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Genome organization of *Magnaporthe grisea*: genetic map, electrophoretic karyotype, and occurrence of repeated DNAs

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Abstract. A genetic map of *Magnaporthe grisea* (anamorph = *Pyricularia oryzae* and *P. grisea*), the causal agent of rice blast disease, was generated from segregation data utilizing 97 RFLP markers, two isoenzyme loci and the mating type locus among progeny of a cross between parental strains Guy 11 and 2539. Of the seven chromosomes of *M. grisea*, three were resolved by contour-clamped homogeneous electric field (CHEF) electrophoresis, while the remaining four migrated as two doublet bands. By utilizing differences between CHEF mobilities of unresolved chromosomes from the parental strains, Southern analysis with selected markers allowed the chromosomal assignment of all linkage groups. A small translocation involving 1 marker was found in the parental strains used to produce the segregating population from which the map was constructed. Nine classes of repetitive DNA elements were found in the genome of a fungal isolate pathogenic to rice. These occurred only a few times or not at all in the genomes of isolates showing reduced virulence on rice. One repetitive DNA was shown to have structural similarity to the *Alu* sequences found in primates, a sequence similarity to the *copia*-like elements of *Drosophila*, and peptide similarity to transposable elements found in *Drosophila*, other fungi, and higher plants.

Key words: Rice blast – RFLP – Retrotransposon

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Introduction

Magnaporthe grisea (Hebert) Barr comb. nov. is the teleomorphic stage of *Pyricularia oryzae* and *P. grisea*, fungi causing rice blast and diseases of many other grass species, respectively. The teleomorphic stage of *P. grisea* was first described in 1971 as *Ceratosphaeria grisea*, the sexual stage of crabgrass-infecting isolates of the fungus (Hebert 1971). Five years later it was reported that *P. grisea* and *P. oryzae*, which are morphologically identical, could be mated to produce perithecia, although the level of fertility was low (Yaegashi and Hebert 1976). Later, *P. grisea* was transferred to the teleomorph *Magnaporthe grisea* Hebert (Yaegashi and Udagawa 1978).

The rice blast fungus is considered to be one of the most important plant pathogens in the world (Ou 1985). Because of its economic importance, considerable efforts have been made to understand the genetics and molecular biology of this fungus. A previous genetic study of *M. grisea* defined five linkage groups based on 11 auxotrophic and fungicide resistance markers (Nagakubo et al. 1983). Other markers have been described (Leung and Williams 1985; Leung and Taga 1988), although little is known about the linkage relationships of those markers. A RFLP map utilizing preliminary data from this study has been reported (Skinner et al. 1990; Budde et al. 1993), and a genetic map utillizing a repetitive DNA sequence as a genetic marker has been constructed (Romao and Hamer 1992) and has yielded eight large linkage groups. Another map has been constructed using cloned genes,

cosmid clones, repeated DNA, and a telomeric repeat (Sweigard et al. 1993).

Several studies have centered on the processes involved in the interaction with the host plant (Chumley and Valent 1990; Hamer et al. 1988; Howard and Ferrari 1989; Leung et al. 1988). Transformation systems based on auxotrophic complementation (Parsons et al. 1987) and a dominant, selectable marker (Leung et al. 1990) have been developed for *M. grisea*, greatly facilitating the cloning and study of genes. To further facilitate the cloning of genes, we have undertaken the construction of a genetic map of *M. grisea* that is based on restriction fragment length polymorphisms (RFLPs) to provide starting points for "chromosome-walks" from RFLP markers to linked genes of interest. The principle of gene isolation based on saturated maps has been discussed elsewhere (Botstein et al. 1980; Michelmore and Hulbert 1987), and the utility of marker-based cloning in fungi has been demonstrated in several studies (Froeliger and Leong 1989; Glass et al. 1988; Giasson et al. 1989; Mutasa et al. 1990; Tzeng et al. 1991; May et al. 1991; Romao and Hamer 1992).

Historically, the construction of conventional genetic maps of fungi has been based solely on the segregation analysis of genetic markers. The routine assignment of linkage groups to fungal chromosomes was not possible. However, the development of pulsed-field electrophoretic techniques has allowed the separation of fungal chromosomes and the assignment of DNA markers to chromosomes by hybridization studies. The resolution of fungal chromosomes was first reported for yeast chromosomes that range in size from about 0.2 megabase pairs (Mgbp) to about 3 Mgbp (Schwartz and Cantor 1984). The larger chromosomes of *Neurospora crassa*, ranging in size from about 4 to 12 Mgbp, also have been resolved (Orbach et al. 1988) using CHEF electrophoresis (Chu et al. 1986). We report here the use of the CHEF system to resolve the chromosomes of *M. grisea* and the subsequent use of the resolved chromosomes for the construction of chromosome-enriched plasmid libraries. Southern analysis of CHEF gels allowed the assignment of all linkage groups to electrophoretically separated chromosomes.

Repeated DNA species occur in all organisms. Some are of known function (e.g. the genes encoding the ribosomal subunits), while others have no known function. DNA elements capable of transposition can be reiterated throughout the genome, hence repeated DNA elements are of interest as possible transposable elements. We report here the occurrence of several unique classes of repeated DNAs in the genome of *M. grisea* and the relationship of one class of this DNA to known transposable elements from other organisms.

Materials and methods

Fungal isolates

M. grisea isolate Guy11 (Mat 1-2), a rice pathogen that was originally discovered in French Guyana was obtained from Dr. J. L. Notteghem, Institute de Recherches Agronomiques Tropicales, Montpellier, France. Isolate 2539 (Mat 1-1) was developed in the laboratory as described previously (Leung et al. 1988). Isolates AR-4, CH40-1, CH104-3, and 6-28 were provided by A. H. Ellingboe (Kolmer and Ellingboe 1988). Isolates O-135 and 4091-5-8 were provided by B. Valent (Valent et al. 1991).

The mapping population was derived from a single cross between Guy11 and 2539 from which 68 random ascospores and ten complete or partial tetrads were obtained (Leung et al. 1988). From this cross, 61 random ascospore progeny were used to determine the segregation of RFLPs and linkage relationships. The segregation of mating type and two lactate dehydrogenase loci (LDH1 and LDH3) were determined as described previously (Leung and Williams 1985).

DNA isolation

DNA was extracted from mycelium grown in 100 ml liquid complete medium (CM) (Valent et al. 1986) in 250-ml Erlenmeyer flasks, at 25°–30 °C with shaking at 100 rpm on an orbital shaker. The flasks were inoculated with mycelia from PDA or oatmeal plate cultures and were harvested when mycelial density reached its maximum but before dark pigments were produced, usually about 3 days after inoculation. Mycelial harvest and DNA extraction was by the CTAB method (Manicom et al. 1987).

Electrophoresis and Southern transfer of genomic DNA

Restriction endonuclease-digested DNA was electrophoresed in 0.7% SeaKem LE agarose gels (1.5 μg/lane) using standard conditions (Maniatis et al. 1982). The electrophoresed DNA was transferred to a positively charged nylon membrane (Schleicher and Schuell, Keene, N.H.) according to standard protocol (Southern 1975).

