Population Structure of *Pyricularia grisea* at Two Screening Sites in the Philippines

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ABSTRACT

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The population structure of the blast pathogen, *Pyricularia grisea*, was analyzed at two field sites used for evaluating blast resistance in rice. During 1992, 1,516 monoconidial isolates of the pathogen were collected from 38 rice cultivars and lines from the blast nursery of the International Rice Research Institute (IRRI-BN) and from the upland screening site at Cavinti. Each isolate was subjected to DNA fingerprinting and phenetic analysis using the probe MGR586. Nine lineages were detected at Cavinti during the wet season. Although the same four lineages were collected from the IRRI-BN during the dry and wet seasons, the relative abundance of lineages differed in the two collections. While the lineage diversity was greater at Cavinti than at the IRRI-BN in either season, the haplotypic diversities of the collections were similar. Genetic differentiation and chi-square analysis indicated that populations of the fungus were differentiated geographically and temporally. The largest proportion of differentiation was attributable to host selection (G_{ST} = 0.39). To assess how well field infection reflected compatibility, a subset of isolates was inoculated on their hosts of origin. Isolates were often unable to reinfect their hosts of origin, especially isolates belonging to the dominant pathogen lineage and when collected from young seedlings and those with low diseased leaf area. Our results provided insight into pathogen population structure, and provided useful information for rice improvement and management.

Additional keywords: *Magnaporthe grisea*, phylogenetics, *Pyricularia oryzae*, rice blast.

Breeding for stable resistance to blast, caused by *Pyricularia grisea* (Cooke) Sacc. (*P. oryzae* Cav., the anamorph of *Magnaporthe grisea* (T. T. Hebert) Yaegashi & Udagawa), has been extremely difficult. Although cultivars with durable resistance have been recognized (2), resistance has often been short-lived (24,26). Inadequate screening methods could be one cause of the rapid breakdown of resistance: cultivars may be considered resistant although they are susceptible to existing pathogen subpopulations that are not represented in the screening nurseries used for varietal evaluation. Characterizing the structure of pathogen populations at screening sites and understanding the relationship between host and pathogen subpopulations can contribute to the design of more efficient screening strategies for blast resistance.

Although the rice-blast pathosystem is believed to follow a gene-for-gene relationship (45,47), virulence analysis has led to conflicting opinions on the degree of variability of *P. grisea* (3,25,37). Recently, DNA fingerprinting has been widely used for studying the population structure of *P. grisea* in various countries (5,13,16,28,29,30,36,50,51,52). In most of these studies, the dispersed repetitive probe MGR586 (14) was utilized. In these studies, populations of *P. grisea* were shown to consist of groups of closely related strains, which were inferred to represent clonal lineages (30).

For the purpose of this paper, "pathogen population structure" refers to the amount of genetic variation among individuals in a population; the ways in which this variation is partitioned in time and space; and the phylogenetic relationships among individuals within and between subpopulations (27). The population structure inferred from a collection of isolates will depend on the sampling strategy employed. The structure of a pathogen population is likely to be strongly influenced by the structure of its host population, and thus the number and diversity of host cultivars sampled in a study will affect the conclusions reached. Xia et al. (49) systematically sampled 113 isolates from one farmers' cultivar, and thus were able to assess the diversity of the pathogen population on a single host. Levy et al. (30) analyzed 151 isolates taken from 15 cultivars at a disease nursery over a 3-year period and analyzed the relationship between host subpopulations (cultivars) and pathogen subpopulations (lineages, pathotypes). For the majority of the hosts sampled, however, fewer than 10 isolates were analyzed.

The present study was undertaken to characterize the population structure of *P. grisea* at two sites used by the International Rice Research Institute for screening resistance sources and breeding lines for blast resistance (18,19,37). Although hundreds of races of the fungus had been identified from these sites (20), a clear picture of the population structuring of *P. grisea* was lacking. We used DNA fingerprinting of 1,516 isolates systematically collected from 38 cultivars to analyze the partitioning of pathogen populations across 38 hosts, two sites, and two seasons.

MATERIALS AND METHODS

Cultivar and site selection. A diverse set of rice cultivars and lines was selected to serve as a trap nursery for the collection of pathogen isolates. The nursery included lowland and upland rice

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cultivars with different origins and a range of field susceptibility, and near-isogenic lines carrying individual known resistance genes (Table 1).

Trap nurseries were sown at the IRRI's main Philippine screening sites for breeding blast-resistant cultivars. These sites were the blast nursery at the International Rice Research Institute's campus in Los Baños, Laguna (IRRI-BN), and the upland rice screening site at Cavinti, Laguna. The IRRI-BN is located in a lowland rice-growing area, where *indica* cultivars predominate. The Cavinti site is located in an upland area, where little rice is grown. Most of the rice grown at the Cavinti site are *japonica* cultivars. The nursery was planted at the IRRI-BN in the 1992 dry season (DS = February to May) and the 1992 wet season (WS = July to November), and at Cavinti in the 1992 WS.

Seeds (10 to 15 g of each entry) were planted in two rows in the nursery beds, with two replications. A mixture of the rice cultivars IR50, IR64, IR66, IR72, UPLRi5, Carreon, Kinarabao 3777, CO39, and IRAT13 was used as spreader rows, sown 1 week ahead in the DS at the IRRI-BN, and simultaneously with test materials in the WS at both sites.

Disease evaluation, isolate collection, and maintenance. Disease progress was evaluated at the sampling times based on the percentage of diseased leaf area. Sampling started 1 week after symptoms appeared in most cultivars and was conducted once a week afterward for 4 weeks, with one extra sampling at the heading stage in the DS experiment at the IRRI-BN. Serial samplings were done weekly in the seedling stage and biweekly in the adult stage, and neck blast, if present, also was collected at the IRRI-BN and Cavinti in the WS. Up to 15 diseased leaves were taken each sampling from each entry, depending on disease severity. An attempt was made to sample infected panicles as well as infected leaves. Only a few infected panicles were obtained, however.

Monoconidial isolates were obtained by picking single germinating spores on 4% water agar from discrete sporulating lesions, with one isolate taken per lesion per leaf. In total, 243, 677, and 596 monoconidial isolates were collected from the IRRI-BN in the DS and the WS, and Cavinti in the WS in 1992, respectively. While the vast majority of the isolates were from infected leaves, a few (n = 54) were taken from infected panicles (especially the neck node). Isolate maintenance for storage and for DNA extraction was as described in Borromeo et al. (5).

DNA manipulations. Total DNA was extracted following the plant DNA extraction procedure of Murray and Thompson (33) or as described by Scott et al. (41). DNA quality and concentration were determined by electrophoresis and fluorimetry (TKO 100 Dedicated Mini Fluorometer; Hoefer Scientific Instruments, San Francisco).

The clone pCB586, which contains the repetitive element MGR586 (14), was kindly provided by J. Hamer and B. Valent (Du Pont Co., Wilmington, DE). The probe was labeled with digoxigenin according to the procedures described in the Boehringer Mannheim nonradioactive DNA labeling and detection kit (Genius Kit; Boehringer Mannheim Corp., Indianapolis, IN).

