Karyotype polymorphism correlates with intraspecific infertility in the homothallic ascomycete *Sordaria macrospora*

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intraspecies polymorphism;
pulsed-field gel electrophoresis.

Abstract

The homothallic fungus *Sordaria macrospora* produces perithecia with meiotically derived ascospores. In most cases, intraspecies crosses between strains from different culture collections generate fertile hybrid perithecia in the contact zone of two mycelia. However, in some of these crosses we observed a significant decrease in the fertility of the hybrid perithecia when strains of different origin were used for mating. Since we assumed that chromosome variability between the culture collection strains might contribute to this reduction in fertility, we performed pulsed-field gel electrophoresis. In the course of our study, we were able to identify two major groups of electrophoretic karyotypes in *S. macrospora* culture collection strains. A quantitative analysis revealed that polymorphic karyotypes contribute to a reduction of fertility in forced crosses between strains carrying differently sized chromosomes. The observed intraspecific chromosome length polymorphism might have consequences on the speciation process of a homothallic fungus capable of sexual but not of asexual spore formation.

Introduction

The ascomycete *Sordaria macrospora* (Pyrenomycetidae, Sordariaceae) is a homothallic fungus, capable of producing only meiotically derived ascospores. In contrast to heterothallic Sordariaceae, asexual spores are absent. Thus in *S. macrospora* karyogamy occurs internally within the ascogenous hyphae by fusion of genetically identical nuclei. Although normal wild-type strains produce self-fertile fruiting bodies (perithecia), fertile hybrid perithecia are formed in intraspecies crosses when nuclei are interchanged by hyphal anastomoses at the contact zone of two mycelia (Esser & Straub, 1958). In some cases, we observed a large decrease in fertility when strains originating from different culture collections were used for mating. In these cases hybrid perithecia with a reduced number of ascospores were formed, most of which were inviable. Low fertility and dead ascospores in crosses of filamentous fungi may be correlated with spore killers (Turner *et al.*, 1987; Nauta & Hoekstra, 1993), or may be the result of the segregation of chromosome rearrangements (Perkins, 1974; Arnaise *et al.*, 1984). In the latter case, progeny of crosses between individuals with different karyotypes are often expected to be inviable due to deletions or duplications. In *S. macrospora* all seven chromosomes can be observed during mitotic and meiotic divisions by means of light microscopy, and also can be recognized with electron microscopy by centromere index and length (Zickler, 1977; Moreau *et al.*, 1985). However, because of the minute size of chromosomes, slight differences in size are difficult to detect. Some 14 years ago electrophoretic karyotyping became an important application of pulsed-field gel electrophoresis (PFGE) for obtaining information on genome size and organization in mycelial fungi. In many investigations, chromosome polymorphisms have been identified among different isolates of the same species (reviewed in Kistler & Miao, 1992; Walz, 1995). In several cases a reduction of fertility in intraspecies crosses could be directly correlated with karyotypic differences of the crossing partners (Pukkila, 1992; Geiser *et al.*, 1996).

In a previous work, Walz & Kück (1995) determined the electrophoretic karyotype of the *S. macrospora* fertile strain K(18). Using PFGE, they were able to resolve five

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chromosomal bands ranging in size from 3.7 to 7.5 Mb, two of which represent two co-migrating chromosomes. To ascertain if chromosomal length variations in wild-type strains are responsible for the reduction in fertility in some of the intraspecies crosses in *S. macrospora*, we determined the electrophoretic karyotypes of several wild-type isolates derived from different strain collections. In the study presented here, we have discovered two different electrophoretic karyotypes in *S. macrospora* wild-type strains. Both karyotypes were characterized by hybridization with three gene-specific probes. A significant reduction in fertility was observed in forced outcrosses between strains harbouring different karyotypes, whereas the fertility of forced outcrosses between strains carrying the same karyotype is comparable to that of homothallic matings. We discuss possible consequences which the variability in chromosome length might have on the speciation process of the homothallic *S. macrospora*.

Materials and methods

Strains and culture conditions

All *S. macrospora* strains investigated in this study are listed in Table 1. For karyotype analysis, all *S. macrospora* wild-type and mutant strains were cultivated on corn meal complete medium (Esser, 1982). Crossing experiments were undertaken as previously described by Esser (1982).

