Grasshopper, a Long Terminal Repeat (LTR) Retroelement in the Phytopathogenic Fungus *Magnaporthe grisea*

Katherine F. Dobinson, Richard E. Harris, and John E. Hamer

Department of Biological Sciences, Purdue University, West Lafayette, IN 47907 U.S.A.
Received 19 June 1992. Accepted 13 October 1992.

The fungal phytopathogen *Magnaporthe grisea* parasitizes a wide variety of gramineous hosts. In the course of investigating the genetic relationship between pathogen genotype and host specificity we identified a retroelement that is present in some strains of *M. grisea* that infect finger millet and goosegrass (members of the plant genus *Eleusine*). The element, designated *grasshopper* (*grh*), is present in multiple copies and dispersed throughout the genome. DNA sequence analysis showed that *grasshopper* contains 198 base pair direct, long terminal repeats (LTRs) with features characteristic of retroviral and retrotransposon LTRs. Within the element we identified an open reading frame with sequences homologous to the reverse transcriptase, RNaseH, and integrase domains of retroelement *pol* genes. Comparison of the open reading frame with sequences from other retroelements showed that *grh* is related to the gypsy family of retrotransposons. Comparisons of the distribution of the *grasshopper* element with other dispersed repeated DNA sequences in *M. grisea* indicated that *grasshopper* was present in a broadly dispersed subgroup of *Eleusine* pathogens, suggesting that the element was acquired subsequent to the evolution of this host-specific form. We present arguments that the amplification of different retroelements within populations of *M. grisea* is a consequence of the clonal organization of the fungal populations.

Additional keywords: filamentous fungus, intraspecific variation, *Pyricularia grisea,* retrotransposon.

Intraspecific variation is a common feature of plant parasitic microorganisms. Many exist as composite species comprised of morphologically indistinguishable forms that infect different host plants. *Magnaporthe grisea* (Hebert 1971) Barr, a filamentous ascomycete that causes one of the most devastating diseases of cultivated rice, provides an interesting example of intraspecific variation. Although in nature members of this species are found on a wide range of monocot hosts, when tested in the laboratory they show a limited infection spectrum, causing the most severe disease symptoms on their host of origin. Isolates that cause disease on rice were once considered to be a separate species from isolates that infect other monocots. With the discovery of the sexual stage it was demonstrated that isolates with different host specificity could be crossed in the laboratory to yield viable progeny (Hebert 1971; Yaegashi and Nishihara 1976; Yaegashi 1977; Yaegashi and Asaga 1981; Kato and Yamaguchi 1982). Although the fertility of field isolates is highly variable, the uniform morphology of the various host-specific forms and their shared sexual stage have led to their reclassification as a single species (Rossman *et al.* 1990).

Despite obvious differences in host specificity, attempts to discriminate among host-specific forms of *M. grisea* have met with limited success. An analysis of enzyme polymorphisms at 18 putative loci in 335 *M. grisea* field isolates failed to discriminate among the host-specific forms (Leung and Williams 1986), suggesting that they were genetically homogeneous. However, repeated DNA sequences in *M. grisea* have been recently shown to have a surprising level of variation. The genomes of *M. grisea* rice pathogens contain a family of dispersed, repeated DNA sequences called MGR (for *Magnaporthe grisea* repeat; [Hamer *et al.* 1989a]). DNA probes derived from MGR sequences hybridize strongly to the DNA of all rice pathogens and weakly to the DNA of pathogens that do not infect rice, a dimorphism that has been observed in worldwide collections of *M. grisea* (Hamer *et al.* 1989a). These data suggest that the copy number of MGR sequences has been highly amplified in the rice pathogens relative to other host-specific forms. The MGR probes have also demonstrated that the rice pathogens are organized into a limited number of genetic lineages (Levy *et al.* 1991). Thus, the rice pathogens of *M. grisea* appear to comprise clonal populations that have become dispersed throughout the world (Hamer *et al.* 1989a; Hamer 1991; Levy *et al.* 1991).

To determine if the conservation of specific repeated DNA in *M. grisea* is a characteristic of host-specific populations, we analyzed repetitive DNA sequences from a form of *M. grisea* that does not infect rice. For this analysis we chose strains that infect the *Eleusine* species *E. indica* (goosegrass) and *E. coracana* (finger millet). Representatives of this group have been used to generate fertile laboratory strains (Valent *et al.* 1986; Valent and Chumley 1991; Valent *et al.* 1991). In an extensive survey, Yaegashi and colleagues demonstrated that among *M. grisea* isolates obtained from 28 genera of host plants, the *Eleusine* pathogens were highly fertile (Yaegashi and

Present address of K. F. Dobinson: Agriculture Canada, London Research Centre, 1400 Western Road, London, Ontario, Canada N6G 2V4.

MPMI Vol. 6, No. 1, 1993, pp. 114-126

Nishihara 1976; Yaegashi 1977). In contrast, isolates infecting rice or other grass hosts were, with minor exceptions, infertile or mated only when paired with isolates from finger millet or goosegrass. The consistently high fertility of the *Eleusine* pathogens suggested that they too may represent a genetically distinct population of *M. grisea*.

We have isolated a repetitive DNA element, designated *grasshopper* (*grh*), from the genome of the *Eleusine* pathogen G-22. This element has features characteristic of retroviral genomes and retrotransposable elements having long terminal repeats (LTRs) and appears to be most closely related to the gypsy family of retrotransposons. We have analyzed a number of *Eleusine* pathogens and other host-specific forms of *M. grisea*, collected from various regions of the world, for the presence of *grh*. Surprisingly, the element was detected in *Eleusine* pathogens obtained from India, Nepal, Japan, and the western African countries of Mali and Burkina Faso but was not present in isolates from other monocot species or from *Eleusine* pathogens from other locations. In contrast, all isolates examined contained various copy numbers of MGR-related sequences. The conservation and distribution of *grh* within a subgroup of *Eleusine* pathogens suggests that *grh* has been recently acquired and provides a striking example of the differential acquisition of a retroelement within one species. We present arguments that the amplification of different families of retroelements within populations of *M. grisea* is a consequence of the clonal organization of this organism.

RESULTS

Structure and genomic distribution of repetitive DNA from the *M. grisea* finger millet pathogen G-22.

To isolate repeated DNA specific to the *Eleusine* pathogen G-22 we used a two-step hybridization strategy to screen a λGEM11 library of genomic DNA from the laboratory strain 4091-5-8 (derived from a cross between G-22 and the weeping lovegrass pathogen G-17). Previous analysis of *M. grisea* has demonstrated that complex genomic DNA probes can be used to identify clones containing sequences represented in multiple copies in the genome (Hamer *et al.* 1989a). We screened approximately 2,000 recombinant bacteriophage by hybridization with ^{32}P-labeled genomic DNA from G-22 and isolated 21 recombinant clones containing repeated DNA. The clones were subsequently screened by hybridization to ^{32}P-labeled genomic DNA from the rice pathogen O-135, to identify clones containing repeated DNA sequences common to both the rice and *Eleusine* pathogen. Fifteen clones hybridized with both O-135 and G-22 DNA and were subsequently shown to contain ribosomal DNA sequences (K. F. Dobinson, J. Romao, and J. E. Hamer, unpublished results). The remaining clones contained repeated DNA sequences specific to G-22. Each of these recombinant phage contained repetitive DNA sequences and adjacent sequences presumed to be single-copy (Fig. 1 and data not shown). The repeated DNA sequences cross-hybridized (data not shown), indicating we had isolated a single type of repetitive DNA element, which we have designated *grasshopper* (*grh*). Restriction maps of four of the clones containing *grh* sequences are shown in Figure 1. Several restriction sites within the repeated DNA, particularly the terminal *Hin*dIII and *Kpn*I sites, were highly conserved between the four clones. λ2 contains two regions of repetitive DNA, oriented as an inverted repeat and separated by approximately 2 kb of single-copy DNA. The left element of λ2 is approximately 8 kb in length and flanked by single-copy sequences. Southern hybridization analysis of genomic DNA probed with restriction fragments subcloned from the 8-kb element demonstrated that the positions of the restriction sites mapped within the element are highly conserved in other genomic copies of *grh* (data not shown), suggesting that the 8-kb sequence represents a full-length element.

In the recombinant lambda clones we characterized, the *grh* sequences appeared to be interspersed with different single-copy sequences. In addition, a subclone of λ2 containing sequences from one end of the 8-kb element

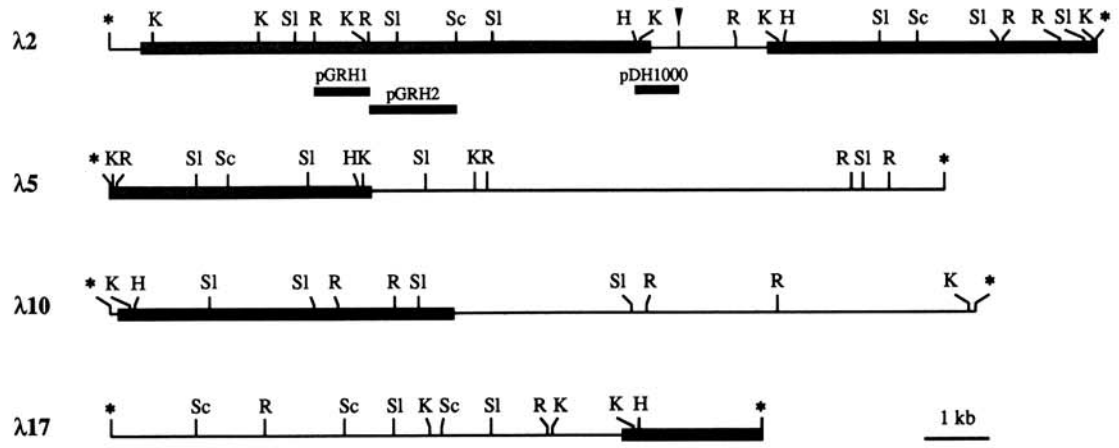

Fig. 1. Restriction maps of recombinant lambda clones containing repetitive DNA specific to the *Magnporthe grisea-Eleusine* pathogen G-22. Single-copy sequences (narrow line) and regions of repetitive DNA (thickened line) in the clones were delimited by hybridization to ^{32}P-labeled genomic DNA. Abbreviations for restriction enzymes are: R, *Eco*RI; H, *Hin*dIII; K, *Kpn*I; S, *Sal*I; Sc, *Sac*I. The *Sac*I sites could not be accurately placed on the map of λ10 and therefore have been omitted. Asterisks denote the cloning sites in λGem11. Hybridization probes used in this study are indicated below the map of λ2; the arrowhead indicates a *Sma*I site used in the construction of plasmid pDH1000.

