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Recombination in crosses between biochemical mutants of *Coprinus lagopus*

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INTRODUCTION

It was believed still quite recently that gene recombination occurs only in the sexual process as a result of chromosome recombination and crossing-over at meiosis.

Experiments with microorganisms revealed the occurrence of what is called parasexual processes leading to gene recombination, e.g. transformation and transduction. According to Pontecorvo (1959) somatic recombination should be also considered a parasexual process, because it leads to gene exchange like the sexual process.

MATERIAL AND METHODS

Biochemical mutants used in this experiment were obtained by Dr. Peter Day in the John Innes Horticultural Institution. These strains do not grow on minimal medium, being unable to synthesize adenine and/or choline. They will grow on minimal media supplemented with one or both of these compounds (in dependence on the genotypes), as well as on natural and complete media.

In the strain of mating-type A_6B_6 the adenine-less gene is linked with A_6, while the choline-less one with the B_6 allele. In the strain A_5B_5 the adenine-less gene is linked with A_5, in the strain A_6B_5 the choline-less one with B_5

Wild-type strains used as testers were of mating types A_5B_7, A_7B_5, A_6B_7, A_7B_6; they had, therefore, one allele fully compatible with the alleles of the mutant strains.

The initial crosses were made on complete medium on Petri dishes 10 cm in diameter. The parental dikaryon was inoculated in the middle of a dish, twelve inocula of the monokaryon being evenly spaced around in a circle 15 mm in diameter. The dishes were kept at 28°C until the entire surface of the medium was covered with the mycelium. Samples were taken from the periphery of the growing mycelium after 4, 8 and 12 days of incubation. Four samples 5×5 mm were taken from each dish each time and transplanted on minimal medium. After 3 days of incubation all samples, either able or unable to grow, were transplanted again on minimal medium. After another 3 days the ability of growing and forming clamps was determined. Monohyphal isolates were taken from strains which could grow and form clamps on minimal medium. The isolates were transplanted on skews and fruited on horse-manure medium at 32°C at the beginning of growth