Preparation of intact chromosomes in agarose microbeads and separation by CHEF electrophoresis

The preparation of intact chromosomes in microbeads was accomplished following the protocol of Koob and Szybalski (1992) with modification. Mycelial cultures were established at 25°–30 °C in 50 ml CM Erlenmeyer flask with shaking at 100 rpm on an orbital shaker. After 72 h of growth, the cultures were ground briefly at full speed in a blender and then added to 200 ml of CM in a 1-l Erlenmeyer flask. The culture was grown for 18 h and was then harvested by filtration through Miracloth (Calbiochem, San Diego, Calif.). The mycelium was suspended in SEC buffer (1 *M* sorbitol, 50 m*M* Na_2 EDTA, 50 m*M* Na_3 citrate, final pH 6.2) and centrifuged at 1600 *g* for 5 min. It was then resuspended in fresh SEC buffer containing 1.5 mg/ml Novozyme 234 (Calbiochem, San Diego, Calif.) and incubated at 30 °C for 2–3 h. The protoplasts were harvested by filtration through Miracloth, rinsed with SEC buffer, and then pelleted by centrifugation (200 *g*, 10 min). A hemacytometer was used to determine that the pellet contained approximately 10^{10} protoplasts/ml. The pellet was diluted ten fold (to a maximum volume of 2 ml) in SEC to give a concentration of approximately 1×10^9 protoplasts/ml. To this was added an equal volume of 1.2% InCert agarose (FMC, Rockland, Me.) in SEC at 55 °C. The solution was mixed by pipetting and then added to 5 ml of mineral oil at 55 °C in a 25 ml Erlenmeyer flask. This solution was mixed vigorously for 1 min at full speed on a vortex mixer

while the flask was held in a horizontal position. The flask was then transferred to salted ice-water for 5 min. When the agarose had solidified, the slurry was poured into a 15 ml Corex tube. The remaining slurry was rinsed in SEC and added to the oil/agarose mixture, which was then centrifuged at 4000 *g* for 5 min to separate the oil from the agarose microbeads. The oil was decanted and the beads were resuspended in an equal volume of PESTS buffer (0.5 *M* Na_2EDTA, 10 m*M* TRIS, 1% Sarkosyl, 1% SDS, 1 mg/ml Proteinase K (Boehringer-Mannheim, Indianapolis, Ind.)) at 55 °C. After incubation at 55 °C for 2–4 h the beads were centrifuged at 4000 *g* for 5 min and the supernatant was discarded. The beads were then resuspended in an equal volume of 0.5 EX (0.5 *M* EDTA, 0.01% TritonX-100), transferred to a 13-ml polypropylene tube, and centrifuged at 4000 *g* for 5 min. The supernatant was decanted, and the beads were stored as a slurry at 4 °C. Chromosomal DNA prepared and stored in this manner was stable for several months.

CHEF electrophoresis was carried out in horizontal slab gels consisting of 0.8% FastLane agarose (FMC, Rockland, Me.) in 0.5 × TBE (Maniatis et al. 1982) using a running buffer of 0.5 × TBE at 14 °C. The CHEF apparatus used was fabricated according to specifications provided by R. Davis (Chu et al. 1986). Switching intervals employed were 90 min for 5 days, then 60 min for 2 days, at 35 V (34 mA). A second apparatus (BioRad DRII) was used with identical switching intervals but was run at 40 V (10 mA) to achieve similar separations.

Construction of random genomic clones

Nuclear DNA was separated from mitochondrial DNA by cesium chloride-bisbenzimide isopycnic centrifugation (Garber and Yoder 1983). Nuclear DNA from isolates 0-135, 4091-5-8, and Guy11 was digested to completion with restriction endonuclease *Bam*HI or *Eco*RI and size-fractionated on a 0.7% agarose gel. Different size fractions were obtained by electroelution from the gel. Fragments 5–10 kb in length were ligated into the appropriate polylinker site of pUC18, and individual clones were established in *E. coli* strain DH5α(F^-,ϕ80*dlac*ZΔM15, Δ(*lac*-ZYA-*arg*F)U169, *rec*A1, *end*A1, *hsd*R17(rk^-,mk^+), *deo*R, *sup*E44λ^-, thi-1, *gyr*A, *rel*A1). Dot blots (Maniatis et al. 1982) of most clones were probed with radiolabeled genomic DNA to identify those which contained moderately to highly repeated DNA (Landry and Michelmore 1985). The clones which did not hybridize were then radiolabeled and hybridized to "survey" blots of *Dra*I, *Eco*RI, and *Bam*HI digests of the parental strains. Around 20% of the clones identified RFLPs and were used for generation of the map. A description of the RFLP clones is presented in Appendix 1.

Preparation of chromosome-enriched probes

Chromosomes from CHEF gels were excised from the gel. The DNA was then digested in situ with *Hin*dIII and the fragments recovered with GeneClean (Bio 101, La Jolla, Calif.). The DNA was size-fractionated in 0.7% agarose, and fragments greater than 2 kb were again recovered with Gene Clean. These fragments were ligated into pUC18 and transformed into *E. coli* strain DH5α. Clones containing repeated DNA and those detecting polymorphisms were identified as above. Clones prepared in this manner (Appendix 1, prefaced with CH followed by a number) that identified RFLPs were used in the generation of the map.

Labeling of probe DNA and hybridization

Plasmid DNA was recovered from *E. coli* using the boiling miniprep method (Maniatis et al. 1982). Whole recombinant plasmids were radiolabeled using either nick translation (Maniatis et al. 1982) or random primer (Feinberg and Vogelstein 1984) labeling techniques. Membranes were washed with 2 × SSPE prior to use. Hybridizations were carried out using a modification of described methods (Amasino 1986). Polyethylene glycol and NaCl were omitted from the hybridization buffer, which was maintained at stringent conditions (50% formamide, 65 °C wash). Autoradiography was done with Kodak OG-1 film and Lanex intensifying screens at −80 °C.

Probe DNA was removed from the membrane prior to re-use by soaking in 0.1 × SSC, 0.1% SDS at 100 °C for 15 min, then washing with agitation in 0.1 × SSC, 0.5% SDS at 65 °C for 16 h. Blots were stored at 4 °C in plastic containers with no added buffer. Blots handled in this way could be used more than 15 times.

Analysis of linkage

Segregation data were analyzed using MAPMAKER (Lander et al. 1987). Parameters for map construction were a minimum LOD (log of the odds) of 4.0 and a maximum recombination fraction of 0.2. The Kosambi mapping function was employed to compute recombination distances in centimorgans (cM). The use of these parameters resulted in a more conservative genetic map compared to the program defaults of a LOD of 3.0 and a recombination fraction of 0.4. Hand-calculations of linkage and a second program, "Surveyor" (Agrigenetics, Madison, Wis.), were employed to verify marker locations assigned by the MAPMAKER program.

Selected probes of each linkage group were hybridized to Southern transfers of CHEF gels of the parental strains to assign chromosome linkages.

Nucleotide sequence determination

All nucleotide sequence determinations were accomplished with the dideoxy-chain termination method using [^{35}S] thio-dATP, the Sequenase kit (US Biochemical, Cleveland, Ohio) and double-stranded template DNA. Clones were in pUC18 and the M13 universal primer was used in all experiments.

The nucleotide sequence of *M. grisea* DNA in clone CH2-8 appears in the EMBL Sequence Database under the accession number X53475.

