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Inheritance of chromosome length polymorphisms in *Coprinus cinereus*

Toshihide Arima and Tsutomu Morinaga

Department of Fermentation Technology, Faculty of Engineering, Hiroshima University, Kagamiyama 1-4-1, Higashi-Hiroshima 724, Japan

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The sexually compatible strains of *Coprinus cinereus* 5302 and Dd 13 revealed chromosome length polymorphisms in their electrophoretic karyotypes. The dikaryon derived from two monokaryons contained a mixture of the two electrophoretic patterns. F_1 progenies were isolated by crossing *C. cinereus* 5302 and Dd 13 strains and it showed unique karyotypes. Chromosome length polymorphisms of both parental strains were inherited at random in the F_1 progenies. As a result, several novel electrophoretic karyotypes which had not been observed in either parental strains were found in the F_1 progeny. The rDNA probe hybridized with one chromosome in both parental strains, with two chromosomes in the hybridization pattern of both parental strains in the dikaryon, and with one to two chromosomes in the F_1 progenies. The relation between mating type and hybridization pattern has thus not been made clear in the case of F_1 progeny.

Key Words——chromosome length polymorphisms; *Coprinus cinereus*; electrophoretic karyotype; F_1 progeny; ribosomal DNA.

Introduction

The ability to fractionate chromosome-sized DNA molecules by pulsed-field gel electrophoresis (PFGE, Schwartz and Cantor, 1984) has led to new approaches for karyotypic analysis of fungi, whose cytogenetics are often intractable. More recently, the development of contour-clamped homogeneous electric field PFGE (CHEF, Chu et al., 1986) has allowed analysis of electrophoretic karyotypes of several yeasts and filamentous fungi, as reviewed by Mills and McCluskey (1990). Electrophoretic karyotypes have been utilized in the determination of chromosome numbers (Merz et al., 1988; Orbach et al., 1988), the confirmation of genetic mapping (Brody and Carbon, 1989), the analysis of dispersed repetitive elements (Hamer et al., 1989), and the identification of minichromosomes (Masel et al., 1990). Though a method for determining electrophoretic karyotypes by chemical treatment of hyphae has recently been reported (McCluskey et al., 1990), high molecular weight DNA for chromosome separation gels has typically been obtained from lysed protoplasts.

The basidiomycetes *Coprinus cinereus* (Schaeffer: Fries) S. F. Gray has proven to be a useful model system for analysis of the meiotic process, as reviewed by Pukkila and Casselton (1991). *C. cinereus* is one of the easiest fungi to grow and fruit in a laboratory, and methods for analysis of this fungus at the molecular level have recently been developed (Binninger et al., 1987) which facilitate cell biological, biochemical, genetic and molecular analyses. A variety of cytological procedures can be conveniently applied to this fungus. Chromosome pairing can be monitored easily using a light microscope (Lu and Raju, 1970; Pukkila and Lu, 1985; Zolan et al., 1988). The well-developed synaptonemal complexes can be characterized by surface spreading and electron microscopy (Pukkila and Lu, 1985). More recently, analysis of the karyotype at the molecular level has been readily accomplished, since chromosomal sized DNA can be prepared and resolved by CHEF gel electrophoresis (Pukkila and Casselton, 1991; Zolan et al., 1992). These techniques, which provide complemetary information, have facilitated analysis of the *C. cinereus* karyotype. As a result, the *C. cinereus* nuclear genome is estimated to be approximately 37,500 kb (Dutta, 1974), with a chromosome number of 13 (Pukkila and Lu, 1985).