P. grisea DNA samples were digested to completion with the restriction endonuclease *Eco*RI according to suppliers' recommendation. The digests (1.5 µg per lane) were separated by horizontal agarose gel (0.8%) electrophoresis at 2 V per centimeter in 0.5 × TBE for 24 h with standard DNA markers as a size reference. DNA fragments were then denatured and transferred to nylon membranes (Hybond-N or Hybond-N$^+$; Amersham Corp., Arlington Heights, IL) by the alkaline transfer method, as recommended by the manufacturer. Blots were prehybridized and hybridized with digoxigenin-labeled MGR586, and a nonradioactive signal was detected using either colorimetric or chemiluminescent substrates for alkaline phosphatase (Genius Kit; Boehringer Mannheim Corp., Indianapolis, IN).

Phylogenetic analysis and designation of lineage and haplotype. Based on visual inspection of MGR586 DNA fingerprints, isolates were assigned to preliminary groups. These preliminary groups of isolates were coelectrophoresed in adjacent lanes to confirm overall haplotypic similarity and particular band identities. For each group, a phenogram was generated, allowing confirmation of the integrity of the group. This process allowed erroneously classified isolates to be identified, and haplotype designations to be assigned to each isolate.

Sixty-six isolates with distinct banding profiles (haplotypes) were selected to represent the 10 major groups (putative lineages). DNA from groups of isolates representing a particular lineage were electrophoresed, together with representatives of other putative lineages, to allow scoring of individual band positions. To facilitate scoring, DNA from an isolate that showed a particularly large number of bands (isolate C921-5, with haplotype 7-16) was run in three to four lanes on each gel.

For each band position between 1.1 and 22 kb, presence or absence of the band was scored manually for each isolate. A data matrix was thus obtained, with "1" indicating the presence of a

TABLE 1. Diseased leaf area on rice cultivars/lines used in three trap experiments conducted at the Blast Nursery of the International Rice Research Institute (IRRI-BN) and the blast screening site at Cavinti, Laguna, Philippines, in 1992

Cultivar/line	Varietal group[a]	Diseased leaf area (DLA %)[b]		
		IRRI-BN/DS	IRRI-BN/WS	Cavinti/WS
Aichi asahi	VI	35.00 ± 5.00[c]	0.07 ± 0.15	100.00 ± 0.00
Akashi	I	10.00 ± 0.00	...[d]	10.00 ± 0.00
Azucena	VI	10.00 ± 0.00	0.00 ± 0.00	2.00 ± 0.00
BL1	VI	2.00 ± 0.50	0.41 ± 0.40	0.10 ± 0.00
CO39[e]	I	95.00 ± 0.00	35.00 ± 0.00	100.00 ± 0.00
C101A51[e]	I	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
C101LAC[e]	I	95.00 ± 0.00	35.00 ± 0.00	97.50 ± 2.50
C101PKT[e]	I	95.00 ± 0.00	32.50 ± 2.50	100.00 ± 0.00
C104PKT[e]	I	60.00 ± 5.00	0.01 ± 0.01	83.00 ± 0.25
C22	I	20.00 ± 0.00	0.00 ± 0.00	56.00 ± 5.00
Carreon	I	2.00 ± 0.00	0.00 ± 0.00	0.06 ± 0.04
CNA4130	VI	10.00 ± 0.00	0.00 ± 0.00	80.00 ± 5.00
Fujisaka 5	VI	10.00 ± 0.00	0.00 ± 0.00	2.60 ± 0.50
IAC165	VI	5.00 ± 0.00	0.01 ± 0.01	...
IAC47	VI	5.00 ± 0.00	0.03 ± 0.02	100.00 ± 0.00
IR36	I	40.00 ± 5.00	2.00 ± 0.00	100.00 ± 0.00
IR442-2-58	I	85.00 ± 5.00	...	...
IR50	I	90.00 ± 5.00	4.00 ± 0.00	100.00 ± 0.00
IR62	I	...	0.01 ± 0.00	54.00 ± 4.00
IR64	I	15.00 ± 5.00	0.07 ± 0.02	1.00 ± 0.00
IR66	I	90.00 ± 0.00	0.50 ± 0.00	0.60 ± 0.40
IR72	I	75.00 ± 5.00	1.50 ± 0.00	100.00 ± 0.00
IRAT104	VI	2.00 ± 0.00	...	...
IRAT2	VI	...	1.00 ± 0.00	0.01 ± 0.00
IRAT13	VI	...	0.00 ± 0.00	0.06 ± 0.03
IRAT208 (1)	VI	25.00 ± 5.00	0.01 ± 0.00	0.45 ± 0.05
IRAT208 (2)	VI	...	...	100.00 ± 0.00
IRAT239 (1)	VI	5.00 ± 0.00	0.00 ± 0.00	100.00 ± 0.00
IRAT239 (2)	VI	...	0.01 ± 0.00	...
Kinandang patong	VI	...	0.03 ± 0.02	0.70 ± 0.10
K59	VI	25.00 ± 5.00	0.29 ± 0.21	100.00 ± 0.00
Kinarabao 19430	VI	...	0.03 ± 0.02	100.00 ± 0.00
Kinarabao 3777	VI	75.00 ± 0.00	0.01 ± 0.01	100.00 ± 0.00
Kusabue	VI	5.00 ± 0.00	0.41 ± 0.40	1.00 ± 0.00
Moroberekan	VI	2.00 ± 0.00	0.00 ± 0.00	1.00 ± 0.00
OS6	VI	85.00 ± 5.00	0.03 ± 0.02	100.00 ± 0.00
Sinam pablo	VI	70.00 ± 5.00	0.43 ± 0.40	100.00 ± 0.00
Tedong	I	5.00 ± 0.00	0.00 ± 0.00	100.00 ± 0.00
Tetep	I	...	0.00 ± 0.00	3.00 ± 0.00
UPLRi5 (1)	I	5.00 ± 0.00	0.00 ± 0.00	1.00 ± 0.00
UPLRi5 (2)	I	...	0.00 ± 0.00	2.00 ± 0.00
UPLRi7	I	...	0.00 ± 0.00	15.00 ± 5.00

[a] Based on isozyme data provided by D. S. Brar (*unpublished*); varietal group I corresponds to *indica* type and varietal group VI corresponds to *japonica* type (12).

[b] Data were evaluated at 31, 33, and 29 days after sowing, respectively.

[c] Mean from two replications.

[d] ... = entry not tested.

[e] A series of near-isogenic lines in the genetic background of CO39 (21,32). C101LAC carries *Pi-1*; C101A51 carries *Pi-z*; C104PKT carries *Pi-3*; and C101PKT carries *Pi-ta*.

band of a particular molecular weight and "0" indicating its absence. The matrix containing the binary data was used for cluster analysis of the strains by the "unweighted pair group method, arithmetic mean" (UPGMA) (46). First, the WINDIST program (I. Yap and R. Nelson, *unpublished*) was used to derive a similarity matrix from the binary data using Dice's coefficient ($F = 2N_{xy}/[N_x + N_y]$, in which N_{xy} is the number of bands shared by a given pair of isolates, and $[N_x + N_y]$ is the total number of bands observed for that pair of isolates). Based on the similarity matrix, phenograms were then constructed using the SAHN program of NTSYS-pc package (40).

