

Systematics, Phylogeny and Trichothecene Mycotoxin Potential of *Fusarium* Head Blight Cereal Pathogens

Takayuki AOKI^{*1}, Todd J WARD^{*2}, H. Corby KISTLER^{*3*4}, Kerry O'DONNELL^{*2}

^{*1} Genetic Resources Center, National Institute of Agrobiological Sciences (Kannondai, Tsukuba, Ibaraki 305-8602 Japan)

^{*2} National Center for Agriculture Utilization Research, ARS-USDA (Peoria, IL 61604, USA)

^{*3} Cereal Disease Laboratory, USDA-ARS (1551 Lindig Street, St. Paul, MN 55108, USA)

^{*4} Department of Plant Pathology, University of Minnesota (St. Paul, MN 55108, USA)

Summary

Economically devastating outbreaks and epidemics of *Fusarium* head blight (FHB) or scab of wheat and barley have occurred worldwide over the past two decades. Although the primary etiological agent of FHB was thought to comprise a single panmictic species, *Fusarium graminearum*, a series of studies we conducted over the past decade, employing genealogical concordance/discordance phylogenetic species recognition (GCPSR)¹⁾, revealed that this morphospecies comprises at least 16 phylogenetically distinct species (referred to hereafter as the *F. graminearum* species complex = FGSC). Results of a multilocus molecular phylogeny, based on maximum parsimony and maximum likelihood analyses of 12 combined genes comprising 16.3 kb of aligned DNA sequence data, suggest that the different species groups within the FGSC radiated in Asia, North America, South America, Australia and/or Africa. The significant biogeographic structure of these lineages, together with evidence of disjunct species in Asia and North America, are consistent with widespread allopatric speciation within the FGSC. In contrast to the results obtained using GCPSR, morphological species recognition using conidial characters and colony morphology was only able to distinguish 6 species and 3 species groups among the 16 species within the FGSC, highlighting the need for sensitive molecular diagnostic tools to facilitate species identification. A validated multilocus genotyping assay was developed to address the need for species determination and trichothecene toxin chemotype prediction, and this assay has been extraordinarily useful in the discovery of novel FGSC species represented in our global FHB surveys. Ongoing molecular and phenotypic analyses are being conducted to elucidate the full spectrum of FHB pathogen diversity, their trichothecene toxin potential and biogeographic distribution. Increased understanding of the distribution and agricultural significance of variation within the FGSC is needed for the development of novel disease and mycotoxin control strategies, including improvements in agricultural biosecurity designed to limit the introduction and spread of non-indigenous FHB pathogens.

Key words : comparative morphology, head scab, multilocus molecular phylogeny, species complex, species delimitation

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Introduction

Outbreaks of scab or *Fusarium* head blight (FHB) of cereals over the past two decades have caused significant losses of wheat and barley worldwide²⁾. These pathogens frequently contaminate grain with trichothecene mycotoxins, such as deoxynivalenol or nivalenol, and estrogenic compounds. Outbreaks and

epidemics of FHB can be economically devastating because heavily toxin-contaminated grain is unsuitable for food or feed³). A series of comparative morphological and molecular phylogenetic studies we conducted over the past decade, employing genealogical concordance/discordance phylogenetic species recognition (GCPSR)¹), demonstrated that the morphospecies *F. graminearum* Schwabe comprises at least 16 phylogenetically distinct species⁴⁻⁸). These species form a genealogically exclusive clade that we designated the *F. graminearum* species complex (FGSC). In this paper, we review recent detailed taxonomic and phylogenetic analyses on the FGSC.

Taxonomic recognition of *Fusarium graminearum* sensu lato based on morphology

Fusarium taxonomy has a long complicated history due to different taxonomic systems and species concepts⁹). The genus *Fusarium* was established by Link in 1809 and was subsequently sanctioned by Fries¹⁰). The type species was originally designated *Fusarium roseum* Link, however, this name is ambiguous nomenclaturally because the specimens that Link left under this name comprise three different species: *F. sambucinum* Fuckel, *F. graminearum*, and *F. graminum* Corda^{11,12}). In 1821 Gray designated one of the three specimens that is equivalent to *F. sambucinum* as the holotype of *F. roseum*. Based on the fact, *F. sambucinum* is designated as the type species by conserving it over *F. roseum* nomenclaturally^{13,14}). Like *F. sambucinum*, the primary causal FHB pathogen of cereals, *F. graminearum*, produces trichothecene mycotoxins; the latter species has experienced relatively little taxonomic change until recently. Prior to the introduction of phylogenetic species recognition, the morphospecies *F. graminearum* was diagnosed by Gerlach & Nirenberg¹²) as producing only fusiform mostly 5- to 6-septate macroconidia typically $41\text{--}60 \times 4.3\text{--}5.5\ \mu\text{m}$ (total range $28\text{--}72 \times 3.2\text{--}6.0\ \mu\text{m}$) and either few or no chlamydospores. Other taxonomists classifying *Fusarium* species morphologically circumscribed *F. graminearum* similarly^{9,15-17}). During the 1980-1990s *F. cerealis* (Cooke) Sacc. (= *F. crookwellense* W. Burgess et al.)^{18,19}) and *F. pseudograminearum* O'Donnell et T. Aoki²⁰) were segregated from *F. graminearum* as morphologically and phylogenetically distinct species. *Fusarium pseudograminearum* was previously recognized as the Group 1 or the putatively heterothallic population of *F. graminearum*²¹). Under the dual naming system of nomenclature the teleomorph of *F. graminearum* is known as *Gibberella zeae* (Schwein.) Petch, but *G. saubinetii* (Mont.) Sacc., a synonym of *G. pulicaris* (Fr.) Sacc. (= *F. sambucinum*) and *G. cyanogena* (Desm.) Sacc. (= *F. sulphureum* Schltdl. 1936) have both been misapplied to this species²²). Subsequent taxonomic studies on *F. graminearum* did not alter the teleomorph synonymy^{15,23}). The dual system allowing separate names for anamorphs and teleomorphs of pleomorphic fungi based on Article 59 of the International Code of Botanical Nomenclature (ICBN) will end on 1 January 2013²⁴). Under the new Melbourne code, we prefer and strongly recommend the exclusive use of *Fusarium* for all of the species within this agriculturally and medically important genus.

