Genetic Differentiation Among Isolates of *Pyricularia* Infecting Rice and Weed Hosts

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ABSTRACT

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DNA polymorphisms among Philippine isolates of the blast fungus from rice and 16 weed species were examined using restriction analysis of mitochondrial DNA (mtDNA) and DNA blot hybridization using ribosomal, single-copy, and repetitive DNA probes. Four rDNA hybridization patterns and six mtDNA restriction profiles were observed. The most frequent rDNA and mtDNA types were shared by isolates infecting rice and a group of weed species. Cluster analysis of the data obtained using probes for single-copy nuclear loci confirmed the phylogenetic relationships suggested by the mtDNA analysis, but differentiated the rice and non-rice-infecting isolates at the 50% similarity level. High levels of polymorphism between weed- and rice-infecting isolates were detected by six repetitive DNA probes. Probe MGR586 showed a consistently high number of hybridizing bands for rice-infecting isolates and low number of hybridizing bands for non-rice-infecting isolates. Subpopulations of the fungus attacking rice and adjacent weeds in two rice fields were found to be genetically differentiated. The results presented strongly suggest that although *Pyricularia* populations infecting rice and many rice-field weeds share a common ancestry, populations of the pathogen-infecting weed hosts do not provide inoculum for the rice crop in the Philippines.

Additional keywords: DNA polymorphisms, *Magnaporthe grisea*, population genetics, *Pyricularia oryzae*.

The blast fungus, *Pyricularia grisea* (Cooke) Sacc. (synonym *Pyricularia oryzae* Cavara, teleomorph *Magnaporthe grisea* (Hebert) Barr; 15) is an important pathogen of rice in many rice-growing countries. The fungus also parasitizes more than 50 other species of grasses and sedges, many of which are common weeds in rice fields (11). Although the host range of the fungus is restricted (5), occasional reports of cross-infection of rice by isolates from weed hosts have led to speculation that the pathogen populations on weeds could be a source of inoculum for rice blast in the Philippines (9,13). A practical concern is whether control of inoculum from weeds is essential for blast management (18).

Recent genetic and molecular analyses of the blast fungus have shed more light on the genetic differences between blast fungus isolates from different hosts. Sexual fertility of field isolates was dependent on host origin (5,23). In crosses between rice and non-rice-infecting isolates, progeny pathogenic to rice were obtained only after repeated backcrossing to the rice-infecting isolates (6,19), suggesting that many genes were involved in determining parasitism on a particular host. On the other hand, host range was found to be conditioned by only a few major genes in some cases, implying that single-gene mutations may potentially alter host specificity (20,23). By DNA hybridization analysis, genomes of rice-infecting isolates were found to contain many copies of repetitive DNA elements that were absent or present only in low copy number in non-rice-infecting isolates (3). These observations suggest that *P. grisea* isolates from different hosts and habitats may be genetically distinct.

Genetic differentiation would suggest that blast fungus populations from weeds do not contribute to rice blast epidemics, and this conclusion would have implications for weed and disease management. In the Philippines, 13 weed species have been reported to be hosts of *P. grisea* (8). Most of these weeds are perennially present in rice fields. To investigate the potential role of weed-infecting *Pyricularia* populations in rice blast epidemics, we conducted molecular characterization of blast fungus populations infecting various hosts in the Philippines. Genetic differentiation was evaluated using several types of molecular markers.

MATERIALS AND METHODS

Isolate collections and maintenance. Table 1 summarizes the host origin, geographic origin, and year of collection of the isolates used in this study. The majority of these isolates were monoconidial cultures obtained as part of this study. Eleven isolates (those obtained before 1987) were from conidial masses from single lesions (9). Five of the hosts, *Paspalum distichum*, *Eragrostis* sp., *Cenchrus echinatus*, *Cynodon dactylon*, and *Cyperus brevifolius*, are reported here for the first time to be infected by *Pyricularia grisea* in the Philippines.

A subset of the isolates listed in Table 1 was used for each experiment. To obtain a general profile of mitochondrial DNA (mtDNA) and repetitive DNA (rDNA) polymorphisms, one or two isolates from each host were randomly selected. For single-copy DNA analysis, multiple isolates from a host collected either from different locations or from the same location in different years were used to maximize diversity of the sample collection. To compare the rice and weed pathogen populations coexisting in a field, six to 23 isolates per host species were examined, depending on the level of infection observed. The fields were an upland rice field in Tanauan, Batangas, and an irrigated lowland rice field in Cabanatuan, Nueva Ecija, both in the Philippines. Collection of isolates from rice and adjacent weeds from the two farmers' fields was done in 1988.

Active cultures of the fungus were maintained on prune agar slants (6). Inactive cultures were preserved on pieces of blotting paper (2 mm^2), and stored desiccated at −20 C. For DNA extraction, the fungus was grown in modified Fries medium (21) supplemented with 0.5 g of casein hydrolysate per liter for 5 days with constant shaking at 28 to 30 C.

DNA extraction. Genomic DNA was extracted from lyophilized mycelia using a CTAB-based procedure (10). Mitochondrial DNA was extracted from mitochondria isolated by differential centrifugation. Freshly harvested mycelium was washed twice with distilled water and suspended in 20 ml of solution containing 0.6 M KCl in 0.2 M sodium phosphate buffer (pH 5.4), 20 mg of lysing enzyme (Sigma L2265), and 20 mg of cellulase (Sigma C2274) for 3–4 h at 30 C. Protoplasts were collected by centrifugation at 800 *g* for 5 min and incubated in 20 ml of solution of 0.2 M mannitol, 50 mM Tris-HCl, pH 7.2, 1 mM EDTA, and 0.1% bovine serum albumin for 30 min at 4 C with occasional vortexing. Nuclei and cell debris were separated by centrifugation at 2,000 *g* for 10 min at 4 C. The supernatant was recovered and centrifuged at 12,000 *g* for 12 min at 4 C to collect the mitochondria. DNA was extracted from mitochondria following the same procedure as for total DNA extraction from ground mycelia.