To evaluate the robustness of the groupings formed, the binary data set was subjected to bootstrapping. Using the program WINBOOT (35), the phenogram was reconstructed 2,000 times by repeated sampling with replacement, and the frequency with which a particular group was formed was considered to reflect the strength of the group. Distinct, robust groups were considered to represent phylogenetic lineages. Each lineage was assigned a numerical designation, and the unique DNA profiles, or haplotype, within each lineage were assigned codes (e.g., 1-2 = second haplotype identified in the first lineage).

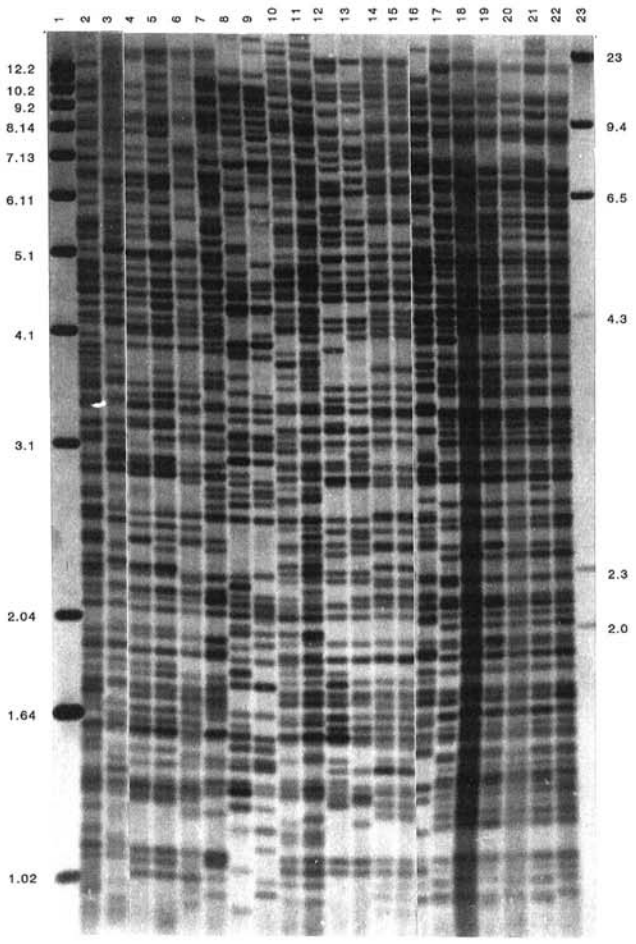

Fig. 1. DNA band profiles of isolates of *Pyricularia grisea* representing different lineages defined by MGR586. Most isolates were collected from the Blast Nursery of the International Rice Research Institute (IRRI-BN) and the upland screening site at Cavinti, Laguna, Philippines, in 1992. Isolates in lanes 8, 9, 11, 12, 13, and 17 were reference strains from the collection of Entomology and Plant Pathology Division, IRRI. Isolate (lineage and haplotype designations): 1, 1 kb ladder; 2, C9214-11 (lineage 7, haplotype 7-16); 3, C9212-23 (4, 4-6); 4, C9228-12 (4, 4-3); 5, C923-1 (4, 4-4); 6, C923-42 (46, 46-1); 7, C9237-11 (17, 17-2); 8, B9064 (9, 9-1); 9, B90065 (11, 11-1,); 10, 92325-1 (14, 14-3); 11, JMB8401 (23, 23-1); 12, B90066 (4, 4-27); 13, Po83-z1-30 (29, 29-1); 14, C9236-7 (46, 46-3); 15, C9236-21 (46, 46-5); 16, C9240-7 (44, 44-1); 17, B90002 (1, 1-13); 18, C923-2 (1, 1-1); 19, C926-11 (1, 1-2); 20, C926-8 (1, 1-1); 21, C926-28 (1, 1-3); 22, C926-12 (1, 1-4); 23, Lambda Hind III.

Measures of genetic variation and differentiation. Diversity indices (based on both haplotypic and lineage data) were calculated using the following equation (34):

$$H = n/(n-1)(1-\Sigma x_i^2),$$

in which n/(n – 1) is a correction for bias in small samples (44) and x_i is the frequency of ith haplotype or lineage in the collec-

Fig. 2. Phenogram derived by UPGMA (unweighted pair group method, arithmetic mean) based on DNA band data obtained using the probe MGR586, depicting similarities of isolates representing different lineages of *Pyricularia grisea* collected from the Blast Nursery and the upland screening site at Cavinti, Laguna, Philippines, in 1992. Values on the branches of the clusters represent the results of bootstrap analysis (the percentage of times the group occurred during 2,000 iterations). Lineage number is indicated by the first letter of the haplotype designation.

TABLE 2. Gametic equilibrium test for populations and subpopulations of *Pyricularia grisea* collected from the two blast nurseries of the International Rice Research Institute, Philippines, in 1992

Population/ subpopulation	Location and collection season	No. of unique haplotypes	No. of total loci	No. of intermediate loci	No. of locus pairs	No. of locus pairs ($P^a > 0.05$)
Cavinti	Cavinti, wet season (WS)	37	139	24	276	169 (61.23%)
IRRI-BN	IRRI-BN, WS and dry season (DS)	22	126	22	231	140 (60.61%)
Cavinti and IRRI-BN	Cavinti, WS; IRRI-BN, WS and DS	59	144	29	406	162 (39.9%)
Lineage 1	Cavinti, WS	10	93	5	10	7 (70%)
Lineage 4	Cavinti, WS	14	100	7	21	7 (33.3%)
Lineage 17	Cavinti, WS	13	79	4	6	3 (50%)
Lineage 46	Cavinti, WS	10	98	9	36	30 (83.33%)

[a] P = Fisher's exact probability for the null typothesis that there is random association between loci.

tion. The potential diversities at each level were calculated by assuming equal frequencies for each detected type (haplotype or lineage).

The partitioning of genetic variation was estimated using the coefficient of genetic differentiation (10,34):

$$G_{ST} = 1 - (H_S/H_T),$$

in which H_S is a weighted average of estimated lineage or haplotypic diversities in the subpopulations, and H_T is an estimate of the lineage or haplotypic diversity of the total population.

The χ^2 statistic was used to test for homogeneity in a $L \times K$ contingency table (17):

$$\chi^2 = \sum_{i=1}^{L} \sum_{j=1}^{K} [(n_{ij} - n_i p_j)^2 / n_i p_j]$$

in which L is the number of localities in the total sample, K is the number of lineages in the total sample, n_i is the sample size in location i, n_{ij} is the observed number of isolates of lineage j from location i, and p_j is the frequency of lineage j in the population. When the number of haplotypes is large, so the expected number of isolates for each haplotype for each location is very small, this test loses power (17). Therefore, the chi-square test was done only for location comparisons using the data from four common lineages (lineages 4, 7, 14, and 17).

Analysis of gametic phase equilibrium. The presence and absence of each MGR586-hybridizing band has been treated as alternate alleles at a locus for gene mapping in *Magnaporthe grisea* (15,39). This single-gene inheritance property was used to evaluate gametic phase equilibrium. MGR586 restriction fragment loci with allelic frequencies of 40 to 60% were selected and pairwise combinations of loci were evaluated for gametic phase equilibrium using Fisher's exact test (43,48).