Contour-clamped homogeneous electric field (CHEF) gel electrophoresis

Intact chromosomes of *S. macrospora* strains were isolated from protoplasts as described by Walz & Kück (1991). Chromosomes were separated in 0.8% agarose (Fast Lane™, FMC, Rockland, ME, USA) gels with 0.5 × TBE buffer (pH 8.3) at 12 °C using the CHEF Mapper™ (BioRad, Richmond, CA, USA). Separation conditions were, unless otherwise specified, identical to those reported by Walz & Kück (1995). *Schizosaccharomyces pombe* chromosomes (BioRad) were used as a size standard.

Table 1 Fungal strains used in this study.

Culture	Culture collection
+Paris	Allgemeine Botanik, Bochum, GER*
CBS 957.73	Centraalbureau voor Schimmelcultures, Baarn, NL†
CBS 396.69	Centraalbureau voor Schimmelcultures, Baarn, NL
DSM 997	Deutsche Sammlung für Mikroorganismen, Göttingen, GER†
IMI 146.455	International Mycological Institute, London, GB
K(18)	Allgemeine Botanik, Bochum, GER†
K(3346)	Allgemeine Botanik, Bochum, GER†
K(1957)	Allgemeine Botanik, Bochum, GER†
K(R)	Read, Edinburgh, UK
pro1 (8871) (*pro*toperithecia)	Allgemeine Botanik, Bochum, GER‡
per5 (*per*ithecia)	Allgemeine Botanik, Bochum, GER‡
spd (*spad*ix)	Allgemeine Botanik, Bochum, GER†
s1 (391) (sterilis)	Allgemeine Botanik, Bochum, GER†

*Heslot (1958); †Esser & Straub (1958); ‡Masloff (1998).

DNA hybridization

Southern blotting was performed according to Sambrook *et al*. (1989). Pulsed-field gels were soaked for 10 min in 0.25 M HCl prior to denaturation and transferred to nylon membranes. Hybridization with radioactively labelled dsDNA probes was performed according to conventional methods (Sambrook *et al*., 1989). Labelling of DNA probes was done with the Decaprime II™ DNA labelling kit (Ambion, Austin, TX, USA).

Quantitative measurements of fertility

In order to estimate the number of asci containing viable and/or inviable ascospores 10-day-old perithecia from wild-type and crossing plates, respectively, were squeezed with a pair of forceps and the contents were spread onto the surface of 5% agar. Rosettes were then examined under a binocular dissecting microscope at about 40× magnification. From each cross and from homothallic wild-type strains 500 asci were analysed. These asci were classified into three categories: asci containing eight viable, black spores; asci containing eight inviable, white spores; and asci containing both black and white spores.

Cytology

Morphology of asci of selfed perithecia from *S. macrospora* wild-type strains and crosses between two mutant strains were examined after cultures grown for 10 days on corn meal agar medium. Rosettes of the perithecia were transferred to water on slides. Pictures were taken with Agfa Technical Pan film through a Zeiss Axiophot microscope at 200× magnification.

For light microscopic observations of chromosomes, rosettes of asci were fixed as described by Lu (1967). After 10 min of hydrolysation at 70 °C in 1 N HCl solution, the asci were stained in propionic iron haematoxylin. Observations were performed with a Zeiss Axiophot microscope. Photographs were taken with AGFAPAN APX 25 (Agfa) at 2000× magnification.

Results

CHEF gel electrophoretic analysis

Previous electrophoretic karyotyping of *S. macrospora* revealed that the karyotype of the *S. macrospora* wild-type strain K(18) consists of seven chromosomes (Walz &

