Curr Genet (1990) 18:81–86

**Current Genetics**



# Highly variable molecular karyotypes in the plant pathogen *Colletotrichum gloeosporioides*

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Received April 19, 1990

**Summary.** Isolates representing two types (A and B) of *Colletotrichum gloeosporioides*, which cause different anthracnose disease on the tropical pasture legumes *Stylosanthes* spp. in Australia, were found to have distinct electrophoretic karyotypes. Type A isolates had five large maxi-chromosomes (2–6 Mb) and eight to ten smaller mini-chromosomes (270–600 kb), while Type B isolates had three maxi chromosomes (4.7–>6 Mb) and three to five mini-chromosomes (330–1200 kb). Extensive chromosomal polymorphisms for both length and number were observed in the mini-chromosomes amongst isolates within both types. Results indicate that chromosomal rearrangements may have a role in generating variation in this pathogen.

**Key words:** Anthracnose – Chromosomes – Genetic variation – Pathogenic specialisation

## Introduction

Recently, pulse field electrophoresis has been developed for the separation and molecular analysis of individual chromosomes in yeasts (Schwartz and Cantor 1984; Smith et al. 1987), filamentous fungi (Howlett 1989; Kinscherf and Leong 1988; Orbach et al. 1988) and other lower eukaryotes (Corcoran et al. 1986; Van der Ploeg et al. 1984). In some studies, variation in both the size and number of chromosomes has been observed between different strains of several organisms pathogenic on humans (Corcoran et al. 1986; Magee and Magee 1987; Suzuki et al. 1988; Polacheck and Lebens 1989). Thus, chromosome rearrangements may have an important role in generating variation, permitting dynamic pathogen populations to overcome host resistance. So far, however, the karyotypes of fungal plant pathogen populations have not been examined in detail.

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In Australia, the fungal plant pathogen *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. causes anthracnose on the tropical pasture legumes *Stylosanthes* spp. This pathogen exhibits extensive pathogenic specialisation and two pathogen types, termed A and B, have been identified (Irwin and Cameron 1978). The two pathogen types can be distinguished by their host range, spore morphology, symptomatology, double-stranded RNA components and genomic RFLP patterns (Irwin and Cameron 1978; Dale et al. 1988; Braithwaite and Manners 1989). In addition, there is further race specialisation within each type with regard to virulence on different host cultivars of *S. scabra* and *S. viscosa*, for the Type A, and *S. guianensis*, for the Type B pathogens (Irwin et al. 1986; Davis et al. 1987). All highly virulent isolates of *C. gloeosporioides* obtained from *Stylosanthes* spp. in Australia are anamorphic (asexual) and, so far, no highly virulent teleomorphic (sexual) strains of *C. gloeosporioides* have been isolated from *Stylosanthes* spp. (Ogle et al. 1986). Because of the lack of a sexual cycle, classical genetic analysis has not been feasible and there is no information available on the possible mechanisms generating genetic variability in this fungus.

We have commenced a study on the molecular genetics of this pathogen with the aim of understanding the molecular basis of type and race specialisation. As a starting point, we have investigated the electrophoretic karyotypes for Type A and Type B isolates. Results indicate that the Type A and Type B pathogens are genetically distinct and that, overall, they have very different electrophoretic karyotypes. Both types carry a number of relatively low molecular weight putative chromosomal structures that we have termed mini-chromosomes. More importantly, however, our results show that individual isolates of this fungus exhibit extensive polymorphisms for the size and number of mini-chromosomes. These results suggest that chromosomal rearrangements may contribute to the considerable variation observed in the *C. gloeosporioides* population and to the ability of this pathogen to adapt to newly introduced resistant host cultivars.