Reinfection of hosts of origin by isolates. To interpret the relationship between field infection and the compatibility of the pathogen to their hosts of origin in the greenhouse, 92 Cavinti isolates from lineages 1, 4, 7, 17, 45, and 46 were selected, based on seedling stages and percentage of diseased leaf area when they were collected and frequency of haplotypes for the host of origin. The isolates were tested for compatibility on their hosts of origin and given susceptible checks CO39 or C22 using randomized design with three replicates. Ten seeds were sown in a plastic pot (9 by 9 cm) supplemented with ammonium sulfate at a rate of 6 g/kg of soil after sowing. Seedlings were grown in a greenhouse for 21 days before inoculation. Inoculation and incubation were as described by Bonman et al. (4).

Disease was scored 6 to 7 days after inoculation. Each seedling was examined and rated using a 0 to 5 scale (32), in which a score of 3.5 or higher was given to expanding sporulation lesions. Readings of 0 to 2 were considered to reflect an incompatible reaction, those of 3 were considered moderate, and those of 3.5 to 5 were taken to reflect compatible reactions. If 20% of the seedlings in a replicate were rated as 3.5 or greater, the reaction was considered to be compatible.

RESULTS

Composition of the overall collection. Using the repetitive probe MGR586, DNA fingerprints were obtained for 243, 677, and 596 isolates collected from 38 entries in the IRRI-BN in the dry and wet seasons and Cavinti in the WS in 1992, respectively. Between 60 to approximately 80 resolvable bands (1 to 22.0 kb) were scored for each isolate (Fig. 1). Based on at least two gel runs per isolate (a preliminary run, followed by coelectrophoresis of those isolates considered to share a similar banding profile), 130 haplotypes (distinct DNA banding patterns) were distinguished. For each group of coelectrophoresced isolates, a "subpopulation" phenogram was derived (6). From this analysis, 10 groups of isolates sharing similar profiles (putative lineages) were identified. Each putative lineage was given a numerical designation. Phenetic analysis was conducted for 66 isolates selected to represent 60 haplotypes drawn from the 10 groups (Fig. 2). Bootstrap analysis showed that the clusters were highly robust: Each was formed in 99.6 to 100% of the 2,000 iterations (Fig. 2). These 10 groups are hereafter termed lineages.

Gametic phase equilibrium among the populations. To assess the possibility of recombination of MGR586-defined loci within and between lineages, MGR586 band data were used for analysis of gametic phase equilibrium. If recombination (sexual or parasexual) was active in the pathogen population, random associations between loci would be expected. Associations between loci can be tested using Fisher's exact probability statistic for loci with intermediate frequencies in the population. The associations between such pairs of loci were examined for isolates from different pathogen populations and subpopulations (isolates from Cavinti, from the IRRI-BN, from both sites, and from four different lineages collected from Cavinti).

Many of the loci tested were randomly associated (Table 2). Collections from the IRRI-BN and from Cavinti showed similar levels of gametic equilibrium: Approximately 60% of the locus pairs showed random association at $P > 0.05$. When the collections from the two sites were pooled for analysis, a lower level of

TABLE 3. Composition of three collections of the blast fungus taken from the Blast Nursery at the International Rice Research Institute (IRRI-BN), Los Baños, Laguna, Philippines, and the upland screening site at Cavinti, Laguna, in 1992

	IRRI-BN/DS[a]		IRRI-BN/WS[b]		Cavinti/WS	
Lineage	No. of isolates	No. of haplotypes	No. of isolates	No. of haplotypes	No. of isolates	No. of haplotypes
1	…[c]	…	…	…	44	8
4	18	3	40	3	178	7
7	55	17	535	54	227	9
14	162	6	94	20	…	…
17	8	1	8	4	76	7
44	…	…	…	…	12	3
45	…	…	…	…	1	1
46	…	…	…	…	55	7
47	…	…	…	…	2	2
48	…	…	…	…	1	1
Total	243	27	677	81	596	45

[a] DS = experiment conducted in the dry season.
[b] WS = experiment conducted in the wet season.
[c] … = the lineage or haplotype was not detected from the collection.

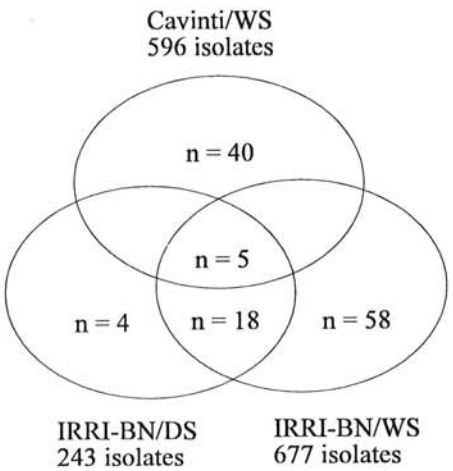

Fig. 3. Distribution of haplotypes of *Pyricularia grisea* collected from Cavinti in the wet season, 1992, and from the blast nursery of the International Rice Research Institute (IRRI-BN) in the dry season and the wet season, 1992, in the Philippines.

gametic equilibrium was obtained (40% of band pairs did not deviate from the expected random association at $P > 0.05$). The level of gametic equilibrium within lineage varied considerably for four lineages analyzed. For lineage 46, 83% of the band pairs did not deviate from the expected random association at $P > 0.05$. For lineage 4, in contrast, only 33% of the bands were randomly associated at $P > 0.05$.

Differentiation among the *P. grisea* collections. The amount of blast on the 38 entries varied widely in each of the three trials (Table 1) and the relative performance of the cultivars/lines were different in the different trials. Correlation analysis of the RAUDPC (relative area under disease progress curve) values between Cavinti and the IRRI-BN showed that the disease profiles of the nursery at the two sites were significantly different (r = 0.35). Some cultivars/lines that were susceptible at Cavinti were resistant at the IRRI-BN. Some entries were scored as highly susceptible in the IRRI-BN/DS, but were resistant, moderate, or susceptible in the IRRI-BN/WS. For instance, the cultivars Aichi asahi, CNA4130, CO39, Sinam Pablo, and Tedong showed substantially more disease at Cavinti than at the IRRI-BN in the 1992 WS, and they showed more disease in the DS than in the WS at the IRRI-BN. These differences in varietal reaction between sites and seasons suggested differences in the composition of the pathogen population.