(pDH1000; see Fig. 1) hybridized to a large number of different-sized restriction fragments of genomic DNA (see below). Taken together, these data suggest that the *grh* elements are dispersed about the genome. To investigate the distribution of *grh* on the chromosomes of G-22 and the laboratory strain 4091-5-8, we hybridized the *grh* subclone pGRH2 to chromosome-sized DNA molecules separated by pulsed-field gel electrophoresis. Previous studies showed that laboratory strains of *M. grisea* have five to six chromosomes, ranging in size from approximately 5–12 Mb (Hamer *et al.* 1989a). Under the electrophoresis conditions used in this study (see Materials and Methods), we were able to resolve four chromosome-sized DNA molecules in both G-22 and the laboratory strain 4091-5-8 (Fig. 2A; partially degraded DNA, of lower mobility than the *Saccharomyces cerivisiae* DNA, is also apparent in lane 1). Figure 2B shows that the *grh* probe hybridized to all resolvable chromosomes, as would be expected for a dispersed, repeated sequence. A ribosomal DNA probe hybridized to only one or two chromosomes (Fig. 2C), demonstrating that the pattern of *grh* hybridization was not an artifact caused by chromosome degradation. From genomic reconstruction experiments, assuming a genome size of 38 Mb (Hamer *et al.* 1989a), we estimate there to be approximately 65 copies of *grh* in the genome of strain 4091-5-8 (K. F. Dobinson and J. E. Hamer, unpublished results).

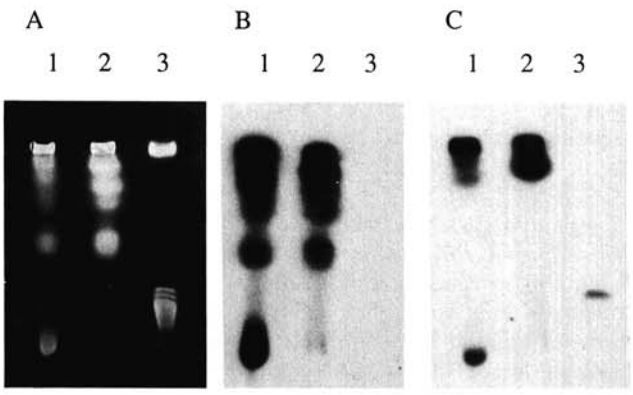

Fig. 2. Hybridization of *grh* sequences to electrophoretically separated chromosomes of *Magnaporthe grisea*. Chromosomal DNA was prepared from strains G-22 and 4091-5-8 and electrophoresed through a 1% agarose gel, as described in Materials and Methods. **A,** Ethidium bromide-stained gel. Lane 1, G-22; lane 2, 4091-5-8; lane 3, *Saccharomyces cerivisiae*. **B,** The gel in A was blotted and hybridized to pGRH2. C, Hybridization with the rDNA probe λrrn8.

grh is an LTR-class retroelement.

Hybridization analysis of the 8-kb element from λ2 indicated that the element was terminally redundant (data not shown), suggesting that *grh* might be related to a family of transposable elements having long terminal repeats. DNA sequencing confirmed that the element terminates in 198 base pair (bp) perfect direct repeats (Fig. 3A). The direct repeats contain the 5′ and 3′ terminal sequences (5′ TG...CA 3′) and short, inverted terminal repeats that are hallmarks of retroviral and most retrotransposon LTRs (see Varmus and Brown 1989; Bingham and Zachar 1989 for review of LTR features). In addition, sequencing of the single-copy DNA adjacent to the 8-kb element showed that the LTRs are flanked by a short, direct repeat (ATAAA; see Fig. 3B), as is characteristic of a target site duplication generated during transposition.

B. Left LTR

atttaaaattaaactttggagtttataaattttaaacaatataaa**TGTTA**CGGATTCGTGCTGATTGCACGTATCCCCG
TATATAGATAGCAGCGGGGACCACGTGATAAGTTGCATTGTATATAAGGGAGGAAGGGTCGCCAAGACCTTTTCCGCACC
CCTTTCTTCTCCTTTTCCTTAGCGATAATAATAACTCCTTTCGGGTACCCAACCGTTGTATGATCGTTGGCCTACCCTAT
AACA*tttattatcgctcattattcttc*

Right LTR

*ggtaggggaggaaggggggcta***TGTTA**CGGATTCGTGCTGATTGCACGTATCCCCGTATATAGATAGCAGCGGGGACCAC
GTGATAAGTTGCATTGTATATAAGGGAGGAAGGGTCGCCAAGACCTTTTCCGCACCCCTTTCTTCTCCTTTTCCTTAGCG
ATAATAATAACTCCTTTCGGGTACCCAACCGTTGTATGATCGTTGGCCTACCCTA**TAACA**ataaaatat

Fig. 3. Restriction map of *grh* and sequences at the 5′ and 3′ termini of the element. **A,** Restriction map of the 8-kb *grh* element from λ2. The direct terminal repeats (darkened areas at each end) and the 3.3-kb internal region that was sequenced (black band in center) are indicated. Abbreviations for restriction enzymes are as for Figure 1. **B,** Nucleotide sequence of the 198-bp long terminal repeats (LTRs) from the element shown in A. The LTR sequences are capitalized, with the 5-bp terminal inverted repeats in bold type and the 5′ TG...CA 3′ nucleotides characteristic of retroviral LTR termini underlined. A potential transcriptional initiation signal (*ct* box) in the left LTR and polyadenylation signal in the right LTR are underlined. Single-copy sequences flanking the LTRs are in lowercase plain type; arrows indicate the 5-bp direct repeat at the borders of the left and right LTR. Sequences within *grh* are italicized, with the polypurine tract immediately upstream of the right LTR underlined.

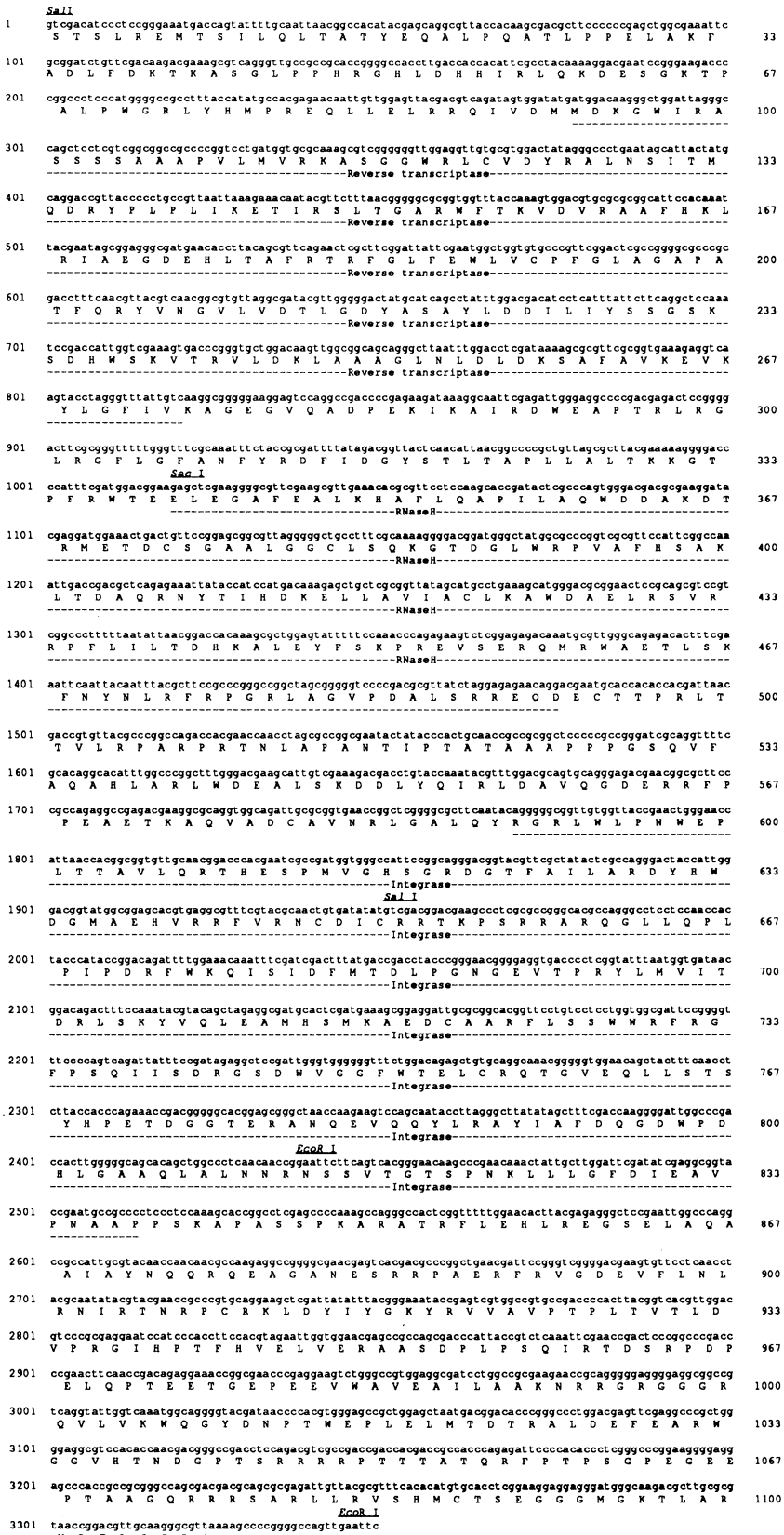

Fig. 4. Nucleotide sequence of a 3.3-kb region from the *grh* element shown in Figure 3. The amino acid sequence of the open reading frame is shown below the DNA sequence in the one-letter amino acid code. The asterisk denotes the termination codon. Regions with similarity to the reverse transcriptase, integrase, and RNaseH domains of LTR retroelement *pol* polyproteins are indicated.

