Mini-chromosomes in *Fusarium sporotrichioides* are mosaics of dispersed repeats and unique sequences

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Variations in trichothecene patterns of 26 *Fusarium sporotrichioides* isolates from different plant and geographic origins showed no correlation with electrophoretic karyotype polymorphisms. When intact chromosomes were examined, interisolate karyotype differences were observed only in the mini-chromosome range. Further polymorphisms were revealed in *NotI*-digested samples. By summing the *NotI* fragments the average genome size of *F. sporotrichioides* was estimated to be 20.4 Mb. Mini-chromosomes shared common sequences with the larger ones; however, clones (RMS-1 and RMS-2) specific to these structures have also been found. These clones contained no coding region and no promising similarities were observed when they were compared to sequences held at GenBank. Mini-chromosomes in *F. sporotrichioides* constitute a mosaic composed of dispersed repeats and unique sequences. This mosaic structure was maintained in all non-interbreeding, genetically isolated strains examined.

Keywords: *Fusarium sporotrichioides*, electrophoretic karyotype, mycotoxin, trichothecenes, mini-chromosome

INTRODUCTION

The filamentous fungus *Fusarium sporotrichioides* occurs on a wide range of cereal plants as a secondary invader or seriously contaminates foods and fodder as a storage organism. This ubiquitous species produces various types of toxic 12,13-epoxy-trichothecenes (Visconti *et al.*, 1985) that may cause losses in animal husbandry and pose public health hazards. Patterns of trichothecene production vary considerably among strains and during long-term culture maintenance; the decline of toxigenic capability after repeated subculturing is frequently observed.

The importance of *F. sporotrichioides* has prompted classical and molecular genetic investigations. As this fungus is strictly asexual, other mechanisms of variability were supposed to exist. Heterokaryons were successfully induced between auxotrophs, but they proved to be unstable; parasexuality was not demonstrated because prototrophic recombinants could not be isolated from the heterokaryons (Cullen *et al.*, 1983). A trichodiene synthase gene (*tox5*) which specifies the key-enzyme of the trichothecene biosynthesis was isolated and sequenced

from this species (Hohn & Beremand, 1989). In a more recent study we have used electrophoretic karyotyping to characterize eight members of the *Fusarium* genus (Fekete *et al.*, 1993). The genome size of *F. sporotrichioides* as represented by one isolate was estimated to be 27.7 Mb and the number of chromosomes appeared to be six; four of them were very similar in size and therefore difficult to separate. One distinct mini-chromosome (1.2 Mb) was also identified in this fungus.

The purposes of the present work were to study the extent of infraspecific karyotype variation in *F. sporotrichioides*, to find correlation, if any, between toxin production patterns and karyotype differences, to provide more exact data on the genome size of this fungus by digesting the chromosomal DNA preparations with octanucleotide-recognizing restriction endonucleases, and to clone DNA fragments specific to mini-chromosomes of this fungus and suitable for tracing the origin of these structures.

METHODS

Fungal strains. The geographic origin, habitat and strain numbers of the 26 isolates of *F. sporotrichioides* are given in Table 1. Other species of the genus, namely *Fusarium avenaceum*, *Fusarium chlamydosporum*, *Fusarium pallidoroseum*, *Fusarium poae* and *Fusarium tricinctum*, were isolated and identified by us; *Fusarium camptoceras* and *Fusarium fusarioides* were kindly sup-

The GenBank accession numbers for the nucleotide sequence data reported in this paper are U10464 for RMS-1 and U10465 for RMS-2.

Table 1. Origins, sources and trichothecene production of *F. sporotrichioides* strains