Results

CHEF separations of chromosomes

Three of the anticipated six chromosomes previously described from cytological studies (Leung and Williams, 1987) were routinely resolved by CHEF electrophoresis (Fig. 1). Chromosomes were numbered from largest to smallest. Slight differences in chromosome mobility between the parental isolates were observed, and these differences revealed a total of seven chromosomes when selected markers were probed to assign chromosome location to linkage groups. The largest two bands in Guy11 represented co-migrating chromosomes 1 and 2 in the top band with chromosomes 3 and 4 in the second band. Chromosomes 1 and 2 were discrete in parental strain 2539, while 3 and 4 migrated as a diffuse single band. Chromosomes three and four were only distinguishable when

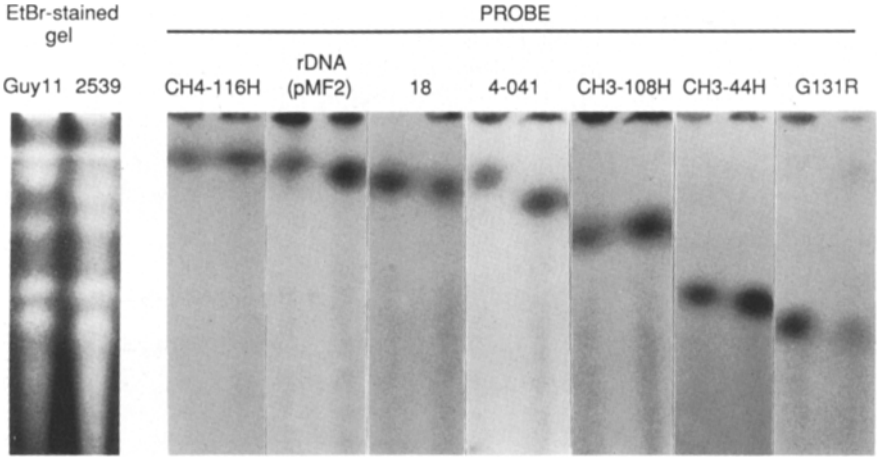

Fig. 1. CHEF electrophoretic separation of *M. grisea* chromosomes. The *first panel* represents an ethidium bromide gel showing the resolution of chromosome-sized DNAs. This gel and duplicates were transferred to nylon membrane as described in Materials and methods. Hybridization of selected markers (Appendix 2) to Southern transfers of chromosomal DNAs define the positions of chromosomes one through seven (*left to right*). The extent of migration in strain 2539 (*right well*) determined chromosomal number. Gels were electrophoresed on a BioRad DRII apparatus using conditions described in Materials and methods

subjected to Southern analysis with selected markers of the mapped linkage groups (Fig. 1).

Comparisons of migration of the *M. grisea* chromosomes to that of the *Schizosaccharomyces pombe* chromosomes (data not shown) indicated that chromosome 6 is about 4.6 Mgbp, because it co-migrated with chromosome II of *S. pombe*, which is currently thought to be that size (Steele et al. 1989). By utilizing the CHEF data and previously published lengths of *M. grisea* chromosomes (Leung and Williams 1987), we would estimate a genome size of 40 Mgbp. However, the fungal isolates used by Leung and Williams (1987) were different from those employed in this study, and their chromosomes may differ both in length and/or number.

Genetic map

Of the 97 RFLP markers (Appendix 1) mapped, 96 segregated in a Mendelian (1:1) fashion. Eleven linkage groups were identified, spanning approximately 460 cM (Fig. 2), and 4 markers were unlinked. Given a minimum distance of 20 cM between each linkage group or unlinked marker on each chromosome (maximum recombination fraction = 0.2), a minimum size estimate of the mapped genome of *M. grisea* would be 620 cM. Clusters of 6 or more markers within a 10 cM span were found on chromosomes 1, 2, and 6. Ordered data showing the inheritance patterns of markers within the progeny are presented in Appendix 2.

Linkage groups were assigned to gel-separated chromosomes based on the hybridization of selected markers to Southern transfers of CHEF gels (Fig. 1). Linkage groups corresponding to chromosomes 1, 2, 3, and 4 could be assigned because of the different mobilities of the chromosomes of the parental strains. A total of 35 markers were hybridized to the CHEF karyotype of the parental strains to confirm marker positions in this map. Close linkage was found between RFLP markers and two genes (Fig. 2), a lactate dehydrogenase locus (LDH3, chromosome 1) and the mating type locus (MAT 1, chromosome 7).

Distribution of polymorphisms

The distribution of polymorphic markers was not random. An apparent clustering of random markers was observed on chromosomes 1 and 2. Furthermore, an abundance of random markers was mapped to chromosome 4, while only 1 random marker was mapped to chromosome 5. An attempt was made to isolate markers from selected gel-separated chromosomes in order to generate a "chromosome-specific" set of markers. The fragments cloned from the resolved chromosomes on CHEF gels were not entirely chromosome specific; only about 32% of the "chromosome-specific" polymorphic probes (designated CH followed by a number and −) actually originated from the intended chromosomes. We attribute this to the extensive smearing of chromosomal DNA observed in the preparative gels used to generate the marker clones.

One clone, CH2-54H, did not segregate as expected. This clone detected a single polymorphic fragment in each parent. Progeny were identified with one fragment, both fragments, or neither fragment, suggesting that CH2-54H detects segments at 2 independent loci in the two parents. When total DNAs from 2 complete

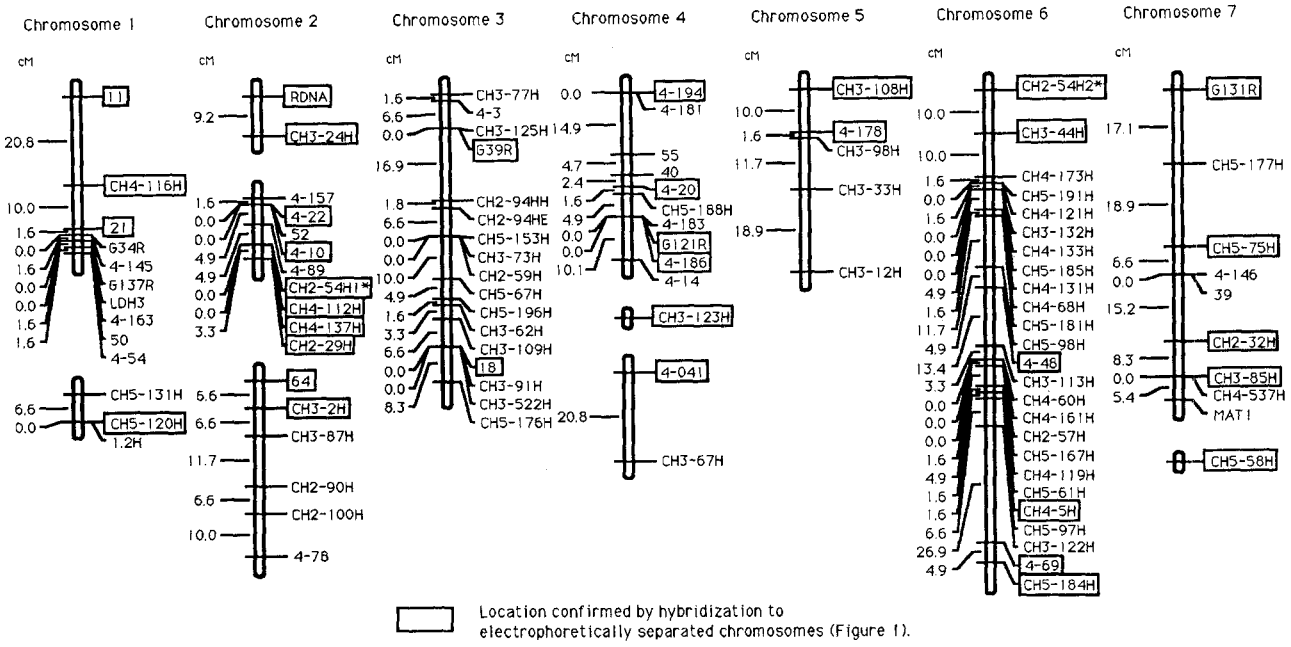

Fig. 2. Genetic map of *Magnaporthe grisea* based on the segregation of 61 progeny from a cross between isolates Guy11 and 2539. Physical linkage of *boxed markers* was confirmed by hybridization to electrophoretically separated chromosomes. The markers involved in translocation are *starred*