We have identified several conserved sequences in the element, including potential transcriptional initiation and polyadenylation signals within the LTR. In particular, the LTR has a 25-nucleotide C+T-rich region (underlined in Fig. 3B) that strongly resembles the *ct* box immediately upstream of the transcription initiation site of the *Aspergillus nidulans gpdA* gene promoter (Punt *et al.* 1990). Similar C+T-rich sequences have been identified in the promoter regions of other genes from filamentous fungi (see Ballance 1986; Gurr *et al.* 1988 for review). Deletion of these sequences results in decreased gene expression and abnormal transcription initiation, indicating that the *ct* box may determine the transcription start point (Ballance 1986; Hamer and Timberlake 1987; Gurr *et al.* 1988; Punt *et al.* 1990).

Two other sequences that are conserved in retroviruses and can be found in many LTR retrotransposons are the primer-binding sites for reverse transcription (Varmus and Brown 1989). A potential tRNA primer-binding site for minus-strand DNA synthesis, located immediately 3′ to the 5′ LTR of most *Drosophila* retrotransposons (Bingham and Zachar 1989) and characterized by a highly conserved 5′ TGG, could not be identified in the *grh* sequence. However, a polypurine-rich sequence, that could correspond to the primer-binding site for plus-strand DNA synthesis, is located immediately upstream of the *grh* 3′ LTR. The conservation of characteristic LTR sequences, and the fact that the two LTRs we have sequenced are identical implies that the LTRs of the 8-kb element are intact and could be functional. In addition, the presence of the target site duplication suggests that multiple copies of *grh* have accumulated via transposition.

Figure 4 shows the DNA sequence of a 3.3-kb internal region of *grh*. Computer analysis identified only one extensive open reading frame (ORF), of 1,107 amino acids. Three regions of the ORF have similarities to polyprotein sequences encoded by *pol* genes from *Drosophila* retrotransposons (Toh *et al.* 1985; Marlor *et al.* 1986; Yuki *et al.* 1986; Lankenau *et al.* 1988), the *del* element of lily (Smyth *et al.* 1989), the recently described *Cf*T1 element from the filamentous fungus *Cladosporium fulvum* (McHale *et al.* 1992) and the *Schizosaccharomyces pombe* element Tf1 (Levin *et al.* 1990). The *grh* sequences have most similarity to the putative reverse transcriptase, RNaseH and integrase domains from *Cf*T-1 and Tf1. Figure 5 shows the alignment of the *grh* sequences with the corresponding sequences from the *Cf*T-1 and Tf1 polyproteins. A 182-amino acid (aa) region of the *grh* ORF contains the seven domains characteristic of reverse transcriptase sequences (Xiong and Eickbush 1988; Xiong and Eickbush 1990) and the highly conserved YXDD sequence that has been proposed to be part of the reverse transcriptase active site (Berg and Howe 1989). Downstream from the putative reverse transcriptase is a 150-aa sequence homologous to the RNaseH domains of *Cf*T-1 and Tf1. This sequence has 39% identity and 57% overall similarity with the Tf1 sequence and contains amino acid residues that are essentially invariant in the RNaseH sequences of other retroelements (Doolittle *et al.* 1989). The putative integrase (endonuclease) region is a 250-aa sequence that shares 30% identity with the integrase

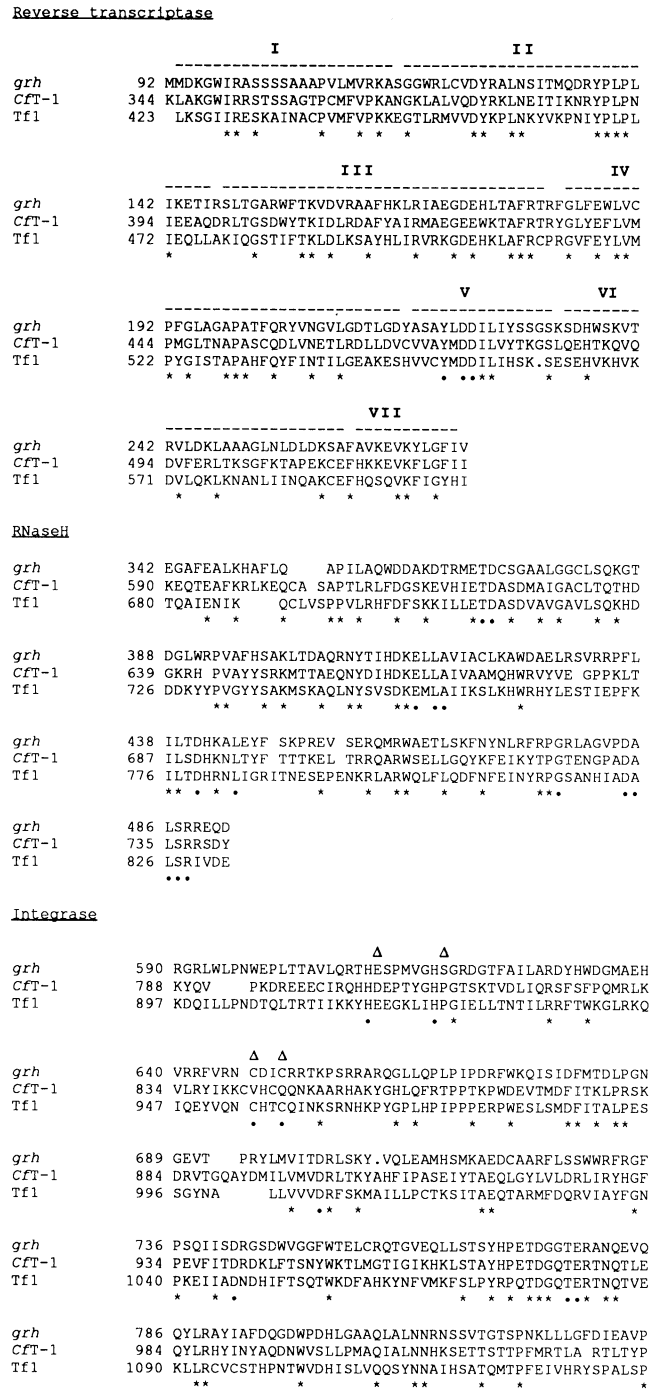

Fig. 5. Amino acid alignment (single-letter amino acid code) of putative reverse transcriptase, RNaseH, and integrase domains from *grh*, with homologous sequences from the *Schizosaccharomyces pombe* element Tf1 (Levin *et al.* 1990) and the *Cf*t-1 element from *Cladosporium fulvum* (McHale *et al.* 1992). Amino acids common to *grh*, *Cf*t-1, and Tf1 are indicated below the Tf1 sequence with an asterisk. Bullets denote amino acids that are highly conserved among *grh* and other gypsy family retrotransposons (Johnson *et al.* 1986; Doolittle *et al.* 1989). Conserved domains within the reverse transcriptase are indicated. Amino acid residues corresponding to the conserved zinc-binding motif in the integrase domain (Johnson *et al.* 1986) are indicated by triangles.

sequence from Tf1 (see Fig. 5). Including conservative amino acid changes, the similarity of the two sequences is 50%. All of the highly conserved amino acids of the gypsy family integrase proteins, including the putative zinc-binding domain proposed to function in binding the integrase to the DNA substrate (Johnson *et al.* 1986), are also present in the *grh* sequence. The similarity of the *grh* sequences to domains of the putative polyproteins from *Cf*T-1 and Tf1 is comparable to the level of similarity between other gypsylike retrotransposons (see Doolittle *et al.* 1989 for review) and strongly suggests that *grh* is a member of this family.

The presence of a single, uninterrupted reading frame encoding putative reverse transcriptase, RNaseH, and integrase proteins suggests that the 8-kb *grh* element of λ2 could produce functional *pol* proteins. The cloned element in λ2 is also of sufficient size to encode a *gag* gene 5′ to the *pol* sequences and, like the *Drosophila* retrotransposons gypsy, 297 and 17.6, could encode another polypeptide 3′ to the *pol* region (see Varmus and Brown 1989 for review). Further sequencing and characterization of the 8-kb *grh* element are in progress to determine the relationship of *grh* to other fungal retroelements and to examine the overall protein coding capacity of the element.

Distribution of *grh* in *Eleusine* pathogens of *M. grisea*.

The *grh* elements of the *Eleusine* pathogen G-22 were first identified as DNA sequences that were present in multiple copies in the G-22 genome but that were not repeated in the genome of the rice pathogen O-135. However, it was not clear if the elements were absent from or simply present in low copy number in the genomes of rice pathogenic strains and other forms of *M. grisea*. To further investigate the extent to which *grh* sequences are conserved in *M. grisea*, we compared the distribution of MGR and *grh* sequences in various host-specific forms of the fungus.

Although the rice pathogen-specific repeated DNA is collectively designated MGR (Hamer *et al.* 1989a), several distinct MGR elements have recently been identified. One MGR sequence encodes a putative protein similar to the reverse transcriptases of the poly(A) retrotransposable elements known as long interspersed nuclear elements (LINEs; Valent and Chumley 1991). Two MGR-LINE specific probes, pCB583 and pCB607, have been used in the experiments described below. Figure 6A shows the hybridization of pCB583 to genomic DNA from various host-specific forms of *M. grisea*. This probe detected conserved restriction fragments of 2.0 and 1.1 kb in all *M. grisea* isolates, although hybridization to DNA from the rice pathogens is typically more intense (Hamer *et al.* 1989a). Hybridization to the DNA of the weeping lovegrass pathogen G-17 (lane 5) was more apparent upon longer exposures of the blots (data not shown).