## Materials and methods

*Fungal isolates.* The isolates of *C. gloeosporioides* used in this study were collected in Queensland, Australia, by either J.A.G. Irwin (The University of Queensland) or R.D. Davis (Queensland Department of Primary Industries). The Type A isolates were obtained from three accessions of *S. scabra* and one of *S. viscosa,* whilst the Type B isolates were obtained from six accessions of *S. guianensis*. The isolates represented four distinct physiological races for each type with regard to specificity on different cultivars of the above host species (Irwin et al. 1986). All isolates were grown from monoconidial cultures. Cultures were maintained on oatmeal agar plates under near UV light at 28 °C. Conidia ($5 \times 10^7$) were used to inoculate 200 ml of clarified liquid V8 juice and subsequently incubated for approximately 48 h at 26 °C with shaking. The mycelium was obtained by filtration and then used for the preparation of protoplasts according to the method of Tilburn et al. (1983). Spores were collected from the filtrate by centrifugation and also used in the preparation of samples. *Saccharomyces cerevisiae* YNN 295 and *Schizosaccharomyces pombe* 972 ($h^-$) strains were used as a source of chromosomal molecular markers. Yeast cells were cultured at 30 °C in 1% w/v yeast extract, 2% w/v bacto-peptone and 2% w/v glucose.

*Preparation of chromosomal samples.* Chromosomal DNA preparations of *C. gloeosporioides* were made from spores or protoplasts according to the method of Orbach et al. (1988), except that agar blocks were set in a commercial sample mould (Biorad Lab Inc, USA). Both *Sa. cerevisiae* and *Sc. pombe* DNA samples were prepared according to Vollrath and Davis (1987). To test for RNA, the agar blocks were incubated in 300 µg/ml RNase A for 18 h at 37 °C prior to electrophoresis. The mobility of mitochondrial DNA was also tested. Intact mitochondria were isolated and treated with DNase I according to the method of Specht et al. (1983). The mitochondria were directly embedded in agarose blocks and treated as for protoplasts (Orbach et al. 1988).

*Gel electrophoresis conditions.* Gel electrophoresis was performed using the contour-clamped homogeneous electric field-dynamically regulated II (CHEF-DR II) system (Biorad Lab Inc. california) adapted from Chu et al. (1986) and Vollrath and Davis (1987). The running buffer was 0.089 M Tris-borate, 0.089 M boric acid and 0.002 M EDTA, pH 8.0 (Maniatis et al. 1982) and was replaced every 48 h during extended electrophoretic runs. Buffer temperature was maintained at 14 °C with constant buffer circulation. All separations were carried out on $14 \times 12.7 \times 1$ cm agarose (Pharmacia), gels under the following conditions; (1) 20 h, 30 sec switch time then 9 h, 90 sec switch time, 200 V, 0.8% agarose, (2) 130 h, 60 min switch time, 40 V, 0.5% agarose, (3) 70 h, ramping switch from 150 sec up to 400 sec, 150 V, 0.8% agarose, and (4) 130 h, 120 min switch time, 50 V, 0.5% agarose. Gels were stained with ethidium bromide (1 µg/ml) for 15–20 min and destained in distilled water for at least 1 h.

*Southern transfer and hybridisation conditions.* The gels were irradiated for 4 min with UV light (254 nm), soaked in 0.25 M HCl for 15 min and finally incubated in 1.5 M NaCl + 0.5 M NaOH for 30 min. The DNA was transfered to Hybond-N (Amersham Int, UK) by capillary action overnight with 1.5 M NaCl + 0.25 M NaOH as transfer buffer. After transfer, membranes were exposed to UV light (300 nm) for 3 min. Hybridisations and washings were carried out according to the manufacturer's instructions for Hybond-N. Autoradiography was carried out using Kodak X-OMAT RP film with intensifying screens for 4–16 h at -70 °C. DNA probes were labelled using an Amersham Multiprime labelling system. A cDNA probe was prepared by first strand cDNA synthesis (Maniatis et al. 1982) from poly $A^+$ RNA isolated as described by Chirgwin et al. (1979) from the mycelium of a Type B isolate.