DNA digestion, electrophoresis, and Southern blot analysis. For Southern blotting, genomic DNA samples were digested with *Eco*RI according to the supplier's specifications (Bethesda Re-

TABLE 1. Geographic origin and year of collection of *Pyricularia* isolates used in this study[a]

Isolate	Host	Origin	Year isolated
Dc8997	*Digitaria ciliaris*	Cabanatuan, Nueva Ecija	1989
Dc88356	*D. ciliaris*	Tanauan, Batangas	1988
Dc88357 to 88364, Dc88420 to 88423, Dc 88428 to 88430, Dc88437 to 88442, Dc88445 to 88447	*D. ciliaris*	Sto. Tomas, Batangas	1988
Dc88439	*D. ciliaris*	Calamba, Laguna	1988
Dc88466	*D. ciliaris*	Caliraya, Laguna	1988
Dc8301	*D. ciliaris*	Los Baños, Laguna	1983
Dc88275	*D. ciliaris*	Los Baños, Laguna	1988
Dc871	*D. ciliaris*	Guimba, Nueva Ecija	1987
BmA8309	*Brachiaria mutica*	Los Baños, Laguna	1983
Bm88315, Bm88324, Bm88508 to 88514	*B. mutica*	Cabanatuan, Nueva Ecija	1988
Bm8946	*B. mutica*	Imus, Cavite	1989
EiA8303	*Eleusine indica*	Los Baños, Laguna	1984
Ei88435	*E. indica*	Tanauan, Batangas	1988
Ei88448	*E. indica*	Tanauan, Batangas	1988
Ei88365 to 88373, Ei88424 to 88426	*E. indica*	Sto. Tomas, Batangas	1988
Ei8927	*E. indica*	Bukidnon	1989
Lh88490	*Leersia hexandra*	Solana, Cagayan	1988
Lh8841 to 8863, Lh88504	*L. hexandra*	Cabanatuan, Nueva Ecija	1988
LhA8401	*L. hexandra*	Los Baños, Laguna	1984
Lh88405	*L. hexandra*	Los Baños, Laguna	1988
Lh89116, Lh8982	*L. hexandra*	Los Baños, Laguna	1989
PrA8202	*Panicum repens*	Los Baños, Laguna	1982
Pr8988	*P. repens*	Los Baños, Laguna	1989
Pr889, Pr886	*P. repens*	Bay, Laguna	1988
Pr88335, Pr88165, to 88188, Pr88342	*P. repens*	Cabanatuan, Nueva Ecija	1988
Pd8816 to 8839, Pd88313	*Paspalum distichum*	Cabanatuan, Nueva Ecija	1988
Pd88413	*P. distichum*	Los Baños, Laguna	1988
BdA8401	*Brachiaria distachya*	Los Baños, Laguna	1984
Ce88454	*Cenchrus echinatus*	Plaridel, Bulacan	1988
Cd88215 to 8827	*Cynodon dactylon*	Cabanatuan, Nueva Ecija	1988
Cd88300	*C. dactylon*	Imus, Cavite	1988
Cb8959	*Cyperus brevifolius*	Los Baños, Laguna	1989
Cr88383 to 88393	*C. rotundus*	Sto. Tomas, Batangas	1988
CrA8401	*C. rotundus*	Los Baños, Laguna	1984
Cr9010	*C. rotundus*	Los Baños, Laguna	1990
Ec883	*Echinochoa colona*	Bay, Laguna	1988
EcA8303	*E. colona*	Los Baños, Laguna	1983
Ec88443	*E. colona*	Los Baños, Laguna	1988
Er88271	*Eragrostis* spp.	Los Baños, Laguna	1988
LcA8401	*Leptochloa chinensis*	Los Baños, Laguna	1984
R88107 to 88130	Rice (IR64)	Cabanatuan, Nueva Ecija	1988
R88374 to 88379	Rice (Kinanda)	Sto. Tomas, Batangas	1988
PpA8202	*Pennisetum purpureum*	Los Baños, Laguna	1982
ReA8401	*Rottboellia exaltata*	Los Baños, Laguna	1984

[a] Isolates obtained in the year 1987 and later were monoconidial cultures, the 11 isolates obtained earlier than 1987 were from conidial masses from single lesions.

search Laboratories, MD). Digested DNA (5 μg per lane) was separated by horizontal agarose gel electrophoresis at 1.5 V/cm in 0.5× TBE for 20 h. Fractionated DNA was transferred to Hybond-N membranes according to the manufacturer's recommendation (Amersham, Chicago, IL). About 3 μg of plasmid DNA was labeled with digoxigenin-dUTP by random priming, and blots were probed with digoxigenin-labeled DNA according to the procedure described (Genius Kit, Boehringer Mannheim, Indianapolis, IN).

Mitochondrial DNA from each isolate was digested with the restriction enzymes *Eco*RI, *Hae*III, *Hin*dIII, *Hpa*II, and *Pst*I. After electrophoresis, gels were stained with ethidium bromide and photographed under UV light. Restriction fragment profiles of the different isolates were compared by recording fragments common or unique among isolates. Approximate mtDNA genome sizes were estimated by summing the sizes of all fragments.

