Gene genealogies reveal global phylogeographic structure and reproductive isolation among lineages of *Fusarium graminearum*, the fungus causing wheat scab

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During the past decade, the plant disease called scab or *Fusarium* head blight of wheat and barley has reached epidemic proportions in North America and elsewhere in the world. Scab is an economically devastating plant disease, not only because it causes significant reduction in seed yields and quality, but also because infested seeds are often contaminated with trichothecene and estrogenic mycotoxins that pose a serious threat to animal health and food safety. To test whether the primary etiological agent of scab, the fungus *Fusarium graminearum*, is panmictic throughout its range, allelic genealogies were constructed from six single-copy nuclear genes from strains selected to represent the global genetic diversity of this pathogen. Excluding one hybrid strain, all six genealogies recovered the same seven biogeographically structured lineages, suggesting that they represent phylogenetically distinct species among which gene flow has been very limited during their evolutionary history. Parsimony analysis of the combined data set comprising 7,120 aligned nucleotide characters resolved most relationships among the seven lineages of the *F. graminearum* clade and related fusaria included in the study. Phylogenetic evidence is also presented for introgressive hybridization and intragenic recombination among lineages of the *F. graminearum* clade in nature.

The *Fusarium* head blight of wheat and barley has reached epidemic proportions in the United States during the last decade. Because of low yields and price discounts as a result of poor seed quality, over 2.6 billion dollars have been lost to U.S. agriculture during wheat scab epidemics in the 1990s (1). Moreover, the disease is a growing threat to the world's food supply because of recent scab outbreaks in Asia, Canada, Europe, and South America (2). Although several species within the filamentous ascomycetous genus *Fusarium* can cause scab, epidemics within North America are caused predominately by *Fusarium graminearum* (sexual state = *Gibberella zeae*). During the asexual phase of this haploid-based species, it grows as a filamentous hypha, producing multiseptate, fusiform macroconidia (i.e., mitotic spores). The homothallic sexual phase consists of a fruiting body containing asci within which ascospores (i.e., meiotic spores) are formed. Both ascospores and macroconidia can function as infectious propagules (3). This pathogen poses a double threat to cereals: (*i*) a significant reduction in seed quality and yields is caused by discolored, shriveled "tombstone" kernels, and (*ii*) scabby grain is often contaminated with trichothecene and estrogenic mycotoxins (4), making it unsuitable for food or feed. The trichothecene toxins produced by this fungus pose a serious hazard to animal and plant health in that these sesquiterpenoids are potent inhibitors of eukaryotic protein biosynthesis. Vertebrate toxicity results in a variety of symptoms including acute dermatitis, hemorrhaging, and diarrhea. In plants, these mycotoxins have been shown to function as virulence factors during pathogenesis (5).

To date, information on the global phylogeographic structure of this pathogen is nonexistent, although such genetic data are necessary for preventing the inadvertent intercontinental introduction of genetically unique foreign populations associated with world trade (6). Given that different trichothecene phenotypes and virulence to wheat have been reported for *F. graminearum* from China and North America (7, 8), we investigated whether gene genealogies inferred from DNA sequence data might reveal underlying population divergence of this pathogen. Furthermore, because closely related species of *Fusarium* (9) and other filamentous fungi are difficult or impossible to distinguish by using conventional phenotypic characters (10–14), we have used allelic genealogies in our study to test whether *F. graminearum* is panmictic over its range that spans six continents. To address these questions, portions of six single-copy nuclear genes totaling 7,120 bp of DNA were sequenced and analyzed phylogenetically from strains selected to represent the worldwide genetic diversity of this pathogen and its near relatives. Herein, we report that, with the exception of one hybrid strain, all six genealogies recovered the same seven biogeographically structured lineages within *F. graminearum* (hereafter referred to as the *Fg* clade), suggesting limited gene flow among these populations. Because several of the lineages seem to occur primarily or exclusively on noncereal hosts, we also determined the trichothecene toxin and estrogen phenotypes for each lineage together with their ability to cause scab on wheat.

Materials and Methods

Biological Materials. The 37 single-spored strains selected for study are listed in Table 1 together with data on their geographic origin and host or substrate. Because *F. graminearum* has been reported to have a broad host range (3), we included strains from a diverse range of hosts. All strains were stored in liquid nitrogen vapor at −175°C in the Agricultural Research Service Culture Collection (National Center for Agricultural Utilization Research, Peoria, IL). Methods for culturing strains and DNA isolation have been described (9, 15).