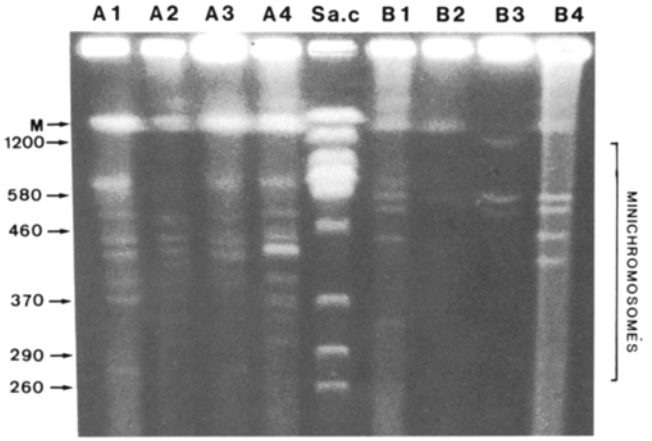


**Fig. 1.** Ethidium bromide stained CHEF gel comparing Type A (*A 1–4*) and Type B (*B 1–4*) isolates of *C. gloeosporioides* using running condition 1. *Sa.c* shows the *Sa. cervisiae* standards, *M* refers to the maxi-chromosomes and the sizes of resolved markers bands are also shown

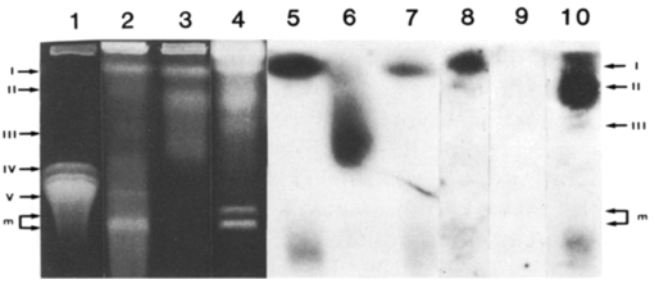


**Fig. 2.** CHEF gel comparing Type A (*lanes 2, 5 and 8*) and Type B (*lanes 4, 7 and 10*) isolates of *C. gloeosporioides* using running condition 2. *Lanes 1–4* indicate ethidium bromide stained DNA. *Lanes 3, 6 and 9* are the *Sc. pombe* standards. *Lanes 5–7* and *8–10* represent hybridisation studies using ribosomal and tubulin probes respectively. *Lane 1* shows the *Sa. cerevisiae* standards. The positions of maxi-chromosomes are *arrowed at the left* (Type A) and *right* (Type B) of the figure: m refers to the mini-chromosomes

## Results

### *Type A and Type B electrophoretic karyotypes*

To resolve chromosomal DNA of *C. gloeosporioides* a number of electrophoretic conditions were tested (see Methods). Typical gels using conditions 1, 2 and 3 are shown in Figs. 1, 2 and 3 respectively. These conditions resolve all the chromosomal bands that we have visualised so far and the results for all isolates that we have analysed are summarized in Table 1.

To compare the Type A and Type B groups, we chose one isolate representing each of the four physiological races identified in each type: A1–A4, B1a, B2a, B3a and B4a (Irwin et al. 1986). The bands obtained with both Type A and Type B fungal isolates fell into two classes. A group of bands of molecular weight lower than 1.3 Megabase pairs (Mb), when compared with the *Sa. cerevisiae* standards, were observed and these were termed mini-chromosomes. Other bands of higher molecular weight (>1.3 Mb), termed maxi-chromosomes, were

**Table 1.** The number and approximate size of putative chromosomal bands observed for Type A and Type B isolates of *C. gloeosporioides*. Data shows the size in Mb of a resolved band when compared to either *Sa. cerevisiae* or *Sc. pombe* standards