Probes. The repetitive sequences PGR613, PGR612, PGR46, PGR6G, and PGR106 were isolated from *P. grisea* isolate PO6-6 and cloned in pUC13 (2). MGR586, described by Hamer et al (3), is a repetitive DNA element of *P. grisea* cloned in pUC18 (provided by Barbara Valent, DuPont, Wilmington, DE). A ribosomal DNA probe was obtained by amplification of the intergenic transcribed spacer region of the nuclear ribosomal gene in *P. grisea* using the ITS1 and ITS4 as primers in a polymerase chain reaction (22). Single-copy and low-copy DNA sequences (provided by S. Leong, University of Wisconsin) were from a collection of clones used in the construction of a genetic map of the fungus (17). The plasmids were maintained in *Escherichia coli* isolate DH5α. Plasmids were isolated by alkaline lysis (1).

Phylogenetic analysis. A phylogenetic tree was constructed based on single copy DNA RFLPs using the NTSYS-pc program (14). The hybridization patterns generated by each probe were considered as alternative character states; for each probe, each band mobility was arbitrarily assigned a number. A similarity matrix based on simple matching coefficients was created using SIMQUAL. Clustering was done using the unweighted pair group, arithmetic mean method (UPGMA), using SAHN. The phenogram with the highest agreement to the similarity matrix based on COPH and MXCOMP was chosen.

RESULTS

mtDNA restriction fragment analysis. Based on restriction analysis of mtDNA, 18 *P. grisea* isolates from 17 host species were divided into six groups. Each of the five endonucleases tested, *Eco*RI, *Pst*I, *Hin*dIII, *Hpa*II, and *Hae*III, revealed the same groupings. The restriction fragment banding patterns generated by *Hin*dIII, *Hae*III, and *Hpa*II are presented in Figure 1. Each pattern is highly distinct, with the RFLP differences between mitochondrial DNA haplotypes averaging more than 90%. Mitochondrial DNA type *A* was shared by isolates from rice and 11 weed species common in rice fields (Table 2). Isolates from *C. echinatus* and *Pennisetum purpureum* had mtDNA type *C*, whereas isolates from *Eragrostis* sp. and *Digitaria ciliaris* had mtDNA type *F*. Two isolates from *Cyperus rotundus* and one from *C. brevifolius* each had unique mtDNA types (*D, E* and *B*, respectively). Using *Hpa*II and *Hae*III, no additional mtDNA polymorphisms were found among an additional 20 isolates from rice and 37 isolates from weed hosts. Isolates from the same host species almost always had identical mtDNA types (data not shown), except for the two isolates from *C. rotundus* having distinct mitochondrial genomes (Fig. 1, Table 2).

The sizes of the different mitochondrial genomes were estimated as the sum of the fragment sizes generated by each of four endonucleases (Table 3). The mitochondrial genome of an isolate from *C. brevifolius* (type *B*, 73 kb) was more than two times larger than that of the rice-infecting isolate (type *A*, 32 kb).

DNA polymorphisms detected by Southern hybridization. *Ribosomal DNA.* Four types of *Eco*RI-rDNA polymorphisms were detected among the 54 isolates tested. Ribosomal DNA was highly conserved among isolates from rice and 14 species of weeds. The isolate from *C. brevifolius* and the two isolates from *C. rotundus* each had a distinct rDNA hybridization pattern (Fig. 2).

Repetitive DNA. Each of the six repeated DNA probes generated distinct DNA hybridization patterns among isolates from different hosts. The hybridization patterns generated by two of the probes, PGR613 and MGR586, are presented in Figure 3. The relative number of bands hybridizing to each probe is summarized in Table 2. Among the isolates tested, only the isolates from *C. rotundus* and *C. brevifolius* consistently showed low copy number or no hybridization with the six probes, although similar amounts of DNA were loaded for these and other isolates (Fig. 3, lanes D–F). MGR586 hybridization profiles of rice- and weed-infecting isolates of *P. grisea* were very distinct (Fig. 3). Lanes representing isolates from rice had many bands (50–60) hybridizing to MGR586, while lanes representing isolates from weeds had significantly fewer hybridizing bands (10–25). Subsequent probing of DNA from six additional isolates from *Leersia hexandra*, one from *C. dactylon*, four from *Brachiaria mutica*, two from *Echinochloa colona*, eight from *D. ciliaris*, six from *Eleusine indica*, three from *Paspalum distichum*, and six from *Panicum repens* showed that isolates from the same host have similar banding patterns with some degree of polymorphism.