Nine lineages of *P. grisea* were detected at Cavinti, while only four were found at the IRRI-BN in both seasons (Table 3). Linea-

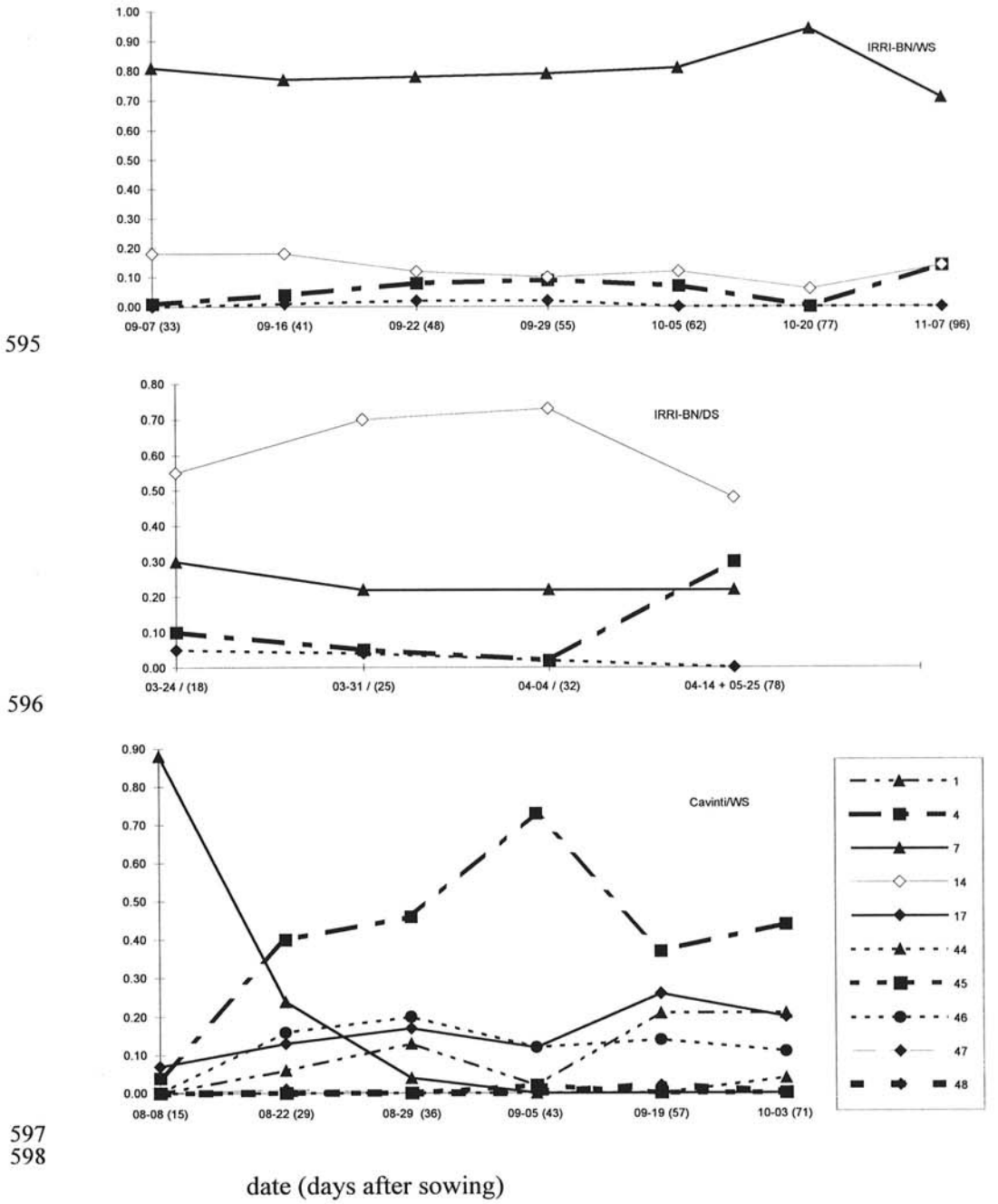

Fig. 4. Changes in frequencies of lineages of *Pyricularia grisea* over time at the Blast Nursery of the International Rice Research Institute (IRRI-BN) and Cavinti in the wet season (WS) and dry season (DS), 1992.

ges 4, 7, and 17 were shared by both sites. While lineage 14 was predominant in the IRRI-BN/DS collection and second in abundance in the IRRI-BN/WS collection, lineage 14 was not detected among the 596 isolates collected from Cavinti in the same year, although the IRRI-BN and Cavinti are only 40 km apart.

Most of the haplotypes detected were found in only one of the three collections (Fig. 3). Only 5 of 130 haplotypes were shared by all three collections. Of the 90 haplotypes detected at the IRRI-BN, only 18 (20%) were recovered in both seasons. The haplotypes of lineage 4 found in the DS were completely different from those detected in the WS, even though most of the isolates were collected from the same cultivars in the two collections.

The predominant lineage shifted between seasons at the IRRI-BN. Although lineages 4, 7, 14, and 17 were found in both seasons, their relative abundance was not constant. In the DS, lineage 14 was predominant, accounting for 67% of the isolates. Lineage 7 was predominant in the WS, accounting for 79% of the isolates. Lineages 4 and 17 were isolated at low frequencies in both seasons (Fig. 4).

The frequency rank order of each lineage was relatively consistent across samplings within each collection. The predominance of lineage 7 at Cavinti, however, changed dramatically. This lineage accounted for 87% of the first collection, but declined sharply in the subsequent two samplings and was not recovered after the third collection. Apparently, entries susceptible to lineage 7 were killed in the early seedling stages and no susceptible entries were available from which lineage 7 isolates could be recovered. This lineage continued to be recovered from different cultivars/lines in a companion experiment in the same field (M. Bronson, *personal communication*) (Fig. 4).

DNA profile data were used to calculate the genetic diversities of the collections at the levels of lineage and haplotype. Based on lineage data, the collection from Cavinti was the most diverse, followed by the IRRI-BN/DS collection and the IRRI-BN/WS collection (Table 4). However, haplotypic diversities for the three collections were similar (Table 4). The potential lineage diversity (supposing each lineage was equally represented) of the collection from Cavinti also was higher than those from the IRRI-BN, while the potential genetic diversities based on the haplotypes were similar for the two sites.

Haplotypic diversity within lineage differed substantially between the collections analyzed (Table 5). The most abundant lineage was not necessarily the most diverse. For instance, lineage 7 exhibited high haplotypic diversity in the dry and wet seasons at the IRRI-BN, but showed low haplotypic diversity at Cavinti although it was the predominant lineage. Similarly, the haplotypic diversity of lineage 4 was much greater in the collection from the IRRI-BN/DS and from Cavinti than from the IRRI-BN/WS.

Approximately 19% of the overall lineage differentiation among the three collections was attributable to differences among locations and between seasons (G_{ST} = 0.19). From the lineage data, pairwise comparisons of the genetic differentiation between collections from the two sites and between collections from different seasons at the IRRI-BN showed highly significant coefficients of genetic differentiation. As expected, the lowest χ^2 value was obtained when the two collections from the IRRI-BN were compared (Table 6).

Differences between locations and seasons accounted for 30 and 20%, respectively, of the overall genetic diversities for lineages 4 and 7. The sampling season contributed 6% of the genetic diversity of lineage 14, since it was only collected from the IRRI-BN. Only 3% of the overall genetic diversity for lineage 17 was due to difference of locations and seasons (Table 5).

Host differentiation of *P. grisea*. Most hosts were infected by multiple haplotypes, and many were infected by multiple lineages (Table 7). Two hosts, however, were found to be attacked by only a single lineage each. C101A51, an isogenic line carrying the gene *Pi-2(t)* (= *Pi-z* [22,32]), was infected only by lineage 44. Tetep, a cultivar widely recognized for its broadly effective resistance to blast (42), was infected only by lineage 17. Both of these lineages were confirmed to be compatible with their hosts of origin in greenhouse inoculation experiments.