| Type A | | | | | Type B | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chr. No. | Race No.[1] | | | | Chr. No. | Race No.[1] | | | | | | | |
| | 1 | 2 | 3 | 4 | | 1 a | 1 b | 2 a | 2 b | 3 a | 3 b | 4 a | 4 b |
| I[2] | >6 | >6 | >6 | >6 | I | >6 | >6 | >6 | >6 | >6 | >6 | >6 | >6 |
| II | 5 | 5 | 5 | 5 | II | 5.2 | 5.2 | 5.2 | 5.2 | 5.2 | 5.2 | 5.2 | 5.2 |
| III | 4.1 | 4.1 | 4.1 | 4.1 | III | 4.7 | 4.7 | 4.7 | 4.7 | 4.7 | 4.7 | 4.7 | 4.7 |
| IV | 3 | 3 | 3 | 3 | | | | | | | | | |
| V | 2 | 2 | 2 | 2 | | | | | | | | | |
| m[3] | 0.6 | 0.6 | 0.6 | 0.6 | m | – | – | – | – | 1.2 | 1.2 | – | – |
| m | 0.5 | – | 0.5 | 0.5 | m | – | – | – | – | – | 0.59 | – | 0.59 |
| m | – | 0.49 | – | – | m | – | 0.58 | – | 0.58 | – | 0.58 | – | – |
| m | – | 0.48 | – | – | m | 0.57 | – | 0.57 | 0.57 | 0.57 | – | – | – |
| m | 0.44 | 0.44 | 0.44 | 0.44 | m | – | 0.56 | – | – | – | – | 0.56 | 0.56 |
| m | – | – | 0.43 | 0.43 | m | 0.55 | – | 0.55 | 0.55 | 0.55 | 0.55 | 0.55 | – |
| m | 0.42 | 0.42 | 0.42 | 0.42 | m | – | – | – | – | – | – | – | 0.45 |
| m | – | – | 0.41 | 0.41 | m | 0.44 | 0.44 | 0.44 | 0.44 | – | – | 0.44 | – |
| m | 0.4 | 0.4 | – | 0.4 | m | – | – | – | – | – | – | – | – |
| m | 0.39 | 0.39 | 0.39 | – | m | – | – | – | – | – | 0.4 | 0.4 | – |
| m | 0.37 | 0.37 | 0.37 | 0.37 | m | 0.33 | – | – | – | – | – | – | – |
| m | – | 0.34 | – | 0.34 | | | | | | | | | |
| m | – | 0.3 | – | 0.3 | | | | | | | | | |
| m | 0.27 | – | 0.27 | – | | | | | | | | | |
| Total m | 8 | 10 | 9 | 10 | | 4 | 3 | 3 | 4 | 3 | 5 | 4 | 3 |
| Total no. | 13 | 15 | 14 | 15 | | 7 | 6 | 6 | 7 | 6 | 8 | 7 | 6 |

[1] Race Nos. are as described by Irwin et al. (1986) and a and b represent separate isolates for each race
[2] The maximum band size resolved was >6 Mb and this band may contain chromosomes of larger size
[3] m refers to mini-chromosomes less than 1.3 Mb in size

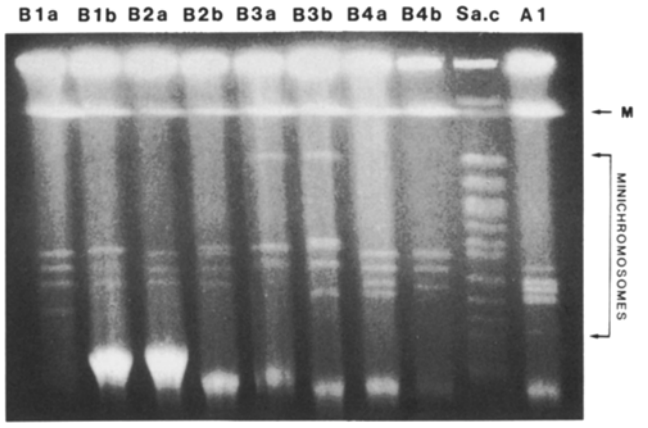


**Fig. 3.** Ethidium bromide stained CHEF gel comparing mini-chromosomes of two isolates of each physiological race of the Type B group of *C. gloeosporioides* using condition 3. Race 1 (*B 1 a–b*), Race 2 (*B 2 a–b*), Race 3 (*B 3 a–b*) and Race 4 (*B 4 a–b*). *Sa.c* shows the *Sa. cerevisiae* size standards and *A 1* is a Type A. *M* refers to maxi-chromosomes

sized by reference to *Sa. cerevisiae* and *Sc. pombe* chromosomes.

Using electrophoretic condition 1 (see Methods), between eight and ten mini-chromosomes in the size range 270–600 Kb were resolved for the Type A isolates, whilst only three to four mini-chromosomes of 330–1200 Kb were observed for the Type B isolates (Fig. 1, Table 1). Using this running condition, chromosomes of a molecular weight greater than 1.2 Mb, including all three of the *Sc. pombe* chromosomes (data not shown), either ran as a large diffuse band, and were not resolved, or did not leave the sample well. Polymorphisms were observed in the number and relative staining intensities of individual mini-chromosome bands between isolates within each type and no two isolates had identical banding patterns (Table 1).