*Sal*I restriction digests were prepared from the same set of genomic DNAs and hybridized with the *grh*-specific probe pGRH1. Figure 6B shows that pGRH1 detected a conserved 1.7-kb restriction fragment (corresponding to a *Sal*I fragment spanning the probe; see Fig. 1) in pathogens of finger millet and goosegrass (*E. coracana* and *E. indica*; lanes 4, 9, and 10). Neither pGRH1 nor other *grh* sequences hybridized detectably to genomic DNA of other host-specific forms of *M. grisea*, even upon prolonged exposures of the blots (Fig. 6B). Nine additional isolates from our collection (six rice pathogens, two pearl millet pathogens, and one isolate obtained from the weed *Rotboellia exal-*

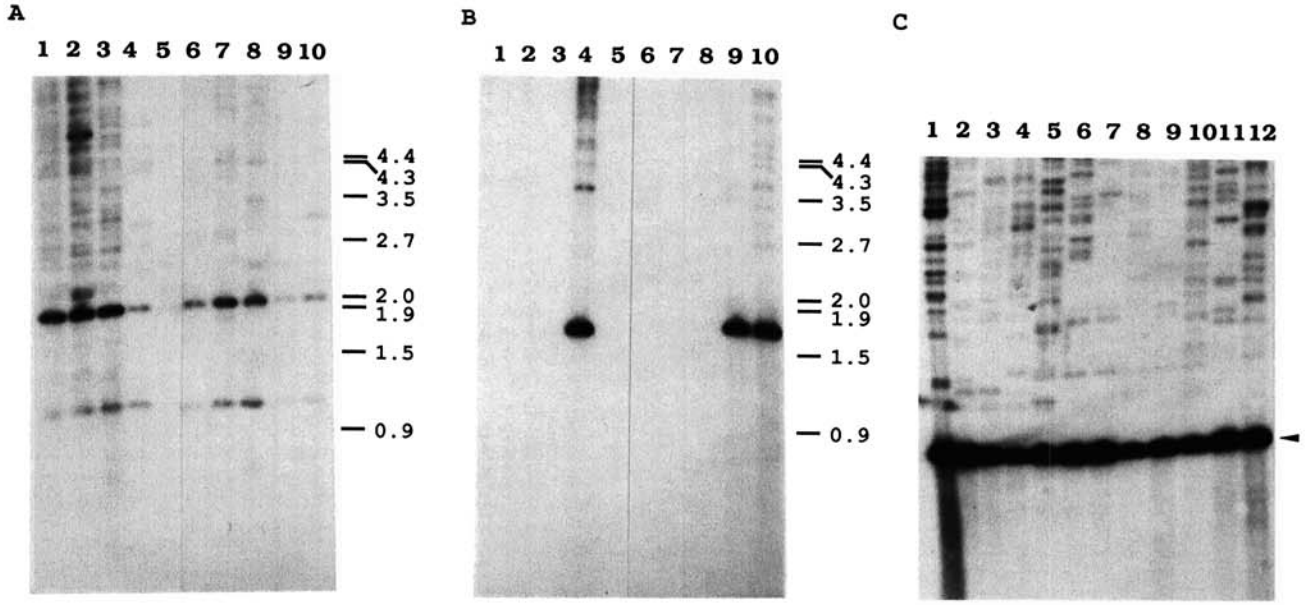

Fig. 6. Southern blot analysis of genomic DNA from *Magnaporthe grisea* field isolates. The origins of the field isolates are described in Table 1. A, Genomic DNA was digested with *Bam*HI and hybridized with the MGR probe pCB583. The lanes contain DNA from: 1, VO-4; 2, VO-110; 3, O-137; 4, G-22; 5, G-17; 6, PM-1; 7, PM-3; 8, VO-107; 9, G-50; 10, G-51. **B,** Genomic DNA was digested with *Sal*I and hybridized with pGRH1. The lanes are loaded as in A. Molecular size standards (kb) are indicated to the right of A and B. **C,** Genomic DNA was digested and hybridized as in B. The lanes contain DNA from: 1, G-22; 2, G-13; 3, G-14; 4, G-18; 5, G-19; 6, G-20; 7, G-21; 8, G-23; 9, G-50; 10, G-15; 11, G-26; 12, G-51. The conserved 1.7-kb *Sal*I fragment of *grh* is indicated by an arrowhead.

tata), were tested and also found to be lacking the *grh* element (data not shown).

We have analyzed a collection of *Eleusine* pathogens from Japan and, as shown in Figure 6C, all contained sequences homologous to *grh*. It was possible that the distribution of strains bearing *grh* could be geographically limited. Alternatively, the element could have accumulated in an ancestral population of *Eleusine* pathogens that has since been dispersed throughout the world. To distinguish between these possibilities, we analyzed a collection of *Eleusine* pathogens from other geographic locations (see Table 1 for origins of strains). Genomic DNA from these isolates was digested with *Bam*HI or *Sal*I, blotted, and hybridized to the MGR-LINE probe pCB607 or the *grh*-

Table 1. *Magnaporthe grisea* field isolates

Isolate	Host species	Origin	
O-135	*Oryza sativa* (rice)	Taiwan	1985[a]
O-137	*O. sativa*	Taiwan	1985[a]
VO-4	*O. sativa*	Louisiana, U.S.A.	1964[b]
VO-110	*O. sativa*	Texas, U.S.A.	1965[b]
VO-107	*Digitaria sanguinalis* (crabgrass)	Texas	1981[b]
G-13	*Eleusine coracana* (finger millet)	Japan	1975[c]
G-14	*E. coracana*	Japan	1975[c]
G-15	*E. indica* (goosegrass)	Japan	1975[c]
G-17	*Eragrostis curvula* (weeping lovegrass)	Japan	1976[c]
G-18	*E. coracana*	Japan	1975[c]
G-19	*E. coracana*	Japan	1975[c]
G-20*	*E. coracana*, *E. indica*	Japan	1977[c]
G-21	*E. coracana*	Japan	1977[c]
G-22*[d]	*E. coracana*	Japan	1977[c]
G-23	*E. coracana*	Japan	1977[c]
G-26	*E. indica*	Japan	1982[c]
G-50*	*E. coracana*	Japan	1982[c]
G-51	*E. indica*	Japan	1990[e]
PM-1	*Pennisetum americanum* (pearl millet)	Georgia, U.S.A.	1990
PM-3	*P. americanum*	Georgia	1990
BF-15*	*E. indica*	Burkina Faso	1990[f]
BR-62*	*E. indica*	Brazil	1991[f]
BR-76*	*E. indica*	Brazil	1991[f]
CD-155*	*E. indica*	Ivory Coast	1989[f]
GD-1*	*E. indica*	Guadeloupe	1991[f]
IN-1	*E. coracana*	India	1985[f]
MD-110*	*E. indica*	Madagascar	1990[f]
ML-27*	*E. indica*	Mali	1990[f]
NP-7*	*E. coracana*	Nepal	1985[f]
OG-1*	*E. coracana*	Uganda	1985[f]
OG-2	*E. coracana*	Uganda	1985[f]
OG-3	*E. coracana*	Uganda	1985[f]
OG-4*	*E. coracana*	Uganda	1985[f]
OG-5	*E. coracana*	Uganda	1985[f]
OG-6	*E. coracana*	Uganda	1983[f]
PH-37*	*E. indica*	Philippines	1983[f]
PH-42	*E. indica*	Philippines	1983[f]
PH-43	*E. indica*	Philippines	1990[f]
PH-44	*E. indica*	Philippines	1990[f]
PH-48*	*E. indica*	Philippines	1990[f]
PH-57	*E. indica*	Philippines	1990[f]
Ph-58	*E. indica*	Philippines	1990[f]
RW-18*	*E. coracana*	Rwanda	1990[f]
RW-20*	*E. indica*	Rwanda	1990[f]

[a] Isolates provided by B. Valent and F. Chumley, DuPont, Wilmington, DE.
[b] M.A. Marchetti (USDA, Beaumont, TX).
[c] Isolates collected by H. Yaegashi.
[d] Asterisk indicates that host specificities were confirmed by inoculating goosegrass seedings with between 0.8–5.0 × 10^5 conidia/ml, essentially as described (Heath *et al.* 1990; Valent *et al.* 1991).
[e] J. Wilson, USDA, Tifton, GA.
[f] J.-L. Notteghem, CIRAD, France.

