ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. XXXV — nr 2
1966

Recombination in crosses between wild-type strains of *Coprinus lagopus*

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INTRODUCTION

Two monokaryotic mycelia of *Coprinus lagopus* carrying different mating-type alleles, inoculated together on an appropriate medium in a way enabling the hyphae to join after about 15 hrs. of incubation will eventually form a dikaryotic hypha. Samples of mycelium taken shortly afterwards from the margin area of one strain will contain the nuclei of the other. This process, known as "Buller's phenomenon", was first observed by this author in *Coprinus lagopus* in 1931. Critical evidence of nuclei migration was provided owing to the experiment by Snider. Snider showed that some substance was transferred from hyphae of wild-type *Schizophyllum commune* to the "puff" mutant strain, causing changes in the growth morphology of the mutant. The transferred substance was involved in the normal meiotic process and showed monofactorial inheritance. Snider concluded that this substance was a wild-type nucleus. This experiment provided both visual and genetic evidence of nuclei migration in *Basidiomycetes*.

Shatkin and Tatum (1959) observed on an electronic micrograph the phenomenon of migration of a *Neurospora* nucleus through a septal pore of 100—200 mμ diameter. The observed nucleus was half-way from one cell to the other in a vegetative hypha.

MATERIAL AND METHODS

Prototrophic, wild-type strains of mating-types A_5B_5, A_6B_5, A_5B_6 and A_5B_1, were provided by Dr. Peter Day from the John Innes Horticultural Institution. A dikaryon was obtained from two strains and then crossed with a monokaryon. Some new dikaryons were obtained this way; they then formed several fruiting bodies. New strains were isolated from individual spores and outcrossed to five testers to determine the mating types. The testers were A_5B_7, A_7B_5, A_6B_7, A_7B_6 and A_7B_1. All new strains selected for experimental purposes were characterized by vigorous growth and normal, uniformly compact, white-coloured mycelium. The initial cross and test crosses were made on complete agar medium. Dikaryons fruited on sterilized horse-manure medium. The dikaryon×monokaryon crosses were made on Petri dishes, 10 cm in diameter. Dikaryotic mycelium was inoculated in the middle of a dish and surrounded by 12 inocula of a monokaryon, evenly

spaced in a circle, 15 mm in diameter. Samples of mycelium were taken after 4, 8 and 12 days of incubation at 28°C. Only these mycelia had fruited which showed dikaryotic growth and had clamps.

Spores collected from fruiting bodies were placed on complete medium, isolated after 24 hrs. of incubation and then tested to determine their mating types.

EXPERIMENTAL RESULTS

The parental dikaryon $A_6B_5+A_5B_6$ was crossed with a fully incompatible monokaryon A_5B_5 (fig. 1). This cross was made in 20 replicates (dishes). Starting from the third day of incubation the diameters of the newly formed mycelia were mea-

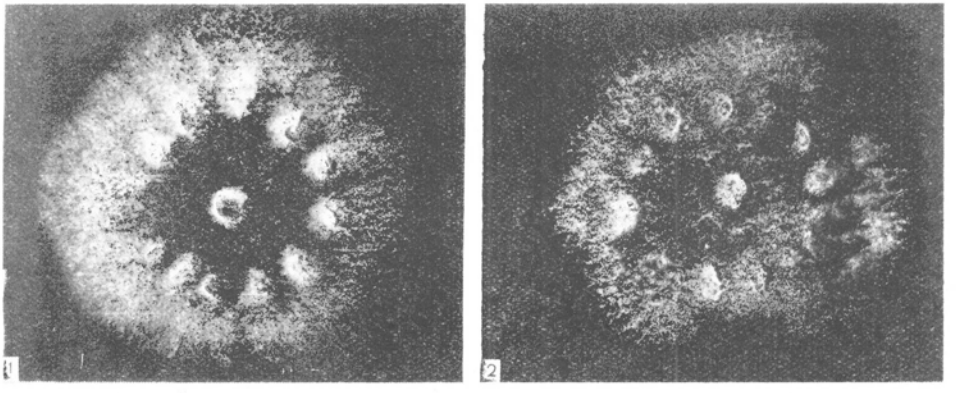

Fig. 1. Cross between the parental dikaryon $A_6B_5+A_5B_6$ with the A_5B_5 monokaryon, after four days of incubation at +28°C

Fig. 2. Cross between a newly isolated dikaryon with the A_5B_1 monokaryon, after four days of incubation at +28°C

sured. Significant differences in growth rate among dishes were found in spite of the uniformity of medium, incubation conditions and genetic composition of the inoculated strains.