Strain number	Origin	Source*	Trichothecenes ($\mu\text{g g}^{-1}$)†								
			MAS	DAS	T-2te	NEO	T-2tr	T-2	HT-2	acT-2	iT-2
A-2	Maize, kernel, Hungary, 1990	Own collection	—	+	7.0	30.0	—	75.3	25.0	+	—
A-5	Maize, cob, Hungary, 1990	Own collection	—	+	6.0	132.2	+	311.0	27.9	6.0	—
A-6	Wheat, kernel, Hungary, 1990	Own collection	—	—	+	2.6	—	6.5	—	—	—
A-16	Wheat, kernel, Hungary, 1990	Own collection	—	—	—	8.0	—	+	—	—	—
A-17	Wheat, kernel, Hungary, 1990	Own collection	—	+	+	47.0	—	21.6	—	—	—
TA-2	Wheat, kernel, Hungary, 1991	Own collection	—	—	—	—	—	119.0	—	—	—
TA-6	Wheat, kernel, Hungary, 1991	Own collection	—	10.6	—	16.2	—	22.0	—	—	—
22-205	Wheat, kernel, Hungary, 1970	Own collection	—	—	—	9.4	—	9.6	+	—	—
KF-1715	Wheat, kernel, Poland, 1992	J. Chelkowski	—	+	15.4	240.0	11.7	520.0	15.0	+	+
M-1-1	—	Y. Ueno	—	—	8.2	56.3	—	32.3	10.0	—	—
I-391	Corn, Italy, 1990	A. Logrieco	—	—	10.0	533.3	10.3	1120.0	393.0	10.6	—
I-707	—	A. Logrieco	—	7.0	+	3.0	—	27.6	+	—	—
I-710	—	A. Logrieco	—	—	—	33.0	—	24.8	11.7	—	—
TASP-1	Wheat, kernel, Hungary, 1991	Own collection	—	—	—	25.0	—	58.4	11.8	—	—
TASP-2	Wheat, kernel, Hungary, 1991	Own collection	—	—	5.7	78.0	10.9	253.2	35.6	4.8	—
TASP-3	Wheat, kernel, Hungary, 1991	Own collection	—	—	—	496.0	—	247.8	247.5	—	—
TASP-4	Wheat, kernel, Hungary, 1991	Own collection	—	—	—	58.6	+	52.5	19.1	—	—
TASP-5	Wheat, kernel, Hungary, 1991	Own collection	—	+	—	4.4	—	7.7	—	—	—
TASP-6	Wheat, kernel, Hungary, 1991	Own collection	—	—	—	4.5	—	11.5	+	—	—
TASP-7	Wheat, kernel, Hungary, 1991	Own collection	+	8.2	+	342.3	12.5	616.8	448.0	—	—
TASP-8	Wheat, kernel, Hungary, 1991	Own collection	—	—	—	25.0	—	21.0	12.0	—	—
TASP-9	Wheat, kernel, Hungary, 1991	Own collection	—	+	15.7	24.2	5.5	29.5	8.5	—	—
TASP-10	Wheat, kernel, Hungary, 1991	Own collection	—	—	—	42.2	—	29.6	13.1	—	—
TASP-11	Wheat, kernel, Hungary, 1991	Own collection	—	10.3	9.6	8.2	4.5	21.3	19.5	—	—
TASP-12	Wheat, kernel, Hungary, 1991	Own collection	—	—	+	167.6	—	183.7	26.0	—	—
TASP-13	Wheat, kernel, Hungary, 1991	Own collection	—	15.6	+	178.6	20.0	162.8	34.3	—	—

—, Not detected.

+, Detected in trace amounts.

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† Abbreviations: MAS, 15-monoacetoxyscirpenol; DAS, 4,15-diacetoxyscirpenol; T-2te, T-2 tetraol; NEO, neosolaniol; T-2tr, T-2 triol; T-2, T-2 toxin; HT-2, HT-2 toxin; acT-2, acetyl T-2 toxin; iT-2, iso-T-2 toxin.

plied by S. Chambers (Victorian Plant Research Institute, Burnley, Australia) and C. Booth (International Mycological Institute, Kew, England), respectively.

Mycotoxin identification. *F. sporotrichioides* isolates were grown on rice grains enriched with peptone solution (Lee *et al.*, 1986). Trichothecenes were directly extracted with an acetonitrile/water mixture (Sugiura *et al.*, 1990); the filtered extract was concentrated by evaporating under vacuum, passed through an LC-18 SPE tube (Supelco), then evaporated again to dryness. The residue was dissolved in dichloromethane, transferred to an LC-CN SPE tube (Supelco) and eluted with acetonitrile. Derivatives were produced by using trimethylsilyl *N,N*-dimethylcarbamate (Fluka) according to the method of Kientz & Verweij (1986) and analysed by gas chromatography (GC-FID, CP 9000, Chrompack) on a CP SIL 5CB capillary column (Chrompack). Mycotoxins were identified and quantified by means of the following standards: 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, 3-acetyl-diacetoxyscirpenol, acetyl T-2 toxin, deoxynivalenol, 4,15-diacetoxyscirpenol, fusarenon-X, HT-2 toxin, iso-T-2 toxin, 15-monoacetoxyscirpenol, neosolaniol, scirpentriol, T-2 tetraol, T-2 toxin, T-2 triol (Sigma) and

nivalenol (Serva). Detection limits of these trichothecenes ranged from 1 to 8 μg (g culture dry weight) $^{-1}$. Each extract was analysed in two separate experiments.

