DNA Fingerprinting to Examine Microgeographic Variation in the *Magnaporthe grisea* (*Pyricularia grisea*) Population in Two Rice Fields in Arkansas

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ABSTRACT

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DNA fingerprinting was used to analyze microgeographic variation in the *Magnaporthe grisea* population in Arkansas. One hundred and thirteen isolates were collected from two commercial rice fields (cv. Newbonnet) in 1991. In addition, several reference isolates representing the predominant pathotypes in Arkansas were examined. Total DNA of each isolate was restricted with *Eco*R1 and probed with a dispersed repeated MGR586 DNA probe. MGR586 DNA fingerprint groups were identified based on DNA restriction fragment length polymorphism similarities. Seven distinct fingerprint groups (designated A through G) were identified among the field and reference isolates. These seven fingerprint groups were very similar to seven of the eight fingerprint groups (lineages) previously reported for *M. grisea*. Four fingerprint groups (A, B, C, and D) were found in both fields. Group A was the predominant group representing 72% and 52% of the isolates collected from the two fields. Hierarchical diversity analysis demonstrated that the majority of genetic variability was distributed within sample locations within a field. Thus, the initial source or sources of inoculum may have been a mixture of the four different fingerprint groups. Isolates representing all of the DNA fingerprint groups were examined for virulence on each of two sets of differential rice cultivars. Although certain fingerprint groups were composed of a single pathotype, the data indicated that other groups in the contemporary population were composed of isolates that are quite heterogeneous with respect to virulence. Furthermore, contemporary isolates in one fingerprint group (group B) were a different pathotype from an older reference isolate of the same fingerprint group. Thus, the relationship between pathotype and DNA fingerprint group in contemporary populations of *M. grisea* in the United States apparently is quite complex.

Knowledge of genetic variability in populations of plant pathogens is important in understanding host coevolution in plant pathosystems (20,28). Progress in the area of population genetics of fungi is notably limited because of a shortage of genetically well-defined and easily scored genetic markers (20). Recently, DNA restriction fragment length polymorphism (RFLP) analysis has been used to measure genetic variation of fungal pathogens (6,18,26). Most investigations have been conducted with a limited number of fungal isolates from widespread geographical origins, thereby providing information of genetic variation on a macrogeographical scale. Only a few recent studies have addressed the amount and distribution of genetic variation in fungal populations on a microgeographical scale (7,19).

The rice blast fungus, *Magnaporthe grisea* (Hebert) Barr. (anamorph, *Pyricularia grisea* (Cooke) Sacc.), is a heterothallic ascomycete that is primarily haploid and reproduces asexually in nature (12,25,27). The rice blast pathogen has become a model

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system for many studies of the molecular basis of host-pathogen interactions (28,29).

Traditional markers used to estimate genetic variability within *M. grisea* populations include pathogenicity, mating type, auxotrophic mutants, melanin deficiency or drug resistance, and isozyme polymorphism (3,12,13,16,29). Investigations also have utilized molecular methods such as gene cloning, fungal transformation, and genomic or mitochondrial DNA RFLP analysis (29). Hamer et al (8) identified a family of dispersed repetitive DNA sequences, called MGR, that was conserved in *M. grisea* and diagnostically useful for genetic analysis of the *M. grisea* fungal population (8,9).

The examination of virulence diversity of the rice blast pathogen continues to be an active area of research. Conflicting results have been reported on the degree of race variability for *M. grisea*. Early studies indicated that single conidial isolates gave rise to so many different races that it was impossible to clearly define a race (23,24,25). However, Bonman et al (2) and Latterell and Rossi (11) reported that races were relatively stable, and altered specificity was detected only in some cases. Levy et al (14) examined DNA fingerprints based on MGR sequence polymorphisms to study the relationship between molecular haplotypes and eight pathotypes in a collection of U.S. isolates of *M. grisea*. Their collection of isolates from macrogeographic origins revealed a close correspondence between fingerprint group and pathotype. Of the eight fingerprint groups they identified, six were composed of a single pathotype and two groups contained two pathotypes each (14).

The overall objective of this study was to examine the molecular and pathogenic variation in *M. grisea* in Arkansas. MGR-DNA fingerprinting was used to examine the microgeographic genetic variability of *M. grisea* from two rice fields in Arkansas. In addition, representative isolates from the various fingerprint groups were characterized for virulence on a set of international differential rice cultivars (15) as well as on a set of differential cultivars commonly grown in Arkansas. A preliminary report of this work has been published (31).

MATERIALS AND METHODS

Collection and culture of fungal isolates. Fungal isolates were collected from the cultivar Newbonnet in two rice fields in Arkansas (Lonoke Co.) in September 1991. Blast was moderate to severe in both fields. Panicles with symptoms of neck blast were sampled from five locations in each field. Each location sampled was approximately 5 × 8 m^2, and locations were approximately 35 m apart. Panicles were returned to the laboratory, trimmed, washed once with sterile distilled water, and placed on moist filter paper in petri dishes at room temperature to induce sporulation. Conidia from the lesion surface were spread onto 3% water agar with a sterile loop and incubated overnight.

