Karyotypic Variation within Clonal Lineages of the Rice Blast Fungus, *Magnaporthe grisea*

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We have analyzed the karyotype of the rice blast fungus, *Magnaporthe grisea*, by using pulsed-field gel electrophoresis. We tested whether the electrophoretic karyotype of an isolate was related to its pathotype, as determined by infection assays, or its genetic lineage, as determined by DNA fingerprinting. Highly reproducible electrophoretic karyotypes were obtained for a collection of U.S. and Chinese isolates representing a diverse collection of pathotypes and genetic lineages. Chromosomes ranged in size from 3 to 10 Mb. Although chromosome number was largely invariant, chromosome length polymorphisms were frequent. Minichromosomes were also found, although their presence was not ubiquitous. They ranged in number from 1 to 3 and in size from 470 kb to 2.2 Mb. Karyotypes were sufficiently variable as to obscure the obvious relatedness of isolates on the basis of pathogenicity assays or genetic lineage analysis by DNA fingerprinting. We demonstrated that the electrophoretic karyotype of an isolate can change after prolonged serial transfer in culture and that this change did not alter the isolate’s pathotype. The mechanisms bringing about karyotype variability involve deletions, translocations, and more complex rearrangements. We conclude that karyotypic variability in the rice blast fungus is a reflection of the lack of sexuality in wild populations which leads to the maintenance of neutral genomic rearrangements in clones of the fungus.

Plant pathogenic fungi continue to elude the efforts of plant breeders to produce durable resistance in major crop species. The generation of newly virulent forms of major crop pathogens can often curtail the use of high-yielding cultivars shortly after their introduction and is a cause of considerable and continuing agricultural expenditure. An example of such a problematic disease is rice blast (5, 12).

Rice blast is caused by the heterothallic, filamentous ascomycete *Magnaporthe grisea* (42). The fungus exists as a number of host-limited forms which collectively parasitize a large variety of grass species (for a review, see reference 31). Isolates of *M. grisea* capable of infecting rice have been shown to be genetically distinct from other host-specific forms (9, 10) and can be classified into pathotypes, or races, using infection assays on a set of differentially resistant rice cultivars (14, 15). Hundreds of pathotypes have been reported to exist in various rice-infesting populations of *M. grisea*, and these produce widespread epidemics, in spite of the introduction of newly resistant rice cultivars (3), presumably due to alterations in the genetic makeup of the pathogen population. The mechanisms that could generate this level of genetic variation are the subjects of much speculation (14, 16, 29, 30).

The molecular karyotype of fungal species may be determined by pulsed-field gel electrophoresis (PFGE). Fungal karyotypes have been shown to be quite variable between strains in several plant and human pathogens (24, 34). Karyotypic variation results from length differences in separate chromosome-sized DNA molecules and has been shown to involve translocation, deletions, and other types of genomic rearrangements (2, 21, 24, 38, 40). It has been speculated that chromosomal rearrangements may be a mechanism for the generation of new virulent forms of plant pathogenic fungi (2). For example, a chromosomal translocation has been shown to cosegregate with the ability to produce a host-specific toxin in the Southern corn leaf blight pathogen, *Cochliobolus heterostrophus* (41), and a dispensable B-like chromosome in the pea wilt pathogen, *Nectria haematococca*, carries a gene for phytoalexin detoxification (22). Both of these gene functions are important in determining virulence in each respective plant pathogen. Variable karyotypes and B-like minichromosomes have also been documented in *M. grisea* (10, 42). However, the organization of this variation and its relationship to pathogenic variation are not known.

Recent population studies of rice-infecting isolates of *M. grisea* have strongly suggested that the sexual stage of the fungus rarely produced in nature (26), and thus populations are clonal. Isolates can be sorted into genetic (clonal) lineages on the basis of a DNA fingerprint generated by Southern blot analysis, using a dispersed repeated DNA sequence, MGR86, as a hybridization probe (18, 19). DNA fingerprinting involves the analysis of MGR-hybridizing EcoRI restriction fragment length polymorphisms which are known to be dispersed along the *M. grisea* chromosomes (11, 32), and thus DNA fingerprints represent multilocus haplotypes. In U.S. blast fungus populations, isolates of the same pathotype belong to either one or two broadly dispersed clonal lineages. Furthermore, genetic similarity between and among pathotypically distinct individuals can be assessed on the basis of DNA fingerprints (19).

