Regional Population Diversity of *Pyricularia grisea* in Arkansas and the Influence of Host Selection

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ABSTRACT

Xia, J. Q., Correll, J. C., Lee, F. N., Ross, W. J., and Rhoads, D. D. 2000. Regional population diversity of *Pyricularia grisea* in Arkansas and the influence of host selections. Plant Dis. 84:877-884.

MGR586 DNA fingerprinting has been widely used to characterize population diversity of the rice blast pathogen, *Pyricularia grisea*. However, the frequency and distribution of particular haplotypes (individuals) within MGR-delimited lineages has not been examined in the United States. MGR586 DNA fingerprinting, mitochondrial DNA (mtDNA) restriction fragment length polymorphism (RFLPs), and virulence phenotyping were used to examine genetic diversity of *P. grisea* in Arkansas. A total of 470 monoconidial isolates were recovered from eight rice cultivars in 18 commercial fields in nine counties in Arkansas. All isolates were examined for nuclear DNA RFLPs with the MGR586 DNA fingerprint probe, and both the MGR lineage (isolates with >80% similarity) and the haplotype frequencies were determined. Four distinct MGR586 DNA fingerprint lineages (designated A, B, C, and D) were identified among the 470 field isolates. All four lineages were found in 9 of the 18 locations. Three lineages were found in four locations, two lineages in three locations, and only a single lineage was found at two locations. In all, 10, 19, 16, and 13 haplotypes (isolates which had MGR586 DNA fingerprints which differed by 1 to 20%) were identified within lineages A, B, C, and D, respectively, among the 470 isolates examined. Within each lineage, a single haplotype (clone) predominated, representing 51 to 71% of the isolates collected for each of the four lineages. Overall, 60% of the 470 isolates belonged to one of only four haplotypes (A1, B1, C1, and D1) and these four predominant haplotypes were recovered from between 7 and 14 of the 18 locations sampled, indicating a widespread distribution of these four clones. These data indicate an exceptionally low level of genetic diversity in the regional rice blast pathogen population in Arkansas relative to several other populations of *P. grisea* examined from tropical environments. In addition, no mtDNA RFLPs were detected among representative haplotypes within each of the lineages, indicating a single mtDNA haplotype was present in the population. Examination of virulence indicated that two races predominated in the regional collection. All 30 isolates in lineages A and C tested had an IB-49 virulence phenotype. Out of 30 isolates in lineages B and D, 29 had an IC-17 virulence phenotype. One isolate in lineage B, isolated from a highly susceptible cultivar (L201), had an IG-1 virulence phenotype. The frequencies of the four lineages varied among the locations sampled and may have been due, in part, to the cultivar from which isolates were recovered. A single lineage was recovered from two cultivars, Mars and Millie. Although only a single field of each of these cultivars was sampled, the data indicate that certain cultivars grown in Arkansas may serve as a "bottleneck", selecting out specific lineages in the regional population. To test this hypothesis, an additional 283 isolates were recovered from replicated plots of cvs. M204 and Mars located within commercial rice fields at two locations during two seasons. All four MGR586 lineages were recovered from each location. However, there was a strong bias for lineage B on cv. M204 (79% of all isolates) and a strong bias for lineage A on cv. Mars (95% of all isolates), indicating some cultivars were effective in excluding certain lineages.

Additional keywords: *Magnaporthe grisea*, MGR586, mitochondrial DNA, races, resistance, rice blast

Rice blast, caused by *Pyricularia grisea* (teleomorph: *Magnaporthe grisea*), is an important disease in most rice-producing areas of the world (31,40). Because of the global importance of rice blast, *P. grisea* has been the focus of extensive research and has emerged as a model system for the study of plant pathogenic fungi and host–pathogen interactions (37,38). Host resistance to *P. grisea* is an effective and economical management practice to reduce the impact of rice blast (17,19,23). However, as with many plant diseases, the durability of resistance to *P. grisea* has been less than desirable (23,28,33,47). Information on regional and global population diversity of *P. grisea* and the mechanisms by which this fungus can overcome host resistance are critical for developing strategies to improve the durability of resistance.

The precision of molecular markers has greatly increased our knowledge of fungal population diversity. Hamer et al. (12) identified a family of dispersed repetitive DNA sequences, designated MGR, that is conserved in rice-infecting isolates of *P. grisea* and could be diagnostically useful for genetic analysis of the rice blast pathogen population (1–4,11,20,21,47). As a result, the MGR586 probe has been used to examine populations of *P. grisea* from throughout the world (5,35,46).

Examination of MGR586 DNA-fingerprint diversity of a collection of *P. grisea* from the United States identified eight lineages (21,44). Examination of microgeographic population diversity in Arkansas revealed that four MGR586 lineages (designated A, B, C, and D) were present on a single cultivar from two fields in a single county (44). Hierarchical diversity analysis in this population demonstrated that the majority (87%) of the genetic variability was distributed among the isolates within a sample location (44). However, the macrogeographical, or regional, diversity of the contemporary *P. grisea* population in Arkansas has not been assessed, nor has it been determined if given haplotypes predominate in the region, on given cultivars, or within fields. Furthermore, little is known on how host selection influences the population structure of *P. grisea*.

Arkansas is the largest rice producing state in the United States, with over 450,000 ha of rice grown annually, which represents approximately 40% of the U.S. rice crop (41). The objective of this study was to examine the regional MGR586 DNA fingerprint group and haplotype diversity of *P. grisea* collected from commonly grown rice cultivars from the major rice-producing counties in Arkansas. Representative isolates of the various DNA lineages and haplotypes were further characterized for mitochondrial DNA (mtDNA) restriction fragment length polymorphisms (RFLP)s and virulence. Field tests also were conducted to determine the effect of certain cultivars on the selection of pathogen MGR586 lineages.