TABLE 1. Reference isolates of *Magnaporthe grisea*

MGR586 DNA fingerprint group	Isolate	Pathotype[a]	Lineage[b]	Collection site and year
A	85L2[c]	IB-1	IB-1	Louisiana 1985
B	85M2	IG-1	IG-1B	Michigan 1985
B	75A49[c]	IG-1	IG-1B	Arkansas 1975
C	85L8	IB-49	IB-49A	Louisiana 1985
D	85A7	IC-17	IC-17	Arkansas 1985
D	75A1[c]	IC-17	IC-17	Arkansas 1975
E	49-D[c]	IB-49	IB-49B	Arkansas 1988
E	793	IB-49	IB-49B	Louisiana 1967
F	75A11[c]	IB-45	IB-45	Arkansas 1975
F	78L5	IB-45	IB-45	Louisiana 1978
G	75A10	IH-1	IH-1	Arkansas 1975
G	74L2	IH-1	IH-1	Louisiana 1974
H	82A1	IG-1	IG-1A	Arkansas 1982

[a]Pathotypes are the international pathotype designations (2,15–17).
[b]Eight lineages previously described by Levy et al (14).
[c]Pathotypes recovered in Arkansas.

Single germinating conidia were isolated and transferred to potato-dextrose agar. Cultures were stored on desiccated filter paper as previously described (12).

A total of 113 isolates were collected: 65 isolates from a field designated "LO" and 48 isolates from field "BM". The two fields were approximately 40 km apart and were separated by nonrice crops. Five isolates representing pathotypes recovered in Arkansas and eight isolates representing eight MGR-DNA fingerprint lineages reported by Levy et al (14) were used as reference isolates (Table 1).

DNA preparation and MGR probe. Cultures were grown in complete medium broth (5) for 4–5 days on a rotatory shaker. Mycelium was harvested, frozen, and lyophilized prior to use. Lyophilized mycelium was ground in liquid nitrogen. DNA was extracted by a modified mini-prep procedure described previously (5,10). Powdered mycelium was dispersed in 1.8 ml of extraction buffer in a 2.0-ml microfuge tube, incubated at 65 C for 1.0 h, and centrifuged for 10 min at maximum speed in a microcentrifuge. The supernatant was extracted with an equal volume of phenol/chloroform (1:1, v/v) and chloroform/isoamyl alcohol (24:1, v/v). Nucleic acids were precipitated by adding an equal volume of isopropanol and centrifuging for 2 min. The nucleic acid pellet was dissolved in 500 μl of TE buffer (1× TE = 10 mM Tris HCl, 1 mM EDTA, pH 7.4) and then precipitated by adding 250 μl of 7.5 M NH_4OAc and 1.0 ml of 95% ethanol. After centrifuging for 2.0 min, the pellet was dissolved in 250 μl of TE, and the nucleic acid was precipitated once more with 95% ethanol. The nucleic acid pellet was dried in a vacuum-desiccator and then dissolved in 50–100 μl of TE.

The dispersed repeated DNA probe (MGR586) was provided by J. E. Hamer (Purdue University). The MGR586 sequence was cloned into a plasmid and was propagated in *Escherichia coli* and isolated using a cleared lysate protocol (8).

Enzyme digestion and DNA fingerprint. Total DNA was digested with the restriction enzyme *Eco*R1 for 2–6 h according to the manufacturer's instructions (Stratagene, La Jolla, CA). Digested DNA (1.5 μg per lane) was separated in an 0.8% agarose TBE (0.5 ×) gel for 54 h at 25 V using a 20 × 30 cm gel electrophoresis apparatus. Capillary transfer (bidirectional) of DNA to nylon$^+$ membrane (Hybond-N+, Amersham, Arlington Heights, IL) was conducted overnight. An ECL gene detection kit (Amersham) was used to label the MGR-DNA probe. Prehybridization and hybridization reactions were performed according to the manufacturer's instructions on a shaker at 42 C in a sealed plastic bag. The MGR sequence probe was covalently labeled with enzyme horseradish peroxidase with glutaraldehyde. Hybridization reactions were allowed to proceed for 12–16 h. Membranes were washed twice at 42 C in primary wash buffer (0.5× SSC, 36% urea and 0.4% SDS) for 20 min each time, followed by two 5-min washes in 2× SSC at room temperature. Film (Hyperfilm-ECL, Amersham) was placed on the membranes and exposed for 10–120 min depending on strength of the enzyme reaction signal.

Virulence tests. Four or five isolates of each DNA fingerprint group were selected at random and used in each of five separate inoculation tests. Three tests were conducted with a set of eight international rice cultivar differentials at Beaumont, TX (15). Two tests were conducted at Stuttgart, AR, on a set of eight differentials representing cultivars commonly grown in Arkansas. The international set of differentials included Raminad Str. 3, Zenith, NP125, Usen, Dular, Kanto 51, Sha Tiao Tsao (S), and Caloro, and the Arkansas differentials included Mars, Newbonnet, Starbonnet, Katy, M-201, Tebonnet, Zenith, and Lemont (Table 2).

The procedure for race identification on the international set of cultivars has been previously published (15,16,17). Eight cultivars (approximately 10 seed per cultivar) were planted in soil in 20 × 30 cm trays and incubated in a greenhouse with temperatures of 24–28 C. Plants were grown until the fourth leaf was half emerged (approximately 2 wk). Conidia were collected from cultures grown on rice-polish agar (17) by washing the agar surface with sterile water. The concentration of conidia was

adjusted to approximate 100,000 per milliliter. Each tray was sprayed with 35 ml of inoculum with a compressed-air sprayer. Plants were incubated in a dew chamber at 20–23 C for 24 h and placed in a greenhouse. Controls in each experiment included plants sprayed with water. The Arkansas differentials were inoculated in a similar fashion but with some modifications. Rice plants were grown in 12 × 12 cm pots, and the fungus was cultured on oatmeal agar (Difco) for conidial production.