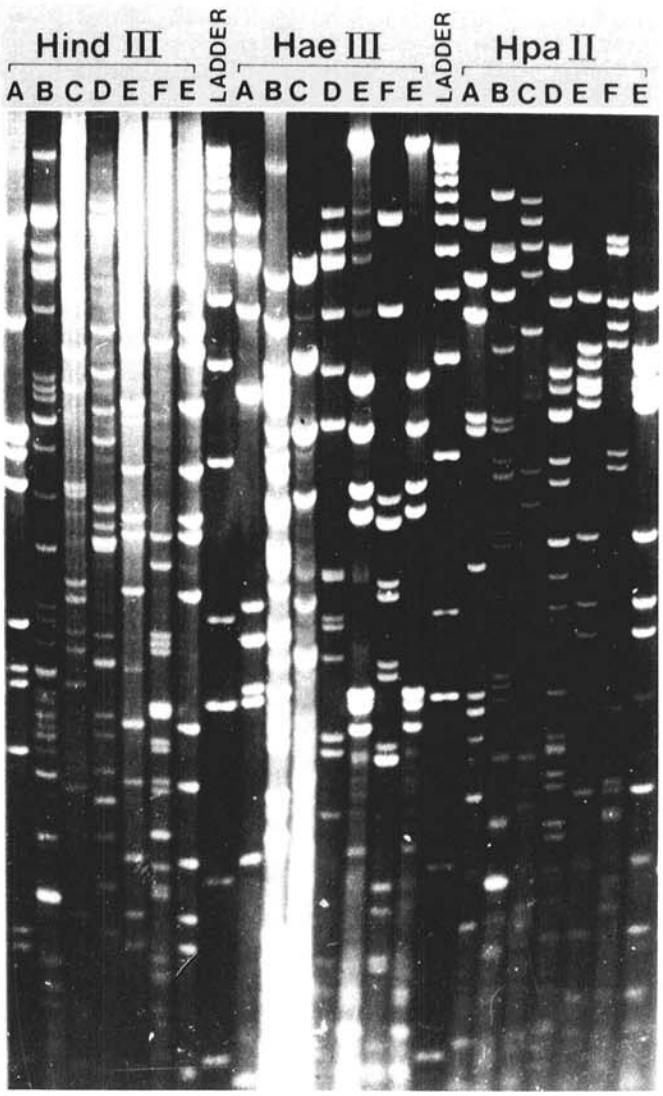


Fig. 1. Restriction fragment patterns of mitochondrial DNA from *Pyricularia grisea* generated by *Hin*dIII, *Hae*III, and *Hpa*II. Among the 18 isolates tested, six distinct patterns were detected by each of the restriction endonucleases used. Isolates representing these types are type *A*, R88107 from *Oryza sativa*; type *B*, Cb8959 from *Cyperus brevifolius*; type *C*, Ce88454 from *Cenchrus echinatus*; type *D*, CrA8401, from *Cyperus rotundus*, type *E*, Cr88383 and Cr9010 from *Cyperus rotundus*; and type *F*, Dc88466 from *Digitaria ciliaris*.

TABLE 2. Molecular profile of *Pyricularia* isolates infecting different host species based on mitochondrial DNA (mtDNA), ribosomal DNA, and repetitive DNA sequences

Isolate	Host	mtDNA type[a]	rDNA type[b]	Hybridizing bands (rel. no.)[c]					
				6g	106	613	612	46	586
R88107	*Oryza sativa*	*A*	1	V	V	H	H	V	V
Cd88215	*Cynodon dactylon*	*A*	1	V	V	H	H	V	I
Lh88504	*Leersia hexandra*	*A*	1	V	V	H	H	H	I
Ec88443	*Echinochloa colona*	*A*	1	V	V	H	H	H	I
Pr88165	*Panicum repens*	*A*	1	V	V	H	H	I	I
LcA8401	*Leptochloa chinensis*	*A*	1	H	H	H	H	L	I
Pd8824	*Paspalum distichum*	*A*	1	H	H	H	H	L	I
Dc88420	*Digitaria ciliaris*	*F*	1	H	H	H	L	L	L
Er88271	*Eragrostis sp.*	*F*	1	H	H	H	L	L	L
Ei88424	*Eleusine indica*	*A*	1	H	H	L	L	L	L
PpA8201	*Pennisetum purpureum*	*C*	1	H	H	N	H	H	L
ReA8401	*Rottboellia exaltata*	*A*	1	L	L	L	L	L	L
Bm88508	*Brachiaria mutica*	*A*	1	H	L	H	H	L	L
BdA8401	*B. distachya*	*A*	1	H	I	I	I	L	L
Ce88454	*Cenchrus echinatus*	*C*	1	I	I	N	N	H	L
CrA8401	*Cyperus rotundus*	*D*	2	L	L	–	–	–	L
Cr88383	*C. rotundus*	*E*	3	–	–	–	–	–	L
Cb8959	*C. brevifolius*	*B*	4	–	–	–	–	–	–

[a] Letters are arbitrary notations corresponding to the six mtDNA banding patterns generated by each of the restriction enzymes used (Fig. 1). There was correspondence among the restriction profiles generated by each of the enzymes.
[b] Numbers correspond to specific rDNA hybridization pattern shown in Figure 3.
[c] Relative number of hybridizing fragments are V = very high, more than 45 bands; H = high, between 30 and 44 bands; I = intermediate, between 15 and 29 bands; L = low, fewer than 15 bands; – = no bands detected, N = not determined.

TABLE 3. Approximate sizes of mitochondrial DNAs in isolates of *Pyricularia* from various host species

Isolate	Host	mtDNA type	Sum of fragment sizes (kb)				
			*Hin*dIII	*Hae*III	*Pst*I	*Hpa*II	Average[a]
R88107	*Oryza sativa*	*A*	32.3	29.9	31.6	33.7	32.3 d
Cb8959	*Cyperus brevifolius*	*B*	71.6	77.0	ND[b]	70.2	72.9 a
Ce88454	*Cenchrus echinatus*	*C*	40.1	40.0	37.3	38.3	38.9 c
CrA8401	*Cyperus rotundus*	*D*	55.3	58.9	56.0	50.6	55.2 b
Cr88383	*C. rotundus*	*E*	37.9	35.8	35.3	35.2	36.0 cd
Dc88466	*Digitaria ciliaris*	*F*	33.4	31.3	37.6	35.7	34.5 cd

[a] Average = sizes of the different mitochondrial genomes estimated as the sum of the fragment sizes generated by each of the four endonucleases. Means followed by the same letter are not significantly different at 5% level by Duncan's multiple range test.
[b] ND = Not determined.