MATERIALS AND METHODS

Field collections. Isolates of *P. grisea* were collected from rice during the 1992 growing season from 18 commercial rice fields in Arkansas (Fig. 1). Approximately

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This work was supported, in part, by the Arkansas Rice Research and Promotion Board. A portion of this manuscript was prepared while J. C. Correll was on sabbatical leave at the International Rice Research Institute, Los Baños, Philippines.

Accepted for publication 2 May 2000.

Publication no. D-2000-0621-01R
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five rice panicles or leaves with blast symptoms were randomly sampled from four to six locations in each field. Sample locations within a field were a minimum of 50 m apart. Monoconidial cultures of the fungus were recovered from blast lesions and maintained by the methods described previously (44).

A total of 470 isolates were collected from eight cultivars from 18 commercial rice fields in nine counties (Table 1). Neck and panicle blast samples were collected from all 18 fields from mature plants late in the growing season (2 September to 1 October). Two fields from Lonoke County (fields 9 and 10) were sampled twice; leaf blast samples were collected 15 July and neck-panicle blast samples were collected 2 September.

MGR586 DNA fingerprints. Cultures for DNA extraction were grown in complete medium broth (6,44) at room temperature for 5 to 7 days on a rotary shaker. Fungal DNA was extracted by a modified "mini-prep" procedure described previously (6,44). For southern blot analysis, 1.5 µg of genomic DNA was digested with *Eco*RI according to the manufacturer's instructions and size fractionated on a 0.8% agarose gel for 58 h at 25 V before transfer to hybond N+ (Amersham Corp., Arlington Heights, IL) hybridization membrane.

The dispersed repeated DNA probe (MGR586) was a gift from J. Hamer (Purdue University). The MGR586 sequence was cloned into a plasmid, propagated in *Escherichia coli*, and isolated by a cleared lysate method (44). The MGR-DNA probe was labeled and hybridization reactions were performed with a direct nucleic acid labeling and detection kit (ECL, Amersham Corp.). The MGR586 sequence probe was covalently labeled with enzyme horseradish peroxidase with glutaraldehyde. After a 12- to 16-h hybridization period, membranes were washed twice at 42°C in primary wash buffer (0.5× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate], 36% urea, and 0.4% sodium dodecyl sulfate) for 20 min, followed by two 5-min washes in 2× SSC at room temperature. Film (Hyperfilm-ECL, Amersham Corp.) was placed on the membranes and exposed for 10 to 120 min depending on strength of the enzyme reaction signals.

Data analysis. Similarities in the MGR586-DNA fingerprints between fungal isolates were calculated using Nei and Li's index of genetic similarity (27) for RFLP comparisons by the formula S_{xy} (%) = $200n_{xy}/(n_x + n_y)$, where n_{xy} is the number of shared MGR586 bands, and n_x and n_y are the number of fragments from isolates x and y, respectively (44).

MGR586 DNA fingerprint groups were defined based on the S_{xy} value. Isolates with an S_{xy} value of >80% were considered to be members of the same fingerprint group. Levy et al. (21) proposed these groups be given lineage designations to

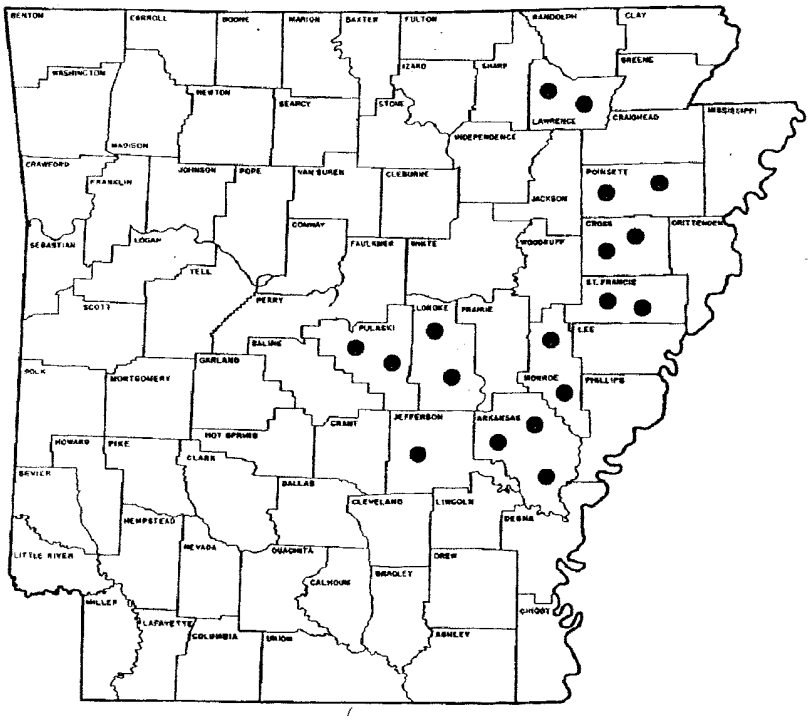

Fig. 1. Eighteen commercial rice fields sampled in Arkansas.