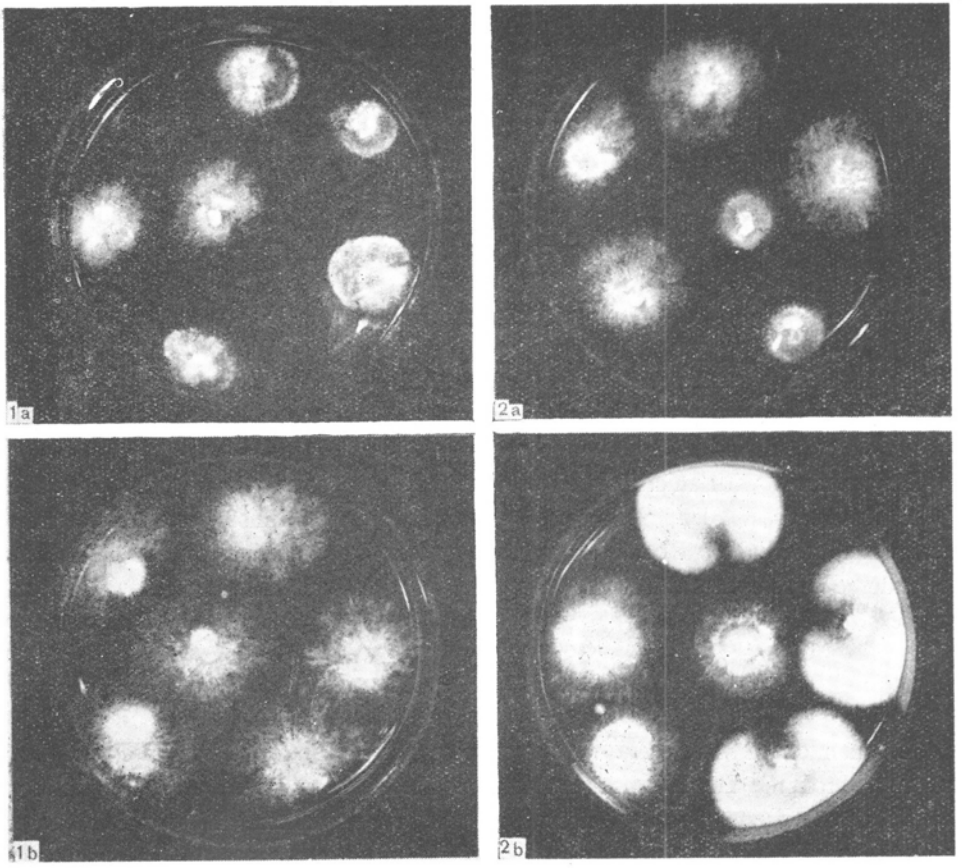

Fig. 1. Test crosses of basidiospores derived from the same fruiting body as the testers: a — A_5 B_7, b — A_7 B_5.

Fig. 2. Test crosses of basidiospores derived from the same fruiting body as the testers: a — A_6 B_7, b — A_7 B_6.

and then at room temperature. Spores collected from fruiting bodies were germinated on complete medium and then isolated. Mating-types of the isolated strains were determined by crossing with four testers (Figs 1 and 2). Nutritional requirements were determined by observation of growth on supplemented media.

EXPERIMENTAL RESULTS

Fourteen fruiting bodies were analysed, formed on dikaryotic mycelia of monohyphal isolates derived from the dikaryon × monokaryon cross. The parental dikaryon A_6a $B_6c + A_5a$ B_5 was formed and then crossed with the monokaryon A_6B_5c. The parental monokaryon had one mating-type allele in common with either of the nuclei of the parental dikaryon; the cross was, therefore, fully incompatible.

The initial cross was made in 3 replicates, i.e. on three separate dishes. Marked

differences were observed among the dishes, although the genotypes of the inoculated strains were identical. The differences became noticeable after 3 days of incubation and increased gradually as the growth proceeded (Table 1).

Table 1

Diameters of mycelia (in mm)
after 4, 5, 6, 8 and 10 days of incubation

Dish No.	Number of days of incubation				
	4	5	6	8	10
I	43	49	64	76	91
II	42	45	50	57	73
III	43	48	63	70	86

Differences in growth rates were also observed between various sectors of the same mycelium. This phenomenon may be interpreted as follows. New dikaryons, as well as different heterokaryons could arise as a result of nuclei migration from dikaryon to monokaryon. The growth rate of dikaryotic sectors was higher than that of heterokaryotic or monokaryotic ones. These differences, due to genetic composition, often exceeded 10 mm after 12 days of incubation. This phenomenon will be discussed in the next section of this paper.

Analysis of spores showed that dikaryotic mycelia derived from the incompatible dikaryon×monokaryon crosses could be classified into four reaction groups (Table 2).

The first group consisted of dikaryons producing spores of mating types $A_6a\ B_6c$, $A_5a\ B_5$, $A_6a\ B_5$, $A_5a\ B_6c$. The parental dikaryon would produce the same types of spores, but it seems hardly possible that dikaryotic hyphae could have grown through the surrounding monokaryotic mycelium. Sometimes dikaryotic hyphae, being more vigorous, will grow over monokaryotic mycelium; however, this easily detectable phenomenon certainly did not occur in this experiment. Dikaryotic sectors occuring outside the inoculation circle could arise in consequence of the migration of both nuclei of the parental dikaryon to the hyphae of the monokaryotic mycelium. Another possibility was, that spores of the same types were produced by the $A_6a\ B_5 + A_5a\ B_6c$ dikaryon. Such dikaryotic mycelium could arise if chromosome recombination would have occurred between nuclei of the parental dikaryon. Such recombination, however, could not be detected with methods of genetic analysis used in this experiment. Moreover, we have established the occurrence of meiotic crossing-over between the chromosomes containing the alleles B_6 and c and the chromosomes with the alleles B_5 and +. (Table 2 group I).

The second reaction group consisted of 3 fruiting bodies, producing basidio--spores of the following genotypes: $A_6\ B_6c$, $A_6\ B_5c$, $A_5a\ B_5c$, $A_5a\ B_6c$. These spores

were presumably produced by a dikaryon of genetic composition $A_6\ B_5c + A_5a\ B_6c$. The mating-type of the first nucleus of such a genotype is identical with that of the parental monokaryon, while the second carries mating-type alleles that are present in both nuclei of the parental dikaryon. The second nucleus, therefore, evidently resulted from chromosome recombination between the nuclei of the parental dikaryon.