To investigate karyotype variability as a mechanism for pathogenic diversification in the blast fungus, we have analyzed clonal populations of *M. grisea* by using PFGE. We expected that if karyotype variability is strongly correlated with pathogenic variation, then isolates of the same clonal lineage would all show the same karyotypes and isolates of different pathotypes would therefore have distinct karyotypes. Conversely, if karyotype variation is frequent but generally neutral in effect, then no strong correlation would be expected.
TABLE 1. Origin of rice blast fungus isolates used for electrophoretic karyotype analysis

<table>
<thead>
<tr>
<th>Isolate*</th>
<th>Yr of isolation</th>
<th>Location</th>
<th>Pathotype*</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO11</td>
<td>1987</td>
<td>Arkansas</td>
<td>IB-49(A)</td>
</tr>
<tr>
<td>VO15</td>
<td>1985</td>
<td>Louisiana</td>
<td>IB-49(A)</td>
</tr>
<tr>
<td>VO104</td>
<td>1987</td>
<td>Puerto Rico</td>
<td>IB-49(A)</td>
</tr>
<tr>
<td>VO115</td>
<td>1982</td>
<td>Florida</td>
<td>IB-49(A)</td>
</tr>
<tr>
<td>VO113</td>
<td>1967</td>
<td>Texas</td>
<td>IB-49(B)</td>
</tr>
<tr>
<td>VO101</td>
<td>1981</td>
<td>Louisiana</td>
<td>IB-49(B)</td>
</tr>
<tr>
<td>VO3</td>
<td>1967</td>
<td>Louisiana</td>
<td>IB-49(B)</td>
</tr>
<tr>
<td>VO109</td>
<td>1967</td>
<td>Louisiana</td>
<td>IB-49(B)</td>
</tr>
<tr>
<td>VO4</td>
<td>1964</td>
<td>Louisiana</td>
<td>IB-54</td>
</tr>
<tr>
<td>VO110</td>
<td>1965</td>
<td>Texas</td>
<td>IB-54</td>
</tr>
<tr>
<td>VO102</td>
<td>1959</td>
<td>Louisiana</td>
<td>IB-54</td>
</tr>
<tr>
<td>VO105</td>
<td>1976</td>
<td>Texas</td>
<td>IB-54</td>
</tr>
<tr>
<td>VO108</td>
<td>1964</td>
<td>Louisiana</td>
<td>IB-54</td>
</tr>
<tr>
<td>VO111</td>
<td>1984</td>
<td>Texas</td>
<td>IB-54</td>
</tr>
<tr>
<td>VO5</td>
<td>1975</td>
<td>Texas</td>
<td>IC-17</td>
</tr>
<tr>
<td>VO9</td>
<td>1978</td>
<td>Arkansas</td>
<td>IC-17</td>
</tr>
<tr>
<td>VO12</td>
<td>1981</td>
<td>Florida</td>
<td>IC-17</td>
</tr>
<tr>
<td>VO13</td>
<td>1985</td>
<td>Texas</td>
<td>IC-17</td>
</tr>
</tbody>
</table>

* Isolate numbers were assigned by J.E.H. Isolates are kindly provided by M. A. Marchetti, Beaumont, Tex.
* Pathotype designations are defined by pathogenicity tests on the Interna-
tional Set of Differential Rice Cultivars (20). Isolates of pathotype IB-49 were subdivided previously into two clonal lineages by MGR fingerprinting (19).
* Reisolation of VO102 after numerous serial transfers (19).