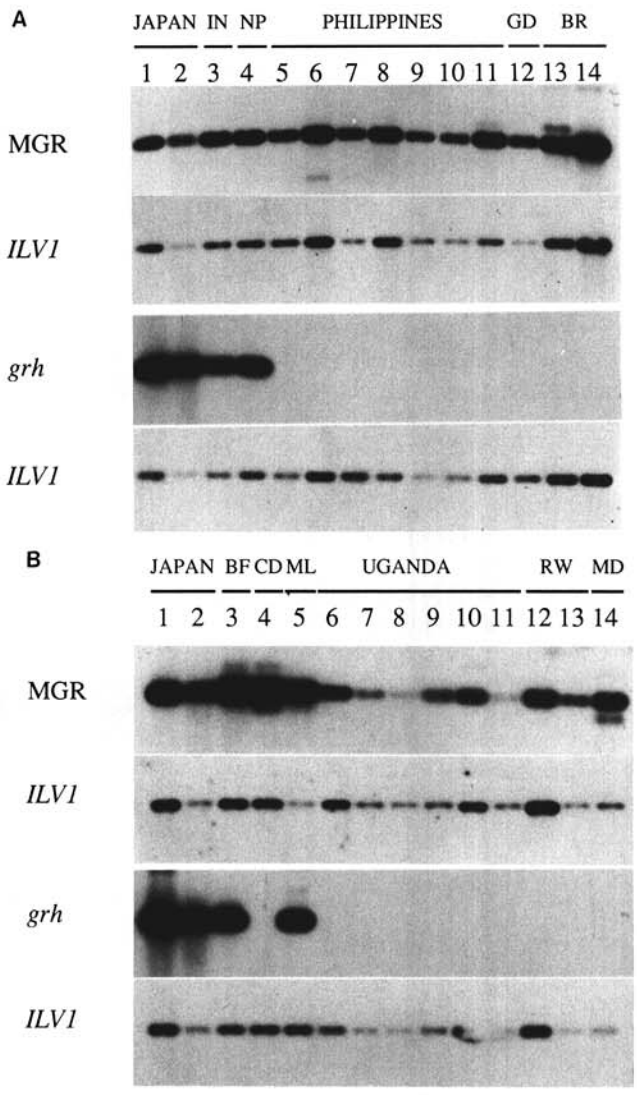

Fig. 7. Southern blot analysis of genomic DNA from *Magnaporthe grisea-Eleusine* pathogens. Genomic DNA was digested and hybridized with MGR or *grh* probes as described for Figure 6, except that the MGR-LINE element probe was pCB607. After hybridization, the probes were stripped from the blots and each blot was hybridized with the *ILV1*-specific probe pCB573. The MGR and *grh* probes hybridize to 2-kb *Bam*HI and 1.7-kb *Sal*I fragments, respectively. The *ILV1* probe hybridizes to a 9.5-kb *Bam*HI fragment and a 6.4-kb *Sal*I fragment. Countries from which isolates were obtained are indicated above the lanes. Abbreviations for the countries are as follows: BF, Burkina Faso; BR, Brazil; CD, Ivory Coast; GD, Guadeloupe; IN, India; MD, Madagascar; ML, Mali; NP, Nepal; RW, Rwanda. **A,** Lanes: 1, G-22; 2, G-50; 3, IN-1; 4, NP-7; 5, PH-37; 6, PH-42; 7, PH-43; 8, PH-44; 9, PH-48; 10, PH-57; 11, PH-58; 12, GD-1; 13, BR-62; 14, BR-76. **B,** Lanes: 1, G-22; 2, G-50; 3, BF-15; 4, CD-155; 5, ML-27; 6, OG-1; 7, OG-2; 8, OG-3; 9, OG-4; 10, OG-5; 11, OG-6; 12, RW-18; 13, RW-20; 14, MD-110. Blots were exposed for the following lengths of time: MGR, 72 hr; *grh* 24 hr; *ILV1*, 96 hr. Due to different specific activities of the hybridization probes and the different lengths of time for which the blots were exposed, only the relative amounts of repeated DNA in different isolates can be compared.

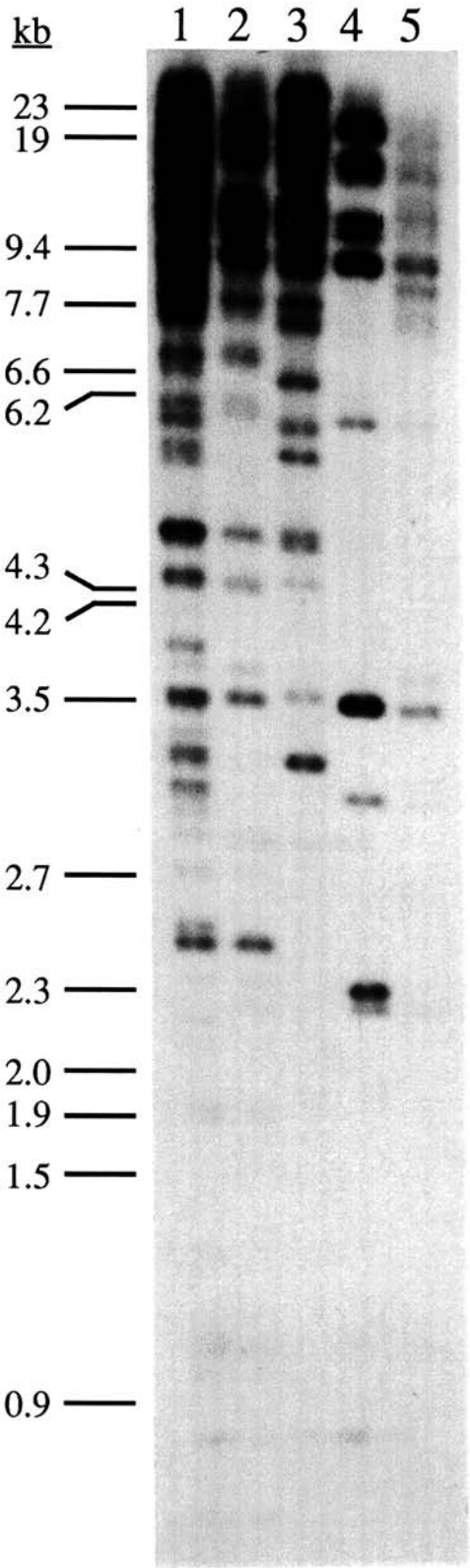

Fig. 8. Southern blot analysis of genomic DNA from *Magnaporthe grisea* laboratory strains. All strains are derived from crosses involving G-22 (see Table 1). Laboratory strains 4091-5-8 and 4136-4-3 are pathogens of weeping lovegrass. Strains 4360-R-12 and 4375-R-26 are pathogens of rice. Genomic DNA was digested with *Hin*dIII and hybridized with the plasmid pDH1000. Lane 1, G-22; lane 2, 4091-5-8; lane 3, 4136-4-3; lane 4, 4360-R-12; lane 5, 4375-R-26.

specific probe pGRH1, respectively. To control for the relative amounts of DNA loaded in each lane, each blot was subsequently stripped of the MGR and *grh* probes and rehybridized with a single-copy gene probe (*ILV1*).

Figure 7A shows the hybridization of pGRH1 and the MGR probe pCB607 to *Eleusine* pathogens from Asia, the West Indies, and South America. Two *Eleusine* pathogens from Japan were also included as positive controls (lanes 1 and 2). The MGR probe detected a 2-kb *Bam*HI restriction fragment in all isolates. Apparent differences in the LINE copy number appeared to be due to differences in the amount of DNA in each lane, as demonstrated by hybridization to *ILV1*. Surprisingly, *grh* sequences were detectable only in the isolates from Japan, Nepal, and India, even after prolonged exposure of the blots. The hybridization of MGR, *grh* and *ILV1* probes to genomic DNA of *Eleusine* pathogens from various African countries is shown in Figure 7B. Again, all isolates contained MGR homologs, although the copy number appeared to vary considerably between isolates (compare *ILV1* and MGR hybridization in lanes 5 and 6, for example). In contrast, the *grh* sequences were detected only in the isolates obtained from the western African countries of Mali and Burkina Faso. Of the 36 *Eleusine* pathogens analyzed in this study, 16 carried *grh* sequences. Thus, on the basis of presence or absence of *grh*, the *Eleusine* pathogens can be divided into at least two genetically distinct subgroups.

Distribution of *grh* sequences in laboratory strains of *M. grisea*.

The *Eleusine* pathogen G-22 has been interbred with weakly fertile rice pathogens in an effort to produce laboratory strains that are pathogenic to rice and sufficiently fertile for genetic studies of pathogenicity and cultivar specificity (Valent *et al.* 1986, 1991; Valent and Chumley 1991). In addition, laboratory strains that are pathogenic to other grasses have also been derived from interbreeding with G-22 (Valent *et al.* 1986; Valent and Chumley 1987). Although all of the laboratory strains contain a number of copies of *grh*, the rice pathogens (Fig. 8, lanes 4 and 5) are more distantly related to G-22 and thus contain fewer copies of the element. It has been demonstrated that at least some genes that determine rice cultivar specificity in these strains must have been derived from the non-rice pathogen parent (Yaegashi and Asaga 1981; Valent and Chumley 1991; Valent *et al.* 1991). *grh* sequences may thus provide convenient genetic landmarks for the mapping and subsequent cloning of these cultivar specificity genes. The presence of *grh* in laboratory strains further demonstrates that these strains differ in genetic makeup from field isolates in that they contain composite genomes derived from genetically distinct forms of *M. grisea*.

Restriction fragment length polymorphisms associated with families of transposable elements have been interpreted as evidence for the presence of mobile elements, in a variety of organisms (Adams and Oeller 1986; Marschalek *et al.* 1989; Moerman and Waterson 1989; Wyman and Blackburn 1991). Similarly, we have detected *grh* associated RFLPs in clonally related isolates of *M. grisea*. We surveyed a number of strains derived from 4091-5-8 by UV mutagenesis or spontaneous mutation (see Table

2), for changes in the array of *grh* sequences. Genomic DNA was digested with *Hin*dIII and hybridized with an LTR-specific probe, to generate a DNA fingerprint for each strain. Figure 9 shows the *grh* fingerprints of three auxotrophic mutants, a melanin biosynthesis mutant, and four morphological mutants. The fingerprints of the mutants were highly similar but not identical to the 4091-5-8 fingerprint. With the exception of the *his* mutant strain CP502 (lane 5), at least one polymorphism was detected in each mutant.

In addition to individual differences between the mutant strains and 4091-5-8, a 2.2-kb band in strain 4091-5-8 was absent in all of the mutants and a 1.7-kb band in 4091-5-8 and the *smo* mutants was absent from the other strains. It seemed unlikely that these sequences would have been systematically lost or altered in the mutants. Additional fingerprint analysis demonstrated that the 2.2- and 1.7-kb bands were not present in genomic DNA prepared from original stocks of strain 4091-5-8 (data not shown), suggesting that these polymorphisms were acquired during the recent clonal propagation of 4091-5-8. In contrast, MGR polymorphisms are rarely observed in clonally propagated laboratory strains (J. E. Hamer, unpublished results). The polymorphisms we have detected could have been generated by transposition or by genetic rearrangement of grh sequences. The availability of *M. grisea* strains lacking *grh* provides the opportunity to identify by functional studies an active element that subsequently could be modified for gene tagging purposes.