DNA Amplification and Sequencing. Primers were designed to amplify and sequence portions of six different single-copy pro-

Abbreviation: WS-R, Wilcoxon signed-ranks.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. AF212435–AF212825).

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Table 1. Strains used in this study

Taxon*	Agricultural Research Service Culture Collection strain no.	Host/substrate	Geographic origin	Trichothecene toxins and estrogens†							Pathogenicity‡
				DON	3-A DON	15-A DON	NIV	4-A NIV	ZONE	ZOL	
F. graminearum (1)	2903	Polypore	Brazil	+	+	−	−	−	−	−	−
F. graminearum (1)	28585	Herbaceous vine	Venezuela	−	−	−	+	+	+	+	++
F. graminearum (1)	28718	Corn	Brazil	+++	+++	−	−	−	++	−	+++
F. graminearum (2)	28436	Orange twig	New Caledonia	−	−	−	−	+	+	−	++
F. graminearum (2)	28723	Corn	Nepal	−	−	−	−	−	+	−	+
F. graminearum (2)	29010	Soil	South Africa	−	−	−	+	+	+	+	++
F. graminearum (3)	29020	Corn	South Africa	+++	−	−	−	−	+++	+	++
F. graminearum (3)	26916	Corn	South Africa	−	−	−	−	−	−	−	−
F. graminearum (3)	29011	—	South Africa	++	−	−	−	−	+	+	++
F. graminearum (3)	29105	Corn	Nepal	++	−	−	−	−	+++	+	++
F. graminearum (4)	25797	Banana	Honduras	+	−	−	−	−	+	−	++
F. graminearum (4)	29148	Grape ivy	Pennsylvania, USA	−	−	−	−	−	−	−	−
F. graminearum (5)	26752	*Acacia mearnsii*	South Africa	+	−	−	−	+	−	−	+
F. graminearum (5)	26754	*A. mearnsii*	South Africa	−	−	−	−	−	−	−	+
F. graminearum (5)	26755	*A. mearnsii*	South Africa	+	−	+	+++	+++	−	−	++
F. graminearum (6)	6101	Barley	Japan	−	−	−	−	−	−	−	−
F. graminearum (6)	13818	Barley	Japan	−	−	−	−	−	+	−	++
F. graminearum (6)	26156	Wheat	China	++	++	−	−	−	−	−	+++
F. graminearum (6)	28720	Corn	Nepal	+	−	−	−	−	++	−	++
F. graminearum (6)	28721§	Corn	Nepal	−	−	−	++	+	++	−	+
F. graminearum (7)	5883	Corn	Ohio, USA	++	−	+	−	−	+++	+	+++
F. graminearum (7)	6394	Millet	Hungary	+	++	−	−	−	+	−	++
F. graminearum (7)	13383	Corn	Iran	−	−	−	−	−	+++	+	++
F. graminearum (7)	28063	Corn	Michigan, USA	+++	−	−	−	−	−	−	++
F. graminearum (7)	28336	Wheat	Ohio, USA	++	+++	−	−	−	+++	−	++
F. graminearum (7)	28439	Leatherleaf fern	Netherlands	−	−	−	+	+	−	−	++
F. graminearum (7)	29169	Wheat	Kansas, USA	−	−	−	−	−	+++	+	+++
F. culmorum	3288	—	—	−	−	−	−	−	−	−	●
F. culmorum	25475	Barley	Denmark	−	−	−	−	−	++	+	●
F. cerealis	13721	Potato	Poland	−	−	−	+	+	+++	+	●
F. cerealis	25491	*Iris hollandica*	Netherlands	−	−	−	−	−	+++	−	●
F. cerealis	25805	Soil	Columbia	−	−	−	−	−	−	−	●
F. lunulosporum	13393	*Citrus paradisi*	South Africa	−	−	−	−	−	+	−	●
F. pseudograminearum	28062	Barley	Australia	−	−	−	−	−	++	−	●
F. pseudograminearum	28065	*Medicago* sp.	South Africa	−	−	−	−	−	+	−	●
F. pseudograminearum	28334	*Medicago* sp.	South Africa	−	−	−	−	−	+++	+	●
F. pseudograminearum	28338	Soil	Australia	+	++	−	−	−	+++	−	●

*Seven phylogenetically distinct, biogeographically structured lineages were identified among the 99 strains of the *F. graminearum* clade sequenced. The putative ancestral area of lineages 1–7 and number of strains of each lineage that were collected in its ancestral area/total number strains of each lineage sequenced follows in parentheses together with data on eight strains that were putatively distributed outside the native region of four lineages on the following agricultural or horticultural plants: (1) South America (4/4); (2) Africa (19/23; one strain from orange and guava twigs from New Caledonia and two from corn in Nepal); (3) Africa (9/10; one strain from Nepal from corn); (4) South Central America (1/2; one strain from *Cissus rhombifolia*, grape or oakleaf ivy, in Pennsylvania, USA; *C. rhombifolia* is native to Central America and northern South America); (5) Africa (3/3); (6) Asia (19/19); and (7) Pan-Northern Hemisphere (36/38); one strain from Argentina from whole wheat and one from Australia from *Oplismenus* sp.).