In this study, we show that the electrophoretic karyotypes of *M. grisea* rice pathogens are uncorrelated with pathotype or clonal relatedness. We examined representatives of three major clonal lineages defined by DNA fingerprinting which resolve three pathotypes commonly found in the United States. Surprisingly, in each case karyotypes appeared to vary within clonal lineages. Our results suggest that karyotype rearrangements are common in *M. grisea* and are not diagnostic for pathotype. The considerable karyotypic variation observed is consistent with the hypothesis that rice-infecting populations of *M. grisea* are clonal.

MATERIALS AND METHODS

**Fungal isolates and culture conditions.** *M. grisea* isolates used in this study are stored in the laboratory of J.E.H. (Purdue University, West Lafayette, Ind.). Isolate histories and descriptions are listed in Table 1. General methods for maintenance of *M. grisea* cultures are described by Crawford et al. (4). Nomenclature for pathotypes is given by Ling and Ou (20). Pathogenicity assays for pathotype determination were performed and recorded by M. A. Marchetti (U.S. Department of Agriculture Rice Research, Beaumont, Tex.), using the International Set of Differential Rice Cultivars. Chinese isolates were obtained from Shen Ying of the China National Rice Research Institute, Hangzhou, People’s Republic of China.

**DNA isolation and analysis.** DNA was isolated from hyphal cultures by using a hexadecyltrimethyl-ammonium bromide (Sigma Chemical Co.) extraction procedure (6). Essentially, hyphal cultures were filtered, and the mycelium was blotted on paper towels to remove excess liquid. Approximately 2 g of mycelium was ground in liquid N2 to a fine powder and resuspended in a solution containing 4 ml of hexadecyltri-

**RESULTS**

Experimental design and MGR-based lineage assignments. Eighteen isolates from three discrete clonal lineages with well-defined pathotypes were chosen for analysis. Representative DNA blots of pairs of isolates of each of the three lineages are shown in Fig. 1. DNA fingerprints (haplotypes) were generated by *EcoR I* digestion and hybridization with the dispersed repeated sequence MGR586. As shown previously (19), each isolate has a distinct MGR586 haplotype; however, obvious similarity can be seen between the hybrid-
virulence phenotype, that of IB-49 isolates and that of IB-54 isolates. All share the same lineage but (19).

Pathotype 4). IB-49(B) V03; 5, V04; 6, V0108; 7, V09; 8; lanes 30-year karyotypes are generated from the restriction fragments shared by all isolates within a given lineage.

Thus, IB-49 isolates would have two karyotype patterns corresponding to the IB-49(A) and IB-49(B) lineages, with the IB-49(B) isolates having the same karyotypes as the IB-54 isolates. This combination of clonal lineages and pathotypes therefore provides a rigorous test of the relationship of karyotypic variation to both clonal lineage and pathotype.

Genetic similarity values within and between the lineages analyzed in this study are given in Table 2. Isolates of the same lineage show greater than 90% DNA fingerprint similarity when calculated by the Nei and Li similarity index (25). Different lineages show similarities of less than 40% by the same analysis. A complete analysis of the U.S. rice blast population structure is in preparation.

Resolution of chromosome-sized DNA molecules. Highly reproducible electrophoretic karyotypes were produced for all M. grisea isolates examined. M. grisea chromosome-sized DNA molecules were resolved by using adaptations of the methods described by Hamer et al. (10) and Talbot et al. (40). Chromosomal length differences were never observed between different preparations of the same isolate. All karyotypes described were found to be highly reproducible, being repeated at least five times from different DNA preparations by independently working coauthors (N.J.T. and Y.P.S.). The karyotypes produced showed chromosome-sized DNA molecules in two ranges, which necessitated different CHEF parameters for their resolution. Conditions for CHEF separations are given in the corresponding figure legends. M. grisea chromosomes varied in size from 3 to 10 Mb, and an average of four to six bands were routinely separated. Sizes were broadly estimated by extrapolation from the reported sizes of Saccharomyces cerevisiae chromosomes (see Fig. 2) and by comparison to Neurospora crassa chromosomal DNA preparations (10). Molecular size markers for the large chromosomes are omitted because these molecules are resolved at the upper limit for CHEF separations (28), and so only relative comparisons of sizes can be made between lanes on the same gel.