Table 2

Results of genetic analysis of basidiospores

Reaction type group	No. of fruiting bodies	Nutritional requirement	No. of spores of mating types				Total
			$A_6\ B_6$	$A_5\ B_5$	$A_6\ B_5$	$A_5\ B_6$	
I	5	ac	87	4*	9*	88	188
		a	5*	89	75	7*	176
		c	—	—	—	—	—
							364
II	3	ac	—	59		51	110
		a	—				
		c	52		44		96
							206
III	3	ac	58	58	54	56	226
		a					
		c					
							226
IVa	2	ac	1*	30	39	2*	72
		a	38	3*	—	28	69
		c					
							141
IVb	1	ac					
		a	9	21	26	16	72
		c					
							72
No. of analyzed basidiospores							1009

The numbers denoted with an asterisk concern the data obtained after meiotic crrossing-over.

The allele A_6 in the parental dikaryon was linked with the adenine-less marker gene, but no adenine-less genes occurred in the second group, which indicates that A_6 descended from the parental monokaryon. The presence of B_5 linked with choline-less gene indicates that the whole nucleus of the parental monokaryon

was involved in the formation of new dikaryons of the second group. The B_5 allele in the parental dikaryon was not linked with the choline-less gene.

The third reaction group consisted of 3 fruiting bodies. Basidiospores were of the following mating types: $A_6a\ B_6c$, $A_6a\ B_5c$, $A_5a\ B_5c$, $A_5a\ B_6c$. The A_6 allele was present in the first nucleus of the parental dikaryon, and also in that of the monokaryon. In the progeny of the third group the allele A_6 was always linked with the adenine-less gene, which indicates that it descended from the first nucleus of the parental dikaryon. The A_5 allele obviously descended from the second nucleus of the parental dikaryon.

The allele B_5 in the third group was always linked with the choline-less gene, which proves its descendance from the nucleus of the monokaryon. A new dikaryon, on which fruiting bodies of the third group were formed had therefore, the composition $A_6a\ B_6c + A_5a\ B_5c$. The author assumes that the $A_5a\ B_5c$ nucleus resulted from the somatic recombination between the second nucleus of the parental dikaryon and the nucleus of the monokaryon. Such recombination could occur only after the migration of both nuclei of the parental dikaryon to the hyphae of the surrounding monokaryon.

The fourth reaction group could be divided into sub-groups (Table 2).

The first consisted of two fruiting bodies, which produced spores of the following mating types: $A_6a\ B_6$, $A_6a\ B_5c$, $A_5a\ B_5c$ and $A_5a\ B_6$. A dikaryon which produced such spores had the genetic composition $A_6a\ B_6 + A_5a\ B_5c$. The genotypes of nuclei of this dikaryon were unlike those of the strains involved in the initial cross. The A_6 allele was linked with the adenine-less gene, which indicates that A_6 descended from the first nucleus of the parental dikaryon. The B_6 allele was present only in the first nucleus of the parental dikaryon; it was, however, linked with the choline-less gene. The second nucleus of the new dikaryon, $A_5a\ B_5c$, could arise by chromosome recombination between the second nucleus of the parental dikaryon and the nucleus of the monokaryon. The occurrence of B_6 not associated with the choline-less gene could be explained as follows: it could arise after crossing-over between chromosomes B_6c (from the nucleus $A_6a\ B_6c$) and B_5 (from the nucleus $A_5a\ B_5$) of the parental dikaryon during their conjugated division. The new nucleus of the constitution $A_6a\ B_6$. could thereafter form with the second recombined parental nucleus a dikaryon of the constitution $A_6a\ B_6 + A_5a\ B_5c$, or with the recombined monokaryon nucleus of the constitution $A_5a\ B_5c$. Besides, we have observed the occurrence of meiotic crossing-over between the chromosomes containing the alleles B_6 and c and the chromosomes with the alleles B_5 and + (Table 2 group IVa). The frequency of the recombinants was low.