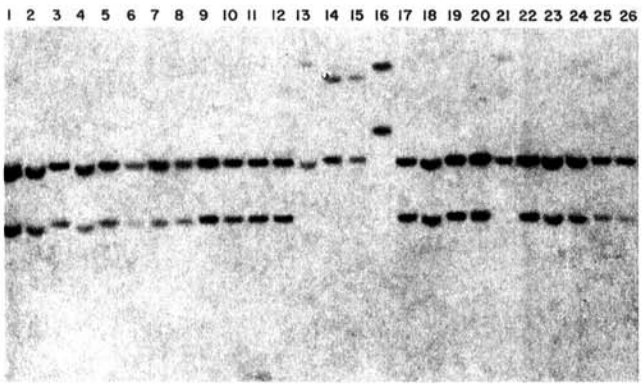


Fig. 2. Ribosomal DNA hybridization patterns for isolates of *Pyricularia grisea* from various host species. Isolates from *Cyperus brevifolius* (lanes 13 and 21) and *Cyperus rotundus* (lanes 14–16) show patterns distinct from those of isolates from rice (lanes 22–26) and from 14 weed species (lanes 1–12 and 17–20).

Single-copy probes. The 10 single-copy DNA probes tested each detected three to nine RFLPs. The five isolates from *Cyperus* showed hybridization with only one of these probes. When the allelic data for each isolate and the rDNA type were combined, 21 multilocus haplotypes were generated (Table 4). The phenogram constructed based on these haplotypes is shown in Figure 4. The phenogram showed good fit to the data, with a matrix correlation of 0.95.

At the 40% similarity level, the phenogram showed four main groups of isolates. These groups corresponded well with the groupings identified by mtDNA analysis. One branch consisted of isolates with mtDNA type *F*, infecting *D. ciliaris* and *Eragrostis* sp.; another branch consisted of an isolate with mtDNA type *C*, from *C. echinatus*; the third cluster was a small group of isolates with mtDNA types *B*, *D* and *E*, from two species of *Cyperus*; the fourth was a large, heterogeneous cluster of isolates from various species, all having mtDNA type *A*. Because 10 of the 11 loci tested showed null alleles for the isolates from *Cyperus*, the apparent homogeneity of that cluster may not reflect the actual diversity of the cluster.

At the 50% similarity level, the rice-infecting isolates were distinguished from the other isolates with mtDNA type *A*. At the 75% similarity level, the clusters defined were associated fairly closely with host of origin: one cluster consisted of isolates from *Brachiaria mutica*; two adjoining clusters consisted of isolates from *Leersia*; one cluster consisted of isolates from *Eleusine* and *Rottboellia*; one cluster consisted of an isolate from *Brachiaria distachya*, and one heterogeneous cluster consisted of isolates from *Paspalum, Panicum*, and *Cynodon*.

Analysis of isolates from two fields. Blast fungus populations on different hosts in the same fields were genetically distinct. In a lowland rice field in Nueva Ecija, Philippines, rice cultivar IR64 and five adjacent weed species, *P. distichum*, *L. hexandra*, *P. repens*, *C. dactylon*, and *B. mutica* were infected with *P. grisea*. The number of *Eco*RI fragments that hybridized with MGR586 was significantly higher in the genomes of rice-infecting isolates than in any of the weed-infecting isolates (Fig. 5). Each host-differentiated group of isolates could also be distinguished by

the banding pattern of hybridizing fragments.

Similarly, *P. grisea* populations infecting different hosts in an upland rice field in Batangas, Philippines, were also distinct. Isolates from the weed species *D. ciliaris* and *E. indica* had significantly fewer hybridizing fragments than the isolates infecting the rice crop, and no hybridizing fragments were detected in isolates from *C. rotundus*.

DISCUSSION

We have used mtDNA restriction patterns and hybridization patterns of rDNA, single-copy, and six repetitive DNA probes to characterize the blast fungus populations from rice and weed hosts. Isolates infecting rice and 14 weed species had identical rDNA hybridization patterns. Analysis of mtDNA restriction patterns subdivided the isolates into four groups. One group consisted of isolates infecting rice and 11 weeds of lowland rice fields. The three other groups were collected from weeds of upland (nonirrigated) rice. These weeds, *C. echinatus*, *D. ciliaris*, and *Eragrostis* sp. are not commonly found in the irrigated lowlands.

Hybridization patterns with the repetitive DNA sequence MGR586 clearly distinguished rice- and weed-infecting isolates. These data confirm and extend the results of Hamer et al (3). Genetic isolation between blast fungus populations infecting different hosts has also been noted in Brazil, where MGR586 hybridization patterns clearly demonstrated the genetic differentiation between pathogens of rice and wheat (19).