†DON, deoxynivalenol; 3-ADON, 3-acetyldeoxynivalenol; 15-ADON, 15-acetyldeoxynivalenol; NIV, nivalenol; 4-ANIV, 4-acetylnivalenol; ZONE, zearalenone; ZOL, zearalenol; −, <5 ppm; +, 5–50 ppm; ++, 51–150 ppm; +++, 151–3000 ppm. Complete numerical data can be viewed on the following web site: http://www.crl.umn.edu/personnel/Kistler.html.

‡Pathogenicity: −, no disease symptoms beyond the inoculated floret; +, disease symptoms present on florets adjacent to inoculation site; ++, disease symptoms on three to six florets; +++, disease symptoms on more than six florets; ●, not tested.

§Strain 28721 is a hybrid strain containing alleles from lineages 2 and 6 of the *F. graminearum* clade.

tein-encoding nuclear genes, and they are publicly available at http://www.crl.umn.edu/Scab/primers.html. PCR and sequencing protocols are described in O'Donnell *et al.* (16). DNA sequences were edited and aligned visually with TSE, a DOS text editor software package (SemWare, Marietta, GA) or SEQUENCER 4.0.5 (Gene Codes, Ann Arbor, MI).

Phylogenetic Analysis. The six individual data sets were compared statistically for incongruence with the nonparametric Templeton Wilcoxon signed-ranks (WS-R) test implemented in PAUP* (17) with 90% bootstrap consensus trees as constraints. Of the 30 tests (data not shown), all but 1 received P values > 0.05, and the one exception, reductase constrained to the ammonia ligase consensus, received a P value of 0.0338. Unweighted maximum parsimony analyses were performed on the individual and combined data sets for the 37 taxon matrix with PAUP*. Analyses used the heuristic search option with 1,000 random addition sequences with MULPARS on and TBR branch swapping. Pub-