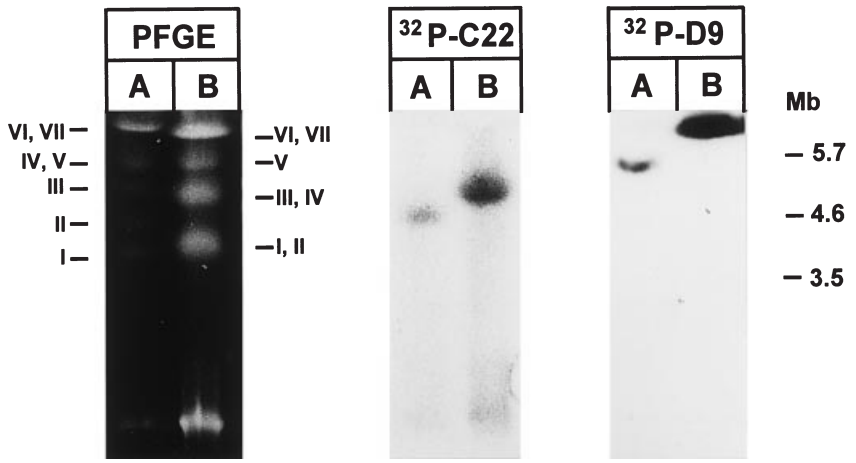

Fig. 1 Karyotype analysis of *Sordaria macrospora.* Pulsed-field gel electrophoresis and Southern hybridization. Chromosomal DNA of the *S. macrospora* strains K(18) (A-karyotype) and K(3346) (B-karyotype) was prepared as described in Materials and methods. The sizes of the *S. pombe* chromosomes are given in the right margin. The relative position and the number of *S. macrospora* chromosomes are indicated for both karyotypes by Roman numerals. Southern hybridization was performed either with cosmid clone C22 containing the α-tubulin gene *tubA* of *S. macrospora* (Pöggeler *et al.*, 1997a) or with cosmid clone D9 containing the *S. macrospora* mating-type locus (Pöggeler *et al.*, 1997b).

Kück, 1995). In analysing different *S. macrospora* wild-type and mutant strains from several sources (Table 1), we are able to distinguish two major electrophoretic karyotypes, which we designated A and B (Fig. 1, Table 2). Fractionation of intact chromosomes of culture collection strains by CHEF gel electrophoresis resulted in the resolution of five and four distinct chromosomal bands in the A- and B-karyotypes, respectively. As can be seen from Fig. 1, the relative intensities of ultraviolet fluorescence suggest that the band with the molecular weight of about 7.5 Mb represents doublets in both karyotypes. The same is true for the band of 5.6 Mb in the A-karyotype and the 4.8 Mb and 3.9 Mb bands of the B-karyotype. From these measurements, the size of the whole genome of the A-karyotype was estimated to be 39.5 Mb, while we calculated a genome size of 38 Mb for B-karyotype strains. To confirm that strains presenting the B-karyotype in the PFGE have the same number of chromosomes as the A-karyotype (Walz & Kück, 1995),

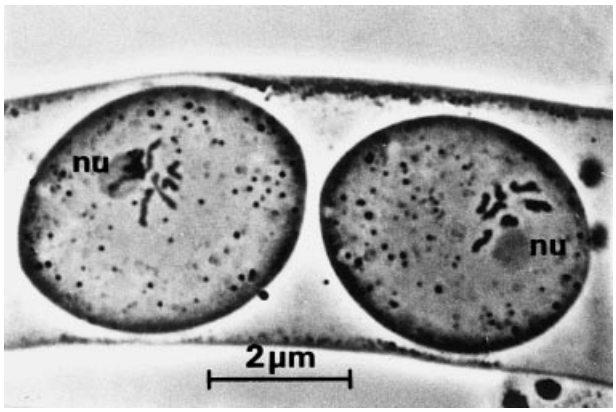

Fig. 2 Light microscopic analysis of *S. macrospora* chromosomes. Microscope picture of the post-meiotic division in an ascus of the *S. macrospora* B-karyotype strain K(3346). nu, nucleolus. ×2000.

we compared the results obtained by PFGE with cytological observations. Figure 2 shows a light microscopic photo of the first post-meiotic metaphase from the B-karyotype found in strain K(3346) of *S. macrospora*. All of the seven chromosomes can be recognized in this micrograph. However, it is difficult to distinguish them by their size. All six culture collection strains shown in Fig. 3 can be assigned to either the A- or the B-karyotype. Although slight variations in chromosome sizes are visible among individual members of the two karyotype groups, each strain can be classified unequivocally. In addition, we have obtained results from PFGE experiments with three strains from type culture

Table 2 Size of the *S. macrospora* chromosomes and estimated genome size of the A- and B-karyotypes.

Karyotype A		Karyotype B	
Chromosome	Mb	Chromosome	Mb
VI, VII	7.5 (2×)	VI, VII	7.5 (2×)
IV, V	5.6 (2×)	V	5.6
III	5.1	III, IV	4.8 (2×)
II	4.5	I, II	3.9 (2×)
I	3.7		
Σ	39.5	Σ	38