Because of the extreme dissimilarity between the MGR586 hybridization profiles of rice- and weed-infecting populations, it was not possible to quantify the degree of genetic similarity be-

TABLE 4. Haplotypes generated by rDNA and 10 single-copy DNA probes for *Pyricularia grisea* isolates from different hosts

Haplotype[a]	Host species[b]
11111111001	*Digitaria ciliaris* (8/8), *Eragrostis sp.* (1/1)
11204840002	*Cenchrus echinatus* (1/1)
20000000000	*Cyperus brevifolius* (2/2)
30000000000	*Cyperus rotundus* (2/3)
40000000000	*C. rotundus* (1/3)
11221735564	*Oryza sativa* (5/5)
12221222122	*Brachiaria mutica* (3/6)
12222222122	*B. mutica* (3/6)
12221333233	*Leersia hexandra* (1/5)
12221334232	*L. hexandra* (2/5)
12221434342	*L. hexandra* (2/5)
12222233132	*Eleusine indica* (5/5)
12222533152	*Rottboelia exaltatata* (1/1)
12221533154	*Paspalum distichum* (1/3)
12221534154	*P. distichum* (1/3), *Echinochloa colona* (1/1), *Panicum repens* (2/7), *Leptochloa chinensis* (1/1)
12221634154	*P. repens* (2/7)
12221534152	*P. repens* (1/7)
12221634152	*P. repens* (1/7)
12221632152	*P. repens* (1/7)
12221932152	*Cynodon dactylon* (2/2)
12223532154	*Brachiaria distachya* (1/1)

[a] For haplotype designations, each digit corresponds to the allele present at one of the 11 RFLP loci identified by rDNA and probes p11, p52, CH4–163H, p55, 4–68, p90, and CH4–133, CH5–58, CH4–116, and p40, respectively (17).

[b] Numbers in parenthesis indicate the proportion of isolates from that host possessing the respective haplotype.

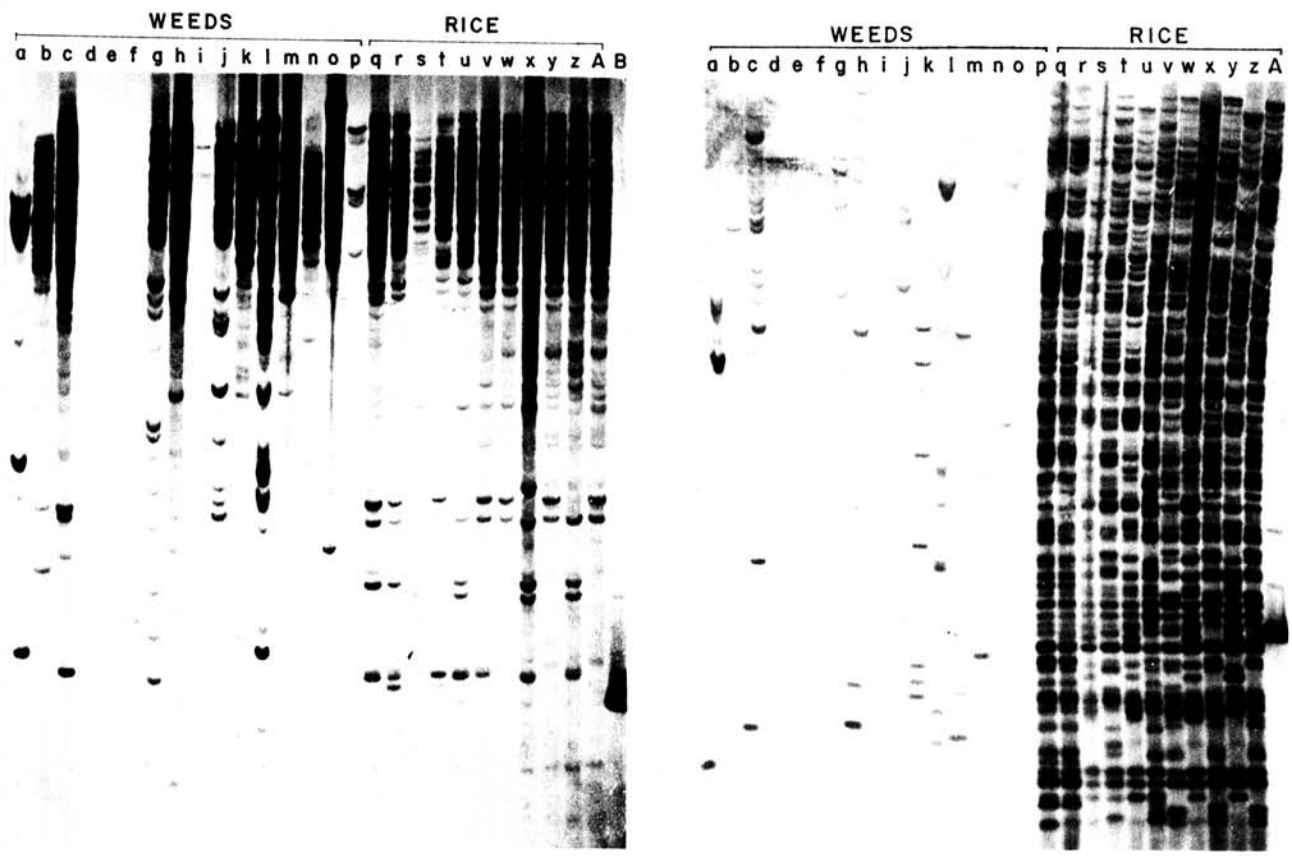


Fig. 3. Restriction fragment patterns generated by PGR613 (a) and MGR 586 (b) for Southern blots of *Eco*RI-digested genomic DNA of *Pyricularia grisea* isolates from various host species. Lane a: Isolate BdA8401 from *Brachiara distachya*; lane b: Isolate Bm88508 from *Brachiara mutica*; lane c: Isolate Cd88215 from *Cynodon dactylon*; lane d: Isolate Cb8959 from *Cyperus brevifolius*; lane e: Isolate CrA8401 from *Cyperus rotundus*; lane f: Isolate Cr88383 from *Cyperus rotundus*; lane g: Isolate Dc88420 from *Digitaria ciliaris*; lane h: Isolate Ec883 from *Echinocloa colona*; lane i: Isolate Ei88424 from *Eleusine indica*; lane j: Isolate Er88271 from *Eragrostis* spp.; lane k: Isolate Lh8840 from *Leersia hexandra*; lane l: Isolate LcA8401 from *Leptochloa chinensis*; lane m: Isolate Pr88335 from *Panicum repens*; lane n: Isolate Pd8824 from *Paspalum distichum*; lane o: Isolate Pp8202 from *Pennisetum purpureum*; lane p: Isolate ReA8401 from *Rottboellia exaltata*; and lanes q-A: Isolates from *Oryza sativa*.