Table 1. Distributions of MGR586 DNA-fingerprint lineages of *Pyricularia grisea* in Arkansas[a]

Location no., county	Cultivar	No. of isolates	Isolates in each MGR586-fingerprint lineage (%)[b]			
			A	B	C	D
1. Arkansas	Newbonnet	16	13	56	12	19
2. Arkansas	L202	20	0	5	75	20
3. Arkansas	Newbonnet	20	10	50	15	25
4. Cross	Alan	12	8	25	42	25
5. Cross	Alan	5	20	40	40	0
6. Jefferson	Newbonnet	19	0	69	26	5
7. Lawrence	Lemont	7	0	0	29	71
8. Lawrence	Millie	9	0	100	0	0
9. Lonoke	Newbonnet	106	2	54	29	15
10. Lonoke	Mars	116	100	0	0	0
11. Monroe	Newbonnet	20	5	25	25	45
12. Monroe	Newbonnet	14	0	79	21	0
13. Pulaski	Lemont	20	5	55	30	10
14. Pulaski	Newbonnet	19	68	11	5	16
15. Poinsett	Alan	13	8	46	31	15
16. Poinsett	Jackson	14	0	29	64	7
17. St. Francis	L201	20	0	40	60	0
18. St. Francis	Lemont	20	5	30	40	25
Total	…	470	30	33	24	13

[a] All isolates were recovered from neck blast samples collected 2 September to 1 October except 49 and 60 isolates recovered from leaf blast samples from cvs. Newbonnet and Mars, respectively, collected 15 July.

[b] Fingerprint lineage designations were those previously described (44).

indicate the evolutionary relationship among isolates. Thus, isolates with >80% similarity were given lineage designations (i.e., A, B, C, and so on). Isolates with an S_{xy} value of <60% were considered different lineages. No intermediate (59% < S_{xy} <81%) isolates were observed. Isolates within a lineage that showed fingerprint variation (80< S_{xy} <100%) were assigned numerical haplotype designations (i.e., A1, A2, A3, and so on).

Lineage and haplotype diversity was calculated based on the Shannon diversity index D, where $D = -\Sigma p_i \ln p_i$ and p_i is the frequency of the *i*th genotype (14,18). The Shannon's index of diversity D was normalized to correct for differences in sample size $D' = D/\ln N$, where N is the sample size (18).

mtDNA RFLPs. No polymorphisms have been identified in the mtDNA among rice-infecting isolates of *P. grisea* outside of the United States, but few isolates from the United States have been examined (35). To examine mtDNA RFLPs from diverse isolates from the United States, two nonoverlapping mtDNA clones from *Colletotrichum orbiculare* (Berk. & Mont.) Arx were used (9). These two clones hybridized to four of five *Eco*RI fragments (9.1, 7.6, 6.9, and 5.5 kb) of purified mtDNA of *P. grisea* restricted with *Eco*RI, but not to a 4.7-kb fragment. Thus, these two clones hybridized to 86% of the mitochondrial genome of *P. grisea*. The two clones were combined in equimolar concentrations and used to probe Southern blots of genomic DNA of *P. grisea* digested with *Eco*RI. Forty isolates of *P. grisea*, representative of the various haplotypes within each of the lineages, were examined.

Race determinations. Sixty isolates, representing multiple haplotypes from each of the four lineages, were characterized for race in greenhouse inoculation tests. Each isolate was inoculated onto an international set and an Arkansas set of differential rice germ plasm (44). The inoculation procedures, incubation conditions, disease ratings, and evaluation of host reactions were conducted as previously described (44). Briefly, 2-week-old rice seedlings were inoculated with a conidial suspension (1×10^5 conidia/ml) prepared by harvesting conidia from the surface of 7- to 10-day-old cultures grown on rice polish agar (44). Inoculated plants were incubated at 24°C at 100% relative humidity for 24 h, then moved to a greenhouse maintained at 23 to 28°C. Plants were scored for disease on a scale of 0 to 9, where a mean disease reaction of 0 to 3 is considered resistant and ≥4 susceptible (44). Each isolate was tested on each of the two sets of differential germ plasm in three separate inoculation tests.

Host selection. An additional 283 isolates were recovered from three experimental sites over two seasons. Each site was located within a commercial rice field. Two of the experimental sites were located in White County and were sampled in 1994 and 1995. These two sites were surrounded by commercial plantings of the cultivars Alan and Cypress in 1994 and 1995, respectively. A third experimental site was sampled in 1995 in Clay County and was surrounded by cv. Katy.

Each experimental site contained 20 rice cultivars or breeding lines. Each site was approximately 30 by 15 m^2 and the 20 cultivars were arranged in a completely randomized block design with three replications per cultivar. Each replicate plot was 1.5 m wide (7 rows, 18-cm spacing) and 5 m long. Each row contained 30 to 60 plants/m of row and 60 to 150 tillers/m of row by the end of the season. Approximately 20 isolates were recovered from each replicated plot from each location in 1994 and 1995 from cvs. M204 and Mars.