Isolates recovered from the same hosts from the three collections were pooled, and lineage and haplotype diversities were calculated for each host (Table 7). While the haplotypic diversities of the collections of isolates from most hosts were high, the

TABLE 4. Lineage and haplotypic diversities of three collections of the blast fungus from the Blast Nursery at the International Rice Research Institute (IRRI-BN) and Cavinti screening site in the Philippines in 1992

	IRRI-BN/DS[a]		IRRI-BN/WS[b]		Cavinti/WS	
	Lineage	Haplotype	Lineage	Haplotype	Lineage	Haplotype
Observed diversity[c]	0.50	0.82	0.35	0.89	0.74	0.83
Potential diversity[d]	0.75	0.97	0.75	0.99	0.89	0.98

[a] DS = experiment conducted in the dry season.
[b] WS = experiment conducted in the wet season.
[c] Observed diversity = genetic diversity calculated from the experimental data.
[d] Potential diversity = theoretical genetic diversity, it was derived if all the types were equally represented in the collections.

TABLE 5. Estimates of genetic diversity and differentiation within lineages of *Pyricularia grisea* in three collections from the Blast Nursery (IRRI-BN) and Cavinti, Laguna, Philippines, in 1992

	Genetic diversity			Diversity statistics		
Lineage	IRRI-BN/DS[a]	IRRI-BN/WS[b]	Cavinti/WS	H_S[c]	H_T[d]	G_{ST}[e]
1	…[f]	…	0.69			
4	0.54	0.10	0.64	0.54	0.76	0.29
7	0.91	0.83	0.11	0.68	0.85	0.20
14	0.60	0.77	…	0.66	0.70	0.06
17	0.00	0.64	0.67	0.61	0.63	0.03
44	…	…	0.44			
45	…	…	0.00			
46	…	…	0.59			
47	…	…	1.00			
48	…	…	0.00			

[a] DS = experiment conducted in the dry season.
[b] WS = experiment conducted in the wet season.
[c] H_S = within-collection lineage diversity is the mean of the diversities for the collections, weighted by the sample size.
[d] H_T = total diversity is calculated from the weighted means of the haplotypic frequencies for the entire sample.
[e] G_{ST} = coefficient of genetic differentiation between collections.
[f] … = the lineage is not detectable.

TABLE 6. Genetic differentiation of pairs of collections of the rice blast fungus collected from the Blast Nursery (IRRI-BN) and Cavinti, Laguna, Philippines, in 1992

	Diversity statistics based on lineage			
Collection	H_S[a]	H_T[b]	G_{ST}[c]	χ^2-test[d] (df)
IRRI-BN(DS)/ IRRI-BN(WS)	0.39	0.51	0.23	272.91**[e](3)
IRRI-BN(DS)/ Cavinti (WS)	0.67	0.78	0.14	419.69**(3)
IRRI-BN(WS)/ Cavinti (WS)	0.53	0.60	0.11	337.38**(3)
IRRI-BN(DS)/ IRRI-BN(WS)/Cavinti (WS)	0.53	0.65	0.19	792.16**(6)

[a] H_S = within-collection lineage diversity is the mean of the diversities for the collections, weighted by the sample size.
[b] H_T = total diversity is calculated from the weighted means of the lineage frequencies for the entire sample.
[c] G_{ST} = coefficient of genetic differentiation between collections.
[d] Chi-square test was based on data from four common lineages (lineages 4, 7, 14, and 17).
[e] ** = significant difference at 0.01 level.

lineage diversities were variable, showing a continuous distribution from 0 to 0.74. Based on pooled lineage data, a coefficient of genetic differentiation ($G_{ST_{host}}$) of 0.39 was obtained. This indicated that host selection exerted a strong influence on structuring the pathogen population.

The chi-square test for independence was used to evaluate the relationship between lineages and hosts. Because of the large number of cells with low expected values, the Monte Carlo option was used to estimate the *P*-value based on 2,000 samples of the dataset (1). Results showed that the distribution of lineages on individual hosts was nonrandom ($P < 0.0023$). This indicated that host selection had a significant effect on the structure of the pathogen population.

Isolates collected from the same host from the three collections were pooled, and the lineage data were used as the basis for cluster analysis of the lines/cultivars. Ten clusters of the cultivars (those with ≥ 10 isolates collected) were formed at the 80% similarity level (Fig. 5). C101A51 (carrying *Pi-2(t)* = *Pi-z*), Tetep, and C104PKT (carrying *Pi-3(t)*) each formed their own clusters. Several groups of hosts with similar or identical genetic backgrounds were clustered together, suggesting that the field collection of the pathogen provides meaningful information on the resistance spectrum of the host. For instance, two entries each of IRAT208 and IRAT239 formed a cluster, together with several other entries. Two entries of UPLRi5 (seed obtained from different sources) were closely clustered, together with UPLRi7. CO39 and its near-isogenic lines, C101LAC and C101PKT, were tightly clustered. Three IRRI cultivars, IR36, IR50, and IR62, also were clustered together.

The cultivars/lines with low lineage diversity (low diversity estimates based on lineage data, or Dv_{lin}; Dv_{lin} = 0.08 to 0.26) were infected by lineages 7 and 14, with some cultivars/lines also attacked by lineage 17. Most (four of seven) of these hosts were IRRI cultivars. Among the 290 isolates collected from IR36, IR50, IR62, and IR72, none were of lineage 4. In contrast, the entries with a somewhat higher lineage diversity (Dv_{lin} = 0.27 to 0.56) were mostly attacked by lineage 4 (10 of 16 entries). These cultivars were each infected by two to four of the following lineages: 7, 14, 17, and/or 4. Among the 52 isolates analyzed from IR64, 38 were of lineage 4, and the remaining 14 were of lineage 14. This suggested that IR64 may lack gene(s) for resistance to lineage 4 that are present in IR36, IR50, IR62, and IR72, but may carry gene(s) for resistance to lineage 7 that are lacking in these cultivars. Isolate collections from 10 hosts showed lineage diver-

TABLE 7. Lineage distribution, genetic diversity, and differentiation of *Pyricularia grisea* calculated from isolates taken from each host, pooled from three collections