tween the various populations using this probe. Results with other repetitive sequences were also unsuitable for phylogenetic analysis. To quantify the evolutionary relationships among host-specific populations of the pathogen, randomly selected single-copy sequences previously mapped in the genome of *P. grisea* (17) were used to analyze a subset of rice- and weed-infecting isolates.

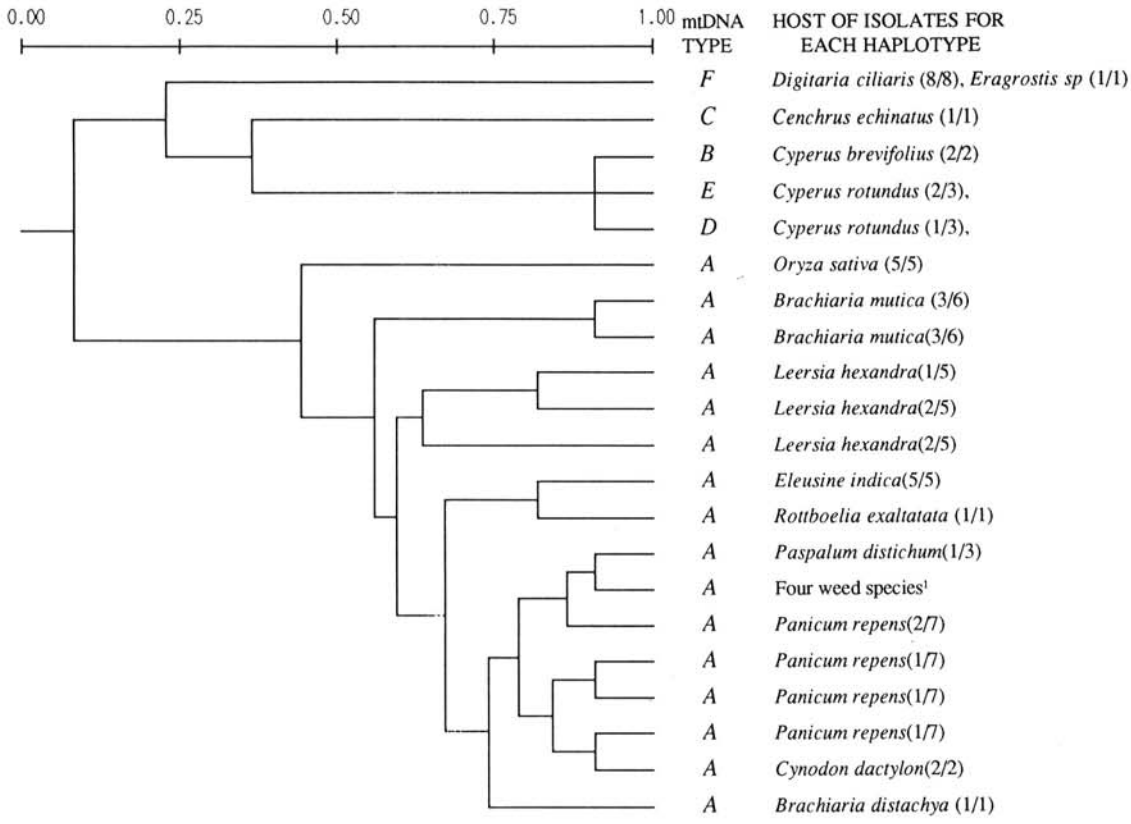
The results of the single-copy analysis were consistent with the results of the mtDNA and repetitive DNA analyses, and further illuminated the relationship between weeds and rice-infecting isolates. The single-copy analysis confirmed the groups defined by mtDNA analysis, except that mitochondrial types *B, D*, and *E* were not resolved, presumably because DNAs from the corresponding isolates did not hybridize with most of the probes used. Isolates with mitochondrial type *A*, including isolates from rice and lowland weeds, formed a large and diverse cluster based on the single-copy analysis, while isolates from other hosts were much more distantly related to the rice-infecting isolates. Within the cluster of isolates with mitochondrial type *A*, the divergence of the rice-infecting strains from the others was apparent.

Based on the results of mtDNA and single-copy DNA analyses, it appears that *Pyricularia* populations infecting *Cyperus* spp., *Cenchrus echinatus* and *P. purpureum*, and *D. ciliaris* could be considered for reclassification as three species, distinct from the species with mtDNA type *A*. Based on conidial morphology and on colony growth on prune agar medium, isolates from different hosts are indistinguishable, except for isolates from *Cyperus*. Isolates from *Cyperus* have relatively slender conidia and very slow growth in culture. Isolates infecting *Cyperus* elsewhere have been described as *Pyricularia higinsii* (7,4) and *P. cyperusae* (16). The isolates infecting different *Cyperus* species further appeared to be quite distinct from one another based on mtDNA and rDNA. However, because DNA from these isolates did not hybridize with most of the repetitive and single-copy DNA probes used, it was not possible to thoroughly evaluate the relationship between these isolates.