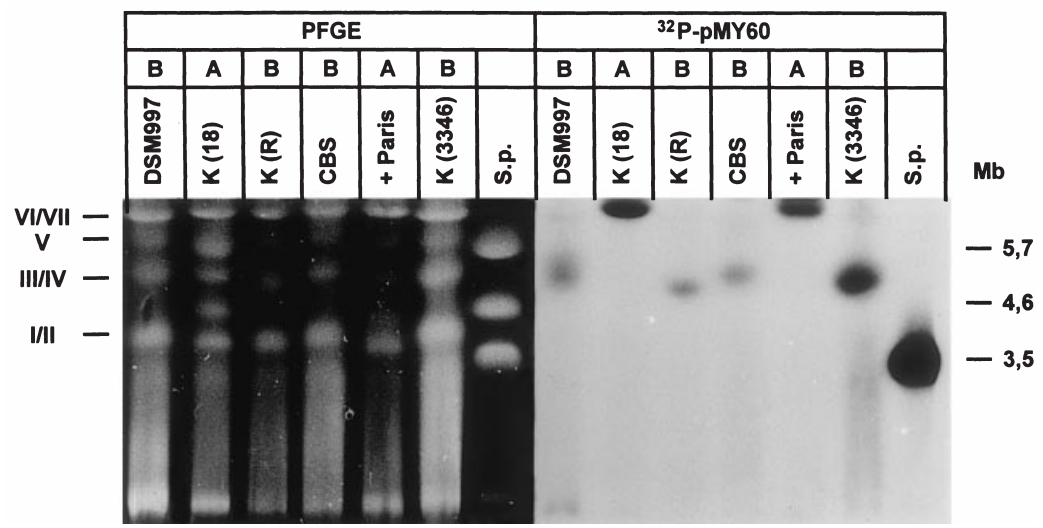

Fig. 3 Pulsed-field gel electrophoresis and Southern hybridization of separated chromosomes from six different *S. macrospora* strains and the *S. pombe* (S.p.) marker strain with a radiolabelled rDNA (pMY60) probe from *Saccharomyces carlsbergensis* (Verbeet *et al.*, 1983). The A- or B-karyotypes are indicated above each lane. CBS designates the *S. macrospora* strain CBS 957.73. The relative position of the chromosomes is indicated for the B-karyotype. The sizes of the *S. pombe* chromosomes are given in the right margin.

collections, namely CBS 396.69, IMI 146.455, which both belong to the A-karyotype, and K(1957), which carries the B-karyotype (data not shown).

Assignment of cloned genes to chromosomes

Southern blot hybridizations with several probes were performed in order to analyse whether similar sized chromosomes comprise homologous sequences. A single representative strain from each karyotype (K(18) A-karyotype and K(3346) B-karyotype) was chosen in order to map the α-tubulin gene *tubA* and the mating-type locus. As shown in Fig. 1, the *tubA* probe (C22) hybridizes to the chromosomal band of 4.5 Mb (chromosome II) in the A-karyotype, but to a double band of 4.8 Mb corresponding to chromosomes III or IV in the B-karyotype. Using the mating-type specific probe D9 containing the entire *S. macrospora* mating-type locus (Pöggeler *et al.*, 1997b), we show that the hybridizing chromosomes also differ in size in the different karyotypes (Fig. 1). In the B-karyotype, the largest chromosomal band (7.5 Mb) gives a signal indicating that the mating-type genes are localized on chromosomes VI or VII (7.5 Mb), while the mating-type locus hybridizes to the second largest chromosomal band (5.6 Mb) in the A-karyotype, which corresponds to chromosomes IV and V. Furthermore, we localize the ribosomal DNA on the fractionated chromosomes of six different *S. macrospora* wild-type strains using plasmid pMY60 carrying the rDNA genes of *Saccharomyces carlsbergensis* (Verbeet *et al.*, 1983) (Fig. 3). In the A-karyotype the largest chromosomal band gave a signal indicating that the rDNA genes are located on chromosome VI or VII, whilst in the B-karyotype the hybridization signal corresponds to chromosome III or IV. The strains belonging to the B-karyotype show a slight difference in size of the chromosomal double band III/IV. In *S. macrospora* strain DSM 997 and CBS 957.73, the corresponding chromosomal band seems to be larger than in strains K(R) and K(3346).