Chromosomal length differences were observed among the majority of isolates examined. An example of this variation is illustrated in Fig. 2, which shows karyotype analysis of isolates belonging to the IC-17 lineage. Three isolates from this lineage, VO5, VO12, and VO13 (lanes 1, 3, and 4), show no overt chromosome length polymorphisms, while one isolate, VO9 (lane 2), has a contrasting karyotype. The karyotypic change in VO9 appears to be due to an increase

<table>
<thead>
<tr>
<th>Clonal Lineage</th>
<th>IC-17</th>
<th>IB-49A</th>
<th>IB-49B</th>
<th>IB-54</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC-17</td>
<td>92</td>
<td>32</td>
<td>32</td>
<td>36</td>
</tr>
<tr>
<td>IB-49A</td>
<td>92</td>
<td>28</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>IB-49B</td>
<td>96</td>
<td>96</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IB-54</td>
<td>99</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Scores are given as percentages based on Nei and Li’s index of similarity for restriction fragment length polymorphism analyses (25). Similarity scores within lineages were calculated by pairwise similarity tests of a large representative group of isolates (19). Between-lineage scores were calculated by comparison to consensus fingerprints defined for each lineage. These were generated from the restriction fragments shared by all isolates within a given lineage.

karyotype is correlated with an isolate’s clonal lineage, then all isolates within a single lineage would share the same karyotype pattern. In this case, IB-49 isolates were found to be highly reproducible, being repeated at least five times from different DNA preparations by independently working coauthors (N.J.T. and Y.P.S.). The karyotypes produced showed chromosome-sized DNA molecules in two ranges, which necessitated different CHEF parameters for their resolution. Conditions for CHEF separations are given in the corresponding figure legends. M. grisea chromosomes varied in size from 3 to 10 Mb, and an average of four to six bands were routinely separated. Sizes were broadly estimated by extrapolation from the reported sizes of Saccharomyces cerevisiae chromosomes (see Fig. 2) and by comparison to Neurospora crassa chromosomal DNA preparations (10). Molecular size markers for the large chromosomes are omitted because these molecules are resolved at the upper limit for CHEF separations (28), and so only relative comparisons of sizes can be made between lanes on the same gel.

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in the size of a chromosome normally resolved as the second largest band in the related isolates, together with a large reduction in size of a smaller chromosome (depicted by the arrow) which is approximately 4 Mb in size in V05, V012, and V013 to give a unique band of 2.2 Mb in V09.

To determine the nature of the genomic rearrangements mediating these karyotypic changes, the CHEF separations were blotted and hybridized with a series of cloned DNA probes. The results of using plasmids pCB606 and pNJT15 as hybridization probes are shown in Fig. 2B and C, respectively. The largest chromosome in V09 contains a sequence found on the second largest chromosome in V05, V012, and V013. The 2.2-Mb band of V09 meanwhile contains a sequence normally found on the 4-Mb chromosome of V05, V012, and V013. This result is consistent with the karyotype variability arising from either a reciprocal translocation involving both of these chromosomes or independent translocation and deletion events.

To further characterize these events in the IC-17 lineage and the potentially diverse nature of chromosomes sharing the same electrophoretic mobility, a two-dimensional analysis of chromosome-sized DNA was performed. CHEF separations of V09 and V012 were excised from the gel in their entirety and digested in situ with EcoRI. The gel slab was then reoriented through 90° and fractionated through a 0.8% agarose gel. The gel was blotted and probed with MGR586 to show the chromosomal locations of MGR-hybridizing EcoRI restriction fragment length polymorphisms. Because the two isolates share almost identical MGR DNA fingerprints but have different karyotypes, the chromosomal locations of shared MGR-hybridizing fragments should be directly comparable. The results of this analysis are shown in Fig. 3. Not all chromosome-specific MGR bands were resolved; however, a small number of shared restriction fragments between 1.8 and 2.3 kb could be compared and are numbered. The chromosomal locations of these shared fragments can be seen in V09 (panel A) and V012 (panel B). The results show that the 2.2-Mb chromosomal band in V09 (panel A) contains MGR sequences found on the largest two chromosomal bands in V012 (panel B, fragments 2, 4, 5, and 7). Furthermore, isolate V09's largest chromosome contains MGR sequences found on the 4-Mb chromosome of V012 (fragments 1 and 6). This suggests that the two chromosome length differences between the isolates may be connected and that other genomic rearrangements may have occurred between the two isolates (for example, the movement of fragment 3). The translocation or deletion events interpreted from probing CHEF separations with single-copy probes may well be oversimplifications of the types of karyotypic differences that actually exist within the IC-17 lineage.