The second sub-group of the fourth group was represented by one fruiting body, which produced spores of the following mating-types (Table 2): $A_6a\ B_6$, $A_6a\ B_5$, $A_5a\ B_5$, $A_5a\ B_6$. The A_5 and A_6 alleles were linked with the adenine-less gene, which points to their descendance from two nuclei of the parental dikaryon. Spores carrying B_5 could grow on adenine-supplemented medium; this indicates that B_5 was not associated with the choline-less gene and, therefore, descended from the second nucleus of the parental dikaryon. On the other hand, B_6 allele

was present only in the first nucleus of the parental dikaryon, but it was linked with the choline-less gene, which was absent in the spores of this sub-group. Here again a separation of genes carried on the same chromosome was observed. The dikaryon which produced spores of this sub-group differed from the parental one only in the absence of the choline-less gene; hence, its genetic composition was $A_6a\ B_6+A_5a\ B_5$.

The germination percentage of each set of spores after 24 hrs was determined. The differences between individual fruiting bodies were pronounced, but no correlation was found between the germination percentage and the frequency of somatic recombination. These data, however, do not prove the complete lack of such correlation.

DISCUSSION

The use of biochemical mutants made it possible to identify the nuclei involved in the recombination process. Linkages between mutant genes and mating-type alleles helped to determine whether the whole chromosomes or their sections were exchanged. Genetic analysis showed that in the progeny from 3 fruiting bodies (Table 2, gr. IV) an exchange of chromosomes sections occurred. It is known, however, that crossing-over can occur only when homologous chromosomes pair with each other. A dikaryon carries a diploid number of chromosomes, but homologues are located in different nuclei which from separate spindles during conjugated divisions and remain separate throughout the whole mitotic process. Thus, of special importance seems to be the establishement of the mechanism, promoting the exchange not only of whole chromosomes, between nuclei of a dikaryon, but also of chromosome sections between homologues, as it was observed in this work.

Somatic crossing-over in *Basidiomycetes* was described by L. Crowe in 1960 in *Schisophyllum commune*. This paper points out its occurrence in *Coprinus lagopus*. The mechanism of somatic recombination has not yet been fully understood. It seems, however, that the diploid status in a cell of a hypha of dikaryotic mycelium is an indispensable condition.

To the authors knowledge, the formation of diploid sectors was not observed in *Coprinus lagopus*. This seems in contradiction to the above stated condition of the occurence of somatic recombination. It is possible, however, that the diploid status in a hyphal cell is very unstable. Hence, there are two possible ways in which the number of chromosomes is reduced. One of them is normal meiosis promoting the exchange of chromosomes or their sections. Crowe (1960) and Kimura (1958) observed reduced germination of spores with recombinations. Since this phenomenon occurred rather often and the observed ratios of segregation were abnormal, recombination could not be attributed to normal meiosis. Pontacorvo (1959) advanced the hypothesis that the reduction of the chromosome number from diploid to haploid may occur as a result of gradual loss of chromosomes in subse-

quent mitotic divisions. This hypothesis fully accounts for the reduced germinability of spores resulting from somatic recombinations.

In the author's opinion, the formation of spores corresponding in mating-type to the nuclei of the parental dikaryon, as it has been observed in this work, as well as in others (Crowe 1960; Świeżyński 1962), does not necessarily mean that they are immediate products of migration of both nuclei of the parental dikaryon to the monokaryon. Such classification may lead to underestimation of the frequency of somatic recombination, because the same types of spores would result from chromosome recombination within the parental dikaryon. The genotypes of nuclei which migrated through the parental monokaryon could be identified by crossing the new dikaryotic mycelium with a monokaryon compatible with only one nucleus of the parental dikaryon and carrying a mating-type allele absent in either nuclei of the dikaryon.

Crowe (1960) showed that the migration of both nuclei of a dikaryon to monokaryotic hyphae can occur also in fully compatible crosses. Thus the formation, within the dikaryotic mycelium, of a new nucleus compatible with that of a monokaryon does not prevent the migration of its nuclei to the monokaryotic mycelium. This is in accord with the author's assumption. The author concludes, therefore, that the actual percentage of fruiting bodies resulting from somatic recombination in this experiment could be still higher than estimated on the basis of genetic analysis.