Chromosome length polymorphisms (CLPs) among strains of *Saccharomyces cerevisiae* Meyen ex Hansen have been demonstrated by PFGE. These CLPs are much more extensive in the brewers', bakers' and wine yeast strains (Casey et al., 1990; Rank et al., 1991; Vezinhet et al., 1990). The CLPs have also been well studied in the pathogenic yeast *Candida albicans* (Robin) Berkhout and other *Candida* species in order to distinguish between clinical strains (Merz, 1990; Iwaguchi et al., 1990; Asakura et al., 1991; Doi et al., 1992). In basidiomycetes, CLPs have also been reported for the edible mushrooms *Agaricus bisporus* (Lange) Singer (Royer et al., 1992) and *Lentinus edodes* (Berkeley) Singer (Arima and Morinaga, 1993), *C. cinereus* (Pukkila and Casselton, 1991; Zolan et al., 1992), *Phanerochaete chrysosporium* Burdsall apud Burdsall et Eslyn (D'Souza et al., 1993), and *Schizophyllum commune* Fries (Horton and Raper, 1991). However, the inheritance of chromo-

some length polymorphisms has been reported for only a few strains, for example, *S. cerevisiae* (One and Ishino-Arao, 1988), *Tilletia caries* (de Candolle) Tulasne, *Tilletia controversa* Kuehn, the F_1 progenies between *T. caries* and *T. controversa* (Russell and Mills, 1993), and *Ustilago hordei* (Persoon) Lagerheim (McCluskey and Mills, 1990).

In this paper, we report the inheritance of chromosome length polymorphisms in *C. cinereus* by using CHEF gel electrophoresis and the use of DNA hybridization on the separated chromosomes to determine the location of a ribosomal RNA gene.

Materials and Methods

***Coprinus cinereus* strains, culture conditions, and plasmid** The strains used in this study are summarized in Table 1. The PR series of F_1 progenies was obtained by crossing strains of *C. cinereus* 5302 and Dd 13. Potato-sucrose medium (Morinaga et al., 1985) was used for vegetative growth and fruiting body formation. Ribosomal DNA (rDNA) was obtained from F. H. J. Schuren (University of Groningen, The Netherlands). This was a 1.9-kb *Eco*RI-*Xba*I insert containing 5.8S and 18S rRNA genes of *S. commune* cloned in pGEM1 (Promega). This plasmid was maintained in *Escherichia coli* (Migula) Castellani et Chalmers JM109, which was grown on Luria-Bertani medium (Maniatis et al., 1982) containing 100 μg/ml of ampicillin. Plasmid DNA was isolated through alkaline lysis according to the method of Birnboim and Doly (1979).

Preparation of intact chromosomal DNA *Coprinus cinereus* chromosomal DNA was prepared by a modification of the procedure developed by Binninger et al. (1991). Protoplasts were prepared from young hyphae, based on the procedure described by Morinaga et al. (1985). Novozym 234 (Novo Labs.) was used at a concentration of 3 mg/ml to degrade the mycelial cell walls. After the enzymatic treatment, the protoplasts were pelleted and washed three times with MS solution (50 mM maleic acid/0.5 M sorbitol, pH 5.8). The protoplasts were then resuspended in MS solution at a concentration of $5.0\text{–}7.0 \times 10^8$ protoplasts per ml. A solution of 1.4% low-melting-point agarose in MS solution was prepared and kept at 50°C. Aliquots of protoplasts were mixed with an equal volume of agarose, and the mixture was immediately poured into a sample plug caster (Pharmacia). The plugs were chilled at 4°C for 30 min, removed with a metal spatula, and added to a large amount of NDS buffer (10 mM Tris, pH 7.5, 0.5 M EDTA, pH 8.0, 1% sodium N-lauroyl sarcosinate containing 1 mg of proteinase K per ml) to completely cover the plugs. The plugs were then incubated for 24 h at 50°C, and the buffer was removed. The plugs were rinsed three times with 50 mM EDTA, pH 8.0, at room temperature and stored in 0.5 M EDTA, pH 8.0, at 4°C until use. Intact chromosomal DNA molecules of *C. albicans* (ATCC 14053) (Clontech Lab.) and *Schizosaccharomyces pombe* Lindner (972) (FMC BioProducts) were used as size markers for chromosomal DNA.