asci were probed with CH2-54H, 1 proved to be the parental ditype (Fig. 3a), while the other was the non-parental ditype (Figure 3B), confirming that 2 independent loci were involved. Southern analysis of a CHEF gel revealed that CH2-54H was located on chromosome 2 in 2539 and chromosome 5 in Guy11 (Fig. 4). The fragment occurred on both chromosomes in 1 progeny of Guy11 and 2539 (Fig. 4), confirming the result from the non-parental ditype tetrad. The scoring of each fragment of as an independent locus revealed a map location consistent with the CHEF gel result (CH2-54H1 and CH2-54H2, Fig. 2). Markers near these fragments segregated normally and also hybridized to the expected single chromosomes.

Deletions or insertional events were identified in a few progeny with 10 of the 97 RFLP markers, resulting in the polymorphic fragments differing from either

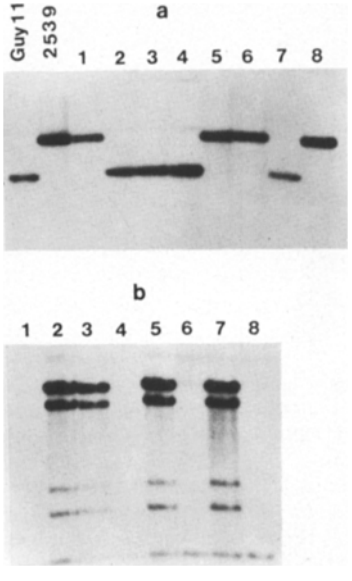

Fig. 3a, b. Identification of an insertional translocation. Southern hybridization analysis of DNA from *Magnaporthe grisea* isolates (**a**) Guy11, 2539, and 8 progeny (*lanes 1–8*) of Guy11 × 2539 obtained from one ascus and (**b**) 8 progeny derived from a second ascus. Genomic DNA was digested with *Hin*dIII, resolved on agarose gels, transferred to nylon membranes, and probed with RFLP marker CH2-54H

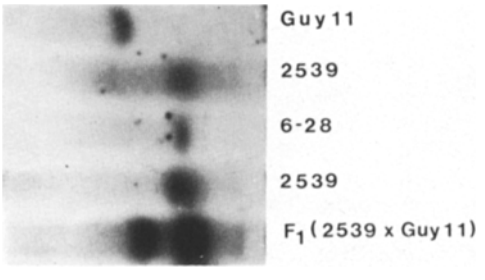

Fig. 4. Southern hybridization analysis of electrophoretically resolved chromosomes of *Magnaporthe grisea* to demonstrate the occurrence of the non-parental phenotype. Electrophoretic karyotypes of isolates Guy11, 2539, 6-28, and 1 progeny of Guy11 × 2539 were transferred to a nylon membrane and probed with RFLP marker CH2-54H

parent or their being missing entirely. Markers CH3-91H and 18 (chromosome 3) were missing in 1 progeny but were present in the other 60, while CH3-522H, which mapped to the same location, showed expected polymorphisms in all of the progeny (Appendix 2). Other markers probed to the same Southern transfer showed normal hybridization, indicating that these findings were not spurious. Marker CH3-24H was absent in 4 of the 61 progeny. Again, other markers hybridized to the same transfer gave normal polymorphisms. Fragments with sizes differing from either parent occurred in 2 of the 61 progeny (Appendix 2), 3 markers in column 4 (chromosomes 3 and 4), and 1 marker in column 48 (chromosome 2). These differences occurred on different membranes, all of which showed expected polymorphisms with other markers.

Repeated DNA

Of the 105 random genomic fragments cloned from DNA of the rice-pathogenic isolate O-135, 24 yielded a complex pattern of hybridization in Southern analyses (Fig. 5), indicating that a substantial portion of the genome of O-135 is comprised of repeated DNA. Repeated DNAs from this isolate were found to be highly repeated in rice-pathogenic isolates, but occurred at a low frequency or not at all in isolates that are pathogenic on grasses other than rice (Skinner et al. 1988; Hamer et al. 1989) (Fig. 5). To determine whether the repeated DNAs found were the same, or if more than one class of repeated DNA was present, cross-hybridization experiments were carried out among cloned fragments known to contain repeated DNA. A total of nine classes of repeated DNAs which did not cross-hybridize at high stringency were identified from the isolates used in this study (data not shown). These nine classes could represent distinct classes of repeated elements or may represent parts of larger repeated elements. Hybridization of MGR 2 (Hamer et al. 1989) to repetitive DNAs representing each of the nine classes revealed homology with two of them. This may indicate that these two classes are different members of the MGR family or that they may be different parts of one of the MGR elements.

One clone (4-045, isolated from 4091-5-8, a non-rice pathogen) was examined in detail by restriction mapping and DNA probing to localize the repeated region of the clone. A small (< 700 bp) region was repeated and flanked by single-copy sequences (data not shown); therefore, the repeated DNA in clone 4-045 is not part of a larger element but represents one of possibly several unique classes of repeated DNA. One of the flanking fragments identified the RFLP 1.2H, which mapped to chromosome 1 (Fig. 2).

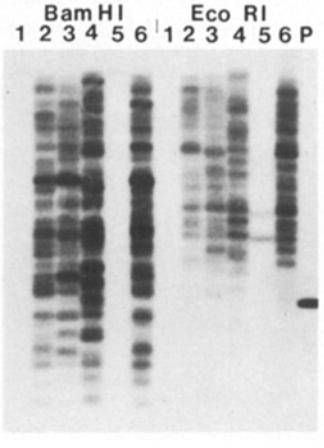

Fig. 5. Occurrence of sequences homologous to repeated DNA clone pMG6 in rice-infecting and non-rice-infecting isolates of *Magnaporthe grisea*. *Bam*HI or *Eco*RI-digested genomic DNA of *M. grisea* was electrophoresed in agarose, transferred to a nylon membrane, and probed with repetitive *M. grisea* DNA clone pMG6. *Lanes 2–4 and 6* contain DNA from rice-pathogenic isolates (CH104-3, 2539, Guy11, and O-135, respectively); *lane 1 and 5* contain DNA from a rice non-pathogenic isolates (AR-4 and 4091-5-8, respectively); *lane P* contains linear pUC18 as a hybridization control