Cultivar	Number of isolates in lineage										No. of haplotypes	Genetic diversity	
	1	4	7	14	17	44	45	46	47	48		lineage	haplotype
IRAT2					2						1	0.00	0.00
C101A51						12					3	0.00	0.44
Tetep					37						4	0.00	0.64
IRAT13			2								2	0.00	1.00
IRAT104		2									2	0.00	1.00
Kinarabao 3777			47	1	1						14	0.08	0.77
IR36			80	4							20	0.09	0.85
IR62			69	4							16	0.11	0.78
IR50			72	4	1						26	0.12	0.91
C101PKT			55	6							13	0.18	0.83
IR72			54	6							19	0.18	0.88
OS6			30	3	2						14	0.26	0.88
Kinarabao 19430		2	18		1						7	0.27	0.71
IAC47		2	18	1							7	0.27	0.76
Carreon		29		7							7	0.32	0.79
Aichi asahi		2	43	6	2						14	0.33	0.88
C101LAC			49	14	2						16	0.39	0.84
IR64		38		14							10	0.40	0.73
CO39		4	56	10	5						21	0.42	0.89
IRAT208		3	18	3	1						13	0.43	0.91
Sinam pablo			37	12	5						19	0.48	0.91
Tedong			18	5	3						13	0.49	0.92
IRAT239		2	21	9	2						17	0.50	0.74
CNA4130			1	3							3	0.50	0.83
IR442-2-58		1		3							3	0.50	0.83
BL1			13	4	2						10	0.50	0.92
K59		1	31	21	1						20	0.53	0.92
C22		35	2	11	7		1	1			11	0.58	0.86
Azucena		1	16	7	2			1			13	0.60	0.92
UPLRi7	2	14		4	5						10	0.64	0.80
UPLRi5	1	38		12	1			26	1	1	15	0.65	0.80
IAC165		1	2	4							6	0.67	0.95
Kusabue			6	9	5						8	0.68	0.86
IR66		13	15	13	1						17	0.70	0.93
C104PKT		24	12	29				7			17	0.70	0.87
Kinandang patong		3	15	4	1			10	1		17	0.72	0.92
Fujisaka 5	26	14	1	9	3			5			15	0.72	0.90
Akashi	25	7	1	8			5				12	0.74	0.88
Moroberekan			1	1							2	1.00	1.00
Weighted mean of diversity (H_S)[a]												0.40	0.84
Total diversity (H_T)[b]												0.65	0.94
Coefficient of genetic differentiation ($G_{ST_{host}}$)[c]												0.39	0.10

[a] H_S = within-collection lineage diversity is the mean of the diversities for the collections from hosts.
[b] H_T = total diversity is calculated from the weighted means of the haplotype or lineage frequencies for the entire sample.
[c] $G_{ST_{host}}$ = coefficient of genetic differentiation among hosts.

sities of 0.58 to 0.74. Each of the hosts in this group were infected by three to seven lineages. IR66, in this group, was infected by isolates of lineages 4, 7, 14, and 17.

Compatibility of collected isolates to their hosts of origin. The majority of the isolates recovered from Cavinti were virulent on one or both susceptible checks, but most could not reinfect their hosts of origin in the greenhouse. Of 92 isolates collected from cultivars with a range of diseased leaf areas (DLA) and in different seedling stages (29 and 15 days after sowing [DAS]), tested for compatibility on their hosts of origin, and given a susceptible check, 69 were compatible to one or both test hosts (Table 8). Overall, however, only 41% of demonstrably virulent isolates were fully compatible to their hosts of origin. Another 17% of the isolates gave intermediate reaction types, with a maximum lesion type of 3 based on the 0 to 5 lesion type scale (32). The remainder (42%) were unable to infect the rice lines or cultivars from which they had been isolated.

The compatibility of virulent isolates to their hosts of origin varied with lineages. The frequency of host compatibility of a lineage decreased with the increasing abundance of lineages in the collection ($r = -0.93$). Of 17 isolates belonging to the rare haplotypes in particular hosts, nine (53%) could reinfect the original hosts. It appeared that when a lesion was caused by a rare lineage for a particular host, it was more likely to reinfect its host of origin. However, when a lesion was caused by a predominant lineage, it was less likely to reinfect its host of origin.

The relationship between DLA and compatibility with the host of origin was compared for isolates taken at 29 DAS. In general, isolates recovered from cultivars with large DLA were most likely to be compatible to their hosts of origin (Table 9). No fully incompatible reaction with the host of origin was observed for isolates collected from cultivars with large DLA at 29 DAS, while 42% were incompatible to their hosts of origin for isolates taken from cultivars with large DLA at 15 DAS. However, the effects of DLA and seedling stages on the compatibility of isolates to their hosts of origin varied among the individual host genotypes. Association of low compatibility with the host of origin for isolates collected from a cultivar with large DLA at 15 DAS could be due to none or partial expression of resistance in the host and some opportunistic factors.

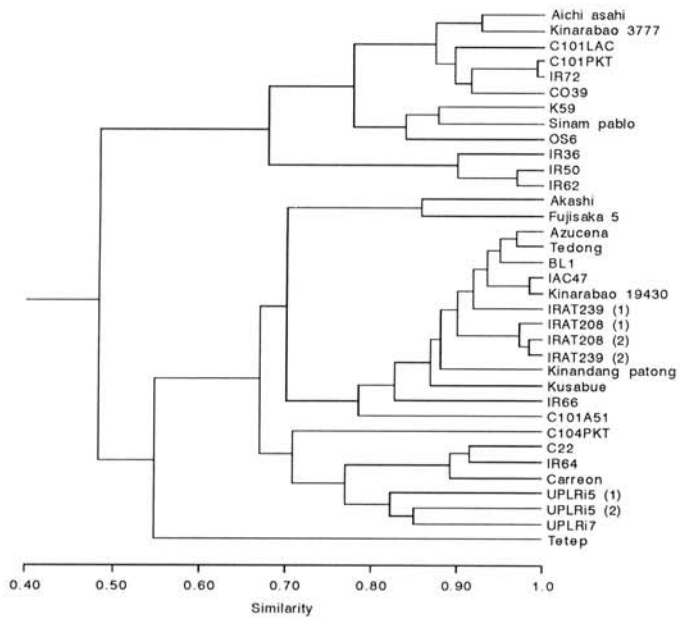

Fig. 5. Phenogram derived by UPGMA (unweighted pair group method, arithmetic mean) based on lineages isolated from each host in three collections.

DISCUSSION

The diversity and differentiation of pathogen populations may be inferred from field disease severities on sets of diverse cultivars or from direct analysis of isolate genotypes and/or virulence. In this study, a large set of diverse cultivars and lines was evaluated and used as a trap nursery for the collection of sets of isolates of the rice blast pathogen from two screening sites used for the evaluation of rice cultivars and lines for blast resistance. Diversity and differentiation of pathogen collections were analyzed according to sites, seasons, and host origins.

Populations of the blast fungus at Cavinti (1992, wet season) and the IRRI-BN (1992, wet and dry seasons) were highly differentiated both geographically and temporally. The entries showed different disease severities between locations in the same season and between seasons at the same site. DNA fingerprinting of collected isolates further illustrated the distinctness of pathogen populations among the three collections. The observed differentiation between pathogen populations from the two screening sites could have been due to agroecological differences between the sites. Cavinti is relatively isolated from commercial rice fields and serves as an upland breeding site in which predominant populations have tropical *japonica* backgrounds. The IRRI-BN is in the midst of lowland *indica* rice production and breeding fields. Other studies have indicated that pathogen populations infecting *japonica* and *indica* cultivars differ (8,16,50,51).

In previous studies, including one conducted at the IRRI Blast Nursery (38), the race structure of field populations of the blast fungus was found to change over time (23). Our DNA fingerprinting data indicated a strong lineage structure, as found in previous studies using repetitive DNA elements for DNA fingerprinting of the blast pathogen. At the lineage level, the composition of the pathogen population at the IRRI-BN remained constant over the two seasons sampled. However, lineage frequencies varied between seasons and tremendous haplotypic change was observed between seasons.