Symptoms were scored by examining the disease reaction on leaves. A disease rating scale of 0–9 was used to score the disease reaction. This is considered a combination of a quantitative and qualitative disease reaction scale (17). Generally, a reaction of 0–3 is considered a resistant reaction and ≥4 a susceptible reaction.

Data analysis. RFLPs were detected by hybridization of the MGR586 probe to *Eco*R1 DNA restriction fragments present on a Southern blot. Similarities between MGR-DNA fingerprints among the fungal isolates were calculated using Nei and Li's index (22) of genetic similarity for RFLP comparisons, which is given by the formula:

$$S_{xy} = 2n_{xy}/(n_x + n_y),$$

where n_{xy} is the number of shared fragments, and n_x and n_y are the number of fragments in isolates x and y.

MGR586 DNA fingerprint groups were identified on the basis of DNA RFLP similarities. Isolates with >80% shared fragments were considered to be members of the same fingerprint group, whereas isolates with <60% shared fragments were considered separate groups; no intermediate isolates (60–80% shared fragments) were observed. Frequencies of the fingerprint groups were tabulated for each location and for the total population from the two fields. Genetic diversity of the *M. grisea* population was measured using the following formula:

$$h = 1 - \Sigma x_j^2,$$

where x_j is the frequency of the fingerprint groups (19,21).

The heterogeneity of fingerprint groups between locations within a rice field was tested using chi square (χ^2) following the formula (30):

$$\chi^2 = N\Sigma\, Q^2P_i/P_i,$$

where N is the number of isolates, P_i is the mean variance of frequencies of groups, and Q^2P_i is the weighted variance of frequencies of groups.

Total diversity of the two populations was partitioned into components within locations, between locations in a field, and between the two fields using hierarchical gene diversity analysis (1).

RESULTS

MGR-based group assignment. One hundred thirteen isolates of *M. grisea* were collected from two rice fields (cv. Newbonnet) in Arkansas in 1991 and tested for MGR586 DNA fingerprints. Examples of the DNA fingerprints among the isolates from two locations in field BM are shown (Fig. 1). Isolates were scored

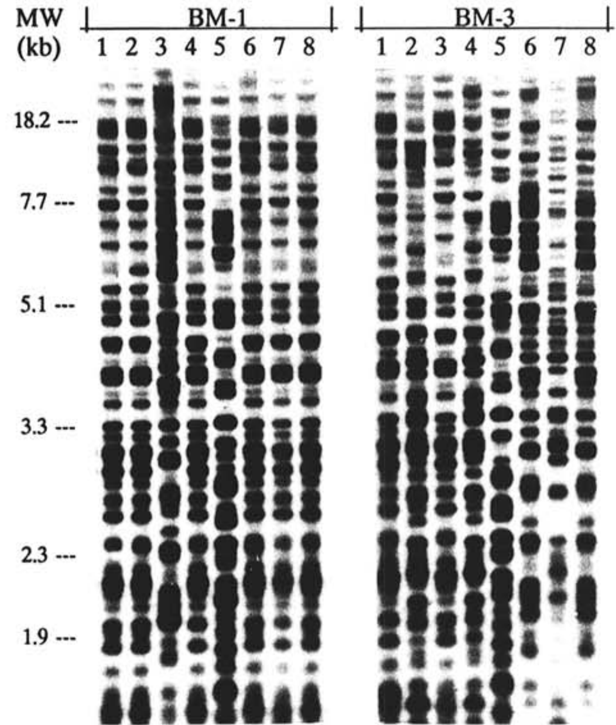

Fig. 1. Differences in MGR-DNA fingerprints of *Magnaporthe grisea* isolates from a commercial rice field. The isolates were sampled from two locations (BM-1 and BM-3) from cv. Newbonnet from field BM1, Lonoke County, AR, in 1991. DNA was digested with *Eco*R1 and hybridized with the MGR-DNA probe. Eight randomly selected isolates (numbered 1 through 8) from each location are shown.

TABLE 2. Summary of reactions of international pathotypes of *Magnaporthe grisea* on two sets of differential rice cultivars

	International races[a]								
Cultivar	IB-1	IB-33	IB-45	IB-49	IB-54	IC-17	IE-1	IG-1	IH-1
Arkansas differentials[b]									
M-201	S[c]	S	S	S	R	S	S	S	S
Starbonnet	S	S	S	S	S	S	S	S	R
Tebonnet	S	S	S	S	R	S	S	R	R
Newbonnet	S	S	R	S	R	S	S	R	R
Lemont	R	S	R	S	R	S	S	R	R
Zenith	S	S	S	S	S	R	R	R	R
Mars	S	S	I	S	R	R	R	R	R
Katy	R	S	R	R	R	R	R	R	R
International differentials									
Raminad Str. 3	R	R	R	R	R	R	R	R	R
Zenith	S	S	S	S	S	R	R	R	R
NP125	S	R	R	R	R	S	R	R	R
Usen	S	S	S	R	R	R	R	R	R
Dular	S	S	R	S	S	S	S	R	R
Kanto 51	S	S	R	S	R	S	S	R	R
Sha Tiao Tsao (S)	S	S	S	S	S	S	S	S	R
Caloro	S	S	S	S	R	S	S	S	S

[a] Pathotype designations on international differentials (15).
[b] Disease reactions on the Arkansas and international differentials are a composite summary from pathogenicity tests conducted at Stuttgart, AR, or Beaumont, TX.
[c] R = Resistant reaction (disease rating 0–3); S = susceptible reaction (disease rating ≥ 4); I = intermediate reaction.

for similarity based on the presence or absence of approximately 50 DNA fragments ranging in size from 1.8 to 20.0 kb. Based on these similarities, four distinct fingerprint groups, designated A, B, C, and D, were identified among all of the field isolates, and each group contained several haplotypes (Fig. 2). DNA similarities among haplotypes ranged from 87 to 98%. The similarities between MGR586 DNA fingerprints for all pairwise comparisons among the main groups and haplotypes are shown in Table 3.

