinants were found in expected frequencies. In isolate 3 the number of B_6 alleles in the progeny was much higher than the number of B_5 alleles (79 : 2 against 1 : 1 expected). The probability of such a result being due to chance is less than $p = 0{,}001$.

In 2 isolates (isolates 10 and 11) apparently the two common B nuclei recombined (the resident one with one of the migrant ones). In both cases the germination of basidiospores was poor (5% and 6% respectively) and in most subsequent growth was abnormal. In isolate 10 of 212 single spore colonies isolated 35 grew and only 24 (about 11% of those isolated) developed sufficiently for determination of their growth requirements and mating types. In isolate 11 of 136 single spore colonies isolated 18 grew and only 8 (about 6%) developed sufficiently to be analysed. In the progeny of both isolates the allele A_6 appeared less frequently than A_5, and moreover in the progeny of isolate 11 it appeared without the gene *a* which is closely linked with it. It appears that strains with A_6 are much less viable in this combination.

The isolates 10 and 11 arose from the same plate and from samples taken near each other so that their common origin is not excluded. Recombinants due to exchange of genetic material between both migrant nuclei were more or less equally frequent on all plates.

In 4 fruitbodies both nuclei of the original dikaryon were found. The basidiospore germination was poor but most isolated colonies were able to develop normally. All colonies isolated required choline (Table 5).

The analysis of recombinants obtained from the fruitbody of isolate 2 indicates that one nucleus of the original dikaryon lost its choline requirement. As spontaneous reversions to wild type were never observed with this mutant (Świeżyński 1961b) it appears likely that a cross over involving loci B and *c* occured between homologous chromosomes of the common A nuclei A_5 *m*. B_5 *c* and A_5. B_6.

In 1 fruitbody the basidiospore analysis confirmed the supposition that it originated from a common B heterokaryotic mycelium. The isolated colonies developed well and contained exclusively the B_6 allele. The A alleles appeared in unequal numbers, A_5 being more frequent than A_6 (proportion 17 : 1 against 1 : 1 expected). The probability of such a result being due to chance is less than $p = 0{,}001$.

In 66 cases the mating type of isolates with abnormal growth was determined. The mating type reaction in these strains was found to be normal (Table 5).

Growth of progeny tested dikaryotic mycelia

Each type of recombinant dikaryotic mycelia with analysed progeny was inoculated to complete medium in Petri dishes in 4 replicates and the growth of the colonies measured after 3 and after 5 days. The original dikaryotic mycelium: (A_5 *m*. B_5 *c* + A_6 *a*. B_6 *c*) the original monokaryotic one (A_5. B_6) and 3 dikaryotic strains corresponding to the recombinant ones found, which were synthesised from cultures available: (A_5. B_6 + A_6 *a*. B_5 *c*), (A_5 *m*. B_5 *c* + A_6 *a*. B_6) and (A_5 *m*. B_5 + A_6 *a*. B_6 *c*) were used as controls. The results are presented in table 6.

Table 6

Growth of recombinant dikaryotic isolates with analysed progeny

Type of dikaryon	No. of isolate	Average radius of the colony (mm)	
		after 3 days	after 5 days
Recombination between compatible nuclei	1	7.75	18.50
	3	10.5	25.25
	4	10,00	20.75
	7	8.75	25.50
	9	11.00	26.00
	13	9.25	22.25
	14	11.75	24.00
	15	11.25	25.50
	16	10.00	24.25
(A_5.B_6+A_6 *a*.B_5 *c*)	control	13.00	29.50
Recombination between common A nuclei	2	9.25	23.25
(A_5 *m*.B_5+A_6 *a*.B_6 *c*)	control	11.25	25.50
Recombination between common B nuclei	10	9.25	24.25
	11	9.25	25.00
(A_5 *m*.B_5 *c*+A_6 *a*.B_6)	control	11.25	22.50
(A_5 *m*.B_5 *c*+A_6 *a*.B_6 *c*)	original dikaryon	13.25	25.00
A_5.B_6	original monokaryon	11.25	27.00

It is apparent that the rate of growth of the recombinants obtained in the incompatible di-mon mating is usually less that the rate of growth of the control mycelia, but the difference is not great.

DISCUSSION

The following is an attempt to elucidate some of the processes which go on when a monokaryotic mycelium is colonised by nuclei of an incompatible dikaryon.

Types of associations of nuclei in incompatible di-mon matings. Both nuclei of the dikaryon migrate in large numbers into the monokaryotic colony (Tables 1 and 2). The resulting mycelium contains three types of nuclei. All these nuclei multiply and may be detected in the new growth (Tables 3 and 4). Resident and migrant nuclei could be sometimes isolated in monokaryons which indicates the occurence of secondary limitation of migration (Świeżyński and Day, 1960b) in such mycelia. The secondary limitation may be connected with changes in the colony causing poor initial growth of isolated samples (cf. Fig. 3 and 4).