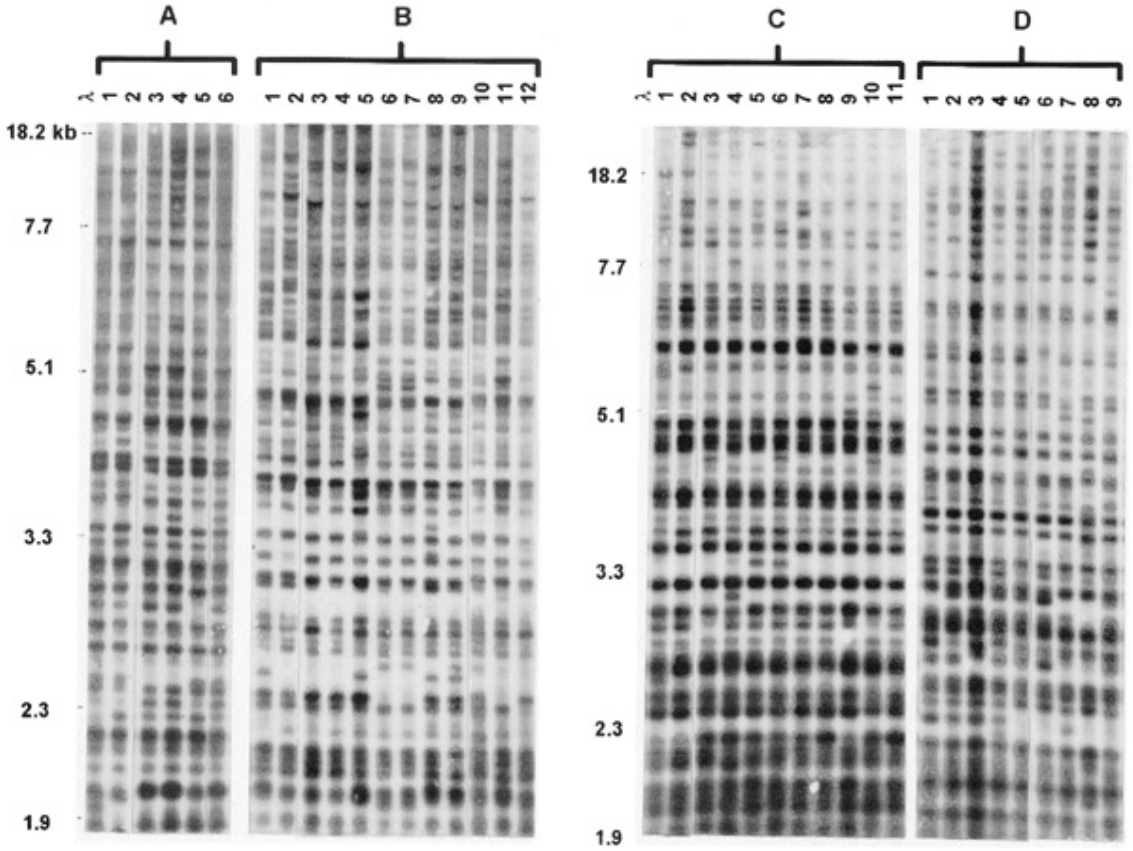

Fig. 2. Composite southern blot showing haplotypes in MGR586 DNA fingerprint lineages A and B of *Pyricularia grisea* collected from Arkansas in 1992. Haplotypes within group A in lanes 1 to 6 are A1 (isolate A182), A2 (A177), A5 (A193), A6 (A300), A7 (A516), and A9(A201). Haplotypes within group B in lanes 1 to 12 are B1 (isolate A611), B5 (A139), B7 (A405), B8 (A355), B9 (A361), B10 (A431), B11 (A436), B17 (A496), B18 (A511), B19 (A601), B2 (A427), and B4 (A416). Haplotypes within group C in lanes 1 to 11 are C1 (isolate A119), C6 (A406), C9 (A362), C10 (A368), C12 (A549), C13 (A599), C14 (A562), C15 (A572), C16 (A482), and C17 (A536). Haplotypes within group D in lanes 1 to 9 are D1 (isolate A142), D2 (A125), D3 (A542), D9 (A561), D5 (A507), D10 (A454), D11 (A493), D12 (A513), and D13 (A531).

Mars was selected as a cultivar because it showed a strong bias for lineage A in previous samples and M204 was selected because it was considered to be one of the more susceptible cultivars.

RESULTS

Distribution of MGR586 DNA-fingerprint lineages. Four distinct lineages, designated A, B, C, and D, were identified among the 470 isolates based on the presence or absence of approximately 50 DNA fragments ranging in size from 1.8 to 20.0 kb (Table 1, Fig. 2). DNA similarity (S_{xy}) among the isolates within a lineage ranged from 81 to 100% and was <60% among isolates between lineages. No isolates with intermediate S_{xy} values (61 to 80%) were found. The regional lineage and haplotype diversity based on the Shannon index of diversity was similar to that calculated for populations in the Philippines and Columbia, but considerably lower than several populations examined in India (Table 2).

The frequency of the four lineages varied with sample location and cultivar (Table 1). The overall mean frequency of lineages A, B, C, and D from all 18 locations was 0.30, 0.33, 0.24, and 0.13, respectively. All four lineages were found at 9 of the 18 locations, three lineages were found at 4 locations, two at 3 locations, and only a single lineage was recovered from 2 locations.

Haplotype diversity within MGR586 DNA fingerprint lineages. Based upon MGR586 DNA fingerprint differences of 1 to 20%, 10, 19, 16, and 13 haplotypes were found within lineages A, B, C, and D, respectively (Table 3, Fig. 1). One haplotype predominated in each of the fingerprint groups; 71, 55, 57, and 51% of the isolates belonged to haplotypes A1, B1, C1, and D1, respectively (Table 3). Thus, 270 of the 470 isolates (60%) recovered belonged to one of four haplotypes. Several haplotypes within each lineage were represented by a single isolate.

The predominant haplotypes (A1, B1, C1, and D1) within each lineage were widely distributed throughout the state and were found in 7, 14, 12, and 9 of the 18 locations sampled, respectively (Table 4). Two locations (fields 9 and 10), which were sampled more intensively, also

Table 3. Haplotypes of *Pyricularia grisea* found within each of four MGR586 DNA-fingerprint lineages[a]

	No. of isolates (%)[b]			
Haplotype	**A**	**B**	**C**	**D**
1	100 (70.9)	86 (54.8)	64 (56.6)	30 (50.8)
2	6 (4.3)	1 (0.6)	1 (0.9)	2 (3.4)
3	3 (2.1)	1 (0.6)	5 (4.4)	6 (10.2)
4	2 (1.4)	2 (1.3)	2 (1.8)	2 (3.4)
5	22 (15.6)	2 (1.3)	8 (7.1)	4 (6.8)
6	3 (2.1)	1 (0.6)	3 (2.7)	3 (5.1)
7	2 (1.4)	41 (26.1)	1 (0.9)	1 (1.7)
8	1 (0.7)	1 (0.6)	2 (1.8)	1 (1.7)
9	1 (0.7)	1 (0.6)	1 (0.9)	2 (3.4)
10	1 (0.7)	3 (1.9)	3 (2.7)	3 (5.1)
11	…	2 (1.3)	6 (5.3)	1 (1.7)
12	…	1 (0.6)	2 (1.8)	1 (1.7)
13	…	1 (0.6)	7 (6.2)	3 (5.1)
14	…	7 (4.5)	3 (2.7)	…
15	…	1 (0.6)	1 (0.9)	…
16	…	3 (1.9)	4 (3.5)	…
17	…	1 (0.6)	…	…
18	…	1 (0.6)	…	…
19	…	1 (0.6)	…	…

[a] Letters indicate MGR586 DNA-fingerprint lineage and numbers indicate haplotypes found within each lineage.
[b] Number and percentage of isolates in each haplotype and lineage, based on MGR586 DNA-fingerprinting.