Karyotype analysis. Protoplasts were isolated as described previously by Nagy & Hornok (1994). Chromosomal DNA bands were separated using the contour-clamped homogeneous electric field dynamically regulated II (CHEF-DR II) system (Bio-Rad) at 9 °C in circulated 0.5 × TAE (40 mM Tris-acetate, 1 mM EDTA, pH 8.3) buffer. Concentrations of agarose (chromosomal grade, Bio-Rad), voltages, pulse rates and run times were varied to obtain optimal separation; these data are given in Results. Size standards were *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* chromosomal DNA (Bio-Rad), lambda concatemer ladder (Boehringer) and lambda DNA/*Hind*III fragments (Amersham). Photographic negatives of the ethidium-bromide-stained gels were scanned with a densitometer (Bosch TYK 92D). Chromosome numbers and sizes were estimated after three independent fractionations.

Restriction enzyme digestion of chromosomal DNA. Agarose plugs containing intact chromosomal DNA were washed twice for 10 min in TE (10 mM Tris/HCl, 1 mM EDTA, pH 8.0).

They were then washed twice in 150 µl restriction enzyme buffers (as recommended by the manufacturers) and incubated for 16 h at 37 °C in the same buffers containing 35 U *Pme*I, *Sfi*I (New England Biolabs) or *Not*I (Amersham). Chromosomal fragments generated from restriction enzyme digestion were separated by using the CHEF-DR II system. Other restriction endonucleases (*Bam*HI, *Eco*RI, *Hind*III, *Sac*I, *Xho*I) were obtained from Amersham and used according to the manufacturer's recommendations.

Blotting and hybridization. Gels were transferred onto Hybond-N nylon membrane (Amersham) according to Southern (1975). Pooled DNA probes as well as a 1.1 kb *Xho*I-*Sac*I fragment of the *tox5* gene (donated by T. M. Hohn, USDA, ARS, Peoria, Illinois, USA) were labelled with [α^{32} P]dCTP by nick-translation (Sambrook *et al.*, 1989) using the Nick Translation System (Gibco-BRL). Random clones from various strains of *F. sporotrichioides* were also labelled with [α^{32} P]dCTP by random priming (Sambrook *et al.*, 1989) using random hexanucleotide primer (New England Biolabs).

Cloning and sequencing of DNA fragments from mini-chromosomes. After separation of chromosome-sized DNAs by pulsed-field gel electrophoresis, they were cut from the gel and isolated as described by Maniatis *et al.* (1982). DNA fragments digested with *Bam*HI, *Eco*RI and *Hind*III were cloned in Bluescript pSK (M13-), Amp^r (Short *et al.*, 1988), according to the procedures outlined by Sambrook *et al.* (1989). Competent cells of *Escherichia coli* DH5 α were prepared and transformed as described by Maniatis *et al.* (1982). Clones RMS-1 and RMS-2 were sequenced by the dideoxy chain-termination method of Sanger *et al.* (1977) using the Sequenase Version 2.0 DNA sequencing kit (USB); [35 S]dATP α S was obtained from Amersham. Conditions used for sequencing were as described in the Sequenase brochure (USB).

RESULTS

Mycotoxin production

The production of trichothecenes by 26 isolates of *F. sporotrichioides* is summarized in Table 1. All strains were able to produce T-2 toxin and all but one synthesized neosolaniol. On the other hand, two compounds, 15-monoacetoxyscirpenol and iso-T-2 toxin, were identified in trace amounts and only in two isolates. Scirpentriol, 3-acetyldeoxynivalenol, deoxynivalenol, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, nivalenol and fusarenon-X could not be detected in any sample. There were great qualitative and quantitative differences in toxigenic capabilities among strains. In the case of the major compounds (neosolaniol and T-2 toxin) the quantitative variations predominated: neosolaniol was produced from 2.6 to 533.3 µg g⁻¹ while levels of T-2 toxin varied between 6.5 and 1120.0 µg g⁻¹. Several strains were unable to synthesize HT-2 toxin, but others accumulated high amounts of this compound. Differences in the trichothecene patterns seem to be independent of the geographic or plant origins of the strains.