In samples of mycelia with clamps (containing paired nuclei) dikaryons with both migrant nuclei and common B heterokaryons could be found. The presence of various types of recombinant dikaryons could also be demonstrated.

Progeny testing of recombinants shows that such nuclei have frequently a decreased viability. It is probable therefore that the classes of recombinants isolated are not all existing in the mycelium but only those which multiply with sufficient efficiency to be recovered from the isolates. It must be also pointed out that only the progeny of 17 isolates was tested and this number may be too small to recover all types of recombinants, which could be found in the mycelium.

These data indicate that as result of an incompatible di-mon mating a mycelium develops containing a mixture of original and recombinant nuclei present singly or in pairs.

Conditions favouring the formation of recombinant nuclei. No recombination of genes between nuclei was found when pairs of dikarytic mycelia were grown jointly (Świeżyński 1961b). On the other hand in compatible di-mon matings made by Kimura (1958), in which mating type relations of the nuclei were similar although not identical with the former, recombinant nuclei were found. The important difference between conditions of both experiments lies apparently in the fact that in the first case (pairs of dikaryotic mycelia) no extensive migration was possible, while in the second (compatible di-mon matings) the nuclei migrated. This indicates that nuclear migration may favour the formation of recombinant nuclei.

This may not be the only factor favouring recombination. It is possible that the very presence in one mycelium of 3 types of nuclei, two of them being mutually compatible and one of them being incompatible

with the other two also favours recombinations. This is suggested by the fact that the proportion of recombinant nuclei seems to increase with time (cf. tables 3 and 4). One would expect the reverse if the recombination is due solely to migration, as no extensive migtation could take place between both sampling dates and the recombinants are likely to multiply slower than parent nuclei (Table 6).

The types of recombinants obtained give some clue to the conditions favouring recombination of nuclei.

In the incompatible di-mon mating performed three types of recombinant dikaryons are expected if it is assumed that recombination between each pair of nuclei is equally likely and if it is assumed that linked genes will usually recombine jointly. These types are: (A_5. B_6 + + A_6 *a*. B_5 *c*), (A_5 *m*. B_5 *c* + A_6 *a*. B_6) and (A_6 *a*. B_6 *c* + A_5. B_5 *c*). The first would involve recombination between compatible nuclei (the migrant ones), the other two would involve recombination between common B and common A nuclei respectively (either of the migrant nuclei and the resident one). The selective pressure applied (inoculation of samples to minimal medium) favours only the first two types of recombinants (between compatible and common B nuclei) the third one producing dikaryotic mycelia requiring choline.

The first two types were obtained. There was also 1 case of recombination of common A nuclei accompanied apparently with recombination of linked genes, what caused the recombinant to be prototrophic.

The existing evidence is summarised as follows:

1. Formation of recombinant nuclei may be favoured:
   a) by nuclear migration,
   b) by the presence of 3 types of nuclei in the mycelium, two of them being mutually compatible and the third one being incompatible with the other two.
2. Apparently compatible, common B and common A nuclei may recombine. Recombination of linked genes is also possible.

More experiments are necessary to define in detail conditions favouring somatic recombination of genes in tetrapolar *Basidiomycetes*.

Mechanism of somatic recombination. All the recombinants tested produced fruitbodies with poor germination of basidiospores and low viability of the colonies obtained. Also the proportions of recombinants appear to be frequently biased (Table 5). Abnormalities in some recombinant mycelia resulting in inability to produce fruitbodies or basidiospores were also found by Crowe (1960) and Kimura (1958). The abnormalities are apparently compensated in dikaryons as the growth of the recombinant dikaryotic mycelium is not seriously handicaped (Table 6). The inheritance of the abnormalities

was not analysed and therefore only speculation is possible as to their nature. Nevertheless their existence indicates that recombination cannot be ascribed to occasional diploidisation and meiosis.

The application of the model worked out by Pontecorvo (1959) for somatic recombination in *Aspergillus nidulans* appears to be more plausible. It would explain the existence of abnormal strains in the progeny as these might be due to the genetic unbalance resulting from gradual haploidisation. It would explain occasional recombination of linked genes observed once in the presented experiments and several times in the experiments of Crowe (1960). This might happen in somatic cross over in the diploid phase. It would explain also the occasional appearance of fruitbodies producing basidospores giving rise to colonies with spontaneous clamp formation, which might be formed by mycelia with diploid nuclei. One such fruitbody was found by Kimura (1958) in a compatible di-mon mating and one by the present author in an incompatible di-mon mating (Świeżyński unpublished).

Although the model of Pontecorvo appears to be useful, 2 objections may be raised against it:

1. Deviations from the expected ratios of recombinants (Table 5) indicate that decreased viability is due not only to lack of certain chromosomes (gradual haploidisation) but is also due to intrachromosomal changes.

2. The existence of recombinants between common B and common A nuclei would imply that diploid common A and diploid common B nuclei may be formed. The difficulty of obtaining normal fruitbodies from such mycelia (Świeżyński and Day 1960a) indicate that such nuclei are not easily formed.