Table 4. Distributions of the predominant haplotypes A1, B1, C1, and D1 in counties in Arkansas

		Haplotype[a]			
Location, county	**Cultivar**	**A1**	**B1**	**C1**	**D1**
1. Arkansas	Newbonnet	1/2	8/9	2/2	1/3
2. Arkansas	L202	…	1/1	10/15	4/4
3. Arkansas	Newbonnet	0/2	9/10	0/3	2/5
4. Cross	Alan	1/1	3/3	4/5	2/3
5. Cross	Alan	1/1	1/2	1/2	…
6. Jefferson	Newbonnet	…	2/13	4/5	0/1
7. Lawrence	Lemont	…	…	0/2	0/5
8. Lawrence	Millie	…	1/9	…	…
9. Lonoke	Newbonnet	1/2	42/57	17/31	8/16
10. Lonoke	Mars	82/116	…	…	…
11. Monroe	Newbonnet	0/1	1/5	2/5	6/9
12. Monroe	Newbonnet	…	0/11	0/3	
13. Pulaski	Lemont	1/1	10/11	2/6	1/2
14. Pulaski	Newbonnet	13/13	1/2	1/1	3/3
15. Poinsett	Alan	0/1	4/6	2/4	0/2
16. Poinsett	Jackson	…	0/4	0/9	0/1
17. St. Francis	L201	…	1/8	12/12	…
18. St. Francis	Lemont	0/1	2/6	7/8	3/5

[a] Number of isolates recovered from each of the four predominant haplotypes/number of isolates in the MGR586 DNA fingerprint lineage. For example, two MGR586 lineage A isolates were recovered from location 1 (cv. Newbonnet), of which 1 isolate was haplotype A1; 8 of 9 lineage B isolates from this location were haplotype B1; … indicates no isolates in that lineage were recovered.

Table 2. Lineage and haplotype diversity of several populations of *Pyricularia grisea* based on MGR586 DNA fingerprints

		Lineages[a]		Haplotypes	
Location	**No. of isolates examined**	**Number**	**Diversity index[b]**	**Number**	**Diversity index[b]**
United States	470	4	0.22	58	0.40
Europe[c]	41	5	…	24	…
Columbia[d]	151	6	0.29	115	…
Philippines[d]	1516	10	0.19	102	…
India[d]	222	45	0.57	157	0.92

[a] Lineages and haplotypes based on MGR586 DNA fingerprinting; … = data not available.
[b] Shannon's normalized diversity index (18).
[c] Isolates were from a collection of isolates and not a sampled population (34).
[d] Data previously calculated (18).

showed a low level of haplotype diversity (Table 3). In field 10, all 116 isolates from cv. Mars were in lineage A. Of these, 82 isolates (71%) were haplotype A1 and 20 isolates (17%) were haplotype A2 (*data not shown*). In field 9, all four lineages were found among the 106 isolates recovered from Newbonnet. Of 57 isolates in lineage B, 42 (74%) were haplotype B1 and 11 (19%) were haplotype B7. Of 31 isolates in lineage C, 17 (55%) were in haplotype C1 and 7 (23%) were in haplotype C5.

Frequency of MGR586 DNA fingerprint lineages on different cultivars and at different sample times. The distribution of the various lineages differed on the various cultivars sampled (Table 5). All four lineages were recovered from cvs. Alan, Lemont, and Newbonnet; 4 to 10% of the isolates from these three cultivars were in group A, 36 to 50% in group B, 24 to 35% in group C, and 16 to 26% in group D. Although some of the sample sizes were small, lineage A isolates were not recovered from cvs. Jackson, L201, L202, and Millie; lineage B isolates were not recovered from cv. Mars; lineage C isolates were not recovered from Mars or Millie; and lineage D isolates were not recovered from L201, Mars, or Millie. Although only a single field of each cultivar was sampled, all 116 isolates recovered from cv. Mars were in lineage A and all 9 isolates recovered from cv. Millie were in lineage B.

The frequency of the lineages recovered at two different sample times from two locations (fields 9 and 10) also were compared (Table 6). From a single field of cv. Mars, all 60 isolates recovered from leaf blast samples on 15 July and all 56 isolates recovered from neck-panicle blast samples from 2 September were in lineage A. From a single field of cv. Newbonnet, lineages B, C, and D were identified among the 49 isolates collected on 15 July and all four lineages were found on 2 September. Lineage C predominated (50 to 72% of the isolates) at two of the three sample locations within the field among isolates collected on 15 July from leaf blast samples, whereas lineage B predominated (58 to 89% of the isolates) at all three sample locations within the field among isolates collected on 2 September from neck-panicle blast samples.

mtDNA RFLPs. Examination of purified mtDNA of reference isolate S1 from lineage B cut with *Eco*RI revealed five fragments (9.1, 7.6, 6.9, 5.5, and 4.7 kb; *data not shown*). The two mtDNA clones employed hybridized to all but the 4.7-kb fragment of the purified mtDNA. No mtDNA RFLPs were detected in 40 isolates representing different haplotypes from the four lineages, indicating that the population of *P. grisea* in Arkansas is composed of a single mtDNA haplotype.