Contour-clamped homogeneous electric field (CHEF) gel electrophoresis conditions CHEF gel electrophoresis was performed using a BS-80 apparatus (Bio Craft, Tokyo) according to the manufacturer's instructions. Gel (100 ml) containing 0.8% chromosomal grade agarose (Bio-Rad) was gently poured into a mould ($10 \times 10 \times 0.5$ cm). The DNA-agarose plugs were inserted into the gel wells and sealed with 1% low-melting-point agarose. The electrophoretic conditions were 60 V for 137 h, with a 20-min pulse time, in 0.5 × Tris-borate EDTA buffer (TBE) (Maniatis et al., 1982), which was maintained at 9–15°C by circulation. The TBE buffer was not changed during these runs. The gels were stained with ethidium bromide (1.5 μg/ml) for 30 min and then destained in distilled water overnight.

Table 1. *Coprinus cinereus* strains used.

Strain	Mating type	Genotype	Used as
5302	A2B2	Wild	Parent
Dd 13	A12B12	Wild	Parent
No. 13	A2B12	Wild	Tester
No. 33	A12B2	Wild	Tester
PR series		Wild	F_1 progeny

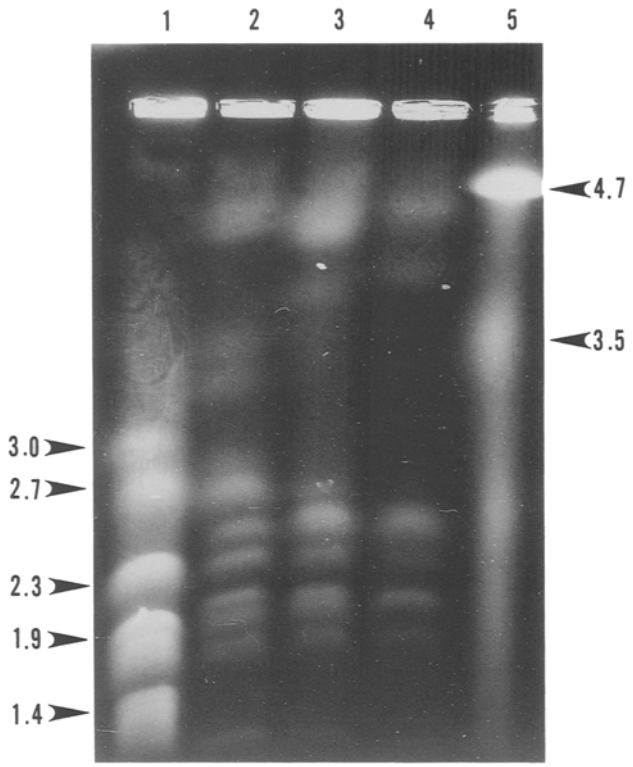

Fig. 1. Electrophoretic karyotypes of *Coprinus cinereus*, parental strains. Lane 1, *Candida ablicans* (ATCC14053); lane 2, 5302; lane 3, 5302 × Dd 13; lane 4, Dd 13; lane 5, *Schizosaccharomyces pombe* (972). The positions of size markers, *C. albicans* and *S. pombe* chromosomes, are shown on the left and right margins of the photograph, respectively. The size is shown in megabase pairs.