Effects of different karyotypes on sexual crossings

As shown above, at least four out of the seven chromosomes of the A- and B-karyotypes differ in length. To address the question of how the karyotypes are inherited and whether the observed polymorphism leads to a reduction in fertility, we crossed strains carrying the A-karyotype with strains carrying the B-karyotype. *Sordaria macrospora* is a homothallic fungus which produces self-fertile perithecia. Thus, it is difficult to distinguish between self-fertile and hybrid perithecia in crosses of wild-type strains. In order to circumvent this problem, we used sterile mutant strains for crossings. In these crosses complementation of the genetic defects results in the formation of fertile perithecia only in the contact zone of two mutant mycelia, all of which are hybrid perithecia. To analyse the involvement of different karyotypes in the reduction of the fertility, we crossed sterile mutants carrying the A-karyotype with sterile mutants harbouring the B-karyotype. The A-karyotype mutants s1 and spd, originally isolated by Esser & Straub (1958), show different developmental blocks. While mutant s1 forms perithecia lacking any ascospores, the spd mutant is unable to generate any perithecia at all.

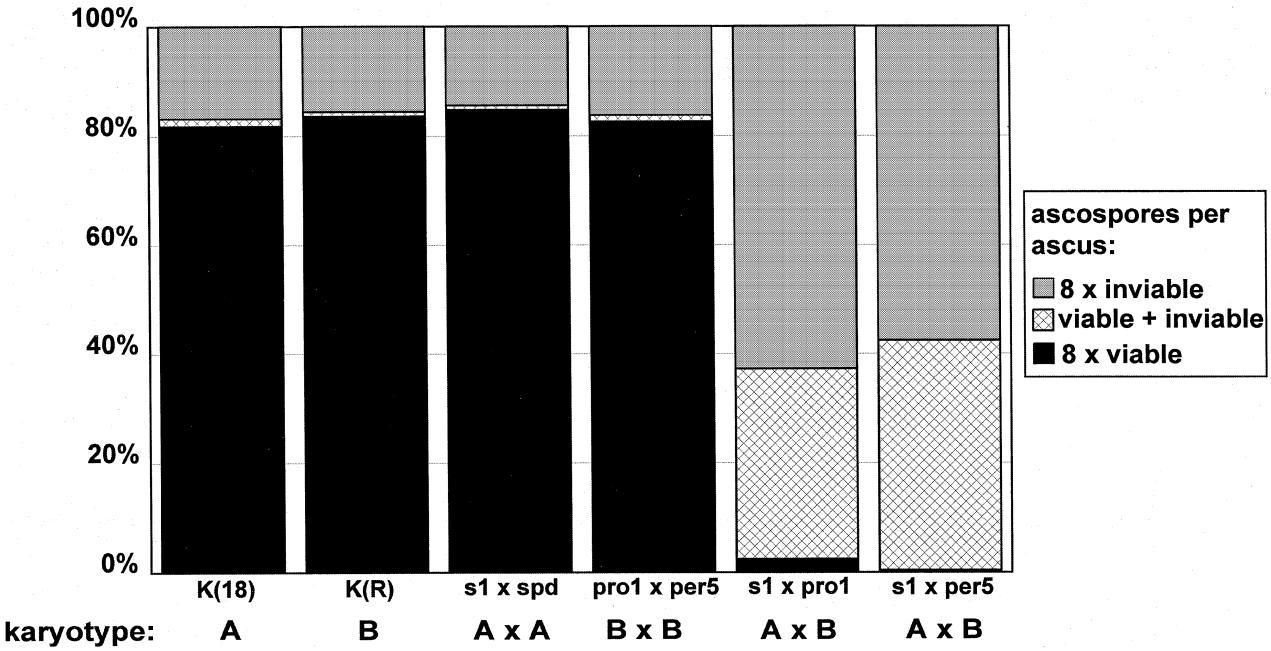

Fig. 4 Determination of the fertility of wild-type strains and sexual crossings of mutant strains carrying different karyotypes. Ratio of asci containing eight black, viable, or eight white, inviable ascospores, or a mixture of viable and inviable ascospores. Analysis of 500 asci in each case was performed as described in Materials and methods.