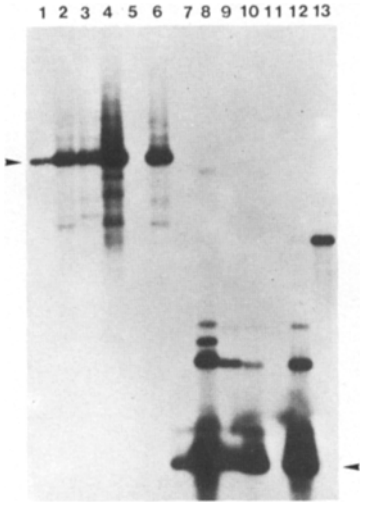

Fig. 6. Occurrence of sequences homologous to repeated DNA clone CH2-8 in rice-infecting and nonrice-infecting isolates of *Magnaporthe grisea*. Genomic DNA from *M. grisea* was digested with restriction endonucleases, electrophoresed in agarose, transferred to a nylon membrane, and probed with repetitive *M. grisea* DNA clone CH2-8. *Lanes 1 and 7* contain DNA from isolate 2539 digested with *Hin*cII or *Eco*RI, respectively; *lanes 5 and 11* contain DNA from a rice non-pathogenic isolate (4091-5-8) digested with *Hin*cII or *Eco*RI, respectively; *lanes 2–4 and 6* contain DNA from rice-pathogenic isolates (CH104-3, CH40-1, Guy11, and O-135, respectively) digested with *Hin*cII; *lanes 8–10 and 12* contain DNA from the previously mentioned rice-pathogenic isolates, but were digested with *Eco*RI; *lane 13* contains linear pUC18 as a hybridization control. *Arrows* indicate the major band of hybridization of this clone; many copies are present in that band

a. RNA Polymerase III promoter structure of repeated element

	"a box "		"b box "
tRNA consensus	TGGCNNAGTNGG	--17-60 bases--	GGTTCGANNCC
CH2-8	TGACCAAGAAAG	--24 bases--	CCTGCGAAACC

b. Sequence of flanking direct repeats

Left repeat --13 bases-- "a box"---"b box" --191 bases-- Right repeat

Left repeat: ATCCGAGATTT..C..TCAAAGA.....TCGTAGCC
Right repeat: TTACGAGATTT$_A$C$_G$ACAAAGA$_A$T$_T$GCTAGCC

Fig. 7a, b. Sequence similarity of repetitive *Magnaporthe grisea* DNA clone CH2-8 and *Alu*-like repetitive DNA elements (**a**) *Bold letters* within the "*a*" and "*b*" box indicate exact match to the consensus RNA polymerase III binding site; *normal intensity* indicates a mismatch. (**b**) Direct repeat structure flanking the putative RNA polymerse III binding site described in (**a**); *bold letters* indicate exact match between the repeat units; normal intensity indicates *mismatch.* Note that the 3′ repeat unit has 4 base pairs not found in the 5′ unit, these are indicated in *subscript*

A second repeated DNA clone (CH2-8, isolated from 2539) detected highly repeated DNA fragments of a similar size in restriction endonuclease digests of *M. grisea* isolates Guy11 and 2539 DNA (Fig. 6). This clone did not hybridize to DNA from *M. grisea* isolated from eight different weed species, was present in isolates from two other weed species, and occurred many times in 25 different isolates from rice (data not shown). Hybridization of CH2-8 to electrophoretically separated chromosomes of strains 2539 and Guy11 revealed that this sequence was present on all chromosome-sized DNAs (data not shown).

The DNA sequence of clone CH2-8 was determined. Comparison of its nucleic acid sequence with those in the EMBL and Genbank databases revealed a similarity to the reverse transcriptase portion of the *copia*-like elements of *Drosophila melanogaster*. The sequence was also found to contain an apparent RNA polymerase III binding site bounded by direct repeats that span a 300 bp region (Fig. 7). This kind of structure is similar to that of *Alu* sequences, commonly repeated elements found in humans and several other animal species (reviewed by Deininger 1989). Comparison of the translated sequence of CH2-8 with the PIR protein database revealed a striking identity with portions of sequences of reverse transcriptases from retrotransposons found in other fungi, *Drosophila*, and higher plants (Fig. 8).

Discussion

We have constructed a genetic map for *M. grisea* that should prove useful for "chromosome-walking" to genes of interest. The current map consists of 11 linkage groups. We were able to assign linkage groups of the physical map to electrophoretically separated chromosomes by probing selected markers to Southern blots of CHEF gels of the parental strains. Even though all chromosomes could not be completely resolved under any of the conditions employed, the differential mobility of chromosomes between 2539 and Guy11 allowed markers to be scored to chromosomes 1, 2, 3, or 4 by comparing the patterns of hybridization to the two karyotypes. Markers from chromosomes 5, 6, and 7 were easily scored because these chromosomes were well resolved. Results from Sweigard et al. (1983) indicate that *M. grisea* contains seven chromosomes and that the chromosome band we had designated as 3 (Budde et al. 1993) actually represents two closely migrating chromosomes distinguishable only by Southern analysis of representative markers. Because of this finding, we have renumbered the chromosomes from largest to smallest, resulting in a numeral change for the three smallest chromosomes. Previous cytological evidence indicated the presence of six chromosomes in *M. grisea* (Leung and Williams 1987). The discrepancy between chromosome numbers may represent strain variation similar to that which has been described in other fungal systems (McClusky and Mills 1990; McDonald and Martinez 1991) or reflect the difficulty in counting chromosomes whether it be by electrophoretic or cytological karyotyping.

The present map spans 480 cM and, by adding a minimal distance of 20 cM between unlinked linkage

	codon	
CH2-8	1	**EF**FVTETK**F**L**G**LLVGVE**G**VKMDPEKITAVLDWQT**P**KKLTDVQA**FLG**FGNFYRR**FI**
Tf-1	599	**EF**HQSQVK**F**I**G**YHISEKGFTPCQENIDKVLQWKQ**P**KNRKELRQ**FLG**SVNYLRK**FI**
CfT-1	512	**EF**HKKEVK**F**L**G**FIISTTGITIDPAKTQSIREWPE**P**KTVKDVQS**FLG**LANYNRK**FI**
del	721	**EF**WMEKVK**F**L**G**HVVSREGIVVDPVKVKAVMNWELP**K**NIFEIRS**FLG**LAGYYRR**FI**
17.6	402	**EF**LKQETT**F**L**G**HVLTPD**G**IKPNPEKIEAIQKYPI**P**TKPKEIKA**FLG**LTGYYRK**FI**
297	401	**EF**LKKEAN**F**L**G**HIVTPD**G**IKPNPIKVKAIVSYPI**P**TKDKEIRA**FLG**LTGYYRK**FI**

Fig. 8. Alignment of translated *CH2-8* sequence with peptide sequences of reverse transcriptases from retroelements. *CfT-1* is from the filamentous fungus, *Cladosporium fulvum*; *Tf-1* is from *Schizosaccharomyces pombe*; *del* is from *Lilium henryi*; and *17.6* and *297* are from *Drosophila*. Sequences were taken from Levin et al. 1990 (Tf-1); McHale et al. 1992; Inouye et al. 1986 (297); Saigo et al. 1984 (17.6); and Smyth et al. 1989 (del). *Bold letters* indicate amino acid identity

groups or markers, the map would represent 620 cM. The current map is not saturated, and the paucity of markers on chromosomes 1 and 5 suggest that these chromosomes represent the bulk of the unmapped DNA. This conclusion is supported by evidence from our lab obtained during determination of the distance of marker 11 from the end of chromosome 1. This study has indicated that chromosome 1 extends another 1.8 Mgbp distal to marker 11 (MLF and SAL, unpublished). Hybridization of markers from chromosome 5 to strain 6–28, which contains a translocation, indicates that 2.0 Mgbp of this chromosome is also not represented (MLF and SAL, unpublished). Romao and Hamer (1992) used a repetitive DNA as a probe to construct a genetic map for a cross between a rice pathogenic isolate and grass pathogenic isolate of *M. grisea*. Using the recombination fraction values obtained in that study, we calculate that the linkages presented by Romao and Hamer would span 802 cM by the Kosambi mapping function. This is similar to the 840 cM estimated for the map generated by Sweigard et al. 1993. While no attempts have been made to share probes between groups, this exchange should result in generation of a nearly saturated map. Such a map would minimize the distance to be covered in a "chromosome-walk" to a gene of interest.