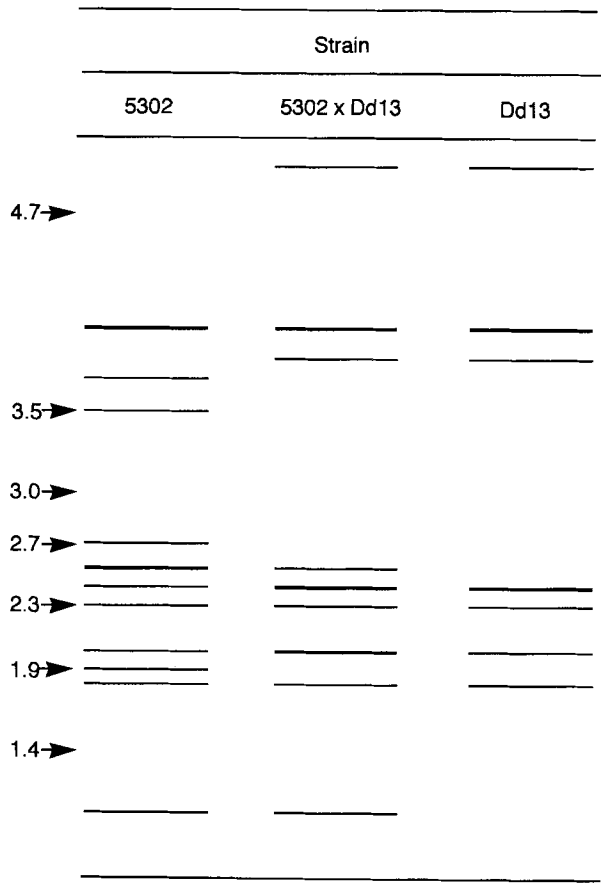

Fig. 2. Scheme of chromosome band profiles of the *C. cinereus* strains 5302, 5302 × Dd 13 and Dd 13. The relative positions of size markers, *C. albicans* and *S. pombe* chromosomes, are given on the left. The size is shown in megabase pairs.

Transfer and hybridization conditions The gels were incubated in 200 ml of 0.5 M NaOH/1.5 M NaCl for 15 min to denature the DNA, then neutralized in 200 ml of 0.5 M Tris, pH 7.5/1.5 M NaCl (two 15-min periods). The DNA was transferred to a nylon membrane Hybond N (Amersham) using a standard method (Maniatis et al., 1982). Hybridization was done using a non-radioactive digoxygenin-dUTP labeled DNA probe. Procedures for hybridization were carried out according to the manufacturer's recommendations (Boehringer, Mannheim).

Results

Electrophoretic karyotype Figures 1 and 2 show the chromosomal patterns resulting from CHEF gel electrophoresis of parental strains (5302 and Dd 13) and of a dikaryon derived from crossing between these parental strains. The numbers of chromosome bands were at least 11 and 7 for 5302 and Dd 13 strains, respectively. Judging from the relative intensity of their ultraviolet fluorescence after staining with ethidium bromide, some of these bands probably contain more than one chromosome. Attempts to resolve these bands containing multiple chromosomal DNAs using altered run conditions (i.e., length of pulse interval, voltage, concentration of agarose gel) did not succeed (data not shown). The patterns are highly reproducible from gel to gel, but strikingly divergent from strain to strain. Thus, chromosome length polymorphisms (CLPs) were repeatedly found in parental strains of *C. cinereus*. For example, the 5302 strain appeared to have the smallest chromosome, 1.0 Mb, but this was not observed in the Dd 13 strain (Figs. 1, 2). These results suggested that in the 5302 strain the chromosome number was at least 13 and that the size of chromosomal DNAs ranged from 1.0 to 4.0 Mb. The chromosome number of this strain is consistent with electron microscopic studies of synap-