Separation of *C. gloeosporioides* chromosomes under running condition 2 resulted in the resolution of chromosomes greater than 1.0 Mb in size (Fig. 2, Table 1). These running conditions resolved the three chromosomes of *Sc. pombe* and these bands, of 3.5, 4.7 and 5.7 Mb (Steele et al. 1989), were used as size markers. The karyotypes of the Type A and Type B isolates were strikingly different under these conditions (Fig. 2). All of the Type A isolates had five bands ranging in size from 2 Mb to greater than 6 Mb whilst the Type Bs had three bands of very high molecular weight ranging in size from 4.7 Mb to greater than 6 Mb. No polymorphisms in maxi-chromosomes were observed among individual isolates of either Type A or B under this running condition (Table 1). To ascertain the size of the largest chromosome, condition 4 (see methods) was used to assess its molecular weight. Under this electrophoretic condition the largest *Sc. pombe* chromosome migrated 30 mm on the gel whilst the largest

*C. gloeosporioides* chromosome only just entered the gel with a mobility of 8 mm (data not shown). Thus the size of the largest *C. gloeosporioides* chromosome resolved cannot be accurately measured but must be greater than 6 Mb.

Further isolates were analysed to determine whether polymorphisms in karyotype could be detected amongst mini-chromosomes within races of the Type B group (Fig. 3). Two separate isolates from different locations in Queensland representing each of the four physiological races of the Type B group (Irwin et al. 1986) were analysed for their electrophoretic karyotype using condition 3. This running condition was used as it most clearly resolves all Type B mini-chromosomes. A number of polymorphisms were observed between pairs of isolates of the same race (Fig. 3, Table 1). Interestingly, both of the isolates representing Race 3 carried the largest mini-chromosome of 1.2 Mb and this was clearly absent from all of the other isolates. No polymorphisms were observed among maxi-chromosomes I, II and III for the eight Type B isolates when running condition 2 was used (data not shown).

The banding patterns for each of the four Type A and eight Type B isolates were reproducible regardless of whether chromosomal blocks were prepared by initially embedding either spores or protoplasts (Orbach et al. 1988) and we have not observed any significant variation in banding patterns of individual isolates in up to five separate preparations. For convenience we now routinely prepare blocks using agarose-embedded spores which are produced in abundance by *C. gloeosporioides* when grown in liquid culture. The banding patterns were unaffected by treatment of the blocks with RNase A prior to electrophoresis indicating that neither single stranded or double stranded RNA (Dale et al. 1988) were producing artefacts when run under conditions 1, 2 and 3. Type A isolate 1a and Type B isolate 1b were subcultured five times on agar plates prior to karyotyping but no alterations in band numbers or positions were observed in single-spored isolates after this treatment. Isolates A1 and B1a (Table 1) were inoculated onto plants of *S. scabra* and *S. guianensis* respectively and recovered from lesions and this process was repeated three times. Single spore isolates were then karyotyped and were found to be identical to that of the original strains. We conclude from these studies that the karyotype of *C. gloeosporioides* is stable once isolated in pure culture from the field.

*Hybridisation studies*

We have used a clone of the tandem repeat of ribosomal DNA from *Aspergillus nidulans* (pAR 1, Lockington et al. 1982) as a probe to locate the ribosomal genes of *C. gloeosporioides*. This probe hybridised to chromosome I in the Type A and Type B isolates using condition 2 (Fig. 2). The tubulin gene of *Neurospora crassa* cloned on plasmid pSV50 (Volmer and Yanofsky 1986) hybridised to chromosome I in the Type A isolates and to chromosome II in the Type B (Fig. 2). Neither of these two