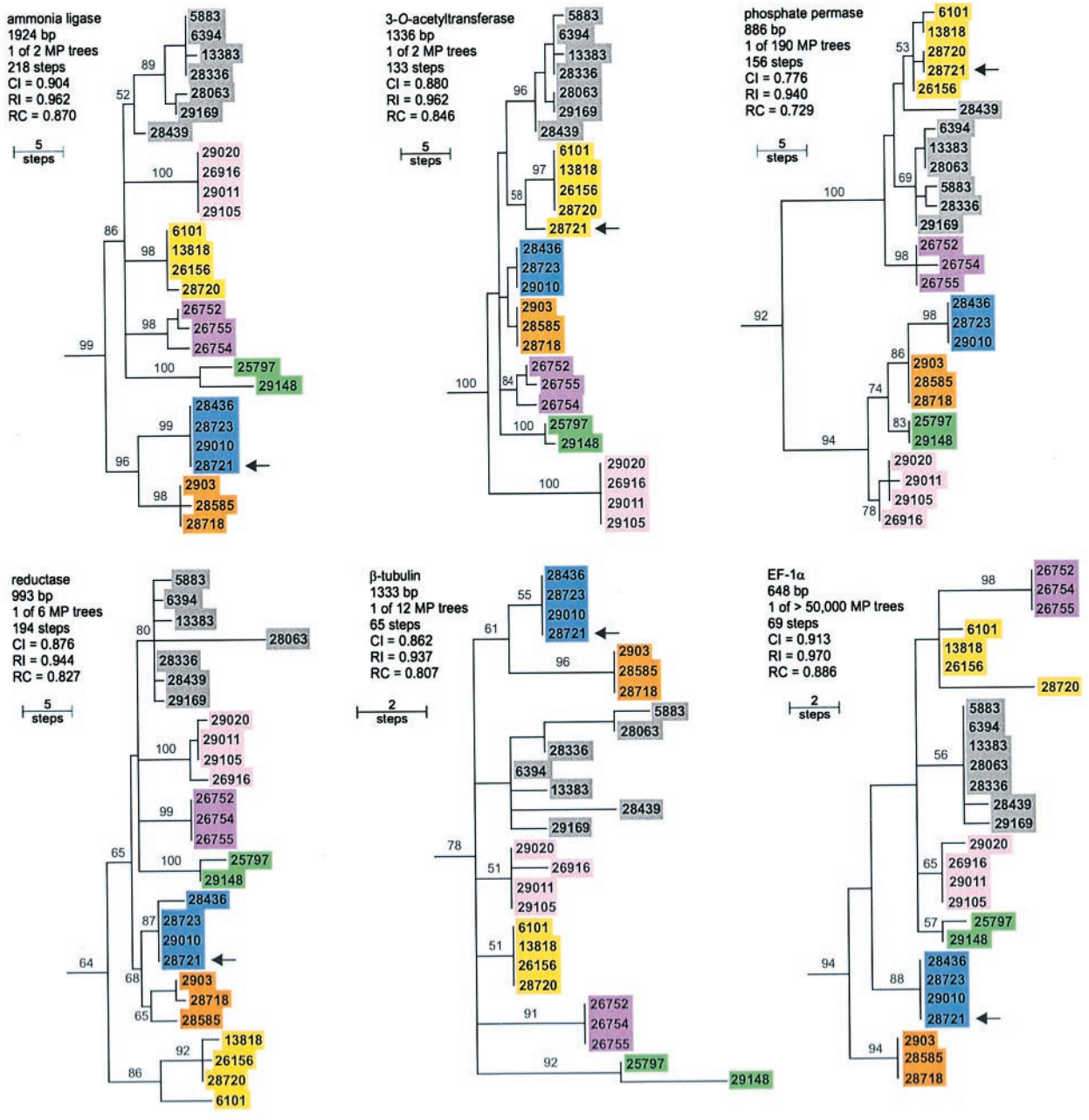

Fig. 1. One maximum parsimony phylogram from each of the six genes sequenced. CI, consistency index; RI, retention index; RC, rescaled consistency index. Numbers by nodes represent bootstrap support ≥50% from 1,000 replications. The seven biogeographically structured lineages of the *F. graminearum* clade are color-coded by origin as shown in Fig. 3. The arrow on each phylogram indicates the position of the hybrid strain 28721, which is resolved cladistically within either Asian (yellow code) or African (blue code) lineages. The 3-*O*-acetyltransferase (i.e., *TRI101*) allele of 28721 is an Asian–African hybrid (see Fig. 2).

lished sequences of *F. pseudograminearum* were selected for rooting the gene trees by the outgroup method based on the results of a previous phylogenetic analysis (18). Stability of clades was assessed by 1,000 parsimony bootstrap replications implemented in PAUP* and by decay indices calculated with TREEROT (19). Unrooted relative apparent synapomorphy analysis (RASA 2.3.7; ref. 20; available at http://test1.bio.psu.edu/LW/rasatext.html) was used to test for potential long-branch taxa. The evolutionary pattern of phylogeography was examined with MACCLADE (21). The Templeton WS-R test implemented in PAUP* was used to compare various constrained topologies with the unconstrained most-parsimonious trees.

Mycotoxin Analyses. Strains were cultured on sterile rice grains as described (15). Mycotoxins were extracted from 1 g of ground culture material with 40 ml of acetonitrile:water (84:16, vol/vol) for 1 h on a horizontal shaker. A 5-ml aliquot of the supernatant was gravity filtered through 1.5 g of C18:alumina (1:1, vol/vol), and a 3-ml aliquot of the eluate was evaporated with air at 55°C for 30 min. Trimethylsilyl-mycotoxin derivatives were separated on a Shimadzu GC-17A gas chromograph with a Restek (Bellefonte, PA) 30-m 35 × 0.25-mm-i.d. × 0.25-μm-phase capillary column and assayed by select ion monitoring with electron ionization in an Shimadzu Qp5000 mass spectrometer, by using two to four ion fragments for identification and quantitation of

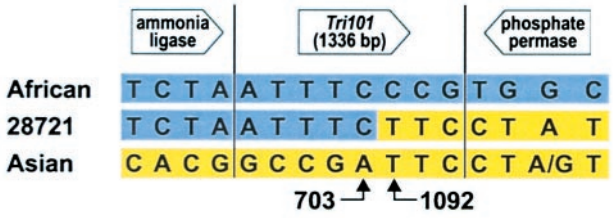

Fig. 2. Evidence for intragenic recombination within *TRI101* in strain 28721. Phylogenetically informative sites are shown within the three adjacent genes and are color-coded according to the legend in Fig. 3. Based on DNA sequence analysis, the recombination breakpoint maps between nucleotide positions 703 and 1,092 (22).

each mycotoxin. The estimated detection limit for each mycotoxin (Table 1) is 5 μg/g.