The B-type mutant pro1 carries a defect in the *pro1*$^{+}$ gene, which is responsible for the perithecial development and therefore produces only protoperithecia, whilst mutant per5 generates perithecia without any ascospores (Masloff, 1998; Masloff *et al.*, 1999). In all crosses between mutant strains carrying different karyotypes, the fertility was greatly reduced. Three different types of asci can be distinguished: (a) asci containing eight, black viable spores, (b) asci containing white, inviable spores and (c) asci containing a mixture of viable and inviable spores. Most asci produced in A × B crosses belong to the types described under (b) and (c) (Fig. 4). White ascospores never germinate, whilst the black ascospores, as expected for such a cross, are able to produce a mycelium exhibiting either a parental phenotype, the recombinant wild-type phenotype, or a double mutant phenotype. To quantify the reduction in fertility, it is not suitable to count the total number of perithecia produced in a cross as this number often varies. Therefore, we decided to examine rosettes contained in wild-type perithecia and in crosses between mutant strains carrying both the same karyotype. In all cases in which mutant strains carry the same karyotype, crosses show a high number of asci with eight viable ascospores (Fig. 4). This is comparable to perithecia produced in self-fertile wild-type strains. Therefore, the reason for the decrease in fertility is most probably not due to incomplete complementation of mutations in genes controlling sexual development, or due to secondary effects of these mutations. In addition, the A × B crosses of spd × pro1 and spd × per5 (data not shown) exhibit the same reduction of viable ascospores as the A × B crosses shown in Fig. 4. Rosettes from homothallic matings and outcrosses of mutant strains carrying either the A- or the B-karyotype are shown in Fig. 5. Perithecia produced in crosses between mutant strains carrying two different karyotypes predominantly carry asci with eight white or deformed ascospores, whilst in rosettes of wild-type matings and A × A and B × B mutant crosses more than 80% of the asci contain eight black, viable ascospores. Moreover, the number of 'mixed' asci with black and white spores is also drastically increased in crosses between strains carrying different karyotypes. In most cases only two black ascospores can be observed in these 'mixed' asci (Fig. 5).

Discussion

Two distinct electrophoretic karyotypes can be distinguished in the homothallic *S. macrospora* culture colletion strains

Comparison of electrophoretic karyotypes of nine *S. macrospora* wild-type strains originating from several culture collections revealed significant differences in chromosome size. In addition to the recently described A-karyotype (Walz & Kück, 1995), we were able to identify a second electrophoretic karyotype (B-karyotype) that differs in size in at least four out of seven chromosomes (Fig. 1; Table 2). Intraspecific polymorphisms in electrophoretic karyotypes were observed in a

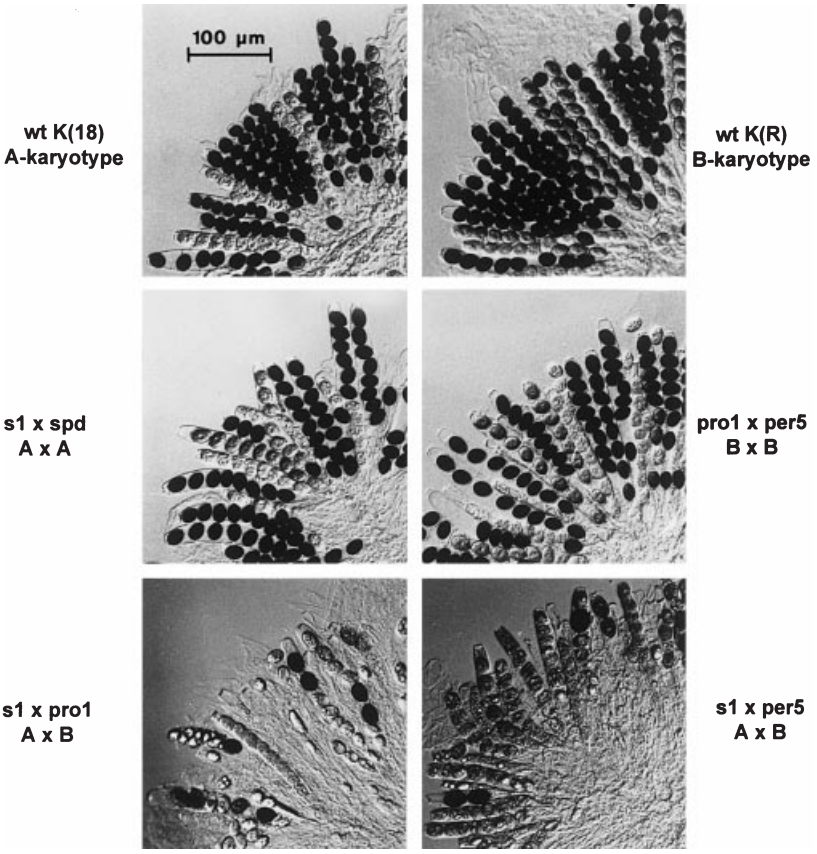

Fig. 5 Light microscopic investigation of rosettes from homothallic matings and outcrosses of mutant strains. Wild-type (wt) and mutant strains, as well as the corresponding karyotype are given in the margin (×200).