Variable electrophoretic karyotypes of isolates from IB-49(A) and IB-49(B) lineages are shown in Fig. 4. Isolates of the IB-49 pathotype had variable karyotypes, showing that electrophoretic karyotype is not correlated with pathotype. Meanwhile, the observation that IB-49(A) and IB-49(B) isolates also had variable karyotypes showed that karyotype was not correlated with the clonal lineage of an isolate. Southern blot hybridization analysis of IB-49(A) and IB-49(B) separations was carried out, using pNJT15 and pCB586 as probes. The results of these experiments are shown in Fig. 4B and C, respectively. Despite the variability in karyotypes, the size of homologous chromosomes was occasionally found to be maintained within clonal lineages, as shown by probing with pNJT15. Probing with pCB586 confirmed the presence of the MGR586 sequences on all chromosomal bands within all isolates. This analysis was carried out for all karyotypes shown in this study (data not shown), confirming the widespread dispersal of MGR sequences in the rice pathogenic populations of M. grisea.

Isolates of the IB-54 lineage showed some degree of karyotypic similarity. VO111 showed the most striking chromosome length differences from the other four isolates (Fig. 5, lanes 5 and 9). Interestingly, isolates VO111 (lane 5) and VO105 (lane 10) are reisolations of VO102 (lane 2) following numerous serial transfers in culture and then passage through a compatible rice cultivar (19). These isolates have identical MGR fingerprints (data not shown; see also reference 19) and pathotypes, and yet each has a distinct karyotype. This suggests that karyotype changes can continue to occur in culture. The karyotypes of IB-54 isolates also varied from those of the IB-49(B) isolates, further showing the lack of correlation between karyotype and clonal lineage. Chro-
mosomal DNA proved hard to extract from isolates of this lineage because of lower than average protoplast production. The results from two independent preparations are thus shown to clarify the karyotypes of these isolates and to demonstrate the reproducibility of the karyotypes.

Variation in minichromosomes. The term minichromosome has been used to describe DNA molecules resolved by PFGE from undigested chromosome plugs that resolve in the size range of 200 kb to 2.2 Mb (24, 42). However, the chromosomal nature of these molecules is still open to investigation as the presence of telomeres or centromeres or genes has not been conclusively shown in *M. grisea*.

The majority of isolates examined contained between one and three chromosomal DNA bands in the same overall size range as *S. cerevisiae* chromosomes. These minichromosomes were observed in 15 of the 19 field isolates from the United States. The presence of these minichromosomes is not ubiquitous because none of 12 Chinese isolates examined appear to contain them (see below). Minichromosomes varied in size from 470 kb to 2.2 Mb, as shown in Fig. 6A. The size and number of minichromosomes were not correlated with pathotype or conserved within MGR-based clonal lineages. Similarities between isolates within the same lineage, however, could be observed. For example, within the IB-54/IB-49(B) lineage, isolates contain two general patterns of minichromosome karyotype. Isolates VO102, VO105, and VO111 share the same minichromosome complement (lanes 12, 13, and 15). Isolate VO4 shares a similar karyotype differing in the size of the larger, approximately 1-Mb-sized band (lane 10). A second distinct karyotype is shared by VO108 and VO110 (lanes 11 and 14), although a difference in the length of the smallest minichromosome can be seen.