Pathogenicity. Relative pathogenicity was determined on wheat cultivars Norm or Pioneer 2375 grown in a greenhouse. Plants were inoculated at early-to-mid anthesis with approximately 1×10^4 conidia added to the wounded lower floret. After inoculation, the plants were placed in a humidity chamber for 72 h and then transferred to a greenhouse maintained at ≈27°C. As a negative control, plants were inoculated with *F. oxysporum* forma specialis *lycopersici*, a tomato pathogen that does not cause scab (head blight) on wheat. Inoculation treatments consisted of five plants within a pot and two pots per treatment, and experimental treatments were repeated at least once on each wheat cultivar. Head blight was rated 14 days after inoculation (Table 1) by the number of florets showing disease symptoms (necrosis and/or bleaching of palea/lemma) as described (5).

Results

To obtain an initial estimate of the genotypic diversity of the *Fg* clade from a worldwide collection ($n = 99$), aligned partial DNA sequences of translation elongation factor (*EF-1α*, 648 bp) and phosphate permase genes (*PHO*, 886 bp) were analyzed by maximum parsimony. Strains from six continents together with 31 strains of four closely related B trichothecene-producing fusaria were included. Based on these preliminary analyses, 27 strains of the *Fg* clade were selected from the global collection to represent the full range of genetic diversity of this taxon together with 10 strains of four closely related fusaria in the *Gibberella zeae* species complex (Table 1). For this 37-taxon matrix, fragments of four additional nuclear genes were sampled, including β-tubulin (*TUB*, 1,333 bp), UTP-ammonia ligase (*URA*, 1,924 bp), trichothecene 3-*O*-acetyltransferase (*TRI101*, 1,336 bp), and a putative reductase (*RED*, 993 bp). *URA*, *TRI101*, *and PHO* are tightly linked (22).

Parsimony analysis of the six individual loci showed strong gene–gene concordance in that the same seven biogeographically structured lineages within the *Fg* clade were resolved as exclusive groups in the gene trees, except that strain 28439 (lineage 7) formed a paraphyletic grade with lineage 6 in the *PHO* gene tree (Fig. 1), and strain 28721 seemed to be of hybrid origin (see below). Although the *Fg* clade was resolved as monophyletic in the six individual parsimony gene trees with bootstrap support ranging from 64 to 100% (Fig. 1), evolutionary relationships among the seven lineages within this clade were poorly resolved except for a possible sister-group relationship among lineages 1 and 2.

Phylogenetic reconstruction also provided strong evidence for intragenic recombination within the *TRI101* locus of strain 28721 (Fig. 1) based on the sequences of markers at *URA-TRI101-PHO*, which are physically linked (Fig. 2). Within strain 28721, *URA* and *PHO* sequences clearly represent African and Asian alleles of lineages 2 and 6, respectively. The *TRI101* sequence, in