Genetic diversity and distribution of the fungal population. Among the four fingerprint groups found among the field isolates, group A was the predominant group in both fields, representing 72% and 52% of the isolates in fields LO and BM, respectively (Table 4). The mean frequency of groups B, C, and D ranged from 5 to 19%.

The frequencies of the DNA fingerprint groups and Nei's measurements of genetic diversity from the two fields are summarized in Table 4. Nei's genetic diversity index, *h*, per location ranged from 0.25 to 0.63 in field LO and from 0.31 to 0.66 in field BM (Table 4). Chi-square tests for heterogeneity of the fungal population within fields showed that group frequencies were significantly different among locations for both fields (Table 4). Hierarchical genomic diversity analysis demonstrated that the majority of variability was distributed within locations (94.2% and 77.3%, respectively), and only a small component of the variability was distributed among locations (average 10.7%) and between the two fields (2.5%) (Table 5).

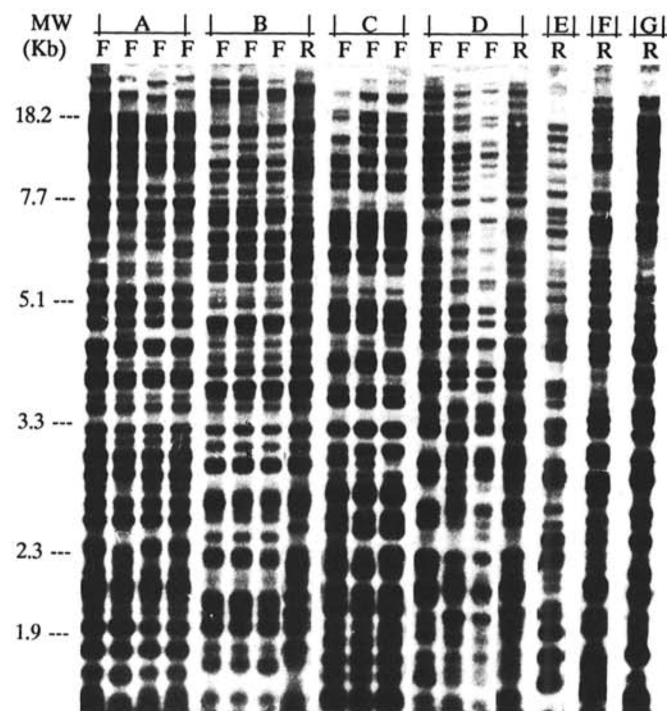

Fig. 2. Composite Southern blot (MGR-DNA fingerprinting) showing seven groups in *Magnaporthe grisea* population from Arkansas. DNA of the isolates from each of seven fingerprint groups was digested with *Eco*R1 and hybridized with MGR-DNA probe labeled with an ECL DNA detection kit. The seven groups are designated A to G. Isolates within a group had >80% shared fragments, and isolates between groups had <60% shared fragments. F = field isolates; R = reference isolates. The isolates in lanes 1–18 are: BM1-22, LO4-18, LO2-12, LO2-24, LO3-8, LO5-5, LO1-10, 75A49, BM5-25, BM5-30, LO4-27, LO1-24, LO3-4, LO3-5, 85A7, 49-D, 75A11, and 75A10.

Comparisons of field isolates and reference isolates. The four fingerprint groups identified among the field isolates (A, B, C, and D) were compared for fingerprint similarities to isolates representing the eight previously reported lineages (14) (Table 6). The results indicated that each of the four groups is similar to one of the reference lineages previously reported (14) (Table 6). In addition, three Arkansas reference isolates, representing races IB-49, IB-45, and IH-1, were similar to three other reference lineages and were therefore designated groups E, F, and G, respectively (Table 6).

Virulence tests. The results of the five inoculation tests on the two sets of differential cultivars are summarized in Table 7. Four reference isolates (75A49, 85A7, 49-D, and 75A10) representing four different races (IG-1, IC-17, IB-49, and IH-1, respectively) were consistent in their disease reactions in all five pathogenicity tests on both sets of differentials.

Field isolates within some of the fingerprint groups were consistently similar in their disease reactions on the differentials, whereas isolates in other fingerprint groups differed in their disease reactions. In addition, some individual isolates differed slightly in their disease reactions from test to test.

For fingerprint group D, all five field isolates and reference isolate 85A7 were consistently characterized as pathotype IC-17 on both the international and the Arkansas differential cultivars (Table 7). Although some variation in disease reactions was observed among field isolates in fingerprint group B, most of the isolates were characterized as pathotype IC-17 for the five tests. In several tests, certain isolates were similar in disease reaction to pathotype IE-1. However, borderline disease reactions (disease rating of 3 or 4) on only one or two cultivars were responsible for the inconsistency in pathotype characterizations. In contrast, reference isolate 75A49 (collected in AR in 1975), which also was in the B fingerprint group, was consistently identified as pathotype IG-1.