The formation of new heterokaryons was expected, resulting from nuclei migration from dikaryotic to monokaryotic mycelium. A new dikaryotic sector could arise in two ways: migration of both unchanged nuclei of the parental dikaryon or migration of a somatically recombined nucleus of the parental dikaryon to monokaryotic mycelium. Either of the non-recombined nuclei of the parental dikaryon would be incompatible with those of the monokaryon. No distinct dikaryotic sectors were found in this experiment. All three strains involved in the initial cross carried either the A_5 or the B_5 allele; it was, therefore, impossible to determine genetically which nuclei were involved in the formation of new dikaryons. To answer this question, samples of dikaryotic mycelia were crossed again with strain A_5B_1 (Fig. 2) which was fully compatible with one of the nuclei of the parental dikaryon. Genetic analysis of samples of this cross enabled to determine whether

recombination between the nuclei of the parental dikaryon had taken place. The percentage of germinating spores from 12 fruiting bodies is given in Table 1.

The highest percentage of germination was found in fruiting bodies Nos 1, 3, 5 and 16. Genetic analysis showed that fruiting bodies Nos 1, 3, 8, 10 and 15 were formed on dikaryons of the same mating type as the parental one. An attempt will be made in the next section to account for the wide differences in spore germinability among fruiting bodies of the same mating type.

Table 1
Percentage of spores germinating after 24 hrs. of incubation

Fruiting body number	Germination %
1	30
3	40
4	18
5	35
6	21
7	29
8	20
9	24
10	5
12	0
15	20
16	30

Other fruiting bodies showed somewhat lower spore germinability. Not a single spore germinated from the fruiting body No. 12, although the technique of collecting and storing of spores was uniform. The fruiting body No. 12, although formed on a typically dikaryotic and clamp-forming mycelium, evidently produced sterile spores. Consequently, only 11 out of 12 fruiting bodies could be genetically analyzed.

According to the results of genetic analysis, the fruiting bodies were classified into 3 groups (Table 2).

The first group consisted of 5 fruiting bodies which produced spores of the following mating types: A_6B_5, A_6B_6, A_5B_5, A_5B_6. The same types of spores would be produced by the parental dikaryon. Since the mycelium of the parental dikaryon was completely surrounded by the monokaryon and could not grow through it, the only way, in which the genotype of the parental dikaryon could penetrate outside the ring of monokaryotic mycelium was the migration of both types of its nuclei to the hyphae of the monokaryon. It should be pointed out that the parental-type dikaryon was crossed with two different monokaryons, the first being fully incompatible, whereas the second was half compatible; namely, the nuclei of the A_5B_1 monokaryon were fully compatible only with the A_6B_5 nuclei of the dikaryon. The results of genetic analysis indicate that the nuclei of the dikaryon migrated through the hyphae of either the compatible or the incompatible monokaryon, remaining in permanent contact and undergoing conjugated divisions.

Spores produced by fruiting bodies of the second reaction-group were of A_5B_1, A_5B_6, A_6B_1 and A_6B_6 mating-types. Thus, the genetic composition of a dikaryon which produced such spores was $A_5B_1+A_6B_6$. The allele B_1 was involved only in the second cross and was present in the nucleus of the monokaryon. This indicates, therefore, that the A_5B_1 nucleus of the new dikaryon is that of the monokaryon used in the second cross. Since no A_6B_6 strain was involved in either cross, it may be concluded that the other nucleus of the new dikaryon arose by recombination. The A_6 allele was present in the first, while B_6 in the second nucleus of the parental dikaryon. Neither of the two monokaryotic strains used in crosses contained these alleles. Basing on these data the author concludes that the recombination occured between the nuclei of the parental dikaryon. Two new nuclei, A_6B_6 and A_5B_5, resulted from this recombination, presumably by an exchange of chromosomes. Monokaryons used in both crosses contained allele A_5; the recombination nucleus A_5B_5 was incompatible with them and was, therefore, eliminated from the cross. The other recombination nucleus was compatible with the monokaryons and after migration to the hyphae of the A_5B_1 monokaryon could form a dikaryon $A_5B_1+A_6B_6$.