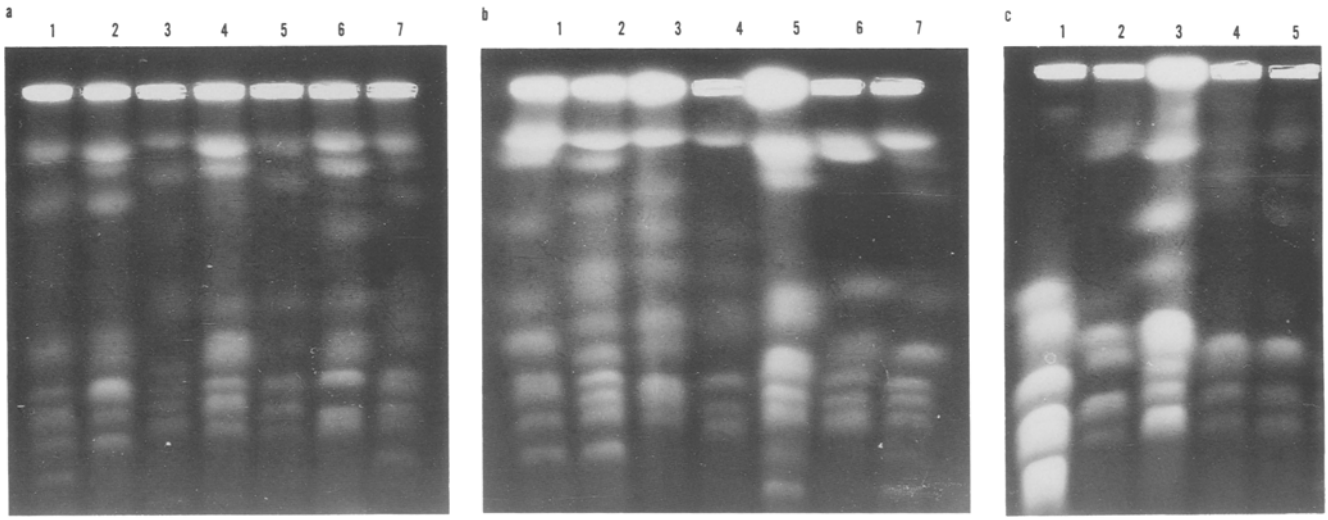

Fig. 3a–c. Electrophoretic karyotypes of *C. cinereus*, F_1 progenies (PR series). a. Lane 1, PR-1; lane 2, PR-2; lane 3, PR-3; lane 4, 5302 × Dd 13; lane 5, PR-4; lane 6, PR-5; lane 7, PR-6. b. Lane 1, PR-7; lane 2, PR-8; lane 3, PR-9; lane 4, PR-10; lane 5, PR-11; lane 6, PR-12; lane 7, PR-13. c. Lane 1, *C. albicans*; lane 2, PR-14; lane 3, PR-15; lane 4, PR-16; lane 5, PR-17.

tonemal complexes in *C. cinereus* by Pukkila and Lu (1985). On the other hand, the Dd 13 strain possessed at the least 9 chromosomes within a range of 1.7-5.0 Mb, as estimated by using chromosomal DNAs of *C. albicans* (ATCC 14053) and *S. pombe* (972) as size markers. As expected, the dikaryon appeared to contain a mixture of the karyotypic patterns of each parental strain (Figs. 1, 2). The total genome size calculated from these data is estimated to be approximately 35 Mb in the 5302 strain, agreeing with results by Dutta (1974). Due to the missing of some chromosomal DNAs, the total genome size was estimated at 28 Mb in the Dd 13 strain.

Inheritance of chromosome length polymorphisms To investigate inheritance patterns of the chromosome length polymorphisms, we crossed the 5302 strain with Dd 13 strain and isolated F_1 progenies from one of the fruiting bodies. The strains derived from F_1 progenies were analyzed by CHEF gel electrophoresis to ascertain whether the electrophoretic karyotype was variable or static. The electrophoretic karyotype of each F_1 progeny was unique (Fig. 3a-c). Also, the chromosome numbers varied between 7 and 13 among the F_1 progenies, whereas parental strains had 13 and 9 chromosomes, respectively (Figs. 1, 2). The chromosomes of the F_1 progenies varied in length from approximately 1.0 to 5.0 Mb, and the total genome size ranged from approximately 25 to 35 Mb.