Distinct inconsistencies in disease reactions were observed

TABLE 3. Percent similarity[a] of MGR-DNA fingerprint and group assignments among field isolates of *Magnaporth grisea* in Arkansas

Haplotype	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1		**96**	**94**	**88**	**90**	**96**	**96**	**98**	28	27	30	31	44	42	43
2			**93**	**89**	**89**	**95**	**97**	**96**	26	27	31	30	45	44	41
3				**87**	**88**	**92**	**93**	**95**	24	26	29	28	43	42	39
4					**98**	**95**	**92**	**92**	22	25	29	27	43	40	43
5						**93**	**91**	**90**	28	26	34	32	47	42	45
6							**96**	**94**	25	25	33	31	46	44	45
7								**95**	27	26	31	32	48	45	44
8									27	28	30	32	45	46	42
9										**93**	26	23	38	36	35
10											25	24	35	34	36
11												**95**	21	27	22
12													23	26	21
13														**91**	**95**
14															**93**
MGR586 fingerprint group[b]	A1	A2	A3	A4	A5	A6	A7	A8	B1	B2	C1	C2	D1	D2	D3

[a] Percent similarity of MGR586 DNA fingerprints was calculated with Nei and Li's formula (22). Bold numbers indicate >80% similarity. Isolates with <60% shared fragments were designated distinct groups; isolates within a group with minor differences (<20%) were considered haplotypes.
[b] Letter indicates fingerprint group and number indicates haplotype within a group.

among isolates within fingerprint groups A and C. For example, fingerprint group A isolates gave disease reactions of pathotypes IB-1, IB-17, or IC-17 on the international differentials but were characterized as IB-49 on the Arkansas differentials (Table 7). Even more variability in disease reactions was observed among isolates within fingerprint group C. On the international set of differential cultivars, isolates were characterized as pathotypes IB-49, IC-17, IB-1, IE-1, or IG-1; these same isolates gave disease reactions characterized as either IB-49 or IC-17 on the Arkansas differentials. Several isolates within fingerprint group C did not give consistent disease reactions from test to test.

TABLE 4. Fingerprint group frequencies and measure of genetic diversity in *Magnaporthe grisea* among locations in two commercial rice fields

Location[a]	No. of samples	Frequency A	B	C	D	h[b]
Field 1[c]						
LO-1	21	0.86	0.10	0.00	0.05	0.25
LO-2	12	0.67	0.00	0.33	0.00	0.44
LO-3	11	0.55	0.09	0.18	0.18	0.63
LO-4	10	0.70	0.10	0.20	0.00	0.46
LO-5	11	0.73	0.18	0.09	0.00	0.43
Mean (%)	...	72	9	14	5	
Field 2						
BM-1	11	0.64	0.09	0.18	0.09	0.55
BM-2	11	0.82	0.00	0.09	0.09	0.31
BM-3	10	0.50	0.20	0.10	0.20	0.66
BM-4	7	0.57	0.00	0.14	0.29	0.57
BM-5	9	0.00	0.22	0.45	0.33	0.64
Mean (%)	...	52	10	19	19	

[a] Fields were in Lonoke County, AR. Five locations were sampled per field.
[b] Nei's measure of genomic diversity for each location based on DNA fingerprint groups (21).
[c] The χ^2 test for heterogeneity of group frequencies among locations calculated a value of 21 for Field 1 data and 17 for Field 2 data, both significant at $P < 0.001$.

TABLE 5. Hierarchical genomic diversity analysis of Arkansas populations of *Magnaporthe grisea* by MGR-DNA fingerprint groups

Diversity components[a]	Field 1 (LO) Calculated value	Field 1 (LO) Percent	Field 2 (BM) Calculated value	Field 2 (BM) Percent	All locations Average value	All locations Percent
D_L	0.553	94.2	0.352	77.3	0.533	86.8
D_F	0.034	5.8	0.103	22.7	0.066	10.7
D_P	...	...	...	...	0.016	2.5
H_T	0.586	100.0	0.456	100.0	0.614	100.0

[a] Total diversity was partitioned into components within locations, between locations in a single field, and between the two fields. D_L is the proportion of total diversity within locations; D_F is the proportion of total diversity among locations within fields; D_P is the proportion of total diversity between populations in the two fields; H_T is total diversity.

DISCUSSION

MGR586 DNA restriction fragment length polymorphisms have been used to examine both the population genetics and phylogenetics of *M. grisea* (8,14,29). Our objective was to examine the molecular and pathogenic variation of *M. grisea* in two populations in Arkansas. Isolates were scored for percent similarity in their DNA fingerprints (22), and DNA fingerprint groups were arbitrarily established as any isolates with more than 80% similarity; different fingerprint groups had less than 60% similarity. Isolates within a fingerprint group were further divided into haplotypes based on minor differences in the DNA fingerprints (1–20% dissimilarity).

On the basis of these criteria, four fingerprint groups, designated A, B, C, and D, were identified among the 113 field isolates from two rice fields in Arkansas. All four groups were found in both fields, but group A predominated, representing 72% and 52% of the isolates collected in both fields. The four fingerprint groups found were distributed in most of the sample locations in each of the two fields. Hierarchical gene diversity analysis indicated that the majority of the RFLP variation was distributed within locations (Table 5). Chi-square tests showed that significant differences in group frequencies existed among the locations from each of the two fields. The relatively low level of genetic diversity found among isolates from the two fields was very different from the high level of genetic diversity found among isolates of *Septoria tritici* in a single wheat field (19) or *Rhyncosporium secalis* from barley populations (18). The sexual stage of *S. tritici* may be an important source of genetic variation on a microgeographic scale. Although the sexual stage of *R. secalis* is not known, a relatively high recombination rate may contribute to the high level of genetic diversity in populations of this pathogen.