number of fungi although the type and dimensions of the variations differed depending on the genera. Possible mechanisms for generating chromosomal polymorphisms are deletions, translocations and duplications. Furthermore, the integration of a DNA fragment of foreign origin or aneuploidy generates length polymorphisms (reviewed in Skinner *et al*., 1991; Kistler & Miao, 1992; Walz, 1995). Calculation of the genome size revealed different values (38 Mb and 39.5 Mb, respectively; Table 2) for both *S. macrospora* karyotypes. Light microscopic investigation of the B-karyotype and the PFGE data clearly show seven chromosomes, indicating that there is no dispensable or supernumerary chromosome (Figs 1 and 2). The existence of small supernumerary chromosomes that lead to variation in the genome size has been shown for several plant pathogenic ascomycetes and for *Aspergillus nidulans* (Miao *et al*., 1991; Valent & Chumley, 1991; Tzeng *et al*., 1992; Geiser *et al*., 1996). Therefore, the differences in the *S. macrospora* genome size may result either from deletions or additions of chromosomal sequences, or from imprecise size estimations based on comparisons of separated chromosomes with those of *S. pombe*. These estimations of chromosome sizes may be imprecise because the two largest chromosomes of *S. macrospora* migrate slower than the *S. pombe* chromosomes used as a size standard. Precise determination of sizes of individual chromosomes, and thus of genome size, awaits the development of high-molecular-weight markers. Suitable markers may become available after completion of genome sequencing projects of filamentous fungi such as *N. crassa*.

Homologous sequences on individual chromosomes were identified by studying the location of the α-tubulin gene (*tub*A), the mating-type genes and the genes encoding rRNA (Figs 1 and 3). Southern hybridization of separated chromosomes revealed that in the A- and B-karyotypes, homologous genes are located on differently sized chromosomes. Thus, we conclude that translocation events might have led to chromosome length polymorphism in *S. macrospora*, such as has been described in several filamentous fungi including *Cladosporium fulvum* (Talbot *et al*., 1991), *Cochliobolus heterostrophus* (Tzeng *et al*., 1992), *Agaricus bisporus* (Kerrigan *et al*., 1993), *Magnaporthe grisea* (Talbot *et al*., 1993) and *Coprinus cinereus* (Zolan *et al*., 1994).

The use of individual chromosomes as templates for preparing probes is a very efficient tool for studying karyotypic polymorphisms derived from translocations. Using the chromosomal band VI/VII of the *S. macrospora* A-karyotype as a probe, we were able to identify in

addition to the expected hybridization signals on chromosome VI and VII signals on chromosomal bands I/II and III/IV of the B-karyotype (data not shown). These data are consistent with those obtained by the hybridization of the rDNA genes. The rDNA genes are located on chromosome VI or VII in the A-karyotype, whilst in the B-karyotype the hybridization signal corresponds to chromosome III or IV (Fig. 3). From our data it seems most likely that in *S. macrospora*, as in the closely related fungus *N. crassa*, the rDNA is located at a single genomic site, the nucleolus organizing region (NOR) (Barry & Perkins, 1969). In *N. crassa* the NOR is arranged as a series of 100–200 repeats and it has been shown that the number of rDNA repeat units changes frequently during the sexual phase of the life cycle (Butler & Metzenberg, 1989, 1990). As can be seen in Fig. 3, sizes of the chromosomal band harbouring the rDNA varies in several B-type strains. It may well be that these slight chromosomal variations are due to rDNA expansion or contraction. Apart from *N. crassa*, such variations of the rDNA harbouring chromosome were reported for the ascomycetes *S. cerevisiae* and *S. pombe* (Pasero & Marilley, 1993) and for the basidiomycete *Coprinus cinereus* (Pukkila & Skrzynia, 1993). The data obtained by the hybridization of the rDNA can be explained by a single translocation event from the largest chromosomes VII or VI to one of the two smaller chromosomes. But, when the differences in hybridization patterns obtained with the α-tubulin gene (Fig. 1) or the mating-type specific probe (Fig. 1) are considered, additional translocation events, large-scale deletions or insertions must have occurred. However, to prove this hypothesis physical mappings have to be carried out. Owing to the lack of a sufficient number of genetic markers, in *S. macrospora* it is currently impossible to verify this hypothesis.