Although all these objections may be evercome by making additional assumptions, it cannot be overlooked that other mechanism might also bring about somatic recombination in tetrapolar *Basidiomycetes*, for instance some kind of abnormal division and formation of restitution nuclei of the type postulated by Quintanilha (1939).

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SUMMARY

1. In the incompatible di-mon mating analysed the monokaryotic mycelium was invaded by both nuclei of the incompatible dikaryon and as result of nuclear migration and formation of recombinant nuclei a mixed colony developed. In sam-

ples isolated from various parts of such a colony the following categories of mycelia could be isolated:

a) monokaryotic mycelium, containing either the resident or one of the migrant nuclei only,

b) dikaryotic mycelium containing both migrant nuclei,

c) heterokaryotic mycelium containing common B nuclei (1 resident and 1 migrant nucleus associated),

d) dikaryotic recombinant mycelia of various types.

2. Three kinds of recombinant nuclei were found:

a) nuclei arising through exchange of genes between compatible nuclei (both migrant nuclei).

b) nuclei arising through exchange of genes between common B nuclei (one migrant and one resident nucleus),

c) nuclei arising through the exchange of genes between common A nuclei (one migrant and one resident nucleus, in this case apparently linked genes recombined).

Out of 14 recombinants analysed, 11 involved both compatible nuclei, 2 involved common B and 1 involved common A ones.

3. A large proportion of the progeny of recombinant dikaryons was unable to normal growth what indicates that recombinant nuclei are frequently genetically unbalanced.

4. Conditions favouring the formation of recombinant nuclei and existing evidence concerning the mechanism of somatic recombination in tetrapolar *Basidiomycetes* are discussed.

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STRESZCZENIE

Skrzyżowano grzybnię dikariotyczną o składzie genetycznym: (A_5 *m*. B_5 *c* + + A_6 *a*. B_6 *c*) z grzybnią homokariotyczną: A_5. A_6. Ta ostatnia inokulowała by na płytce Petriego dokoła pierwszej (ryc. 1). Po zetknięciu się grzybni równocześnie z dalszym wzrostem nastąpiła migracja obu typów jąder dikarionu do grzybni homokariotycznej. Oprócz tych trzech typów jąder (dwa imigrujące oraz jądra pierwotnego homokarionu) pojawiły się również jądra rekombinacyjne, tj. jądra, których skład genetyczny wskazywał, że pochodzą od dwóch różnych jąder poprzedniej grupy.

Z rozrastającej się grzybni pobierano próbki po 2, 3, 4, 5 i 8 dniach od chwili inokulacji. Analiza tych próbek pozwala wyciągnąć poniższe wnioski:

1. W próbkach można wyróżnić następujące rodzaje grzybni:

a) grzybnia homokariotyczna zawierająca albo wyłącznie jądra homokarionu wyjściowego, albo wyłącznie jedno z jąder wyjściowego dikarionu,

b) grzybnia dikariotyczna, zawierająca obydwa jądra wyjściowego dikarionu,

c) grzybnia heterokariotyczna, w której obydwa jądra składowe zawierają ten sam allel determinującego typ koniugacyjny czynnika B (heterokarion ze wspólnym czynnikiem B, w skład którego wchodzi jądro wyjściowego homokarionu i odpowiednie jądro wyjściowego dikarionu),

d) różnego rodzaju rekombinacyjne grzybnie dikariotyczne, w których jedno z jąder powstało przez rekombinację.

2. Znaleziono 3 rodzaje jąder rekombinacyjnych:

a) jądra powstałe przez wymianę genów między jądrami o różnych allelach czynników A i B, determinujących typ koniugacyjny (powstałe w wyniku wymiany genów między obydwu jądrami wyjściowego dikarionu),

b) jądra powstałe przez wymianę genów między jądrami ze wspólnym czynnikiem B (wymiana nastąpiła między jednym z jąder wyjściowego dikarionu i jądrem wyjściowego homokarionu),

c) jądra powstałe przez wymianę genów między jądrami ze wspólnym czynnikiem A (wymiana nastąpiła między jądrem wyjściowym homokarionu a jednym z jąder wyjściowego dikarionu, w tym wypadku stwierdzono również wymianę genów sprzężonych).

Wśród 14 dikarionów, u których określono pochodzenie jąder rekombinacyjnych poprzez analizę potomstwa (tabela 5), wymienione 3 rodzaje wystąpiły odpowiednio w ilości 11 (wymiana między jądrami o różnych allelach czynników determinujących typ koniugacyjny), 2 (wymiana między jądrami o wspólnym czynniku B) i 1 (wymiana między jądrami o wspólnym czynniku A).

3. Duża część potomstwa dikarionów rekombinacyjnych nie była zdolna do normalnego wzrostu. Wskazuje to, że jądra rekombinacyjne są często genetycznie nie zrównoważone.

W dyskusji omówiono warunki, które mogą sprzyjać powstawaniu jąder rekombinacyjnych oraz rozważono, jaki może być przebieg procesu rekombinacji.

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