DISCUSSION

We have identified in the genome of *M. grisea,* a retroelement that is related to the gypsy class of LTR-containing transposons and that could potentially be used to enhance genetic and molecular approaches to studying fungal-plant interactions in *M. grisea*. Repeated DNA sequences in *M. grisea* have proven useful for genetic mapping. The MGR sequences common to rice pathogens have been used to map pathogenicity genes (Hamer and Givan 1990; Orbach *et al.* 1991; Valent *et al.* 1991), construct a genetic map for *M. grisea* and develop marked strains for mapping new mutations (Romao and Hamer 1992). Given the distribution of *grh* in fertile laboratory strains it is obvious that *grh* could be used for similar purposes. In addition, promoters from *M. grisea* genes have not been characterized and thus transformation vectors currently rely on *Neurospora* and *Aspergillus* promoters (Parsons *et al.* 1987; Parsons 1988; Leung *et al.* 1990). The LTRs from *grh* are a convenient source of transcription initiation signals that could potentially enhance the expression of drug resistance genes on trans-

Table 2. Laboratory strains of *Magnaporthe grisea*

Strain (genotype)[a]	Reference or source
4091-5-8	Valent *et al.* 1986
4136-4-3	Valent and Chumley 1987
4360-R-12	Valent and Chumley 1991
4375-R-26	Valent and Chumley 1991
CP270 (*ilv3*)	F. G. Chumley and B. Valent[b]
CP435 (*ilv3*)	F. G. Chumley and B. Valent
CP485 (*rsyl-1*)	Chumley and Valent 1990
CP502 (*his8*)	F. G. Chumley and B. Valent
CP777 (*smo1-4*)	Hammer *et al.* 1989
CP778 (*smo1-5*)	Hammer *et al.* 1989
CP785 (*smo1-9*)	Hammer *et al.* 1989
CP787 (*smo1-10*)	Hammer *et al.* 1989

[a] All strains designated CP, except CP785, were derived from 4091-5-8 by UV mutagenesis. CP785 was a spontaneous mutant. Strains 4360-R-12 and 4375-R-26 are pathogenic on rice.
[b] Dupont, Wilmington, DE.

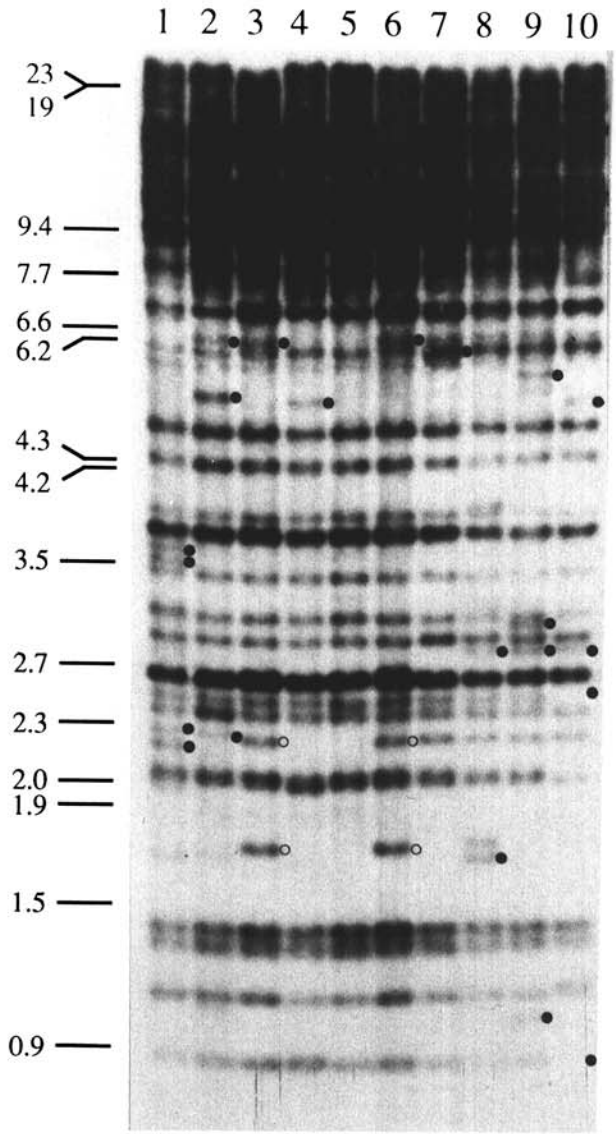

Fig. 9. Southern blot analysis of genomic DNA from mutant strains derived from *Magnaporthe grisea* laboratory strain 4091-5-8. Genomic DNA was digested with *Hin*dIII and hybridized with the LTR-specific probe pDH1000. The lanes are as follows: 1, CP270 (*ilv* mutant); 2, CP435 (*ilv* mutant); 3, 4091-5-8; 4, CP485 (*rsy* mutant); 5, CP502 (*his* mutant); 6, 4091-5-8; 7, CP777 (*smo* mutant); 8, CP778 (*smo* mutant); 9, CP785 (*smo* mutant); 10, CP787 (*smo* mutant). Open circles indicate the restriction fragment polymorphisms acquired during propagation of 4091-4-8. Closed circles indicate individual polymorphisms between 4091-5-8 and each of the mutants. Identical restriction fragments were generated in three separate experiments, using a single DNA preparation from each strain.

forming plasmids and thus improve transformation efficencies.

LTR-class retroelements that have been previously identified in fungi include the well-characterized *S. cerevisiae* Ty elements (reviewed in [Boeke 1989]), the Tf families of elements from *S. pombe* (Levin *et al.* 1990) and the *Cf*t-1 element from the tomato pathogen *C. fulvum* (McHale *et al.* 1992). In each of these fungi the associated retroelements are widely distributed in the strains examined thus far, although the copy number of the elements in different strains can vary substantially. Similarly, the MGR sequences of *M. grisea*, although amplified in rice pathogens, can be detected in isolates that infect other host species (Hamer *et al.* 1989a; see also Figs. 6 and 7). In contrast, we observed an exclusive distribution of *grh* in a subgroup of *M. grisea-Eleusine* pathogens. These results suggest that *grh* was acquired more recently than MGR sequences. Our current hypothesis is that the element was acquired by an interspecific horizontal transfer event, subsequent to the emergence of this particular host-specific form of *M. grisea*.

The distribution of *Eleusine* pathogens harboring *grh* is unusual. An analysis of *Eleusine* pathogens from geographically diverse regions demonstrated the presence of *grh* in isolates collected from Japan, India, Nepal, and the west African countries of Bukina Faso and Mali, while other isolates lacked the element. It would seem unlikely that the introduction of *grh* into the *Eleusine* pathogens occurred more than once in several geographical locations, or that all copies of the element have been systematically lost from some *Eleusine* pathogens. The most straightforward explanation is that isolates that contain *grh* are members of a clonal population that has been dispersed throughout the aforementioned countries. We speculate that this dispersal occurred with the transport of finger millet germplasm between these countries, in much the same manner that the *M. grisea* rice pathogens have become dispersed throughout the rice-growing regions of the world (Levy *et al.* 1991). Broader sampling of the *grh* containing and *grh*-deficient groups of *Eleusine* pathogens will be needed to determine if these populations coexist or are strictly geographically limited.

By many criteria the host-specific forms of *M. grisea* constitute a single species. At the microscopic level they are morphologically indistinguishable (Rossman *et al.* 1990). Although culture morphology of field isolates can be highly variable, these differences are not correlated with host specificity and likely reflect subtle physiological differences between isolates. An exhaustive analysis of isozyme polymorphisms also failed to reveal significant differences between isolates that infect different hosts (Leung and Williams 1986). In light of our findings, an important feature of the repetitive elements of *M. grisea* is that they provide insight into the evolution of the host-specific forms of this fungus. For example, on the basis of having amplified copies of MGR, the rice pathogens have been shown to represent a genetically distinct group descendent from a single ancestral founder population (Hamer *et al.* 1989a). MGR probes have also demonstrated the clonal organization of these rice-infecting populations (Levy *et al.* 1991). Our study demonstrates that at least one other population of *M. grisea* has recently acquired a retrotransposable element. A summary of the distribution of known repeated DNA elements in *M. grisea* is shown in Figure 10. Given the relative ease with which repeated sequences were identified in rice and finger millet pathogens, it seems likely that other repeated DNA exists in *M. grisea* and may mark different populations and/or host-specific forms.

The amplification in *M. grisea* of specific retroelements and the acquisition of novel elements is reminiscent of the patterns of genome evolution observed in animals. For example, the amplification of specific retroelements has been documented in members of the order Salmonidae (Kido *et al.* 1991). Similarly, LINE elements found in extant murine species appear to have arisen by amplification of an ancestral LINE homolog at or near the time of murine species radiation (Pascale *et al.* 1990). It has been suggested that the accumulation of different elements within populations may result in changes in genome organization and structure that are prerequisites for the emergence of new species. Despite the differences we have observed in the distribution of retroelements within populations of *M. grisea*, the intrusion and amplification of different families of elements in the genome of this organism have not resulted in the evolution of separate species.

In nature, the predominant mode of reproduction in *M. grisea* appears to be asexual. Two observations support this hypothesis: First, many field isolates are infertile; second, the sexual stage has not been observed in the field. In sexually reproducing eukaryotes, intraspecific genomic differentiation is difficult to document. In a clonal eukaryotic organism like *M. grisea*, in which genetic exchange is likely to be minimal or nonexistent, changes in genome structure, such as the acquisition and amplification of different transposable elements, would persist and be observed only within descendant clones. Recently it has been suggested that many eukaryotic microorganisms, including some known to have functional sexual stages, may exist in nature as clonal populations (Tibayrenc *et al.* 1991). Thus, we speculate that population-specific repeated elements, which have not been documented in sexually reproducing eukaryotes, may be a common feature of other clonal organisms.