Race identification. Three virulence phenotypes, or races, designated IB-49, IC-17, and IG-1, were identified among 60 isolates examined (Table 7). All 30 isolates in lineages A and C were characterized as race IB-49 on both the international and the Arkansas differentials in the repeated test (Tables 7 and 8). Of the 30 isolates in lineages B and D, 29 were characterized as race IC-17 on the two sets of differentials. A single isolate in fingerprint group B (A431), originally recovered from cv. L201 from St. Francis County, was consistently characterized as race IG-1.

Host selection. All four lineages (A, B, C, and D) were detected at the experimental sites in White County in 1994 and Clay County in 1995 (*data not shown*; 34). However, disease pressure was low in 1995 and only lineages A and B were detected at the White County site in 1995. A total of 159 isolates were recovered from cv. M204 and 124 from cv. Mars. Of the 159 isolates recovered from M204 from all three sample sites, 125 (78%) belonged to lineage B (Table 9). The bias for the B lineage was consistent for all three replications at all three locations. Between 85 and 100% of the isolates were in lineage B for six of the nine subplots (i.e., replications) and 54 to 69% for the other three subplots.

The bias for lineage A on cv. Mars was very evident. Of the 124 isolates collected from the three sample sites in both years, 118 (95%) belonged to lineage A (Table 9). The bias for the A lineage was evident for all nine subplots; 88 to 100% of the isolates belonged to the A lineage.

DISCUSSION

Examination of genetic diversity of the *P. grisea* population has been the focus of a number of investigations in many rice-growing regions throughout the world. Earlier studies have focused on characterizing race diversity within populations of *P. grisea* (25,30), whereas more recent studies have focused on the assessment of population structure using DNA fingerprinting, race characterization, and the relationship between the two (2,3,20,21,44,47). These studies have revealed several key features about the population structure of *P. grisea*, including the presence of distinct lineages within a given country or growing region, the absence of a given lineage in more than one country or growing region, and varying degrees of virulence diversity among isolates within a given lineage. In this study, only four lineages were recovered among the 470 isolates sampled. The four lineages have previously been recovered from a microgeographic collection in the state

Table 5. Distribution of MGR586 DNA-fingerprint lineages of *Pyricularia grisea* on cultivars sampled in Arkansas

			Isolates in each MGR586 DNA-fingerprint lineage (%)			
Cultivar	**No. of isolates**	**No. of fields**	**A**	**B**	**C**	**D**
Alan	30	3	10	39	35	16
Jackson	14	1	0	29	64	7
L201	20	1	0	40	60	0
L202	20	1	0	5	75	20
Lemont	47	3	4	36	34	26
Mars	116	1	100	0	0	0
Millie	9	1	0	100	0	0
Newbonnet	214	7	9	50	24	17

Table 6. Frequency of MGR586 DNA-fingerprint lineages at two sample times in two rice fields in Lonoke County, Arkansas, in 1992[a]

			Isolates in each MGR586 DNA-fingerprint lineage (%)			
Cultivar, date	**Site[b]**	**No. of isolates**	**A**	**B**	**C**	**D**
Newbonnet						
15 July	1	18	0	11	72	17
	2	17	0	59	6	35
	3	14	0	43	50	7
Total	…	49	0	37	43	20
2 September	1	20	5	60	25	10
	2	18	0	89	11	0
	3	19	4	58	16	21
Total	…	57	3	68	18	11
Mars						
15 July	1	21	100	0	0	0
	2	19	100	0	0	0
	3	20	100	0	0	0
Total	…	60	100	0	0	0
2 September	1	19	100	0	0	0
	2	17	100	0	0	0
	3	20	100	0	0	0
Total	…	56	100	0	0	0

[a] Field 9 (Newbonnet) and field 10 (Mars) were 10 km apart.
[b] Each site was separated by a minimum of 100 m.

(44). Although a total of eight lineages have been identified in the U.S. population (21,44), only lineages A, B, C, and D have been recovered in contemporary populations in Arkansas. Furthermore, 60% of all of the isolates recovered from throughout the rice-growing region of the state belonged to one of only four haplotypes. These four haplotypes, A1, B1, C1, and D1, were widely distributed throughout the state, having been recovered from 7, 14, 12, and 9 fields sampled, respectively. In addition, it was not uncommon for one or all of these four haplotypes to predominate within a given field (Table 4). For example, at location 9, all four lineages were recovered, and 42 of 57 lineage B isolates, 17 of 31 lineage C isolates, and 8 of 16 lineage D isolates were haplotypes B1, C1, and D1, respectively. Also, 82 of 116 lineage A isolates recovered from location 10 were haplotype A1. Thus, although mutations which can cause minor changes in MGR fingerprints in a given haplotype have been observed under both laboratory and field conditions (42,43,45), the distribution and frequencies of several common haplotypes would indicate that they are relatively stable over the course of a growing season.

Geographically distinct populations of the rice blast pathogen show considerable variation in the degree of MGR586 DNA fingerprint diversity (Table 2). Based on Shannon's diversity index for lineages, values of 0.19 and 0.29 where recorded for populations in the Philippines and Columbia, respectively, and comparable to a 0.22 value for the U.S. population sampled in the current study. These values are considerably lower than the 0.57 value calculated for populations in India, where the fungus was suspected to be sexually reproducing (18). The diversity index based on haplotype diversity also was low in the United States (0.40) compared to India (0.92) (Table 2).