FIG. 3. Two-dimensional analysis of CHEF separations of IC-17 isolates, (A) VO9 and (B) VO12, to show chromosomal distribution of MGR sequences in isolates which share very similar MGR fingerprints but have contrasting electrophoretic karyotypes. CHEF separations of chromosome-sized DNA molecules were performed as described in the legend to Fig. 2. The entire CHEF separation was then excised from the gel and digested with EcoRI. The gel slab was reoriented through 90° and fractionated in 0.8% agarose. The gel was blotted to nylon membranes and probed with pCBS86. Seven of the MGR-hybridizing fragments shared by the two isolates are labelled, and their chromosomal band of origin is indicated.

FIG. 4. CHEF separations of isolates from pathotype IB-49 showing variable electrophoretic karyotypes. These isolates are subdivided by MGR fingerprinting into two clonal lineages, IB-49(A) and IB-49(B). Chromosomal DNA preparations were separated by electrophoresis at 3 V/cm in 0.5x TBE at 4°C with a pulse interval of 90 min for 168 h. Lanes contain DNA from the following isolates: IB-49A—1, VO11; 2, VO15; 3, VO104; 4, VO115; IB-49B—1, VO113; 2, VO101; 3, VO3; 4, VO109. (A) Ethidium bromide-stained gel. (B) Southern blot analysis with pNJT15. (C) Southern blot analysis with pCBS86 (10). The light hybridization signal on the left of the gel was due to poor DNA transfer in this area, and hybridization of both probes to VO11 and VO15 was independently confirmed (data not shown).
analyze karyotypic variation within and between clonal lineages of the rice blast fungus that had previously been deciphered by DNA fingerprinting (19). The experimental design of this study was adopted to test whether the electrophoretic karyotype of an isolate was correlated with its pathotype or its clonal lineage. Our results show that electrophoretic karyotype is not correlated with either of these features. Electrophoretic karyotypes were as variable within pathotype groups as between them and also as variable within clonal lineages as between them. The IB-49(A) and IB-49(B) isolates have distinct karyotypes, with the IB-49(B) isolates showing differences from the IB-54 isolates. The degree of karyotype variation observed shows that chromosomal length polymorphisms probably occur frequently in rice pathogenic populations of *M. grisea* and are sufficiently common to obscure the obvious genetic relatedness between isolates as defined by MGR fingerprinting.

Although karyotypes are extremely variable, the overall chromosome number in *M. grisea* appears to be constant. The small variation in the number of resolved bands between different isolates is probably due to comigration of similarly sized chromosomal DNA molecules. Previous cytological studies suggested that the chromosome number of field isolates of *M. grisea* varied from two to six in cells, both within the same mycelium and between different isolates (8, 43). This variation was proposed as a potential mechanism for the promiscuous generation of new virulent forms of the fungus (31). The reproducibility of electrophoretic karyotypes from different preparations of the same isolate is inconsistent with the early cytological studies. Increasing evidence from genetic studies and recent cytological examinations of fertile laboratory strains of *M. grisea* has established that its haploid chromosome number is six (17, 27, 32, 35, 36). Our study suggests that the same is true for rice-infecting field isolates of the fungus.

The precise mechanism for the observed karyotypic variation in *M. grisea* is unclear but could involve large chromosomal deletions and translocations. Translocations have been identified in laboratory strains of *M. grisea* by restriction fragment length polymorphism mapping studies (42) and in earlier studies of hybridization to CHEF separations. Genomic rearrangements of this type are evident in the karyotypes of IC-17 isolates, which were hybridized with single-copy DNA probes and analyzed by MGR586 two-dimensional karyotype analysis. Our results suggest that similarly sized chromosomal DNA molecules resolved from different isolates are not necessarily homologous. They also suggest that the full extent of genomic rearrangement between two isolates may be underestimated by examining the karyotype pattern alone or by Southern blot analysis with single-copy DNA probes. Hybridization of dispersed repeated DNA probes to chromosome-sized DNA bands of related isolates may clarify the types of karyotypic rearrangements that exist in natural populations.