Table 2

Results of genetic analysis of individual basidiospores

Reaction type group	No. of fruit. bodies	Number of spores in mating-type groups						Total
		A_6B_6	A_5B_5	A_6B_5	A_5B_6	A_6B_1	A_5B_1	
I	5	57	54	58	56	—	—	225
II	4	55	—	—	53	42	45	195
III	2	—	26	23	—	19	24	92
No. of analysed basidiospores								512

The third reaction group consisted of dikaryons producing spores A_5B_1, A_6B_1, A_6B_5 and A_5B_5. There were two fruiting bodies in this group. Spores of these types could be produced by a dikaryon with genetic composition $A_6B_5+A_5B_1$. A dikaryon of this type could arise by dikaryotization of monokaryon A_5B_1 with the A_6B_5 nucleus of the parental dikaryon without recombination between its nuclei.

DISCUSSION

The products of somatic recombination were found in 4 out of 11 fruiting bodies which were genetically analysed. It should be emphasized that, although all strains involved in this experiment were prototrophic, complete media were used to provide all essential components available in natural media on which the fungus usually grows. Temperature and humidity were kept at optimum level.

These facts seem to be of particular importance. In other experiments of this kind auxotrophic mutant strains were mostly used. The high frequency of somatic

recombination in such materials might have been attributed to selection pressure favouring recombinant genotypes better suited to grow on given media. The high frequency of somatic recombination in wild-type strains grown on complete media at optimum temperature and humidity indicates that this phenomenon occurs also without any environmental pressure. It is, therefore, a genetically conditioned physiological property of the mycelium, leading to the formation of new genotypes, apart from the sexual process.

Somatic recombination which occurred in this experiment resulted from the exchange of whole chromosomes or their sections between the nuclei of the parental dikaryon. Świeżyński (1961) and many other authors assume that chromosome exchange may occur as a result of sporadic diploidization of the nuclei. Diploidization, however, may not necessarily lead to chromosome exchange, but also to haploidization by means of a gradual loss of chromosomes in subsequent mitoses. If we assume a random course of this process, the occurrence of aneuploid nuclei in dikaryotic mycelia is to be expected. Aneuploid hyphae may be completely or partly incapable of growing in dependence on the kind and number of missing chromosomes

The author presumes that this might be the cause of sterility of the spores from the fruiting body No. 12.

According to the author's view, chromosome exchange can occur also during conjugated mitotic divisions of nuclei of a dikaryon, as a result of non-parallel situation of the mitotic spindle. In this experiment, the most numerous were fruiting bodies formed on dikaryons which resulted from migration of both nuclei of the parental dikaryon to the hyphae of a monokaryon. In this case there was presumably a double migration of both nuclei of the parental dikaryon to the mycelia of monokaryons, one of them being fully incompatible, the other semi-compatible. According to Świeżyński and Day (1960) such migration is quite possible. The most striking phenomenon is the high frequency of double migration.

The spores in this group may not necessarily originate from one dikaryon, but from three separate dikaryons carrying the same mating-type alleles. Spores A_5B_5, A_5B_6, A_6B_6, A_6B_5 may be produced by a dikaryon of parental type, A_6B_5+ $+A_5B_6$, and also by other type, namely, $A_5B_5+A_6B_6$.

The $A_5B_5+A_6B_6$ type could result from chromosomal recombination within the parental dikaryon. Such recombination could occur either before migration to the first monokaryon, or during migration to the second. In both cases the cross would have occured between semi-compatible strains.

The $A_6B_6+A_5B_5$ type could result from migration of a recombined A_6B_6 nucleus of the parental dikaryon to the mycelium of the A_5B_5 monokaryon.