A previous estimate of 38 Mgbp as the genome size of *M. grisea* (Hamer et al. 1989) was by quantitative dot blot analysis. Using CHEF electrophoresis of *M. grisea* chromosomes with size standards and extrapolating this data to the published lengths of the chromosomes for this fungus (Leung and Williams 1987), we can estimate a genome size of approximately 40 Mgbp. However, the strains involved in these three studies differ, and the lengths we calculated are for only the six chromosomes reported (Leung and Williams 1987) instead of the seven which have been identified in the parental strains used in this study. If we include the seventh chromosome in the estimation, the genome size might be as large as 49 Mgbp. A size range estimation of 35–50 Mgbp would probably most accurately reflect the true genome size for this fungus.

By tracing the descent of genetic markers, it was possible to determine what portions of the parental chromosomes were inherited as a unit in any particular progeny (Appendix 2). Despite limited resolution due to the number of markers, it appears that recombination was low in this cross. Many progeny appear to have inherited whole chromosomes from one parent. Moreover, recombination events involving more than four crossovers per chromosome were rarely detected. This may reflect substantial genomic variation between the parental strains leading to a low frequency of chiasma formation.

Repeated DNAs are common in rice-infecting isolates of *M. grisea*, and at least one of the repeated elements (CH2-8) shows sequence similarities to *Alu* sequences and retroelements. This element was found to be highly repeated in rice-infecting isolates but was lacking from most isolates pathogenic to wild grass species. This contrasts somewhat to the MGR sequences (Hamer et al. 1989) that are highly repeated in rice-infecting isolates but which are also found to a lesser extent in the grass pathogenic isolates. To our knowledge there are no other filamentous fungi where the presence or absence of repeated DNAs in sexually compatible isolates is correlated with their host ranges. An element-sharing sequence homology to CH2-8 has been shown to transpose in *M. grisea* (V. Shull and J. Hamer personal communication and M. Lebrun and B. Valent personal communication), suggesting that transposition may have played a role in adaptation of *M. grisea* to the rice host. Hyphae from a single conidium give rise to numerous new races upon passage through the host (reviewed by Ou 1985), suggesting that this fungus in genetically unstable. The electrophoretic karyotype has also been shown to differ when a serially cultured isolate is passed through the host (Talbot et al. 1993). These kinds of instability could be accounted for by the transposition of repeated element and/or by unequal crossover between dispersed elements and will be the subject of future investigations.

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Appendix 1. RFLP markers used to construct a genetic map of *Magnaporthe grisea*. DNA fragments were cloned from the indicated isolates using the fragment enzyme. Polymorphisms between isolates Guy11 and 2539 were identified with the polymorphic enzyme

Marker	Isolate of origin	Fragment enzyme	Polymorphic enzyme
11	O-135	*Bam*HI	*Eco*RI
CH4-116H	2539	*Hin*dIII	*Dra*I
21	O-135	*Bam*HI	*Hin*dIII
G34R	Guy11	*Eco*RI	*Eco*RI
4-145	4091-5-8	*Bam*HI	*Eco*RI
G137R	Guy11	*Eco*RI	*Eco*RI
4-163	4091-5-8	*Bam*HI	*Eco*RI
50	O-135	*Bam*HI	*Hin*dIII
4-54	4091-5-8	*Bam*HI	*Hin*dIII
CH5-131H	2539	*Hin*dIII	*Dra*I
1.2H	4091-5-8	*Hin*dIII	*Hin*dIII
CH5-120H	2539	*Hin*dIII	*Dra*I
RDNA (pMF2)	*Neurospora crassa*		*Eco*RI

Appendix 1. (*Continued*)

Marker	Isolate of origin	Fragment enzyme	Polymorphic enzyme
CH3-24H	2539	*Hin*dIII	*Hin*dIII
4-157	4091-5-8	*Bam*HI	*Eco*RI
4-22	4091-5-8	*Bam*HI	*Hin*dIII
52	O-135	*Bam*HI	*Hin*dIII
4-10	4091-5-8	*Bam*HI	*Hin*dIII
4-89	4091-5-8	*Bam*HI	*Hin*dIII
CH2-54H1	2539	*Hin*dIII	*Hin*dIII
CH4-112H	2539	*Hin*dIII	*Dra*I
CH4-137H	2539	*Hin*dIII	*Eco*RI, *Hin*dIII
CH2-29H	2539	*Hin*dIII	*Eco*RI
64	O-135	*Bam*HI	*Eco*RI
CH3-2H	2539	*Hin*dIII	*Eco*RI
CH3-87H	2539	*Hin*dIII	*Dra*I
CH2-90H	2539	*Hin*dIII	*Eco*RI
CH2-100H	2539	*Hin*dIII	*Dra*I
4-78	4091-5-8	*Bam*HI	*Eco*RI
CH3-77H	2539	*Hin*dIII	*Hin*dIII
4-3	4091-5-8	*Bam*HI	*Eco*RI
CH3-125H	2539	*Hin*dIII	*Eco*RI
G39R	Guy11	*Eco*RI	*Eco*RI
CH2-94H	2539	*Hin*dIII	*Eco*RI, *Hin*dIII
CH5-153H	2539	*Hin*dIII	*Dra*I
CH3-73H	2539	*Hin*dIII	*Hin*dIII
CH2-59H	2539	*Hin*dIII	*Hin*dIII
CH5-67H	2539	*Hin*dIII	*Hin*dIII
CH5-196H	2539	*Hin*dIII	*Hin*dIII
CH3-62H	2539	*Hin*dIII	*Hin*dIII
CH3-109H	2539	*Hin*dIII	*Eco*RI
18	O-135	*Bam*HI	*Eco*RI
CH3-91H	2539	*Hin*dIII	*Eco*RI
CH5-176H	2539	*Hin*dIII	*Dra*I
4-194	4091-5-8	*Bam*HI	*Hin*dIII
4-181	4091-5-8	*Bam*HI	*Hin*dIII
55	O-135	*Bam*HI	*Eco*RI
40	O-135	*Bam*HI	*Eco*RI
4-20	4091-5-8	*Bam*HI	*Hin*dIII
CH5-188H	2539	*Hin*dIII	*Hin*dIII
4-183	4091-5-8	*Bam*HI	*Eco*RI
G121R	Guy11	*Eco*RI	*Eco*RI
4-186	4091-5-8	*Bam*HI	*Hin*dIII
4-14	4091-5-8	*Bam*HI	*Hin*dIII
CH3-123H	2539	*Hin*dIII	*Hin*dIII
4-041	4091-5-8	*Bam*HI	*Hin*dIII
CH3-67H	2539	*Hin*dIII	*Dra*I