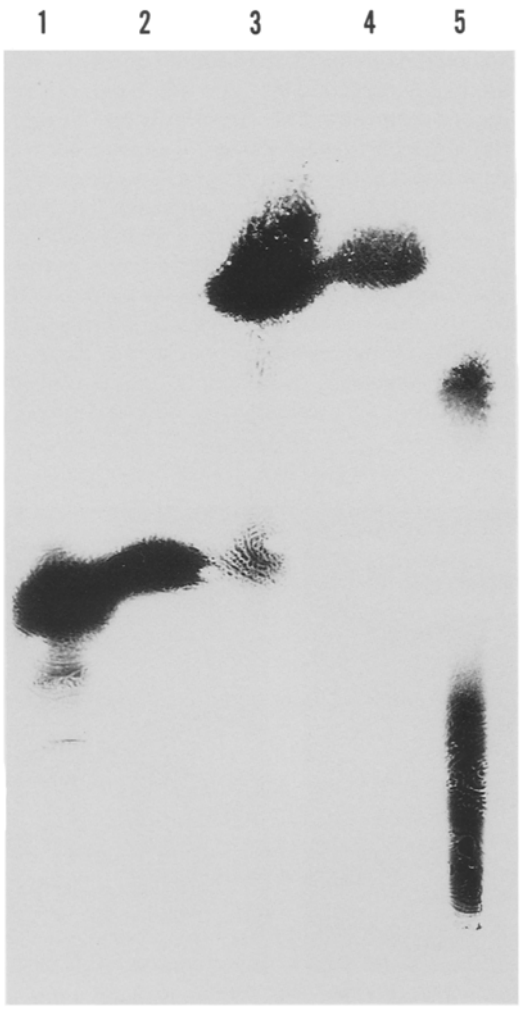

Fig. 4. Hybridization analysis of the gel from Fig. 1 probed with *Schizophyllum commune* rDNA.

Mapping of gene onto the chromosomes of *C. cinereus* Resolving the chromosomal DNAs makes it possible to map a specific gene to the individual chromosomal DNAs of *C. cinereus* by hybridization. To demonstrate this, the 1.9-kb rDNA repeat unit of *S. commune* containing 5.8 S and 18 S ribosomal RNA gene was mapped to the chromosomal DNAs of *C. cinereus*. Figs. 4 and 5 and Table 2 show the hybridization patterns of *C. cinereus* chromosomal DNAs separated by CHEF gel electrophoresis with rDNA probe. The probe hybridized with one chromosome in both parental strains. Dikaryons resulting from a cross between the two monokaryones (5302 × Dd 13 strains), as expected, hybridized with two chromosomes of both parental strains (Fig. 4). The F_1 progenies, however, hybridized with one to two chromosomes and hybridization patterns were variable (Fig. 5; Table 2). Some of these strains revealed a new hybridization pattern. They were divided into two classes. In the PR-4 and PR-16 strains, the probe hybridized with one chromosome that was smaller than that of other strains. In the PR-11 strain, the probe hybridized with two chromosomes. The relation between mating type and hybridization pattern is indeterminate in the F_1 progenies (Table 2).

Discussion

CHEF gel electrophoresis was used to examine the electrophoretic karyotypes of strains of *C. cinereus* 5302, Dd

Table 2. Relation between mating type and hybridization patterns of F_1 progenies (PR series) in *C. cinereus*. (Probe: rDNA.)

F_1 progeny	Mating type	Hybridization pattern
PR- 1	A2B2	5302
PR- 2	A12B2	Dd 13
PR- 3	A2B2	No. 33
PR- 4	A2B12	New
PR- 5	A12B2	Dd 13
PR- 6	A12B12	Dd 13
PR- 7	A12B12	Dd 13
PR- 8	A12B2	5302
PR- 9	A2B12	No. 13
PR-10	A12B12	5302
PR-11	A12B2	New
PR-12	A2B12	Dd 13
PR-13	A2B12	Dd 13
PR-14	A2B2	Dd 13
PR-15	A2B12	Dd 13
PR-16	A2B12	New
PR-17	A12B2	No. 33