Electrophoretic karyotype analysis

When preparations of chromosomal DNA of *F. sporotrichioides* isolates were subjected to pulsed-field gel electrophoresis under conditions where *S. pombe* chromosomal DNAs were clearly resolved (0.6 % agarose, voltage

Fig. 1. Separation of chromosome-sized DNAs of selected *F. sporotrichioides* strains. Lanes: 1, *S. pombe* (*S. cerevisiae*) size standards; 2, TA-2; 3, KF-1715; 4, TASP-1; 5, TASP-2; 6, TASP-3; 7, TASP-4; 8, TASP-5; 9, TASP-6; 10, TASP-7. (a) Electrophoretic conditions for separation of the larger chromosomes. (b) Conditions to separate mini-chromosomes.

45 V, switching time 3 s for 30 h, then ramped from 1500 to 3600 s for 162 h at 40 V), each gave a similar overall karyotype (Fig. 1a). One weak and diffuse band appeared at around 7 Mb, another strong and thick band was observed at around 6 Mb and one or two bands were detected in the lower region. Recently we compared single isolates of several closely related species of the genus *Fusarium* (Fekete *et al.*, 1993) and the same karyotype was obtained for *F. sporotrichioides*; based on these comparisons the largest band was identified as one chromosome and the thick, bright band around 4.6–5.4 Mb was estimated to be four co-migrating chromosomes of the same or similar sizes. The present investigation showed that this chromosomal pattern is typical of *F. sporotrichioides* as no interstrain variation was detected in the large chromosome range.

Chromosomes smaller than 2.0 Mb were separated in 0.8 % agarose by using switching times of 120 s for 12 h then 180 s for another 12 h at 160 V (Fig. 1b). This

Fig. 2. Scheme of mini-chromosome band profiles of the *F. sporotrichioides* strains. The chromosome bands stained with ethidium bromide are presented as bars positioned according to their molecular size in kb.

Fig. 3. *NotI*-digested chromosome-sized DNAs of selected *F. sporotrichioides* strains. Lanes: 1, lambda *HindIII* fragments; 2, lambda ladder; 3, M-1-1; 4, KF-1715; 5, I-707; 6, A-2; 7, TASP-2; 8, TASP-3; 9, TASP-10; 10, *S. cerevisiae*. (a) Ethidium-bromide-stained gel. (b) The same gel probed with *tox5*. Separation conditions: agarose concentration 1.0% (w/v); 165 V; ramped switching time from 20 to 90 s for 23 h.

electrophoresis revealed a dissimilar banding pattern for each strain. The mini-chromosomes resolved by means of these parameters ranged from approximately 700 kb for the smallest band in strain TASP-2 to a 1650 kb band in strain TASP-13 (Fig. 2).

Thus, if intact chromosomes of different isolates were compared, infraspecific polymorphisms could be detected only in the mini-chromosome range. Further polymorphisms were expected to be resolved by digesting the agarose-embedded DNA with restriction enzymes that

recognize an 8 bp sequence. Digestion with *PmeI* and *SfiI* resulted in a strong smear, therefore the exact identification of the bands was impossible. However, *NotI* generated 31–32 fragments ranging from 40 to 2380 kb in size and revealed detectable polymorphisms among strains (Fig. 3a). Polymorphisms were more evident when gels were hybridized with a probe containing a 1.1 kb *XhoI*–*SacI* fragment of the *tox5* gene (Fig. 3b).