It is not known why group A isolates predominated in both fields, because the cultivar Newbonnet is apparently susceptible to isolates in all four of the fingerprint groups. Although it is possible that isolates in fingerprint group A are better adapted to these environmental conditions, the distribution of all four fingerprint groups at almost all locations sampled indicates that the source or sources of initial inoculum was a mixture of the four different fingerprint groups. Consequently, both a founder effect and surrounding cultivars may influence which fingerprint groups predominate. Ongoing investigations in this area may provide valuable insight into the influence of host genotype on the population diversity of the rice blast pathogen. In addition, yearly sampling is in progress and should provide information on the regional distribution of the various fingerprint groups.

Four additional fingerprint groups (E, F, G, and H) were identified among references isolates from Arkansas (Table 1). These data add additional support to the hypothesis that the rice blast fungus population in the United States is composed of a limited number of distinct lineages with similar haplotypes within each lineage (14,29). Within the four fingerprint groups found among the Arkansas field isolates, there were eight haplotypes within group A, three in D, and two each in B and C. Investigations on the stability of the fingerprint groups during epidemics and the cause and rate of appearance of the haplotypes

TABLE 6. Percent similarity[a] between MGR586-DNA fingerprint groups from Arkansas and those previously reported

MGR586 Fingerprint group (isolate)	Lineage[b] (reference isolate) IB-1 (85L2)	IG-1B (85M2)	IB-49A (85L8)	IC-17 (75A1)	IB-49B (793)	IB-45 (78L5)	IH-1 (74L2)	IG-1A (82A1)
A1 (LO1-4)	**82**	37	32	52	39	31	52	47
B1 (LO3-8)	32	**85**	25	42	31	27	22	35
C1 (BM1-24)	29	34	**81**	33	25	39	31	26
D1 (BM5-11)	46	41	28	**93**	28	28	53	59
E1 (49-D)	34	36	21	27	**92**	39	36	32
F1 (75A11)	37	33	37	30	23	**97**	34	37
G1 (75A10)	48	38	29	47	32	32	**98**	39

[a] Nei and Li's (22) percent similarity. Bold numbers indicate >80% similarity.
[b] Lineage designations for the isolates are shown in Table 1 (14).

within fingerprint groups are in progress. Because the sexual stage of this pathogen is not considered functional under field conditions, these minor differences in fingerprints may be caused by mutations, heterokaryosis, and/or parasexuality (25,29).

Although this study supports the hypothesis that there are a limited number of distinct fingerprint groups (i.e., lineages), our data indicate that the relationship between pathotype and DNA fingerprint group may not be as diagnostic as was originally reported (14), particularly when field isolates with a microgeographic origin are examined. In the study by Levy et al (14), six fingerprint groups were composed of a single pathotype, and two groups had multiple pathotypes. Examination of our field isolates and reference strains indicate that this relationship is not so straightforward (Table 8). For example, in this study, pathotype diversity was detected in three of the six fingerprint groups earlier reported to contain a single pathotype (14). Since only four or five isolates per fingerprint group were examined, this may even represent the lower limit of pathogenic variation within a group. Furthermore, relatively few rice cultivars are grown in rice-producing areas of the United States; in locations with more cultivar diversity, we would predict an even greater degree of pathotype diversity within a fingerprint group when microgeographic populations are examined.

The isolates used in the previous study represent multiple locations and were collected over a number of years (14). Moreover, they probably represent isolates that could be readily characterized for pathotype. Isolates from field collections that are not readily characterized for pathotype often are not stored for future use. Consequently, some collections may be somewhat biased toward isolates that "fit" a distinct pathotype and may not be representative of the actual virulence diversity in the population. Interestingly, one reference isolate collected in 1975 was in fingerprint group B (75A49) and was consistently characterized as race IG-1. However, all of the contemporary field isolates that were in fingerprint group B were characterized as pathotype IC-17. It is possible that virulence in this fingerprint group has changed from an IG-1 pathotype to IC-17.

Virulence diversity of *M. grisea* is a difficult trait to characterize and is completely dependent on the differential rice cultivars used. Although it is necessary to use the international set of rice differentials for comparison with international pathotype nomenclature for this pathogen, there is justification for using cultivars that are, or have been, commonly grown in a geographical area. Although some discrepancies in pathotype were observed between the international differentials and the Arkansas differentials, most of the pathotype identifications were consistent. However, caution is warranted when using the Arkansas differentials for pathotype identification. For example, on the Arkansas differentials, IB-49 and IB-1 were separated by the reaction on cv. Lemont; Lemont is resistant (disease reaction ≤ 3) to IB-1 and susceptible (disease reaction ≥ 4) to IB49. Resistance of Lemont to IB-1 is controlled by a single recessive gene (*pi-d*) (16), and frequently a disease reaction of 3 is observed under greenhouse conditions. Minor modifications in the protocol for testing virulence could increase this disease reaction to 4 and thereby change the pathotype identity. In contrast, the international differentials separate IB-1 and IB-49 by the reactions of two cultivars, Usen and NP125; both cultivars are susceptible to IB-1 and resistant to IB-49. In addition, pathotypes IE-1 and IC-17 cannot be differentiated on the Arkansas differentials (Table 7).