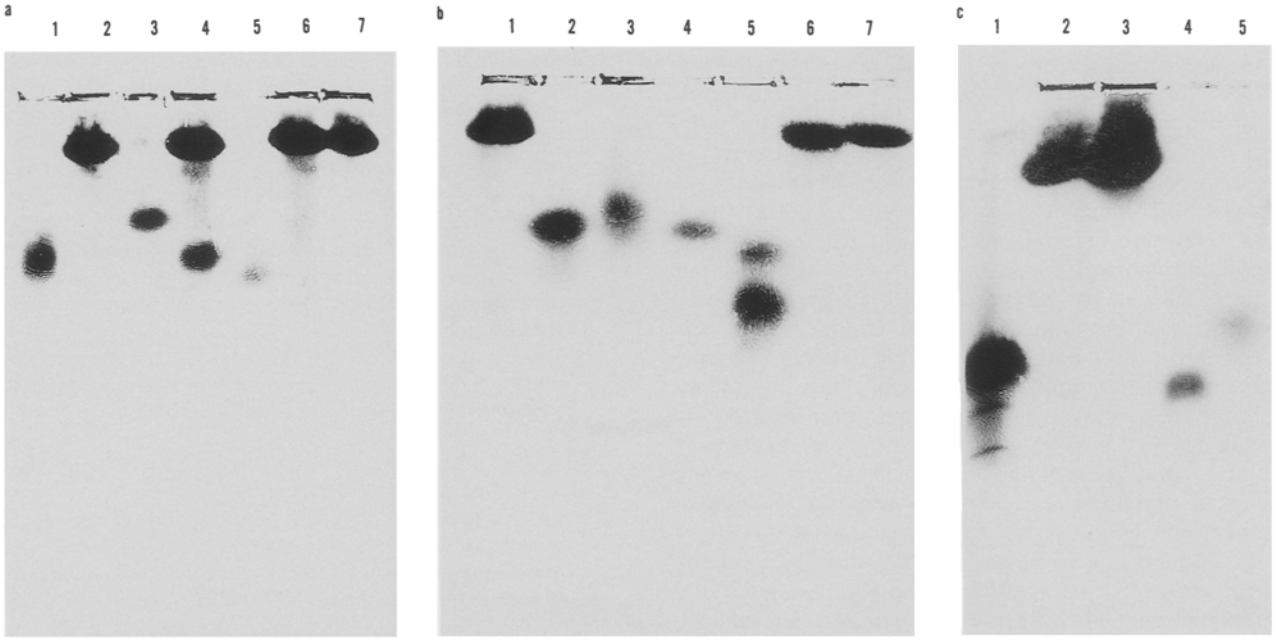

Fig. 5a–c. Hybridization analysis of the gel from Fig. 3a–c probed with *S. commune* rDNA.

13, the dikaryon between 5302 and Dd 13, and their F_1 progenies. The electrophoretic karyotype of each strain varied with respect to both the number and length of chromosome-sized DNA bands. Under typical conditions (137 h duration, 20 min pulse interval, 60V), 7–11 chromosome-sized DNA bands were completely resolved, and intensity differences implied that some of these bands contain more than one chromosome.