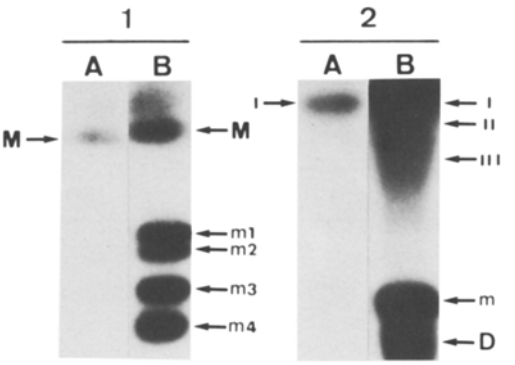


**Fig. 4.** Southern DNA hybridization analysis of the electrophoretic karyotype of a Type A and a Type B of *C. gloeosporioides* using the dispersed repeat probe pKB2. *A and B* indicate isolate A 1 and B 4a (Table 1) respectively, *Panels 1 and 2* represent blots prepared from gels run under conditions 1 and 2 respectively. *Arrows* indicate position and number of maxi-chromosomes and mini-chromosomes. *M* refers to maxi-chromosomes and *m 1–4* refer to the mini-chromosomes of isolate B 4a; *D* refers to degraded DNA

probes revealed polymorphisms amongst Type A and Type B isolates (data not shown). A cDNA probe was made using poly $A^+$ RNA isolated from Type B isolate 1a and this hybridised to all the chromosomes and mini-chromosomes (data not shown). A probe (pKB2) for a dispersed repeat sequence of *C. gloeosporioides* has been previously isolated in a study on the phylogenetic relationship of *C. gloeosporioides* using RFLPs (Braithwaite et al. 1990). The pKB2 probe hybridised to both the mini and maxi-chromosomes in Type B isolates. In contrast, this probe did not hybridise to any of the mini-chromosomes of Type A but did hybridise weakly to one maxi-chromosome (Fig. 4). The maxi-chromosomes and mini-chromosomes of *C. gloeosporioides* did not hybridise to a probe for mitochondrial DNA (data not shown). When purified intact DNase I-pretreated mitochondria were electrophoresed using condition 1, there was strong hybridisation in the wells. This is consistent with the results of Kinscherf and Leong (1988) who showed that mitochondrial DNA of *Ustilago maydis* did not migrate into the pulse field gels.

## Discussion

The most important finding we have made is that polymorphisms exist in the karyotype of individual isolates within each type of *C. gloeosporioides*. These polymorphisms were only observed in the lower molecular weight mini-chromosomes. In this study we have analysed field isolates of a fungus which has only recently been diagnosed in a localised geographical area (Irwin and Cameron 1978) and our results suggest that, in the field, the genome of *C. gloeosporioides* populations is very variable. At present we do not know how these polymorphisms arise but our results suggest that chromosomal rearrangements should be seriously considered as a means of generating variability for this fungus. The highly variable karyotype observed for *C. gloeosporioides*

may explain its considerable pathogenic specialisation and rapid rate of adaption to newly introduced resistant cultivars of its host *Stylosanthes* (Irwin et al. 1986).

There were few obvious correlations of karyotype with race specificity in the Type B group although the 1.2 Mb mini-chromosome was unique to the two isolates representing race 3. Further correlations with larger numbers of isolates, leading to an increased understanding of the homologies between polymorphic chromosomes in separate isolates, together with inter-race chromosome transfers will be used in future to determine whether polymorphic chromosomes carry genes encoding race and type specificity.

Type A and Type B forms of *C. gloeosporioides* infecting the tropical pasture legumes *Stylosanthes* spp. clearly have distinct karyotypes. Considerable differences have also been recently found in DNA sequence between these two types using RFLP analysis (Braithwaite and Manners 1989; Braithwaite et al. 1990). The clear genetic distinction between the two types indicates that they have probably evolved independently over a considerable period of time. Anthracnose caused by many diverse *C. gloeosporioides* pathotypes is prevalent on *Stylosanthes* spp. in South America (Lenne and Calderon 1984), the centre of origin of the genus, whilst the disease was first observed on *Stylosanthes* spp. in Australia only as recently as 1973. *Stylosanthes* spp. were introduced to Australia earlier this century (Pont and Irwin 1976); thus the Type A and B pathogens are most probably the result of at least two separate introductions. The existence of such distinct karyotypes within two members of the group species *C. gloeosporioides* is not surprising as there are few reliable taxonomic criteria for the separation of fungi within this complex. Our results suggest that electrophoretic karyotyping has considerable potential for assessing genetic and taxonomic relationships within this group and perhaps also in other fungal groups (Steensma et al. 1988).