The degree of virulence diversity within a population from a given geographical area also can vary. Several studies have shown that virulence diversity within a lineage can be rather high (3,4,20,44). Three virulence phenotypes, or races, were identified among the isolates examined in the current study. Thirty geographically diverse lineage A and C isolates, representing 7 and 10 different haplotypes, re-

Table 7. The relationship between MGR586 DNA-fingerprint lineage and race among isolates of *Pyricularia grisea* in Arkansas

		Origin		
Isolate	**Lineage-haplotype[a]**	**County**	**Host**	**Race[b]**
A598	A1	Cross	Alan	IB-49
A603	A1	Cross	Alan	IB-49
A491	A1	Arkansas	Newbonnet	IB-49
A343	A1	Pulaski	Newbonnet	IB-49
A359	A1	Pulaski	Newbonnet	IB-49
A233	A1	Lonoke	Newbonnet	IB-49
A177	A2	Lonoke	Mars	IB-49
A274	A2	Lonoke	Newbonnet	IB-49
A187	A3	Lonoke	Mars	IB-49
A193	A5	Lonoke	Mars	IB-49
A213	A4	Lonoke	Mars	IB-49
A290	A5	Lonoke	Mars	IB-49
A400	A5	Monroe	Newbonnet	IB-49
A516	A7	Arkansas	Newbonnet	IB-49
A201	A9	Lonoke	Mars	IB-49
A264	B1	Lonoke	Newbonnet	IC-17
A358	B1	Pulaski	Newbonnet	IC-17
A624	B2	Lonoke	Newbonnet	IC-17
A631	B3	Lonoke	Newbonnet	IC-17
A139	B5	Lonoke	Newbonnet	IC-17
A405	B7	Monroe	Newbonnet	IC-17
A355	B8	Pulaski	Newbonnet	IC-17
A361	B9	Pulaski	Lemont	IC-17
A431	B10	St. Francis	L201	IG-1
A460	B12	St. Francis	Lemont	IC-17
A552	B13	Lawrence	Millie	IC-17
A560	B15	Poinsett	Jackson	IC-17
A496	B17	Arkansas	Newbonnet	IC-17
A511	B18	Arkansas	Newbonnet	IC-17
A601	B19	Cross	Alan	IC-17
A119	C1	Lonoke	Newbonnet	IB-49
A345	C1	Pulaski	Newbonnet	IB-49
A434	C1	St. Francis	L201	IB-49
A475	C1	Arkansas	L202	IB-49
A136	C2	Lonoke	Newbonnet	IB-49
A395	C3	Monroe	Newbonnet	IB-49
A406	C6	Monroe	Newbonnet	IB-49
A281	C7	Lonoke	Newbonnet	IB-49
A362	C9	Pulaski	Lemont	IB-49
A461	C11	Arkansas	L202	IB-49
A549	C12	Lawrence	Lemont	IB-49
A559	C13	Poinsett	Jackson	IB-49
A575	C17	Poinsett	Alan	IB-49
A536	C17	Jefferson	Newbonnet	IB-49
A482	C…	Arkansas	Newbonnet	IB-49
A142	D1	Lonoke	Newbonnet	IC-17
A143	D1	Lonoke	Newbonnet	IC-17
A347	D1	Pullaski	Newbonnet	IC-17
A542	D3	Lawrence	Lemont	IC-17
A242	D5	Lonoke	Newbonnet	IC-17
A513	D12	Arkansas	Newbonnet	IC-17
A267	D6	Lonoke	Newbonnet	IC-17
A366	D8	Pulaski	Lemont	IC-17
A381	D9	Monroe	Newbonnet	IC-17
A454	D10	St. Francis	Lemont	IC-17
A493	D11	Arkansas	Newbonnet	IC-17
A531	D13	Jefferson	Newbonnet	IC-17
A561	D9	Poinsett	Jackson	IC-17
A592	D9	Cross	Alan	IC-17
A385	D4	Monroe	Newbonnet	IC-17

[a] Lineage was determined with the MGR586 DNA probe. Letter refers to MGR586 DNA fingerprint lineage; number refers to the haplotype within a lineage; … indicates data not available.

[b] Races were determined by repeated inoculations on an Arkansas set and an international set of rice differentials (44).

Table 8. Summary of disease reactions of international pathotypes of *Pyricularia grisea* on differential rice cultivars

	International races[a]		
Cultivar	**IG-1**	**IC-17**	**IB-49**
M201	S	S	S
Starbonnet	S	S	S
Tebonnet	R	S	S
Newbonnet	R	S	S
Lemont	R	S	S
Zenith	R	R	S
Mars	R	R	S
Katy	R	R	R
Raminad Str. 3	R	R	R
Zenith	R	R	S
NP125	R	S	R
Usen	R	R	R
Dular	R	S	S
Kanto 51	R	S	S
Sha Tiao Tsao (S)	S	S	S
Caloro	S	S	S

[a] R = resistant reaction (disease rating 0 to 3) and S = susceptible reaction (disease rating ≥4).

spectively, had an IB-49 virulence phenotype. Of 30 geographically diverse lineage B and D isolates, representing 14 and 12 different haplotypes, respectively, 29 had an IC-17 virulence phenotype. Although virulence diversity has been identified among selected isolates in the U.S. population (7,8,21,44), in this study, 59 of 60 isolates selected to represent the geographic and haplotype diversity showed a consistent relationship between race and lineage. Based on both the prevalence and distribution of certain haplotypes, combined with the association of virulence and lineage, the results in this study indicate that *P. grisea* is predominantly, if not exclusively, reproducing asexually, and that only a few haplotypes predominate in the population in this geographic region. These data are consistent with the reproductive biology of many other asexual organisms in an epidemic phase of development (36).