MGR-DNA sequence probes have been successfully used as genetic markers in population biology (14) and genetic mapping (9) of *M. grisea*. In this study, the MGR586 probe was used to measure the amount and distribution of the genetic diversity of the fungus on a microgeographic scale. The application of DNA RFLP analysis for the study of genetic variation of fungal populations has usually required multiple single-copy DNA probes (18,19). With the MGR586 sequence, a single probe can detect 50–60 restriction fragments, making it possible to determine minor genomic changes. It is anticipated that the sensitivity of the MGR586 fingerprint probe will make it valuable for studies on the epidemiology of rice blast. In addition, the MGR586 probe will continue to be a useful tool to examine the population diversity of *M. grisea* on both macro- and microgeographic scales as well as changes over time.

The evolution of virulence in *M. grisea* and other plant pathogenic fungi may be independent of changes at the DNA level currently detectable. Indeed, virulence may be more of a promiscuous characteristic whereby, over time, multiple pathotypes become associated with a particular "genotype" (4,14). Consequently, the utility of these molecular tools to identify pathotypes must be cautiously interpreted, particularly when this

TABLE 7. Virulence tests on two sets of rice differential cultivars

			Pathotype identification[b]				
			International differentials			Arkansas differentials	
Isolate	MGR586 Fingerprint group	Lineage[a]	Test 1	Test 2	Test 3	Test 1	Test 2
75A49[c]	B	IG-1B	IG-1	IG-1	IG-1	IG-1	IG-1
85A7[c]	D	IC-17	IC-17	IC-17	IC-17	IC-17	IC-17
49-D[c]	E	IB-49B	IB-49	IB-49	IB-49	IB-49	IB-49
75A10[c]	G	IH-1	IH-1	IH-1	IH-1	IH-1	IH-1
LO1-4	A1	IB-1	IB-17	IB-1	IB-1	IB-1	NT[d]
LO3-16	A2	IB-1	IB-1	NT	NT	IB-49	IB-49
BM1-7	A1	IB-1	IB-1	IB-1	IB-1	IB-49	IB-49
BM3-13	A2	IB-1	IB-1	IC-17	IC-17	IB-49	IB-49
LO5-9	A1	IB-1	NT	IC-17	IB-1	IB-49	NT
LO1-10	B1	IG-1B	IC-17	IC-17	IC-17	IC-17	IC-17
LO5-7	B2	IB-1B	IC-17	IC-17	IC-17	IC-17	IC-17
BM4-27	B2	IG-1B	IC-17	IC-17	IE-1	IC-17	NT
BM4-17	B2	IG-1B	IC-17	IC-17	IE-1	IC-17	IC-17
LO3-8	B1	IG-1B	NT	NT	NT	IC-17	NT
LO2-27	C1	IB-49A	IB-1	IE-1	IE-1	IB-49	IB-49
LO4-8	C1	IB-49A	IE-1	IE-1	IG-1	IC-17	IC-17
BM1-24	C1	IB-49A	IC-17	IE-1	IB-49	IC-17	NT
BM5-27	C2	IB-49A	IB-49	IB-49	IE-1	IB-49	IB-49
LO3-14	C1	IB-49A	NT	NT	NT	IB-49	NT
LO1-24	D3	IC-17	IC-17	IC-17	IC-17	IC-17	IC-17
LO3-4	D2	IC-17	IC-17	IC-17	IC-17	IC-17	IC-17
BM4-10	D3	IC-17	IC-17	IC-17	IC-17	IC-17	IC-17
BM5-11	D1	IC-17	IC-17	IC-17	IC-17	IC-17	NT
BM5-30	D1	IC-17	NT	NT	NT	IC-17	IC-17

[a] Previously reported DNA fingerprint lineage (14).
[b] A disease rating scale of 0–9 for leaf blast disease was used. A reaction of 0–3 was considered a resistant reaction and ≥4 a susceptible reaction. Tests on international differential cultivars were conducted at Beaumont, TX, and on Arkansas differential cultivars at Stuttgart, AR.
[c] Reference isolate from Arkansas.
[d] NT = not tested.

TABLE 8. Summary of fingerprint groups, pathotype identification on two sets of differential rice cultivars, and previously reported lineage

	Pathotype identification[a]		
MGR586 Fingerprint group (isolate)	International differentials	Arkansas differentials[b]	Lineage[c]
A[d] (85L2)	NT[e]	NT	IB-1
A	IB-1, IB-17, IC-17	IB-49	IB-1
B[d] (75A49)	IG-1	IG-1	IG-1B
B	IC-17	IC-17	IG-1B
C[d] (85L8)	NT	NT	IB-49A
C	ID-1, IE-1, IC-17, IB-49	IC-17	IB-49A
D[d] (85A7)	IC-17	IC-17	IC-17
D	IC-17	IC-17	IC-17
E[d] (49-D)	IB-49	IB-49	IB-49B
F[d] (75A11)	NT	NT	IB-45
G[d] (75A10)	IH-1	IH-1	IH-1

[a] Race identification according to Ling and Ou (15).
[b] See Table 2 for disease reactions on Arkansas differentials.
[c] Lineage reported by Levy et al (14).
[d] Reference isolates.
[e] NT = not tested.

information is to be used in a disease-resistance breeding program. As probes to identify specific virulence factors in plant pathogens become available, it may be possible to rely on molecular data to identify pathotypes with greater confidence.