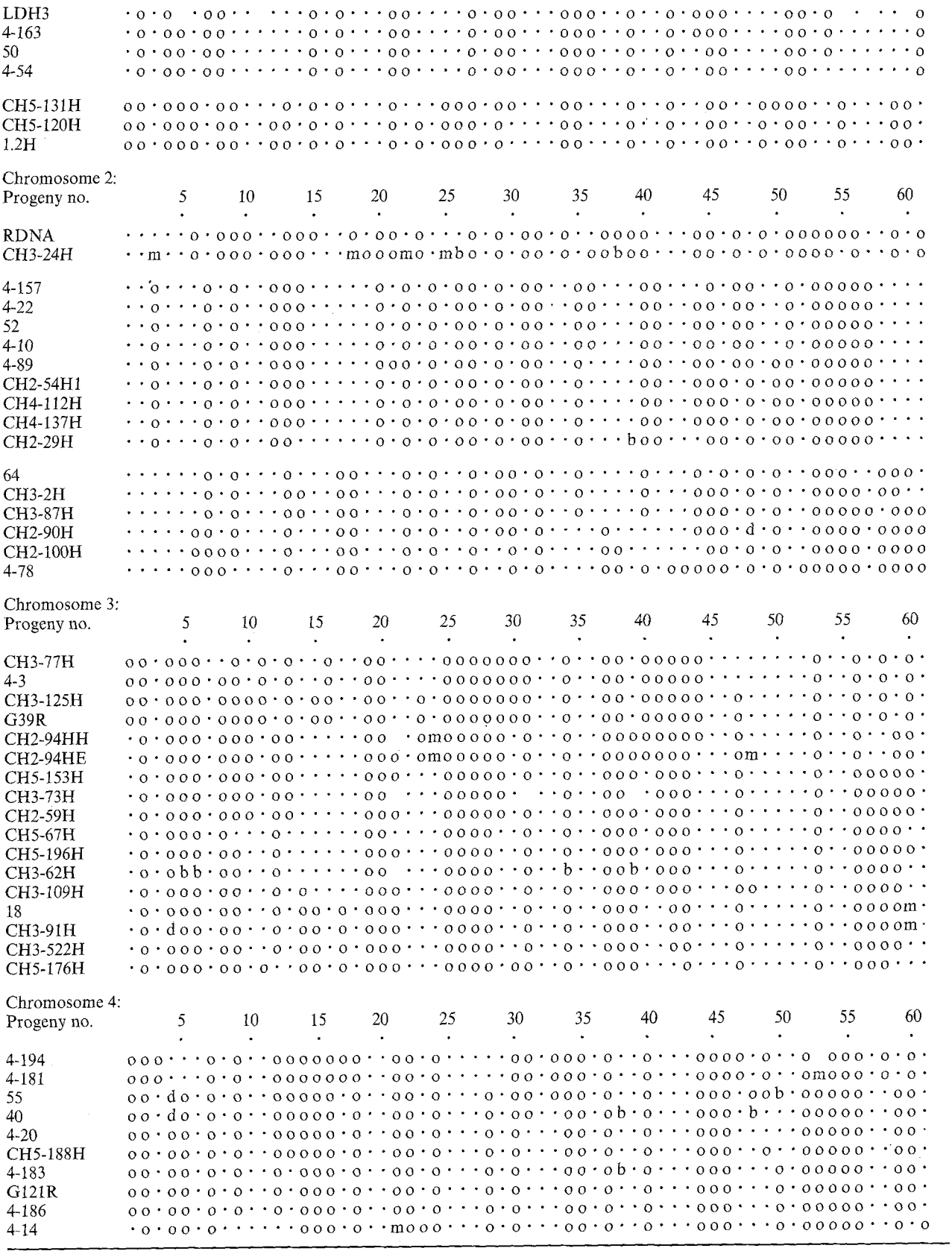
(*Continued*)

Marker	Isolate of origin	Fragment enzyme	Polymorphic enzyme
CH3-108H	2539	*Hin*dIII	*Eco*RI
4-178	4091-5-8	*Bam*HI	*Eco*RI
CH3-98H	2539	*Hin*dIII	*Dra*I
CH3-33H	2539	*Hin*dIII	*Hin*dIII
CH3-12H	2539	*Hin*dIII	*Dra*I
CH2-54H2	2539	*Hin*dIII	*Hin*dIII
CH3-44H	2539	*Hin*dIII	*Dra*I
CH4-173H	2539	*Hin*dIII	*Dra*I
CH5-191H	2539	*Hin*dIII	*Hin*dIII
CH4-121H	2539	*Hin*dIII	*Dra*I
CH3-132H	2539	*Hin*dIII	*Hin*dIII
CH4-133H	2539	*Hin*dIII	*Dra*I
CH5-185H	2539	*Hin*dIII	*Hin*dIII
CH4-131H	2539	*Hin*dIII	*Hin*dIII
CH5-181H	2539	*Hin*dIII	*Eco*RI
CH4-68H	2539	*Hin*dIII	*Eco*RI
CH5-98H	2539	*Hin*dIII	*Hin*dIII
4-48	4091-5-8	*Bam*HI	*Eco*RI
CH3-113H	2539	*Hin*dIII	*Hin*dIII
CH4-60H	2539	*Hin*dIII	*Dra*I
CH4-161H	2539	*Hin*dIII	*Dra*I
CH2-57H	2539	*Hin*dIII	*Dra*I
CH5-167H	2539	*Hin*dIII	*Eco*RI
CH4-119H	2539	*Hin*dIII	*Dra*I
CH5-61H	2539	*Hin*dIII	*Hin*dIII
CH4-5H	2539	*Hin*dIII	*Dra*I
CH5-97H	2539	*Hin*dIII	*Hin*dIII
CH3-122H	2539	*Hin*dIII	*Eco*RI
4-69	4091-5-8	*Bam*HI	*Hin*dIII
CH5-184H	2539	*Hin*dIII	*Hin*dIII
G131R	Guy11	*Eco*RI	*Dra*I
CH5-177H	2539	*Hin*dIII	*Dra*I
CH3-75H	2539	*Hin*dIII	*Hin*dIII
39	O-135	*Bam*HI	*Eco*RI
4-146	4091-5-8	*Bam*HI	*Eco*RI
CH2-32H	2539	*Hin*dIII	*Dra*I
CH3-85H	2539	*Hin*dIII	*Dra*I
CH4-537	2539	*Hin*dIII	*Eco*RI
CH5-58H	2539	*Hin*dIII	*Eco*RI
Unassigned marker			
CH5-581H	2539	*Hin*dIII	*Eco*RI

Appendix 2. Chromosomal molecular marker constitutions of 61 progeny from a single cross of *Magnaporthe grisea* isolates Guy11 and 2539. Only data representing the parental phenotypes was used for construction of the genetic map (Fig. 2). All markers are ordered as they appear on the map. Each column of data represents a single progeny