A more precise estimation of the genome size of *F. sporotrichioides* was attempted by summing the *NotI*

Table 2. Sizes of *NotI* fragments of the chromosomes of selected *F. sporotrichioides* strains

Fragment number	Strains				
	KF-1715	I-707	M-1-1	TASP-3	TASP-7
1	2150	2380	1920	2310	2110
2	1470	730	1570	580†	1660
3	650*	650	710	550	600
4	550	600	610	540	580
5	530	580*	580	520	570
6	520	540	540	500*	550
7	490*	500*	510†	470*	540*
8	470*	480*	480	440†	520
9	460	460*	460	420	500*
10	440*	450	440	400*	480
11	420	440*	410†	370†	470*
12	400†	430	400*	350†	450
13	390*	400†	380*	340	430†
14	370†	390	370*	320†	410*
15	350†	370†	350*	310	380†
16	330*	360	340	300†	350*
17	300*	350	330	290	330†
18	290*	330	320†	280*	310*
19	270†	310*	310	270†	290†
20	260	290*	300*	260*	270†
21	250*	270†	290	250†	250†
22	240*	260	280†	240†	240*
23	230*	250*	260*	220*	225*
24	215*	240*	240†	200†	205*
25	200*	230*	230†	180*	170
26	175*	210	205	130*	150
27	140*	195*	190*	120*	140
28	100	170*	160*	100	125*
29	80	140*	125*	85*	110
30	70	110*	90*	70*	90
31	60	90	75	50*	80
32		80	50	40	
Genome size (kb)	20460	19760	20130	20640	20860

* Two co-migrating fragments present.

† Three co-migrating fragments present.

fragments of selected strains (Table 2). Several bands were identified as doublets or triplets on the basis of ethidium bromide staining intensity as measured by video scanning densitometry. Estimates of total nuclear genome size ranged from 19.76 to 20.86 Mb with an average of 20.37 Mb. These values were somewhat smaller than that (27.7 Mb) determined earlier for this species (Fekete *et al.*, 1993) and remained under the various values (22.5–44.0 Mb) published so far for other filamentous fungi (Skatrud & Queener, 1989; Debets *et al.*, 1990; Cooley & Caten, 1991; Talbot *et al.*, 1991; Carter *et al.*, 1992). However, Kim *et al.* (1993) recently reported a genome size of *Fusarium oxysporum* f.sp. *niveum* ranging between 15.9 and 26.0 Mb; these values seem to support our estimation.

Cloning and sequencing DNA fragments specific to mini-chromosomes

Interisolate karyotype differences were observed only in the mini-chromosome range. In order to obtain further information on these structures a 1.43 Mb mini-chromosome of strain A-17 was isolated from the gel and its 3–20 kb *Bam*HI fragments were nick-translated and hybridized as a pooled DNA probe to Southern blots of the pulsed-field gels. The same experiment was performed using *Hind*III fragments of the 1.13 Mb mini-chromosome of strain TASP-3. These probes hybridized strongly both to the large chromosomes and to the small ones of the 26 *F. sporotrichioides* strains. Several fragments were cloned and used as probes and the majority of them gave the same results obtained with the pooled DNA probes. However, two clones, a 0.16 kb *Bam*HI fragment of the 1.43 Mb mini-chromosome of A-17, and a 0.56 kb *Eco*RI fragment of the 1.25 Mb mini-chromosome of strain A-2, named as RMS-1 and RMS-2, respectively, hybridized exclusively to mini-chromosomes (Fig. 4). In those strains that harboured two small chromosomes the probes always hybridized to the larger ones. Several other *Fusarium* species (*F. avenaceum*, *F. camptoceras*, *F. chlamydosporum*, *F. fusarioides*, *F. pallidroseum*, *F. poae*, and *F. tricinctum*) closely related to *F. sporotrichioides* were also probed with the pooled DNA probe, as well as with RMS-1 and RMS-2, but no signal was observed.

Consequently, RMS-1 and RMS-2 are mini-chromosome-specific clones. When their sequences were analysed no coding region was revealed and no promising similarity was observed when they were compared to sequences held at GenBank.

DISCUSSION

All geographically different *F. sporotrichioides* isolates were able to synthesize several A type trichothecenes in various patterns and amounts. As this species is a well documented trichothecene producer (Thrane, 1989), our results were not unexpected. In a similar survey 11 strains of this fungus were all found to synthesize T-2 toxin (Thrane, 1986) and all 17 isolates were identified as producers of T-2 toxin, HT-2 toxin, and neosolaniol by other workers (Logrieco *et al.*, 1990). On the other hand, Chelkowski *et al.* (1984) stated that only 40% of the *F. sporotrichioides* isolates from Poland were able to produce the major trichothecene mycotoxins.