The possibility exists that some of the six fruiting bodies which produced spores of the types mentioned above contained recombination nuclei. This problem might be solved by using biochemical mutants, but this would be beyond the scope of this work.

CONCLUSIONS

Experiments with prototrophic strains of *Coprinus lagopus* lead to the following conclusions:

1. Somatic recombination occurs spontaneously; its course and frequency is not influenced by environmental pressure, conditioned by nutritional requirements.

2. Chromosome exchange between nuclei of the parental dikaryon occurred with high frequency. The percentage of recombined nuclei in 11 fruiting bodies analyzed in this experiment amounted to at least 25%.

3. Somatic recombination may lead to the formation of mycelia which can form fruiting bodies and produce sterile spores. (fruiting body No. 12).

The above described investigations were carried out in the years 1962—1963 at the Department of Genetics of the Warsaw Agricultural University.

Thanks are due to Dr. Helena Bańkowska for valuable advice in planning the experiment and preparing this paper.

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(Entered: 6.IV.1965.)

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Rekombinacje w krzyżówkach wykonanych w obrębie dzikich szczepów Coprinus lagopus

Streszczenie

Do pracy użyto szczepów prototroficznych otrzymanych z pracowni P. R. Daya z John Innes Horticultural Institution. Pracę wykonano w Zakładzie Genetyki SGGW w Warszawie.

Dikarion o typie koniugacyjnym $A_6 B_5 + A_5 B_6$ skrzyżowano z monokarionem o typie koniugacyjnym całkowicie niezgodnym, zawierającym allele $A_5 B_5$. Krzyżówkę wykonano w ten sposób, że na środku płytki Petriego naniesiono dikarion, a wokół niego dwanaście monokarionów. Następnie z peryferii rozrastającej się grzybni pobrano próbki które wytworzyły nowe grzybnie. Te grzybnie, które były dikarionami, skrzyżowano ponownie z monokarionem o typie koniugacyjnym $A_5 B_1$. Z rozrastającej się grzybni pobierano próbki, które następnie były odowocowywane. Otrzymane w ten sposób pojedyńcze zarodniki były krzyżowane z pięcioma szczepami znakującymi w celu ustalenia ich typu koniugacyjnego. Otrzymane wyniki można zebrać w trzy następujące grupy (Tab. 2):

Do pierwszej z nich zaliczono owocniki, które wykształciły zarodniki o typach koniugacyjnych: $A_6 B_5$, $A_6 B_6$, $A_5 B_6$, $A_5 B_5$. Na podstawie tych wyników można stwierdzić, że oba jądra wyjściowego dikarionu migrowały poprzez strzępki dwóch różnych grzybni monokariotycznych pozostając ze sobą w stałym kontakcie i ulegając sprzężonym podziałom.

Drugą grupę stanowią owocniki z których otrzymane zarodniki miały typy koniugacyjne $A_5 B_1$, $A_5 B_6$, $A_6 B_1$, $A_6 B_6$. Wyniki tej analizy genetycznej wskazują, że w obrębie jąder dikarionu wyjściowego musiała nastąpić rekombinacja polegająca na wymianie chromosomów. Zrekombinowane jądro wraz z jądrem drugiego monokarionu utworzyło dikarion o typie konuugacyjnym $A_5 B_1 + A_6 B_6$. Drugie z rekombinacyjnych jąder miało allele typu koniugacyjnego niezgodne zarówno z jądrem pierwszego, jak i drugiego monokarionu w związku z tym nie utworzyło nowego dikarionu.

Do trzeciej grupy zaliczono dikariony, które poprzez owocniki utworzyły zarodniki o typach koniugacyjnych: $A_5 B_1$, $A_6 B_1$, $A_6 B_5$, $A_5 B_5$. Utworzenie takich zarodników jest dowodem, że w obrębie jąder wyjściowego dikarionu nie nastąpiła rekombinacja.

W pracy tej otrzymano również dikarion, który wytwarzał zarodniki całkowicie sterylne co wskazuje, że w wyniku somatycznej rekombinacji mogą powstawać jądra zawierające mniejszą od normalnej ilość materiału genetycznego.