LITERATURE CITED

1. Beckwith, R., and Chakraborty, R. 1980. Genetic structure of *Pileolaria pseudomilitaris* (Polychae:Spirobidae). Genetics 96:711-726.
2. Bonman, J. M., Vergel De Dios, T. I, Bandong, J. M., and Lee, E. J. 1987. Pathogenic variability of monoconidial isolates of *Pyricularia grisea* in Korea and in the Philippines. Plant Dis. 71:127-130.
3. Chumley, F. G., and Valent, B. 1990. Genetic analysis of melanin-deficient nonpathogenic mutant of *Magnaporthe grisea*. Mol. Plant-Microbe Interact. 3:135-143.
4. Correll, J. C. 1991. The relationship between formae speciales, races, and vegetative compatibiity groups in *Fusarium oxysporum*. Phytopathology 81:1061-1064.
5. Correll, J. C., Gordon, T. R., and McCain, A. H. 1992. Genetic diversity in California and Florida populations of the pitch canker fungus, *Fusarium subglutinans* f. sp. *pini*. Phytopathology 82:415-420.
6. Goodwin, S. B., Spielman, L. J., Matuszak, J. M., Bergeron, S. N., and Fry, W. E. 1992. Clonal diversity and genetic differentiation of *Phytophthora infestans* populations in northern and central Mexico. Phytopathology 82:955-961.
7. Gordon, T. R., and Okamoto, D. 1992. Population structure and the relationship between pathogenic and nonpathogenic strains of *Fusarium oxysporum*. Phytopathology 82:73-77.
8. Hamer, J. E., Farrall, L., Orbach, M. J., Valent, B., and Chumley, F. G. 1989. 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.
9. Hamer, J. E., and Given, S. 1990. Genetic mapping with dispersed repeated sequences in the rice blast fungus: Mapping the SMO locus. Mol. Gen. Genet. 223:487-495.
10. Jacobson, D. J., and Gordon, T. R. 1990. The variability of mitochondrial DNA as an indicator of relationships between populations of *Fusarium oxysporum* f. sp. *melonis*. Mycol. Res. 94:734-744.
11. Latterell, F. M., and Rossi, A. E. 1986. Longevity and pathogenic stability of *Pyricularia oryzae*. Phytopathology 76:231-235.
12. Leung, H., and Taga, M. 1988. *Magnaporthe grisea* (*Pyricularia grisea*), the blast fungus. Adv. Plant Pathol. 6:175-188.
13. Leung, H., and Williams, P. H. 1986. Enzyme polymorphism and genetic differentiation among geographic isolates of the rice blast fungus. Phytopathology 76:778-783.
14. 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.
15. Ling, K. C., and Ou, S. H. 1969. Standardization of the international race numbers of *Pyricularia oryzae*. Phytopathology 59:339-342.
16. Marchetti, M. A., Lai, X., and Bollich, C. N. 1987. Inheritance of resistance to *Pyricularia oryzae* in rice cultivars grown in the United States. Phytopathology 77:799-804.
17. Marchetti, M. A., Rush, M. C., and Hunter, W. E. 1976. Current status of rice blast in the southern United States. Plant Dis. Rep. 60:721-725.
18. McDermott, J. M., McDonald, B. A., Allard, R. W., and Webster, R. K. 1989. Genetic variability for pathogenicity, isozyme, ribosomal DNA and colony color variants in populations of *Rhynchosporium secalis*. Genetics 122:561-565.
19. McDonald, B. A., and Martinez, J. P. 1990. DNA restriction fragment length polymorphisms among *Mycosphaerella graminicola* (anamorph *Septoria tritici*) isolates collected from a single wheat field. Phytopathology 80:1368-1373.
20. McDonald, B. A., McDermott, J. M., Goodwin, S. B., and Allard, R. W. 1989. The population biology of host-pathogen interactions. Annu. Rev. Phytopathol. 27:77-94.
21. Nei, M. 1975. Molecular Population Genetics and Evolution. American Elsevier, New York. 288 pp.
22. Nei, M., and Li, W.-H. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA 76:5269-5273.
23. Ou, S. H. 1980. A look at worldwide rice blast disease control. Plant Dis. 64:439-445.
24. Ou, S. H. 1980. Pathogen variability and host resistance in rice blast disease. Annu. Rev. Phytopathol. 18:167-187.
25. Ou, S. H. 1985. Pages 109-201 in: Rice Diseases. 2nd ed. Commonwealth Mycological Institute, Kew, England.
26. Rodriguez, R. J., and Yoder, O. C. 1991. A family of conserved repetitive DNA elements from the fungal plant pathogen *Glomerella cingulata* (*Collectotrichum lindemuthianum*). Exp. Mycol. 15:232-242.
27. Talbot, N. J., Salch, Y. P., Ma, N., and Hamer, J. E. 1993. Karyotypic variation within clonal lineages of the rice blast fungus, *Magnaporthe grisea*. Appl. Environ. Microbiol. 59:585-593.
28. Valent, B. 1990. Rice blast as a model system for plant pathology. Phytopathology 80:33-36.
29. Valent, B., and Chumley, F. G. 1991. Molecular genetic analysis of the rice blast fungus, *Magnaporthe grisea*. Annu. Rev. Phytopathol. 29:443-467.
30. Workman, P. L., and Niswander, J. D. 1970. Population studies on southwestern Indian tribes. II. Local genetic differentiation in the Papago. Am. J. Hum. Genet. 22:24-29.
31. Xia, J. Q., Correll, J. C., Lee, F. N., and Rhoads, D. D. 1992. DNA fingerprint (MGR) analysis of two local rice blast populations in Arkansas. (Abstr.) Phytopathology 82:1124.