Effects of intraspecific karyotype polymorphism on the speciation process

So far, the reason is not known for the observed intraspecific karyotype variation in the homothallic fungus *S. macrospora*. Sexual crossings of strains harbouring different karyotypes resulted in a low viability in the ascospore progeny (Figs 4 and 5), which may be due to the inability to balance translocations. Similar to our observations made with *S. macrospora*, Zolan *et al*. (1994) also described a low viability of tetrads derived from sexual outcrosses between *C. cinereus* strains carrying different karyotypes. In *N. crassa*, it has been suggested that chromosomal duplications confer sterility due to repeat-induced point mutations (RIP) (Selker, 1990). Such a mechanism for the meiotic inactivation of duplicated sequences has not been identified in *S. macrospora* (Le Chevanton *et al*., 1989). In the closely related genera Neurospora and Podospora the reason for a reduced amount of viable ascospores can be due to a special form of segregation distortion. In these cases crosses between different wild-type strains results in only half the normal number of viable ascospores as a consequence of the action of spore killer genes. Then half of the ascospores in the ascus are killed, while the surviving spores have the killer genotype (Raju, 1979; Turner *et al*., 1987; Nauta & Hoekstra, 1993). Most probably the reduced fertility we observed in A × B crosses of *S. macrospora* is not caused by spore killers, since in the rosettes produced in A × B crosses most of the asci contain eight abortive spores or a mixture of viable and inviable spores. However, most of the latter have only two viable spores, whereas asci with four black and four inviable spores were rarely detected (Figs 4 and 5).

Kistler & Miao (1992) suggested that the occurrence and extent of chromosome length polymorphism is inversely correlated with the frequency of meiosis. This hypothesis is based on the observation that extreme karyotypic variation was frequently found in species which only reproduce asexually. In addition, it assumes that meiosis is a process that would select against aberrations which lead to detectable chromosome polymorphisms, which would impair correct chromosomal pairing. However, although A × B crosses only generate a few viable ascospores in *S. macrospora*, our findings indicate that the meiotic process can sometimes lead to the generation of progeny. PFGE analysis of isolated chromosomes from the black ascospore progeny of an A × B cross revealed a novel third karyotype, which comprises a mixture of chromosomes of the two parental karyotypes (data not shown). Similar observations were made in other meiotically reproducing fungi, for example in the ascomycetes *Leptosphaeria maculans* (Plummer & Howlett, 1993), *Aspergillus nidulans* (Geiser *et al*., 1996) and *Ophiostoma ulmi* (Dewar & Benier, 1995) or the basidiomycetes *C. cinereus* (Zolan *et al*., 1994) and *Agaricus bisporus* (Kerrigan *et al*., 1993), and the oomycete fungus *Phytium sylvaticum* (Martin, 1995). Plummer & Howlett (1993) postulated that the meiotic variability of the phytopathogenic ascomycete *L. maculans* allows the fungus to adapt to new resistance genes in its host, whereas Zolan *et al*. (1994) do not come to a conclusion as to whether chromosome length polymorphism among interbreeding strains of *C. cinereus* is only tolerated or, in fact, exploited by the fungus. In contrast, chromosome rearrangements are rare in natural populations of *Neurospora crassa* (Perkins & Turner, 1988), a heterothallic Sordariaceae, which is closely related to *S. macrospora*. This might be not surprising since considerable karyotype variations would cause more problems in strictly outbreeding heterothallic species than in homothallic species. The progeny of an intraspecific cross analysed in this study was the result of a laboratory cross which leaves the question open as to whether meiotic variability in homothallic fungi is involved in genome evolution under natural conditions. In nature, the propagation of *S. macrospora* strains primarily occurs as a result of selfing, and the ability and frequency of this homothallic species

to outcross is not known. Thus, we only have demonstrated the potential for intraspecific crosses of strains harbouring a different karyotype under laboratory conditions. However, in nature the different genomes might no longer be compatible and outcrossing might never occur among such strains or if it occurs the drastically reduced amount of viable ascospores is not sufficient for propagation of the fungus. Therefore, chromosome polymorphism might promote genetic isolation of two strains and might be the first step in a speciation process. On the other hand, if sexual outcrossing occurs even on an infrequent basis, it may be enough to generate some levels of intraspecific karyotypic variation. Under laboratory conditions mycelia derived from the ascospore progeny of an A × B cross show the same growth rates as the parental strains (data not shown). Meiosis between different genomes then contributes to significant levels of recombination in natural populations and may therefore play an important role for a homothallic fungus in adapting to new environmental conditions.

Acknowledgments

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