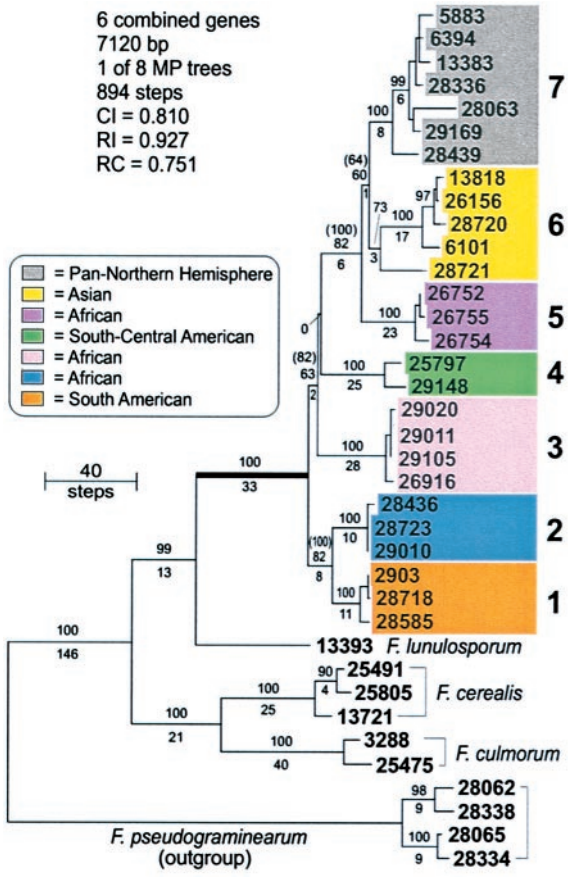

Fig. 3. One of eight most-parsimonious phylograms inferred from the combined data set for the seven color-coded lineages (numbered 1–7) of the *F. graminearum* clade (supported by bold node) and related B trichothecene-producing fusaria. The color-coded key indicates putative geographic origin of the seven lineages as defined in Table 1. Sequences of *F. pseudograminearum*, formerly known as *F. graminearum* Group 1 (18), were used to root the tree by the outgroup method. Decay indices are indicated below nodes; bootstrap intervals from 1,000 replicates are indicated above nodes. Numbers in parentheses above four nodes represent bootstrap support after excluding the hybrid strain 28721.

contrast, is an African–Asian hybrid in which the recombination breakpoint is between nucleotides 703 and 1,092 (22, Fig. 2). As is typical of hybrids, the recombinant strain 28721 within the *TRI101* gene tree was resolved by maximum parsimony analysis as a basal member of the clade that includes its most derived parent (i.e., lineage 6). Strain 28721 was nested within African lineage 2 in the *RED*, *TUB*, and *EF-1α* gene trees (Fig. 1, see arrow).

By using a conditional combination approach (23), results of the Templeton WS-R test indicated that the six individual nuclear data sets could be combined. The 37 sequences in the combined data set comprised 7,120 bp of aligned sequence of which 692 were variable (9.7%) and 560 were synapomorphic (7.85%). When considering only the *Fg* clade, 82 and 191 of 273 variable sites were autapomorphic and synapomorphic, respectively. Of the 191 parsimony-informative sites within the seven lineages of the *Fg* clade, 156 were fixed; 32 were polymorphic but only within one lineage; and 3 represent shared polymorphisms among two lineages. Lineages 6 and 7, the two most recently evolved lineages, collectively account for 25 of the 32 polymorphisms restricted to a lineage. Based on RASA analysis (20), no long-branch taxa were present in the individual and combined data sets. Unweighted maximum parsimony analysis of the combined data yielded eight equally most-parsimonious trees of 894 steps that differed only in minor rearrangements within

lineages 2, 4, and 7 and in the branching order of lineages 3 and 4 (Fig. 3). Phylogenetic relationships among all seven lineages within the *Fg* clade and its affinities with the other B trichothecene-producing fusaria were resolved nearly completely with the monophyly of each lineage receiving 100% bootstrap support. Three internodes showed dramatic increases of 18–19% bootstrap support (shown in parentheses, Fig. 3) after the removal of the hybrid strain 28721.

Results of the molecular phylogeny indicate that *F. lunulosporum* from grapefruit from South Africa is the sister of the *Fg* clade. The five most basal lineages within the *Fg* clade all seem to be endemic to the Southern Hemisphere with two lineages in South/Central America and three in Africa. In contrast, lineages 6 and 7, which represent the two most recently derived lineages, seem to be endemic to the Northern Hemisphere with lineage 6 being restricted to Asia. Topological constraints that forced the monophyly of the two South/Central American lineages and the three African lineages in separate analyses were 11 and 30 steps longer, respectively, and significantly worse than the unconstrained most-parsimonious trees (Templeton = $P < 0.005$ and < 0.0001, respectively). Collectively, these results suggest that either Africa or South America represents the ancestral area of the *Fg* clade. MACCLADE (21) identified Africa as its most-parsimonious geographic origin when the biogeographic origin of the seven lineages was optimized onto the combined gene tree (Fig. 3).

To determine their pathogenic potential, strains within the seven lineages were used to inoculate single basal florets of wheat spikelets (Table 1). Surprisingly, all lineages contained strains that could spread up the spike and cause considerable disease in florets above the point of inoculation, including those isolated from nongramineous hosts. In contrast, the tomato pathogen *F. oxysporum* forma specialis *lycopersici* caused little necrosis that never spread beyond the inoculated floret, similar in reaction to some strains of the *Fg* clade considered nonpathogenic to wheat (e.g., 2903 and 29148). Every species tested was able to produce trichothecene or estrogenic mycotoxins, including strains from all seven lineages of the *Fg* clade.