Extensive karyotype variability within fungal species has frequently been reported. According to a recent hypothesis (Kistler & Miao, 1992) asexual fungi are more likely to show great chromosomal polymorphisms due to the absence of meiosis, which allows the maintenance of these aberrations. In our experiments electrophoretic karyotypes of the different *F. sporotrichioides* isolates proved to be rather uniform as far as the large chromosomes are concerned. In no way would we explain this finding by the genetic uniformity of the species. The lack of major infraspecific chromosome polymorphisms is more probably due to the unusual karyotype of *F. sporotrichioides*, i.e. the large chromosomes constituting

Fig. 4. Electrophoretic karyotypes of selected *F. sporotrichioides* strains probed with the mini-chromosome-specific clone, RMS-1. (a) Ethidium-bromide-stained gel. (b) Southern blot of the same gel. Lanes 1–10: 22–205, I-391, I-707, I-710, TASP-8, TASP-9, TASP-10, TASP-11, TASP-12 and TASP-13, respectively. Separation conditions are as given in Fig. 1(a). L, large chromosomes; M, mini-chromosomes.

more than 90 % of the genome are all above 4.5 Mb and fall into the least resolvable region. At the same time, mini-chromosomes greatly varied both in numbers and sizes presenting thus strain-specific patterns. Interisolate karyotype differences restricted to mini-chromosomes have also been identified in the type B isolates of the plant pathogen *Colletotrichum gloeosporioides* (Masel *et al.*, 1990).

Changes in chromosome structures may result in phenotype modifications. Chromosomal rearrangements were found to be associated with morphological mutations in *Candida tropicalis* (Suzuki *et al.*, 1991), polymorphic karyotypes were observed in *Acremonium chrysogenum* strains differing in β -lactam antibiotic production (Smith *et al.*, 1991; Walz & Kück, 1991) and considerable karyotype variation was apparent between and within wheat- and barley-adapted isolates of *Septoria nodorum* (Cooley & Caten, 1991). In the present study, however, no correlation was found between toxigenic variability and chromosome polymorphisms.

Many fungi harbour mini-chromosomes. Because of their minuteness and variability they were formerly regarded as the equivalent of the B-chromosomes present in animals or higher plants. Although no essential genes have been detected on these structures, functional genes involved in phytoalexin detoxification were mapped on such a chromosome of *Nectria haematococca* (Miao *et al.*, 1991). Several mechanisms are hypothesized to explain the origin of mini-chromosomes. They could have arisen by genome rearrangements or through horizontal transfers among different strains of the species. The chromosomal rearrangement theory is supported by homologies found between mini-chromosomes and large chromosomes in *Ustilago maydis* (Kinscherf & Leong, 1988), *C. gloeosporioides* (Masel *et al.*, 1990), and *Aspergillus flavus* (Keller

et al., 1992). The contribution of horizontal transfer is indicated by a recent finding of Masel *et al.* (1993), who identified a unique mini-chromosome in race 3 isolates of *C. gloeosporioides*; this chromosome was most probably derived through addition from a genetically distinct strain of this fungus.

In order to investigate the origin of the mini-chromosomes in *F. sporotrichioides*, pooled DNA fragments of the 1.43 and 1.13 Mb mini-chromosomes of strains A-17 and TASP-3, respectively, were hybridized to karyotypes of the 26 strains. The strong hybridization between these DNA probes and the large chromosomes of each isolate indicates that mini-chromosomes share common sequences with the large ones in this species. However, sequences specific to mini-chromosomes also exist. Since neither sexual nor parasexual recombination has until now been proven in *F. sporotrichioides*, the reality of chromosome transfers between different isolates of this fungus is very small. The probability of an interspecific transfer is even more unlikely if we consider the complete nonhomology that we found when related species of the genus *Fusarium* were probed with pooled mini-chromosome DNAs, as well as with RMS-1 and RMS-2. The most possible explanation of the origin of the mini-chromosomes is that they are results of major genome rearrangements. During these processes mini-chromosomes were formed as mosaics of dispersed repeats and unique sequences. These rearrangements probably occurred earlier in the evolution of this species as their results were detected in all strains. The universal occurrence of mini-chromosomes in *F. sporotrichioides*, as well as the finding that the mosaic structure composed of dispersed repeats and unique sequences was maintained in many non-interbreeding, genetically isolated strains,

strongly suggests that these structures are useful components of the genome.

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