TABLE 8. Percentage of compatibility of virulent isolates of *Pyricularia grisea* to their hosts of origin in greenhouse inoculation

Lineage	No. of cultivars	Frequency in collection	Hosts of origin C[a]	Hosts of origin M[b]	Hosts of origin I[c]	Susceptible check (CO39/C22)
1	3	0.07	100 (1)[d]	0 (0)	0 (0)	100 (1)
4	11	0.3	32 (8)	20 (5)	48 (12)	100 (25)
7	6	0.38	30 (7)	26 (6)	43 (10)	100 (23)
17	7	0.13	50 (7)	7 (1)	43 (6)	100 (14)
45	1	0.002	100 (1)	0 (0)	0 (0)	100 (1)
46	6	0.92	80 (4)	0 (0)	20 (1)	100 (5)
Overall			41 (28)	17 (12)	42 (29)	100 (69)
Rare type	12		53 (9)	6 (1)	41 (7)	100 (17)

[a] C = compatible reaction, seedlings with score reading 3.5 to 5.
[b] M = moderate reaction, seedlings with score reading 3.
[c] I = incompatible reaction, seedlings with score reading 0 to 2.
[d] Number inside the parenthesis is the number of apparent virulent isolates based on their compatibility to hosts of origin and susceptible checks.

TABLE 9. Relationship between compatibility of virulent isolates of *Pyricularia grisea* with their hosts of origin and seedling stages and the percentage of the diseased leaf area at which isolates were collected

Days after sowing	High diseased leaf area (10-85%) C[a]	High diseased leaf area (10-85%) M[b]	High diseased leaf area (10-85%) I[c]	Low diseased leaf area (1-3%) C	Low diseased leaf area (1-3%) M	Low diseased leaf area (1-3%) I	Very low diseased leaf area (< 1%) C	Very low diseased leaf area (< 1%) M	Very low diseased leaf area (< 1%) I
15	26 (5)	32 (6)[d]	42 (8)	...[e]	...	...	...	...	...
29	78 (7)	22 (2)	0 (0)	75 (6)	0 (0)	25 (2)	6 (1)	23 (5)	67 (12)

[a] C = compatible reaction, seedlings with score reading 3.5 to 5.
[b] M = moderate reaction, seedlings with score reading 3.
[c] I = incompatible reaction, seedlings with score reading 0 to 2.
[d] Number inside parenthesis is isolates.
[e] ... = no isolates available.

Such a population structure (distinct lineages with variable haplotypes) could have resulted from a clonal mode of reproduction coupled with mutation, migration, and drift/selection. It is possible that genetic recombination (parasexual or sexual) also could have contributed to the diversity and variability of the population. Parasexuality has been demonstrated to generate variation morphologically and genetically (9;11; R. S. Zeigler, R. P. Scott, H. Leung, A. A. Bordeos, and R. J. Nelson, *unpublished*). Compatible mating types of the fungus have been isolated from rice fields in the same location (31; H. Leung, *unpublished*; J. Kumar, R. J. Nelson, and R. S. Zeigler, *personal communication*), suggesting the possibility of sexual recombination in nature. In this study, a high percentage of the locus pairs analyzed was found to be in gametic phase equilibrium in both the Cavinti and the IRRI-BN collections. This was consistent with, but did not conclusively indicate, the occurrence of recombination in the field. Other processes (e.g., mutation and migration) could have contributed to gametic phase equilibrium. For instance, when the Cavinti and the IRRI-BN collections were pooled for analysis of gametic phase equilibrium, the percentage of the locus pairs in gametic phase equilibrium declined greatly. This was likely due to genetic differentiation of the collections from the two sites. Further studies are needed to address the potential role of recombination for field populations of *P. grisea*.

Genetic drift may have played a significant role in structuring the pathogen population. At Cavinti there is a severe dry season during which little, if any, rice is present in the field. In the IRRI-BN, very small plant populations in one period of the year may result in "bottlenecks" for the pathogen population. At least one lineage that was previously detected from the IRRI-BN was not detected in the present study. Lineage 15 was detected in abundance on traditional rice cultivars such as Carreon and Kinarabao in 1990 at the IRRI-BN (R. J. Nelson and M. Bernardo, *unpublished*), but was not detected in this study, although Carreon and Kinarabao were included in this trap nursery and Carreon was planted continuously at the nursery. Available isolates of lineage 15 have very narrow virulence spectra (51), so alternate hosts for survival may be relatively rare.

Host selection appeared to play a major role in structuring the pathogen population. Strong host selection was inferred using chi-square analysis, coefficients of differentiation, and cluster analysis. Only one or a few lineages were recovered from some of the hosts, while many lineages were isolated from other hosts. When data on the lineages collected from each host were used for cluster analysis of the hosts, groups of hosts with similar infection spectra were identified. Many of the groups of cultivars/lines identified were consistent with known or suspected genetic similarities among the hosts. The specific pathogen subpopulations infecting each host subpopulation (cultivar/line) appeared to provide insight into the resistance phenotype of the hosts.

While the different resistance genes present in the hosts would be expected to exert selection on the pathogen population, it was interesting to see that this effect could be clearly observed when sampling from miniplots, in spite of the possible interplot interference. Our results underscored the importance of considering host selection when designing sampling strategies aimed at characterization of pathogen populations. Absence of a suitable trap plant in the experiment could lead to a given lineage never being collected. Although a high genetic diversity was detected for the *P. grisea* population at Cavinti in this study (in which samples were taken from 38 cultivars/lines), a low genetic diversity was estimated for Cavinti in the same season from a set of lines derived from a single cross (M. Bronson, *personal communication*).

In attempting to infer the resistance phenotype of a cultivar based on the isolates collected from it, it is important to know whether field infection accurately indicates compatibility (susceptibility of the cultivar to the test isolate). Therefore, we conducted greenhouse inoculation studies to determine the extent of compatibility of isolates with their hosts of origin. Fewer than half (41%) of the apparently virulent isolates were able to reinfect their hosts of origin. This corroborated similar observations by others (7,30), and may have been due, at least in part, to interplot interference and opportunistic infection. The frequency of host incompatibility increased with the increasing abundance of a lineage in the collection, probably because opportunistic infection was more likely to involve a more abundant lineage than a rare one. The more resistant hosts showed a higher frequency of infection by incompatible isolates, probably due to interplot interference from the more susceptible cultivars. This observation suggested that caution must be observed in inferring the susceptibility spectrum from the field infection spectrum. Data on the pathogen subpopulations infecting a cultivar or line should be complemented with controlled greenhouse inoculations before strong inferences on the resistance spectrum are made. Similarly, early infection of seedlings may or may not reflect true susceptibility and should be interpreted with caution in a screening program.

The results of this study have direct implications for resistance breeding. Information on the interactions between host subpopulations (cultivars) and pathogen subpopulations (lineages) can be used to improve the diversity of pathogen populations at screening sites. Further, this analysis provides insight into the resistance spectra of rice cultivars, which can be used to make informed selection of parents for a breeding program.

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