Chromosome 1: Progeny no.	5	10	15	20	25	30	35	40	45	50	55	60
11	oo····o···o···o·oo··oo·o··o·oo···ooo··oo·o·o·o·····oooooooo·o											
CH4-116H	·o····o···o···o·oo··oo·o··o·oo···ooo··o···o····o····oo·o·····o											
21	·o·oo·oo·····o·oo··oo····o·oo···ooo··o···o··oo····oo·o·····o											
G34R	·o·oo·oo·····o·oo··oo····o·oo···ooo··o···o·ooo····oo·o·····o											
4-145	·o·oo·oo·····o·oo··oo····o·oo···ooo··o···o·ooo····oo·o·····o											
G137R	·o·oo·oo·····o·o···oo····o·oo···ooo··o···o·ooo····oo·o·····o											

Appendix 2. *(Continued)*



Appendix 2. (*Continued*)

CH3-123H	·o·ooo··ooooooooo· ··ooo··o·····o··o·o·o·o·o····oo·····o
4-041	·o·ooo··oooo·oooooo·ooo··o··o·o·····o··o···o·o·····oo··o··o
CH3-67H	·o·ooo·ooo·o·o·oooo·ooo··oo···o··ooo··oo···o·o·····o·oo··o···
Chromosome 5:	
Progeny no.	5 10 15 20 25 30 35 40 45 50 55 60
CH3-108H	ooo·oo·o·oooo·ooo··ooo·oo···oo·oo··oo·····oo·o·····oo·o·oo·o
4-178	oo·ooo·o·oooo·ooo··ooo··o···oo·oo··oo·····oo·o·····oo·o··oo·
CH3-98H	oo·ooo·o·oooo·ooo··ooo··o···oo·oo··oo·····o·o·····oo·o··oo·
CH3-33H	oo·ooo·o·oooooooo··ooo·oo·o·oo·oo·ooo·····o·o·····oo·b·o···
CH3-12H	·o·ooo·o·oooooooo··ooo·ooo·ooo·o··o·o·····o···o···oo·o·o··o
Chromosome 6:	
Progeny no.	5 10 15 20 25 30 35 40 45 50 55 60
CH2-54H2	··ooo···oo·o··oooo·o·oo···oo·ooo···ooo·o··o·o·ooo··oo··o·oo
CH3-44H	··ooo···ooo·o·oo·oo·o·oo···oo·ooo···oo···oo·o·o·ooo··ooo·o·oo
CH4-173H	··oooo·oooo·o·oo·oo·o·ooo··oo·ooo···oo·o·oooo·o·ooo··oo··o·oo
CH5-191H	··oooo·oo·o·o·oo·oo·o·ooo··oo·ooo···oo·o·oooo·o·ooo··oo··o·oo
CH4-121H	··oooo·oo·o·o·oo·oo·o·ooo··oo·ooo···oo·o·oooo·o·ooo··oo··o·oo
CH3-132H	··oooo·oo·ooo·oo·oo· ·ooo··oo·ooo···oo·o·oooo·o·ooo··oo··o·oo
CH4-133H	··oooo·oo·ooo·oo·oo·o·ooo··oo·ooo···oo·o·oooo·o·ooo··oo··o·oo
CH5-185H	··oooo·oo·ooo·oo·oo·o·ooo··oo·ooo···oo·o·oooo·o·ooo··oo··o·oo
CH4-131H	··oooo·oo·ooo·oo·oo·o·ooo··oo·ooo···oo·o·oooo·o·ooo··oo··o·oo
CH4-68H	···ooo·oo·ooo·oo·oo·o·ooo··oo·ooo···oo·o·oo·o·o·ooo··oo··o ·o
CH5-181H	··ooo·oo·ooo·oo·oo·o·ooo··oo·ooo···oo·o·oo·o·o·ooo··oo··o·oo
CH5-98H	··o··oooo·o·o·oo·oo·o·ooo··oo·o·o···oo·o·oooo·o·ooo··oo··o·oo
4-48	··o··ooo··o·o·oo·oo·o·ooo··oo·o·o···oo·o·oo·o·o·o·o··oo··o·oo
CH3-113H	o·o··ooo··o·o·ooooooo·ooo··oo··o·o·····oo·o·o·o·o··oo··o·oo
CH4-60H	··o··ooo··o·o·booooo·o·ooo··oo···o·o·····oo·o·o·o·o··oo··o·oo
CH4-161H	··o··ooo··o·o·ooooo·o·ooo··oo···o·o·····oo·o·o·o·o··oo··o·oo
CH2-57H	··o··ooo··o·o·ooooo·o·ooo··oo···o·o·····oo·o·o·o·o··oo··o·oo
CH5-167H	··o··ooo··o·o·ooooo·o·ooo··oo···o·o·····oo·o·o·o·o··oo··o·oo
CH4-119H	···o··ooo··o·o·ooooo·o·ooo··oo···o·o·····oo·o·o···o··oo··o·oo
CH5-61H	··o··ooo··o·o·ooooo·o·ooo··oo·····o·····o··o·o·····o··oo··o·o·
CH4-5H	··o··ooo··o·o·ooooo·o·ooo··oo·····o·····o··o·o·····o··oo··o·oo
CH5-97H	··o··ooo··o·o·ooooo·o·ooo··oo·····o·····oo··o·o···o··oo··o·oo
CH3-122H	··o·oooo··o·o·oooo··o·ooo··oo·····o·····ooo·o·o·o·o··oo··o·oo
4-69	·oo··oooo·o·o·ooooo·o·ooo·oo····oo···o·o···oo····o··ooo·o·o·
CH5-184H	·oo··o·oo·o···ooooo·o·ooo·oo····oo···o·o···oo····oo·ooo·o·o·
Chromosome 7:	
Progeny no.	5 10 15 20 25 30 35 40 45 50 55 60
G131R	oo·oo·o··oo··ooo·oo·o·o·oo··o·oo···oo···ooo··o··o·ooo···oo·o·
CH5-177H	oo·ooo···oo··oooo·o·oo·o·o··o·oo···oo···ooo··b····ooo···o··o·
CH5-75H	·o·ooo··ooo··ooo·oo·o·····o·o·oo·o·oo···ooo··o····o·o·o·o··o·
4-146	·o·ooo··o·o··ooo·oo·····o···oo·o·oo···ooo··o····o·o·ooo··o·
39	·o·ooo··o·o··ooo·oo·····o···oo·o·oo···ooo··o····o·o·ooo··o·
CH2-32H	·o·ooo·oo·o··ooo·oo·····oo··oo·o·oo···oo···o·o·····o·····
CH3-85H	·o·ooo·oo·o··ooo·oo·o····oo··oo·ooo·o···o···o·o·····o·····
CH4-537H	·o·ooo·oo·o··ooo·oo·o····oo··oo·ooo·o···m··mo·o····m·o·····
MAT	·o·ooo·oooo·· oo· ooo····oo··oo·oo ·o···o··· · ·····.····
CH5-58H	oo····o·ooo·oooo·o·o ·o·o··o·····o···o·····o·b····o···o··
Unassigned markers:	
Progeny no.	5 10 15 20 25 30 35 40 45 50 55 60
CH5-581H	···oo·o····oo·oooo·o··oooo···ooo·oo·····o·o·o··oo···oo ·o
LDH1	·o·oo·o··o ·oooo·o··oo·o···oo·····o··o·····o·o·o·ooo o ··oo

·, Allele inherited from Guy 11; o, allele inherited from 2539; m, polymorphic band was missing; d, polymorphic band different from either parent; b, allele shows inheritance from both parents; ( ), blank space indicates data not done

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