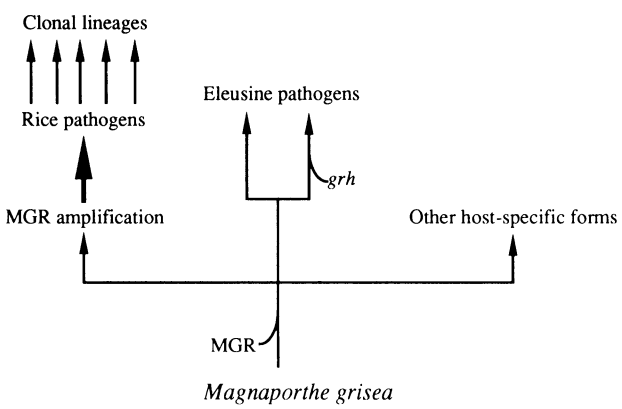

Fig. 10. Proposed clonal history of host-specific forms of *Magnaporthe grisea*, based on the distribution and amplification of the dispersed repeated sequences MGR and *grh*.

MATERIALS AND METHODS

Strains and recombinant DNA.

The origins of *M. grisea* field isolates used in this study are shown in Table 1. The analysis included 36 *Eleusine* pathogens (collected from 12 countries between 1975 and 1991), four rice pathogens, two isolates from pearl millet, and one isolate each from crabgrass and weeping lovegrass. Strain 4091-5-8 is a progeny from a cross between two field isolates, G-22 (WGG-FA40) and G-17 (K76-79). Procedures and media for growing *M. grisea* axenically have been described previously (Crawford *et al.* 1986).

Plasmid pCB583 carries a 5-kb *Sal*I fragment of *M. grisea* repetitive DNA, cloned into the vector pUC18. The *Sal*I fragment hybridizes to a highly conserved 2-kb *Bam*HI fragment in the genomes of *M. grisea* rice pathogens and contains sequences encoding a putative LINE-like reverse transcriptase (Hamer *et al.* 1989a; Valent and Chumley 1991). Plasmid pCB607 contains the conserved 2-kb *Bam*HI fragment, cloned into pUC18 (Hamer *et al.* 1989a). λrrn8 contains *M. grisea* ribosomal DNA sequences, cloned into λGEM11 (K. F. Dobinson, J. Romao, and J. E. Hamer, unpublished results). Plasmids pGRH1, pGRH2, and pDH1000 contain restriction fragments from λ2 (see Fig. 1), cloned into the phagemid vector pBluescript II KS+ (Stratagene, Inc., La Jolla, CA). Plasmid pCB573, obtained from F. G. Chumley and B. Valent (DuPont, Wilmington, DE), contains a 6.4-kb *Sal*I fragment from the *M. grisea ILV1* gene, cloned into pBR322. *Escherichia coli* strain KW251 (Promega Corporation, Madison, WI) was used for propagation of bacteriophage lambda; strains DH5α (BRL Life Technologies, Inc., Gaithersburg, MD) and XL1-Blue (Stratagene, Inc., La Jolla, CA) were used for propagation of plasmids.

Construction and screening of *M. grisea* genomic DNA library.

Genomic DNA was isolated from purified nuclei of *M. grisea* strain 4091-5-8, as previously described (Hamer *et al.* 1989a). The DNA was partially digested with the restriction enzyme *Sau*3A1 and cloned into λGEM11 *Xho*I half-site arms (Promega Corporation), using procedures suggested by the supplier. The library was screened by plaque hybridization with ^{32}P-labeled genomic DNA as described previously (Hamer *et al.* 1989a). Hybridization probes were labeled by the random primer method (Feinberg and Vogelstein 1983) using the Stratagene Prime-It kit. Hybridizations were done at 65 C in 6× SSPE (1× SSPE is 0.18 M NaCl, 10 mM NaH_2PO_4, pH 7.4, 1 mM EDTA), 5× Denhardt's buffer, 0.5% sodium dodecyl sulfate (SDS). Blots were washed at 65 C, with the final wash in 0.2× SSPE, 0.1% SDS, 0.1% sodium pyrophosphate.

Nucleic acid analysis.

Genomic DNA was isolated from *M. grisea* protoplasts by the procedure of Sweigard *et al.* (1990) with modifications previously described (Hamer and Givan 1990), or from frozen mycelia using a CTAB (hexadecyltrimethylammonium bromide) lysis procedure (K. Zeller and M. Levy, Purdue University) modified from Murray and Thompson (1980) and Zolan and Pukkila (1986). Briefly, the mycelia were frozen in liquid nitrogen and ground to a fine powder with a mortar and pestle. The powder was suspended in 4 ml of 65° C CTAB lysis buffer (2% CTAB [Sigma Chemical Co.], 0.1 M Trizma base, 10 mM EDTA, 0.7 M NaCl) with 1% 2-mercaptoethanol, incubated at 65° C for 30 min and extracted twice with an equal volume of chloroform/iso-amyl alcohol (24:1). The DNA was precipitated from the aqueous phase with 1 volume isopropanol and resuspended in 10 mM Tris-HCl pH 8.0, 1 mM EDTA. DNA was digested to completion with the appropriate restriction enzymes, fractionated in 0.8% agarose gels, and transferred to nylon membranes (Hybond-N, Amersham Corporation, Arlington Heights, IL) using conditions suggested by the manufacturer. DNA was fixed to the membranes by ultraviolet irradiation and hybridizations with radioactively labeled probes were carried out as described above.

DNA sequences were determined from double-stranded templates by the dideoxy chain termination method (Sanger *et al.* 1977), using Sequenase (United States Biochemical Corporation, Cleveland, OH) or Taq polymerase (Promega). Restriction fragments from λ2 were subcloned into pBluescript II KS+ and sequence data from one strand were obtained from nested deletions generated by Exonuclease III digestion (Henikoff 1984). The sequence from nucleotide positions 1025 to 3348 was confirmed by sequencing the opposite DNA strand with primers synthesized by the Laboratory for Macromolecular Structure at Purdue University. Amino acid alignments were done by eye or with the University of Wisconsin Genetics Computer Group BESTFIT and ALIGN programs (Devereux *et al.* 1984) made available by the Aids Center Laboratory for Computational Biochemistry at Purdue University.

Protoplasts were prepared for pulsed-field gel electrophoresis from hyphal fragments of *M. grisea*, as previously described (Parsons *et al.* 1987; Hamer *et al.* 1989a). Purified protoplasts were washed, embedded in agarose, and prepared for pulsed-field gel electrophoresis as described by Orbach *et al.* (1988). Chromosome-sized DNA molecules were fractionated in 1% agarose gels prepared from FastLane agarose (FMC). Contour-clamped homogeneous electric field (CHEF) gel electrophoresis was carried out for 120 hr at 45 V, with a switch time of 90 min. The DNA was subsequently transferred to nylon membranes and hybridized with radioactive probes, as described above.

Nucleotide sequence accession numbers.

The nucleotide sequence data reported in this paper have been submitted to GenBank and assigned the accession numbers M77661 and M77662.

ACKNOWLEDGMENTS

We thank Katey Einterz for assistance with restriction enzyme mapping and members of the Hamer laboratory for many valuable discussions. We also thank Morris Levy, Nick Talbot, Steven Harris, and Miriam Zolan for critical reading of the manuscript. This work was supported by a grant to J.E.H. from the National Science Foundation. J.E.H. is a David and Lucille Packard Fellow. R.E.H. was a recipient of a Howard Hughes Summer Undergraduate Research Award.

LITERATURE CITED

Adams, J., and Oeller, P. W. 1986. Structure of evolving populations of *Saccharomyces cerevisiae*: Adaptive changes are frequently associated with sequence alterations involving mobile elements belonging to the Ty family. Proc. Natl. Acad. Sci. USA 83:7124-7127.

Ballance, D. J. 1986. Sequences important for gene expression in filamentous fungi. Yeast 2:229-236.

Berg, D. E., and Howe, M. M., ed. 1989. Mobile DNA. American Society for Microbiology, Washington, DC.

Bingham, P. M., and Zachar, Z. 1989. Retrotransposons and the FB transposon from *Drosophila melanogaster*. Pages 485-502 in: Mobile DNA. D. E. Berg and M. M. Howe, ed. American Society for Microbiology, Washington, DC.

Boeke, J. D. 1989. Transposable elements in *Saccharomyces cerevisiae*. Pages 335-374 in: Mobile DNA. D. E. Berg and M. M. Howe, ed. American Society for Microbiology, Washington, DC.

Chumley, F. G., and Valent, B. 1990. Genetic analysis of melanin deficient, nonpathogenic mutants of *Magnaporthe grisea*. Mol. Plant-Microbe Interact. 3:135-143.

Crawford, M. S., Chumley, F. G., Weaver, C. G., and Valent, B. 1986. Characterization of the heterokaryotic and vegetative diploid phases of *Magnaporthe grisea*. Genetics 114:1111-1129.

Devereux, J., Haeberli, P., and Smithies, O. 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. 12:387-395.

Doolittle, R. F., Feng, D.-F., Johnson, M. S., and McClure, M. A. 1989. Origins and evolutionary relationships of retroviruses. Q. Rev. Biol. 64:1-30.

Feinberg, A. P., and Vogelstein, B. 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. 132:6-13.

Gurr, S. J., Unkles, S. E., and Kinghorn, J. R. 1988. The structure and organization of nuclear genes in filamentous fungi. Pages 93-109 in: Gene Structure in Eukaryotic Microbes. J. R. Kinghorn, ed. IRL Press, Oxford.

Hamer, J. E. 1991. Molecular probes for rice blast disease. Science 252:632-633.