The exceptionally low level of genetic diversity assessed by MGR586 DNA fingerprints and virulence and the widespread distribution of four apparent clones throughout the region is likely due to several factors. Rice cultivation in the United States is a relatively recent endeavor, being successfully introduced into South Carolina in 1694 and into Arkansas in the late 1800s (10). Relatively few cultivars make up the majority of the commercial rice acreage in the United States, and they possess relatively few major genes for resistance (23,24). Furthermore, in the mid-1980s, approximately 70% of the 450,000 ha of rice grown in Arkansas were planted with cv. Newbonnet (19). This extensive monoculture, the relatively short growing season, and environmental conditions that only sporadically favor blast epidemics likely exerted strong selection pressure within the *P. grisea* population for distinct fitness characteristics and a narrow spectrum of virulence. All four lineages with a virulence phenotype of IB-49 and IC-17 can readily infect Newbonnet. Isolates in lineage B with an IG-1 virulence phenotype cannot infect Newbonnet. Thus, the extensive monoculture of Newbonnet in Arkansas in the mid-1980s likely led to the selection of pathotypes IC-17 and IB-49 among the four lineages identified. Historical data, MGR586 population data, and direct virulence changes of certain IG-1 isolates under greenhouse conditions support this hypothesis (7). The fact that the IC-17 pathotype was not found among lineage A or C isolates and the IB-49 pathotype was not found among the B or D lineage isolates also may indicate a limitation of the virulence spectrum within a lineage as has been proposed by others (47).

Host genotype has clearly been shown to exert a strong selection pressure on *P. grisea* (2,4,44,47,49). In this study, there also were apparently strong host influences on the isolates recovered. For example, all 116 isolates recovered from cv. Mars were in lineage A. This may partially be explained based on the close relationship between lineage and race in the Arkansas population; all 30 isolates examined in lineage A and C had an IB-49 virulence phenotype and 29 of 30 isolates in lineage B and D had an IC-17 virulence phenotype; one isolate in fingerprint group B had an IG-1 virulence phenotype. Consequently, a cultivar such as Mars, which contains the *Pi-z* major gene for resistance (15,26), is resistant to IC-17 and would therefore be expected to exclude the majority of the isolates in lineages B and D if the race–lineage relationship identified is consistent throughout the blast pathogen population. However, the original data from Mars were based on isolates from a single field and could be subjected to a number of experimental errors, such as the influence of neighboring cultivars, founder effects, or sampling vagaries. Consequently, this hypothesis was tested at three additional experimental sites with replicated subplots. The strong bias (95%) on Mars for isolates belonging to the lineage A fingerprint was clearly evident in the replicated field plots (32; Table 9). Lineage C isolates can readily infect Mars in the greenhouse; therefore, there apparently are other factors, such as fitness characteristics, associated with lineage A (i.e., sporulation ability) or host characteristics (i.e., minor resistance genes) which may influence the predominance of lineage A on cv. Mars.

Under greenhouse conditions, cv. M204 is highly susceptible to all isolates of lineages A, B, C, and D which have been tested (*unpublished data*). Also, field observations indicate that M204 is one of the more susceptible cultivars grown in Arkansas. However, in the replicated field experiment, there was a consistent bias for recovering the B lineage from M204.

Lineage exclusion, a means of using complementary resistances to prevent or greatly reduce infection by specific lineages of *P. grisea*, has been proposed as a means of improving the durability of resistance in rice (49). Such an approach may be feasible to improve the durability of resistance in rice cultivars grown in the United States because of the relatively simple lineage and race structure. However, for such an approach to be fully effective, more complete sampling to determine how effectively lineages are excluded by specific cultivars is needed. Also, the rate by which isolates within certain lineages can mutate to overcome effective resistance genes is needed. Virulence diversity has been identified within several of the lineages in the United States (8,44) and virulence changes have been documented for given isolates of some lineages (7).

The study of fungal population structure over time can provide information on the evolutionary history of an organism and, more importantly, on its ability to change and evolve. This information is imperative if we are to continue to utilize disease resistance genes effectively. Mechanisms that can potentially influence population diversity of the rice blast pathogen include sexual recombination, asexual recombination (parasexual events), mutation, gene flow, migration, and random genetic drift (46). *P. grisea* has the potential to sexually reproduce under laboratory conditions (29) and recent evidence indicates that recombination may occur under field conditions in certain geographical areas (18,48). Data from the current study indicate that, within a narrow time frame of one growing season, asexual reproduction is the predominant if not the exclusive mode of reproduction. However, further examination of archival isolates with additional genetic and molecular markers may reveal cryptic or historical evidence of sexual or asexual recombination events in the population of *P. grisea* in the United States. Future efforts to better understand the nature of genetic variability and gene flow in populations of *P. grisea* will likely benefit from the direct use of marked field strains (13,22) combined with following the movement of specific pieces of DNA (i.e., avirulence genes; 16,39) within and between field populations.

Table 9. Number and percentage of isolates in each MGR DNA-fingerprint lineage recovered from each of three replications from cvs. M204 and Mars from three experimental sites

	No. (%) of MGR586 DNA lineages			
Cultivar, county, year	**A**	**B**	**C**	**D**
M204				
White, 1994	11 (18)	42 (69)	1 (2)	7 (11)
White, 1995	5 (10)	43 (90)	0 (0)	0 (0)
Clay, 1995	2 (4)	40 (84)	5 (10)	3 (6)
Total	18 (11)	125 (79)	6 (4)	10 (6)
Mars				
White, 1994	52 (93)	4 (7)	0 (0)	0 (0)
White, 1995	16 (94)	1 (6)	0 (0)	0 (0)
Clay, 1995	50 (98)	1 (2)	0 (0)	0 (0)
Total	118 (95)	6 (5)	0 (0)	0 (0)

ACKNOWLEDGMENTS

We thank R. Cartwright and the numerous Arkansas Extension personnel who helped in collecting the rice samples.

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