Discussion

The primary objective of this study was to use molecular phylogenetics to test whether the etiological agent of *Fusarium* scab or head blight of small cereals, *F. graminearum*, is panmictic worldwide. The major result of this study was the discovery of seven biogeographically structured lineages within the *Fg* clade, indicating a long evolutionary history of reproductive isolation. Similar phylogenetic studies on agriculturally (12, 24, 25) and medically important fungi (10–14) over the past half-decade have demonstrated the utility of multiple-gene genealogies for investigating species limits and reproductive mode among morphologically cryptic species. Assuming that reciprocal monophyly and fixation of parsimony-informative sites are indicative of an advanced state of biological speciation (12, 26), excluding hybrid strain 28721, we found that all seven lineages in the six genealogies were monophyletic except for one paraphyletic lineage in the phosphate permase gene tree, and most phylogenetically informative sites show fixed differences (81.7%) within the *Fg* clade. In contrast, the two cryptic species identified within *Coccidioides immitis* (10, 11, 27) and *Aspergillus flavus* (12) are paraphyletic in two of the five nuclear genealogies, whereas only 36% of the parsimony-informative sites within *A. flavus* are fixed in both taxa. Nevertheless, all of these cryptic taxa were resolved as monophyletic or exclusive groups in the combined multiple allelic genealogies, indicating that they are behaving as distinct evolutionary lineages among which gene flow in nature must be extremely limited or absent. On this basis, we follow the authors cited above in accepting genealogical concordance as an objective conceptual and empirical criterion on which phylogenetically based genealogical species (28) such as the seven lineages of the *Fg* clade can be circumscribed. Although these genealogical species presently seem to represent seven independent evolutionary trajectories, genetic data from two independent sources indicate the allelic genealogies of the *Fg* clade may be evolutionarily dynamic. Outcrossing in the laboratory among three of the lineages within the *Fg* clade was reported (29), but a more thorough discussion awaits a detailed genetic analysis in view of the evidence of a history of reproductive isolation detected in the present study. Furthermore, transglobal transposition of lineages associated with movement of agricultural and horticultural plants worldwide seems to have contributed to the partitioning of allopatric lineages onto monocultured cereals where introgressive hybridization has given rise to novel genotypes. One or both of these processes could reduce or replace the bifurcating, hierarchic allelic genealogy with a reticulate tree structure as exemplified by the hybrid strain 28721. Although the hybrid strain 28721 does not seem to have a fitness advantage over strains from the parental lineages (i.e., lineages 2 and 6; Table 1), the novel *Phytophthora* pathogen of alder in Europe is theorized to have evolved from the hybridization of native and introduced species (6), neither of which is apparently pathogenic to this host. Although fungal hybrids are considered rare in nature, a DNA sequence-based molecular phylogeny has elegantly identified natural interspecific hybrids between asexual *Neotyphodium* grass endophytes and their closely related *Epichloë* sexual relatives (30). Phylogenetic evidence suggesting hybridization among plant pathogens has also been reported for the Dutch elm pathogens in Europe (31), the *Gibberella* clade of *Fusarium* (9), and the wheat rust *Puccinia* in New Zealand (32).

To our knowledge, the present study is the first to document intragenic recombination in a natural fungal population. As predicted by McDade (33), the hybrid strain 28721 was resolved by phylogenetic analysis as a basal member of the clade that includes its most apomorphic parent (i.e., Asian lineage 6) in both the *TRI101* (Fig. 1) and combined gene tree (Fig. 3). It is instructive to note that the recombination event within *TRI101* that transferred three synapomorphies across the lineages (Fig. 2) provided enough phylogenetic signal to group 28721 cladistically with the Asian lineage. A similar grouping was also obtained in the combined analysis even though four of the six alleles sampled are African (lineage 2), as are five of the eight synapomorphies at the *TRI101* locus (Fig. 2).

Two significant methodological findings to emerge from these species-level studies include (*i*) the greater phylogenetic utility of protein-encoding nuclear genealogies over ribosomal genes and (*ii*) the importance of a conditional combination approach (23) to assess whether independent partitions should be combined. Based on results of the Templeton WS-R test implemented in PAUP* (17), which statistically supported combining the six individual partitions, maximum parsimony analysis of the combined gene genealogies (Fig. 3) provides a robust initial hypothesis of phylogenetic relationships among these agronomically important fusaria as judged by high bootstrap values (100% for all seven lineages within the *Fg* clade) and decay indices. Furthermore, although not suggested by previous studies (15, 18), the African endemic *F. lunulosporum* was resolved as sister to the *Fg* clade. The sister relationship of these taxa and the reconstruction by MACCLADE (21) suggest that the ancestral area of the latter clade may be in Africa rather than in South America.