There is some interest amongst population geneticists in using DNA probes for the identification of single independent isolates of a fungus. Such isolate-"fingerprinting" would be useful for monitoring pathogen populations in the field. Studies of RFLPs in *C. gloeosporioides* have demonstrated very little variation amongst isolates within each type (Braithwaite and Manners 1989; Braithwaite et al. 1990). In contrast, no single isolate has the same mini-chromosome complement and electrophoretic karyotyping may be more powerful than RFLP analysis for population studies with this pathogen.

All the cultures of *C. gloeosporioides* studied here were derived from single mononucleate, and presumably haploid, conidia. Because the sexual stage is thought to be either rare or non-existent for highly virulent forms of *C. gloeosporioides* infecting *Stylosanthes* spp. (Ogle et al. 1986), chromosomal rearrangements are unlikely to result from unequal crossovers at meiosis. Chromosomal rearrangements in *C. gloeosporioides* would not have the constraint of stability brought about by pairing in meiosis and thus asexuality may allow this pathogen to tolerate such wide variation in karyotype. Processes generating variable karyotypes are probably active either during somatic growth or parasexual recombination. Haploidisation can produce aneuploids by mitotic non-disjunction. Studies aimed at the generation of novel karyotypes via the parasexual cycle are currently underway in our laboratory.

In this study, high molecular weight DNA bands observed on pulsed field gels have been termed either maxi-chromosomes or mini-chromosomes. These terms should be treated with caution until a nuclear origin, mitotically-linked replication and disjunction, and the presence of chromosomal elements such as telomeres and centromeres, have been established. Because the mini-chromosomes do not stain very intensely with ethidium bromide they are not present in high copy number as demonstrated for mini-chromosomes in *Trypanosoma brucei* (Van der Ploeg et al. 1984). Our results indicate that the mini-chromosomes are not mitochondrial DNA. We have considered the possibility that the mini-chromosomes may be small circular molecules. Circular molecules are known to electrophorese with aberrant mobilities under different conditions of pulsed field electrophoresis (Hightower and Santi 1989; Simske and Scherer 1989). A number of circular molecules (10–50 Kb) co-electrophorese with the mini-chromosomes under condition 1 but not under condition 2 (data not shown). In addition, electrophoresis of chromosomal blocks under normal non-pulsed electrophoretic conditions does not reveal any molecules in the 10–50 Kb range. Furthermore, mini-chromosomes respond to different conditions of pulsed field electrophoresis in a manner expected of linear molecules (Figs. 1, 2, and 3). It is possible that they are linear plasmids as observed in other fungi (Hashiba et al. 1984; Kim et al. 1988; Samac and Leong 1989) although they must be much larger than the linear fungal plasmids so far identified.

Our hybridisation studies indicate that the mini-chromosomes share some sequence homology with the larger chromosomes and thus would appear to be a part of the same genetic system. At present it is not possible to determine whether they are dispensable in the life cycle of this fungus, as are the small, variable B-chromosomes found in higher eukaryotes (Jones and Rees 1982), or whether they carry genes related to pathogenicity. In relation to the latter, a gene encoding an enzyme responsible for phytoalexin detoxification has been reported to reside on a meiotically unstable, small dispensable chromosome of *Nectria haematococca* (Van Etten et al. 1989). The mini-chromosomes of *C. gloeosporioides* hybridised to a cDNA probe from total poly $A^+$ RNA and thus would appear to carry genes that are transcribed in culture, although no defined structural gene has yet been assigned to a mini-chromosome. We have recently been able to tag mini-chromosomes of *C. gloeosporioides* with introduced selectable marker genes (Masel and Manners, unpublished) and this will greatly facilitate future studies of their functions.

In conclusion, we report here that the karyotype of field isolates of *C. gloeosporioides* from *Stylosanthes* spp. is highly variable. The significance of this variation in relation to pathogenic specialisation currently remains unclear but gross chromosomal rearrangement must now

be considered as a possible mechanism influencing the adaptation of this fungal pathogen to its host in the field.

*Acknowledgements.* The comments of Dr. R. G. Birch during manuscript preparation and the technical assistance of Ms. H. Way are acknowledged. We are grateful to the Australian International Development and Assistance Bureau and The University of Queensland Foundation for support.

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Communicated by F. Kaudewitz