Hamer, J. E., Farrall, L., Orbach, M. J., Valent, B., and Chumley, F. G. 1989a. Host species-specific conservation of a family of repeated DNA sequences in the genome of a fungal plant pathogen. Proc. Natl. Acad. Sci. USA 86:9981-9985.

Hamer, J. E., and Givan, S. 1990. Genetic mapping with dispersed repeated sequences in the rice blast fungus: Mapping the SMO locus. Mol. Gen. Genet. 223:487-495.

Hamer, J. E., and Timberlake, W. E. 1987. Functional organization of the *Aspergillus nidulans trpC* promoter. Mol. Cell. Biol. 7:2352-2359.

Hamer, J. E., Valent, B., and Chumley, F. G. 1989a. Mutations at the SMO genetic locus affect the shape of diverse cell types in the rice blast fungus. Genetics 122:351-361.

Heath, M. C., Valent, B., Howard, R. J., and Chumley, F. G. 1990. Interactions of two strains of *Magnaporthe grisea* with rice, goosegrass and weeping lovegrass. Can. J. Bot. 68:1627-1637.

Hebert, T. T. 1971. The perfect stage of *Pyricularia grisea*. Phytopathology 61:83-87.

Henikoff, S. 1984. Unidirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing. Gene 28:351-359.

Johnson, M. S., McClure, M. A., Feng, D.-F., Gray, J., and Doolittle, R. F. 1986. Computer analysis of retroviral *pol* genes: Assignment of enzymatic functions to specific sequences and homologies with nonviral enzymes. Proc. Natl. Acad. Sci. USA 83:7648-7652.

Kato, H., and Yamaguchi, T. 1982. The perfect state of *Pyricularia oryzae* Cav. from rice plants in culture. Ann. Phytopathol. Soc. Jpn. 48:607-612.

Kido, Y., Aono, M., Yamaki, T., Matsumoto, K.-I., Murata, S., Saneyoshi, M., and Okada, N. 1991. Shaping and reshaping of salmonid genomes by amplification of tRNA-derived retroposons during evolution. Proc. Natl. Acad. Sci. USA 88:2326-2330.

Lankenau, D.-H., Huijser, P., Jansen, E., Miedema, K., and Hennig, W. J. 1988. Micropia: A retrotransposon of *Drosophila* combining structural features of DNA viruses, retroviruses and non-viral transposable elements. J. Mol. Biol. 204:233-246.

Leung, H., Lehtinen, U., Karjalainen, R., Skinner, D., Tooley., P., Leong., S., and Ellingboe., A. 1990. Transformation of the rice blast fungus *Magnaporthe grisea* to hygromycin B resistance. Curr. Genet. 17:409-411.

Leung, H., and Williams, P. H. 1986. Enzyme polymorphism and genetic differentiation among geographic isolates of the rice blast fungus. Phytopathology 76:778-783.

Levin, H. L., Weaver, D. C., and Boeke, J. D. 1990. Two related families of retrotransposons from *Schizosaccharomyces pombe*. Mol. Cell. Biol. 10:6791-6798.

Levy, M., Romao, J., Marchetti, M. A., and Hamer, J. E. 1991. DNA fingerprinting with a dispersed repeated sequence resolves pathotype diversity in the rice blast fungus. Plant Cell 3:95-102.

Marlor, R. L., Parkhurst, S. M., and Corces, B. 1986. The *Drosophila melanogaster* gypsy transposable element encodes putative gene products homologous to retroviral proteins. Mol. Cell. Biol. 6:1129-1134.

Marschalek, R., Brechner, T., Amon-Bohm, E., and Dingerman, T. 1989. Transfer RNA genes: Landmarks for integration of mobile genetic elements in *Dictyostelium discoideum*. Science 244:1493-1496.

McHale, M. T., Roberts, I. N., Noble, S. M., Beaumont, C., Whitehead, M. P., Seth, D., and Oliver, R. P. 1992. *Cft*-I: an LTR-retrotransposon in *Cladosporium fulvum*, a fungal pathogen of tomato. Mol. Gen. Genet. 233:337-347.

Moerman, D. G., and Waterson, R. H. 1989. Mobile elements in *Caenorhabditis elegans* and other nematodes. Pages 537-556 in: Mobile DNA. D. E. Berg and M. M. Howe, ed. American Society for Microbiology, Washington, DC.

Murray, M. G., and Thompson, W. F. 1980. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res. 8:4321-4325.

Orbach, M., Sweigard, J., Walter, A., Farrall, L., Chumley, F., and Valent, B. 1991. Stategies for the isolation of avirulence genes from the rice blast fungus *Magnaporthe grisea*. (Abstr.) Fungal Genet. Newslett. 38:16.

Orbach, M. J., Vollrath, D., Davis, R. W., and Yanofsky, C. 1988. An electrophoretic karyotype of *Neurospora crassa*. Mol. Cell. Biol. 8:1469-1473.

Parsons, K. A. 1988. The development of genetic transformation in the ascomycete *Magnaporthe grisea* and the cloning of the LYS1+ gene. Ph.D. thesis. University of Colorado, Boulder.

Parsons, K. A., Chumley, F. G., and Valent, B. 1987. Genetic transformation of the fungal pathogen responsible for rice blast disease. Proc. Natl. Acad. Sci. USA 84:4161-4165.

Pascale, E., Valle, E., and Furano, A. V. 1990. Amplification of an ancestral mammalian L1 family of long interspersed repeated DNA occurred just before the murine radiation. Proc. Natl. Acad. Sci. USA 87:9481-9485.

Punt, P. J., Dingemanse, M. A., Kuyvenhoven, A., Soede, R. D. M., Pouwels, P. H., and Hondel, C. A. M. J. J. v. d. 1990. Functional elements in the promoter region of the *Aspergillus nidulans gpdA* gene encoding glyceraldehyde-3-phosphate dehydrogenase. Gene 93:101-109.

Romao, J., and Hamer, J. E. 1992. Genetic organization of a repeated DNA sequence family in the rice blast fungus. Proc. Natl. Acad. Sci. USA 89:5316-5320.

Rossman, A. Y., Howard, R. J., and Valent, B. 1990. *Pyricularia grisea,* the correct name for the rice blast disease fungus. Mycologia 82:509-512.

Sanger, F., Nicklen, S., and Coulson, A. R. 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA 74:5463-5467.

Smyth, D. R., Kaltsis, P., Joseph, J. L., and Sentry, J. W. 1989. Plant retrotransposon from *Lilium henryi* is related to Ty3 of yeast and the gypsy group of *Drosophila*. Proc. Natl. Acad. Sci. USA 86:5015-5019.

Sweigard, J. A., Orbach, M. J., Valent, B., and Chumley, F. G. 1990. A miniprep procedure for isolating genomic DNA from Magnaporthe grisea. Fungal Genet. Newslett. 37:4.

Tibayrenc, M., Kjellberg, F., Arnaud, J., Oury, B., Breniere, S. F., Darde, M.-L., and Ayala, F. J. 1991. Are eukaryotic

microorganisms clonal or sexual? A population genetics vantage. Proc. Natl. Acad. Sci. USA 88:5129-5133.

Toh, H., Kikuno, R., Hayashida, H., Miyata, T., Kugimiya, W., Inouye, S., Yuki, S., and Saigo, K. 1985. Close structural resemblance between putative polymerase of a *Drosophila* transposable genetic element 17.6 and *pol* gene product of Moloney murine leukaemia virus. EMBO J. 4:1267-1272.

Valent, B., and Chumley, F. G., eds. 1987. Genetic analysis of host species specificity in *Magnaporthe grisea*. Pages 83-93 in: Molecular Strategies for Crop Protection. Alan R. Liss, Inc., New York.

Valent, B., and Chumley, F. G. 1991. Molecular genetic analysis of the rice blast fungus, *Magnaporthe grisea*. Annu. Rev. Phytopathol. 29:443-467.

Valent, B., Crawford, M. S., Weaver, C. G., and Chumley, F. G. 1986. Genetic studies of fertility and pathogenicity in *Magnaporthe grisea (Pyricularia oryzae)*. Iowa State J. Res. 60:569-594.

Valent, B., Farrall, L., and Chumley, F. G. 1991. *Magnaporthe grisea* genes for pathogenicity and virulence identified through a series of backcrosses. Genetics 127:87-101.

Varmus, H., and Brown, P. 1989. Retroviruses. Pages 53-108 in: Mobile DNA. D. E. Berg and M. M. Howe, ed. American Society for Microbiology, Washingtion, DC.

Wyman, C., and Blackburn, E. H. 1991. Tel-1 transposon-like elements of *Tetrahymena thermophila* are associated with micronuclear genome rearrangements. Genetics 128:57-67.

Xiong, Y., and Eickbush, T. H. 1988. Similarity of reverse transcriptase-like sequences of viruses, transposable elements, and mitochondrial introns. Mol. Biol. Evol. 5:675-690.

Xiong, Y., and Eickbush, T. H. 1990. Origin and evolution of retroelements based upon their reverse transcriptase sequences. EMBO J. 9:3353-3362.

Yaegashi, H. 1977. On the sexuality of blast fungi, *Pyricularia* spp. Ann. Phytopathol. Soc. Jpn. 43:432-439.

Yaegashi, H., and Asaga, K. 1981. Further studies on the inheritance of pathogenicity in crosses of *Pyricularia oryzae* with *Pyricularia* sp. from finger millet. Ann. Phytopathol. Soc. Jpn. 47:677-679.

Yaegashi, H., and Nishihara, N. 1976. Production of the perfect stage in *Pyricularia* from cereals and grasses. Ann. Phytopathol. Soc. Jpn. 42:511-515.

Yuki, S., Inouye, S., Ishimura, S., and Saigo, K. 1986. Nucleotide sequence characterization of a *Drosophila* retrotransposon *412*. Eur. J. Biochem. 158:403-410.

Zolan, M., and Pukkila, P. J. 1986. Inheritance of DNA methylation in *Coprinus cincereus*. Mol. Cell. Biol. 6:195-200.