Thus, each strain examined revealed a unique karyotype. Chromosome length polymorphisms may prevail in this fungus, as they do in other strains of *C. cinereus* (Pukkila and Casselton, 1991; Zolan et al., 1992; data not shown). The extensive chromosome length polymorphisms make it difficult to identify a presumed linkage group by size alone. This is because linkage group III is probably involved in the translocation between chromosome 3 and 5 that was mapped by light and electron microscopy (Holm et al., 1981; Pukkila and Lu, 1985). Each basidiospore isolated from crossing strains of *C. cinereus* 5302 and Dd 13, and F_1 progenies also exhibited different chromosome length polymorphisms (Fig. 3a–c). Why are chromosome length polymorphisms observed in fungi? We think that they occur as a result of chromosomal rearrangments such as insertions (for example, by transposable elements, plasmids, viral DNA, or DNA of a completely heterologous origin), deletions, duplications on the same chromosome, duplications on a different chromosome, or translocations (transfer of a DNA segment from one chromosome to another). In particular, DNA translocations may occur by recombination between DNA sequences belonging to any one of the several dispersed repeated DNA families. On the other hand, chromosome length polymorphisms may indicate a characteristic of each strain. For instance, the 5302 strain produced abundant oidia, but not the Dd 13 strain. The strains used in this study differed in their morphology and growth rate of mycelium. From the above, it will be important to ascertain whether the observed chromosome length polymorphisms in *C. cinereus* involved essential, dispensable, or both regions of the genome.

Inheritance of chromosome length polymorphisms has been described for sexually compatible strains of *S. cerevisiae* (Ono and Ishino-Arao, 1988), and for strains of *T. caries, T. controversa*, and their F_1 progenies (Russel and Mills, 1993). In *S. cerevisiae*, homologous chromosomes with a length polymorphism produced recombinant chromosomes that differed in size from either of the parental chromosomes. The polymorphism which segregated 1 : 1 has been mapped. In *T. caries, T. controversa*, and their F_1 progenies, the electrophoretic karyotypes of their F_1 progenies were unique, being different from each other and the parental strains. The number of chromosomes in these strains ranged from 19 to 22, whereas each parental strain had 20. This bears a striking resemblance to our results. In the present study, the chromosome numbers of the F_1 progenies (7–13) indicated that the reduction division stage of meiosis had occurred. The apparent variability in chromosome number among these strains suggested that there had been a need to maintain a disomic state for some chromosomes. The variability in size of individual bands may be a physical manifestation of genome rearrangements that require the maintenance of disomic chromosomes. This result indicated that chromosome length polymorphisms of both parental strains are inherited at random in the F_1 progenies, while several novel electrophoretic karyotypes which had not been observed in either parental strain were also found in the F_1 progenies. Some of these strains may be thought of as aneuploid. It is of in-

terest to know whether chromosome length polymorphisms are restricted to a few specific chromosomes, and whether they are due to a few large structural alterations (i.e., additions and deletions) or to many small ones.

A probe made of the ribosomal DNA repeat hybridized with one chromosome in both parental strains. In the dikaryon obtained from crossing a strain of *C. cinereus* 5302 and Dd 13, as expected, hybridization occurred with two chromosomes having a pattern of both parental strains and also with one to two chromosomes in the F_1 progenies. Some of these strains revealed a new hybridization pattern. Such changes were observed in the chromosomes containing rDNA of F_1 progenies that had undergone mating, fruit body formation, basidiospore formation or basidiospore germination. In the case of PR-4 and PR-16 strains, it may be deleted in a repetitive array such as ribosomal DNA, subtelomere and telomere. It may have occurred as a meiotic recombination or translocation within rDNA locations in the PR-11 strain. This was a surprising result, since Cassidy et al. (1984) reported that meiotic recombination was suppressed within the rDNA array in *C. cinereus*, as had been shown for many eukaryotes (Petes and Botstein, 1977; Dvorak and Appels, 1986; Russell et al., 1988). The relation between mating type and hybridization pattern has not been clarified in F_1 progenies (Table 2). The variations in rDNA hybridization patterns had no obvious phenotypic effect. This result suggested that mating type genes were stable, but the location of the rDNA gene may be unstable. According to the results of CHEF gel electrophoresis, the 5302 strain had more chromosomes than the Dd 13 strain. If the rDNA gene was encoded on an excess chromosome of the 5302 strain, the chromosome might be able to translocate freely. Most of the changes might occur following fertilization but prior to meiosis. The identification and characterization of the translocated segments will be of great interest. From such studies, we should be able to obtain better insights into the organization of the *C. cinereus* genome.

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