The presence of minichromosomes in *M. grisea* has been reported previously (27, 42). They were described as being highly polymorphic and meiotically unstable, leading to their classification as B-like chromosomes (24). This classification is analogous to the class of supernumerary chromosomes found in higher eukaryotes (13) and, more recently, in other plant pathogenic fungi such as the pea wilt pathogen, *N. haematococca* (22, 23). In this study, we observed that minichromosomes are not ubiquitous in all rice pathogenic field isolates. Although the minichromosome complement of an isolate is often reflective of its genetic similarity to another strain, it is not correlated with its pathotype.
FIG. 6. CHEF separation of chromosomal DNA preparations of *M. grisea* field isolates to show the presence of minichromosomes. Chromosomal DNA preparations embedded in agarose plugs were loaded on a 1% agarose gel (FMC) and fractionated at 10 V/cm in 0.5× TBE at 4°C, with pulse intervals of 90 s for 28 h and then 60 s for 12 h. Lanes contain DNA from the following isolates: 1, laboratory strain 4375-R-26; 2, VO11; 3, VO15; 4, VO104; 5, VO115; 6, VO113; 7, VO101; 8, VO3; 9, VO109; 10, VO4; 11, VO110; 12, VO102; 13, VO105; 14, VO108; 15, VO111. Lane 16 is laboratory strain CP919. (A) Ethidium bromide-stained gel. (B) Southern blot analysis of the gel blot with pCB586, showing the presence of MGR586 sequences on the minichromosomes.

The fact that minichromosomes contain MGR sequences may suggest their derivation from the larger chromosomes or that they are reservoirs for the transpositional and/or recombinational events that may mediate MGR propagation. A detailed study to assess the role of minichromosomes in the pathogenicity of *M. grisea* is in progress.

The cytological observation of lagging chromosomes during mitosis of *M. grisea* has been interpreted as a reflection...
of a possible mitotic or parasaexual mechanism for generating the high degree of pathotypic variation (33). It has also been suggested that the dispersed repeated sequences in *M. grisea* could act as dispersed sites of homology around the genome mediating ectopic recombination events (2). These events would bring about the observed karyotypic variation with the potential to generate new virulent forms of the fungus. This view is supported by the study of Tzeng et al. (41), which suggests that a genomic rearrangement is capable of bestowing a selective advantage on a plant pathogenic fungus. Our results suggest that, although genomic rearrangements are common in rice-infecting isolates of *M. grisea*, they appear to represent neutral variation with respect to pathogenicity. The high frequency of chromosome length polymorphisms would make it difficult to test the hypothesis that these events were responsible for the generation of new virulent forms. The existence of *M. grisea* as individual clones, due to the absence or rarity of the sexual cycle in natural populations, is likely to lead to the maintenance of any genomic rearrangements which are not deleterious to the fungus. This would seem the most likely cause of the observed variable karyotypes, rather than it being a manifestation of a primary adaptive mechanism for the generation of new virulent forms. The use of specific probes for avirulence or pathogenicity genes may clarify the genetic mechanisms that lead to pathogenic variation.

FIG. 7. CHEF separations of rice-infecting *M. grisea* isolates from China showing variable electrophoretic karyotypes. Chromosomal DNA preparations were separated by electrophoresis at 3 V/cm in 0.5 x TBE at 4°C with a pulse interval of 90 min for 192 h. Lanes contain DNA from the following isolates: 1, 87-120-2 (IV); 2, 87-29 (III); 3, 89-524-2 (I); 4, 87-92-2 (VI); 5, 87-132-2 (V); 6, 87-0835 (II); 7, 88-212 (IV); 8, 83-38 (II); 9, 89-95-4 (I); 10, 89-524-4 (I); 11, 87-75 (II); 12, 90-4-1 (VII). The 12 isolates represent 12 distinct pathotypes which were sorted into seven clonal lineages by MGR fingerprinting. Clonal lineage allocation is given in Roman numerals (in parentheses) as described previously (44).

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