It was expected that in crosses involving the $A_6a\ B_6c$ strain, all segregates carrying the B_6 allele would require also choline for growth. The occurrence of wild-type B_6 forms could be explained by reverse mutation of the choline-less gene. It should be pointed out, however, that B_6c strain has been maintained and transplanted on slopes for many years and no wild-type mutants were ever found in it. The author tends, therefore, to attribute the occurrence of normal B_6 segregates to somatic crossing-over between B and choline-less loci.

CONCLUSIONS

The experiments with auxotrophic mutants of *Coprinus lagopus* lead to the following conclusions:

1. The formation of new genotypes in fully incompatible dikaryon×monokaryon crosses may be attributed in many cases to somatic recombination.

2. The occurrence of wild-type B_6 segregates in the progeny of the strain, containing the B_6c chromosome gives evidence of somatic crossing-over between homologous chromosomes.

3. Somatic recombination of whole chromosomes can occur not only between the nuclei within the parental dikaryon, but also between dikaryon and monokaryon nuclei.

The above investigations were carried out in the years 1961—1962 at the Department of Genetics of the Warsaw Agricultural University. Thanks are due to Dr. Helena Bańkowska and Dr. Kazimierz Świeżyński for valuable advice in planning the experiment and preparing this paper.

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Rekombinacje w krzyżówkach wykonanych między mutantami biochemicznymi Coprinus lagopus

Streszczenie

W pracy tej, przeprowadzonej w Zakładzie Genetyki SGGW w Warszawie, użyto mutantów biochemicznych otrzymanych w pracowni Dr P. Daya w John Innes Horticultural Institution.

Do krzyżówki wyjściowej wzięto dikarion o typie koniugacyjnym A_6 a B_6c+A_5 a B_5 oraz monokarion A_6 B_5 c. Krzyżówkę tą przeprowadzono na pożywce kompletnej nanosząc pośrodku szalki dikarion, a wokół niego dwanaście monokarionów. Z rozrastającej się następnie grzybni pobierano próbki z których wyizolowywano pojedyńcze strzępki dikariotyczne. Te jednostrzępkowe izolaty były następnie odowocowywane, a u otrzymanych z nich zarodników badano typ koniugacyjny i wymagania pokarmowe. Przeprowadzona analiza genetyczna zarodników wykazała, że w tych niezgodnych krzyżówkach dikarionu z monokarionem można wyróżnić cztery zasadnicze rodzaje reakcji (Tab. 2).

1. Migrację obu jąder dikarionu rodzicielskiego do strzępek grzybni monokariotycznej w wyniku której otrzymano zarodniki: A_6 a B_6 c, A_5 a B_5, A_6 a B_5, A_5 a B_6 c.

2. Wymianę chromosomów między jądrami dikarionu rodzicielskiego w wyniku której powstało nowe jądro dające wraz z jądrem monokarionu wyjściowego dikarion wytwarzający zarodniki A_6 B_6 c, A_6 B_5 c, A_5 a B_5 c, A_5 a B_6 c.

3. Wymianę chromosomów między jednym jądrem dikarionu, a jądrem monokarionu w wyniku której otrzymano zarodniki A_6 a B_6 c, A_6 a B_5 c, A_5 a B_5 c, A_5 a B_6 c, a więc nowe jądro utworzyło dikarion z pierwszym jądrem wyjściowego dikarionu.

4. a) Somatyczny crossing over oraz wymianę chromosomów między drugim jądrem dikarionu, a jądrem monokarionu. W wyniku tych reakcji otrzymano dikarion produkujący zarodniki o następujących typach koniugacyjnych: A_6 a B_6, A_6 a B_5 c, A_5 a B_5 c, A_5 a B_6.

b) Somatyczny crossing over w wyniku którego powstał dikarion różniący się od wyjściowego tym, że nie wystąpił w nim gen wymagania choliny. Dikarion ten wytwarzał zarodniki o typach koniugacyjnych: A_6 a B_6, A_6 a B_5, A_5 a B_5, A_5 a B_6.

Tak więc w wyniku migracji jąder dikarionu do strzępek grzybni monokariotycznej otrzymano nowe jądra rekombinacyjne co umożliwiło powstanie dikarionów o różnym od wyjściowego składzie genetycznym.