Although the case for at least one separate species is particularly strong for the isolates from *Cyperus*, mtDNA, rDNA, and single-copy DNA, analyses also indicated that isolates from *C. echinatus* and *P. purpureum*, and *D. ciliaris* are only remotely related to those infecting rice and many weed species. Isolates from *Pennisetum* have been designated *P. penniseti* (12). Isolates from *Pennisetum* share mtDNA type *C* with those from *Cenchrus*.

There is substantial genomic differentiation between rice- and weed-infecting isolates of mtDNA type *A*, detected using both repetitive and single-copy DNA probes. As noted by Hamer et al (3), there is evidence to support the classification of these isolates as one and as two species. It is clear that gene flow between rice and weed-infecting populations has been restricted, and this might support the previous classification of the rice-infecting isolates as *P. oryzae*, and the weed-infecting isolates as *P. grisea*. The fact that fertile crosses can be made between field isolates infecting rice and weeds, on the other hand, might support their classification as a single species.

The molecular data presented here show that populations of the blast fungus infecting most hosts are genetically distinct. This conclusion is supported by field observations suggesting host specificity of the weed- and rice-infecting populations. In most of the rice fields surveyed in the course of this work, the rice crop was rarely attacked by the blast fungus, even when blast was common among adjacent weeds. In some cases, a single *P. grisea* population may infect more than one host species. For example, isolates from *D. ciliaris* and *Eragrostis* sp. were collected



[1]*Paspalum distichum*(1/3), *Echinocloa colona*(1/1), *Panicum repens* (2/7), *Leptochloa chinensis* (1/1)

Fig. 4. Phenogram constructed from RFLP data based on single-copy probes, indicating the relationships among *Pyricularia grisea* isolates from different host species. All procedures were done using the NTSYS microcomputer program (14). A similarity matrix was calculated using SIMQUAL based on simple matching coefficient. The tree was generated from the similarity matrix by unweighted pair group method, arithmetic mean (UPGMA), using the SAHN procedure. The correlation between the phenogram and similarity matrix was 0.95. The number of isolates in each group, and the total number of isolates from each host, are noted in parentheses. The mitochondrial genotype designation of each group is also noted.

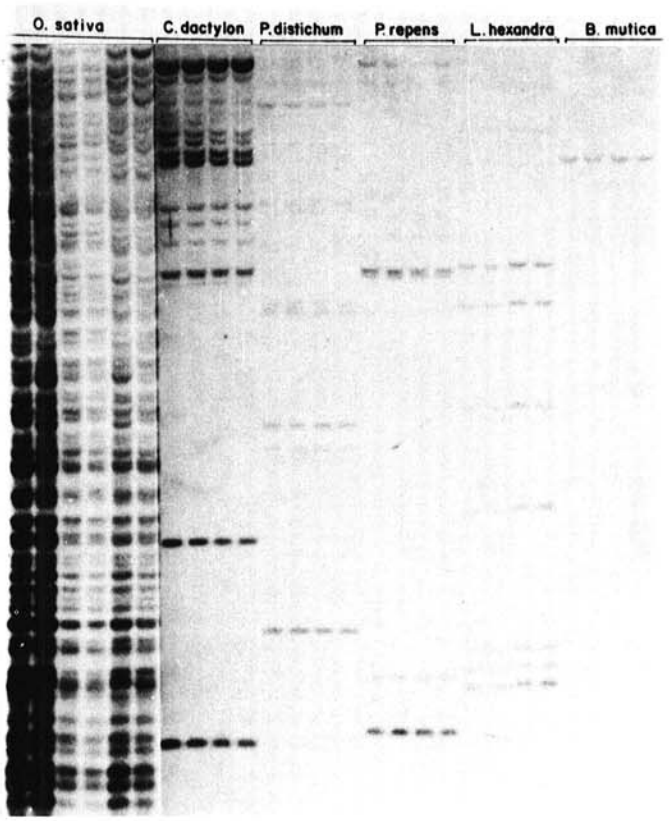


Fig. 5. Differences in hybridization pattern of MGR586 among host-differentiated subpopulations of *Pyricularia grisea* in a ricefield in Cabanatuan, Nueva Ecija, Philippines. Isolates from the weeds *C. dactylon, P. distichum, P. repens, L. hexandra*, and *B. mutica* had significantly fewer hybridizing fragments and showed a weaker hybridization signal with MGR586 probe than did the isolates from rice. Equal amounts of DNA were loaded.

from adjacent plants in the IRRI Blast Nursery and appear identical by all methods of analysis. However, the isolates collected from rice were always distinguishable from those collected from adjacent weeds, and populations from most weed hosts were distinct.

The observed genetic isolation between rice- and weed-infecting isolates suggests that blast populations on weeds do not play a significant role in rice blast epidemiology in the Philippines. Our Philippine survey supports the conclusion reached from broad geographical survey of Hamer et al (3) and the observations of Valent et al (20) from Brazil, that the *P. grisea* populations are strongly delimited by host range. Nonetheless, it should be emphasized that *P. grisea* is found in very diverse ecologies with different local flora. It remains possible that some *P. grisea* strains are capable of cross infecting weeds and rice. A broader sampling of isolates from weed flora in different rice production environments is needed before we can generalize that weed-infecting populations of *Pyricularia* do not pose a threat to rice in most parts of the world.

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