Results of the molecular phylogeny provide a foundation for understanding the mode of speciation in the *Fg* clade within a phylogeographic context. Speciation within this clade seems to be associated with geographic and possibly also host-mediated isolation. Based on our phylogeographic hypothesis (Fig. 3), allopatric speciation seems to account for genetic divergence among geographically isolated lineages in both hemispheres. Because three lineages in Africa (lineages 2, 3, and 5), two lineages in South/Central America (lineages 1 and 4), and two lineages in China (lineages 6 and 7) may have evolved in sympatry, host preferences may have functioned as isolating mechanisms contributing to the speciation of these taxa. As evidenced by the relatively recent

alteration in the distributional patterns of four of the lineages (Table 1), international agricultural and horticultural trade in plants and plant products has the very real potential to bring previously allopatric species together (6). However, because our data indicate very low levels of migration and gene flow between the lineages over the evolutionary history of the *Fg* clade, the geographic origin of strains can still be predicted from the genotype of a single locus (27). The phylogeographic distribution of the nuclear gene lineages suggests that the five most basal lineages within the *Fg* clade are endemic to the Southern Hemisphere, whereas the two most recently evolved lineages seem to have a geographic origin in the Northern Hemisphere. The African–South American disjunctions very likely reflect transoceanic jump dispersals after the fragmentation of West Gondwana. Because the Templeton WS-R test allows us to reject statistically a single origin of the three African and the two South/Central American lineages, at least two independent long-distance dispersals, most likely from Africa to South America, are required to explain the complex phylogeographic pattern in the Southern Hemisphere (21). Graminivorous migratory birds carrying infected seed are plausible dispersal vectors. A third transcontinental dispersal is needed to explain the Northern Hemisphere distribution of lineages 6 and 7.

Given the great importance of mycotoxins and estrogenic metabolites to plant and food safety, we were interested in determining whether representatives of all seven lineages of the *Fg* clade could elaborate these toxins and whether lineage-specific mycotoxin profiles exist. Our results (Table 1) demonstrate that the four trichothecene chemotypes previously described for the *Fg* clade (7) are not lineage-specific. Although the toxin phenotypes do not strongly correlate with the *Fg* clade phylogeny, every lineage can produce trichothecenes, and all lineages except lineage 5 can produce the acetate-derived estrogenic mycotoxin zearalenone. Moreover, the fusaria included in this study seem to be unique in that no other organisms are known that produce both B trichothecenes and zearalenone. It has been theorized that these fusaria have acquired the *TRI101* trichothecene gene through horizontal gene transfer (34). The *TRI101* gene product confers resistance to trichothecenes by the fusaria that produce these toxins through 3-*O*-acetylation of the trichothecene ring (22). Given that *TRI101* tracks with the other nuclear genealogies, except in the hybrid strain 28721, our results indicate that acquisition of the *TRI101* gene, by whatever means, predates speciation of the B trichothecene-producing fusaria. Because mycotoxin production is theorized to be under strong selection (24), we are currently testing whether the other known trichothecene genes, which are all part of a gene cluster (35) separate from *TRI101* (22), are tracking with the *Fg* clade multigene genealogy.

Results of the present study have important practical implications for the control and reduction of *Fusarium* head blight and mycotoxin contamination of cereals worldwide. First, disease management, quarantine regulations, and plant breeding strategies need to appreciate the tremendous genetic diversity and global phylogeographic structure exhibited by the *Fg* clade. Secondly, recognizing that there are at least seven pathogenic/toxigenic lineages within the *Fg* clade, rather than one, requires greatly increased vigilance so that these lineages can be effectively monitored by an integrated international disease control program that uses the tools of molecular population genetics and phylogenetics (14). We further interpret our results to indicate that plant breeding efforts may benefit from including representatives of each lineage of the *Fg* clade when testing new varieties for scab resistance. By doing so, these efforts may increase the likelihood that broad-based resistance, effective against all lineages of the *Fg* clade, is achieved. Finally, the electronically portable multilocus database developed in the present study currently provides the only means by which the pathogenic lineages within the *Fg* clade can be identified unambiguously; moreover, it provides a robust phylogeographic framework for additional comparative studies urgently needed to understand the ecology, epidemiology, and population dynamics of *Fusarium* head scab of cereals worldwide.

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