Phylogenetic and morphological species recognition within the *Fusarium graminearum* species complex

Using an extensive collection of isolates from six continents^{4-8,25,26}), species limits within the putatively panmictic FHB species *F. graminearum* were evaluated using GCPSR¹). Phylogenetic analyses were conducted on DNA sequences from portions of 12 genes totaling 16.1 kb (Table 1). The GCPSR analyses revealed that the B clade of trichothecene toxin-producing FHB pathogens comprised a genealogically

Table 1. Loci sequenced for the GCPSR-based analyses of the *Fusarium graminearum* species complex (FGSC).

Locus	Number bp (PIC ^a)
α -Tubulin (α - <i>TUB</i>)	1,686 (76)
β -Tubulin (β - <i>TUB</i>)	1,337 (62)
translation elongation factor-1 α (<i>EF-1α</i>)	648 (21)
histone H3 (<i>HIS</i>)	449 (16)
mating type (<i>MAT1-1-1</i> , <i>MAT1-1-2</i> , <i>MAT1-1-3</i> , <i>MAT1-2-1</i>) ^b	6,592 (251)
ammonia ligase (<i>URA</i>), 3-O-acetyltransferase (<i>Tri101</i>), phosphate permease (<i>PHO</i>) ^c	4,124 (160)
reductase (<i>RED</i>)	1,273 (76)
Combined	16,109 (595)

^a PIC, parsimony informative character.

^b The four genes at the *MAT* locus are tightly linked.

^c *URA-Tri101-PHO* are contiguous in the FGSC genomes.

exclusive lineage including five early diverging self-sterile or heterothallic species (i.e., *F. cerealis*, *F. culmorum* (W.G. Smith) Sacc., *F. lunulosporum* Gerlach, *F. pseudograminearum* and an undescribed *Fusarium* sp. represented by NRRL 29298 and 29380 from orchard grass (*Dactylis glomerata* L.)) and 16 self-fertile or homothallic species within the *F. graminearum* species complex (FGSC) (Fig. 1). The 16 phylogenetically distinct species within the FGSC were inferred to represent genealogically exclusive species lineages based on strong bootstrap support for their genealogical exclusivity under GCPSR⁸⁾.

Intensive comparative morphological analyses were also conducted on a large global collection of the FGSC. These analyses included detailed comparisons of conidial morphology, sizes (length and width), widest position, conidial curvature and presence or absence of a narrow apical beak when isolates were cultured on synthetic nutrient-poor agar (SNA), together with growth rate and colony morphology on potato dextrose agar (PDA)^{4, 6)}. Based on morphological species recognition (MSR), only 6 species and 3 species groups were resolved within the FGSC (Table 2), reflecting their morphological simplicity and overlapping conidial characters (Figs. 2, 3). The 3 groups of morphological species were represented by two groups each comprising two species and one group comprising six phylogenetic species. This discovery is important because it revealed that morphological/phenotypic characters cannot be used to distinguish two-thirds of the species within FGSC^{4, 6, 8)}.

Description of novel species within the FGSC and their host range and geographic distribution

In addition to *Fusarium graminearum*, fourteen novel species within the FGSC have been formally described (Table 3), and one additional species was informally recognized, based on genealogical exclusivity (Fig. 1) and conidial morphology on SNA (Table 2, Figs. 2, 3)⁴⁻⁸⁾. We retained the name *F. graminearum* for the primary FHB pathogen in North America, Europe and several other regions of the world primarily because this species accounts for close to 100% of the FGSC isolated from cereals in North America and in Europe where this species was originally described from a collection of a graminean flower in Germany²⁷⁾. In addition, *F. graminearum* is commonly isolated from various cereals in northern Asia⁷⁾, South America²⁸⁾ and South Africa²⁹⁾. Because most of the FGSC species exhibit significant biogeographic structure, we hypothesize that independent allopatric species radiations may have taken place in Asia, North and South America, Central America, Australia and possibly Africa.

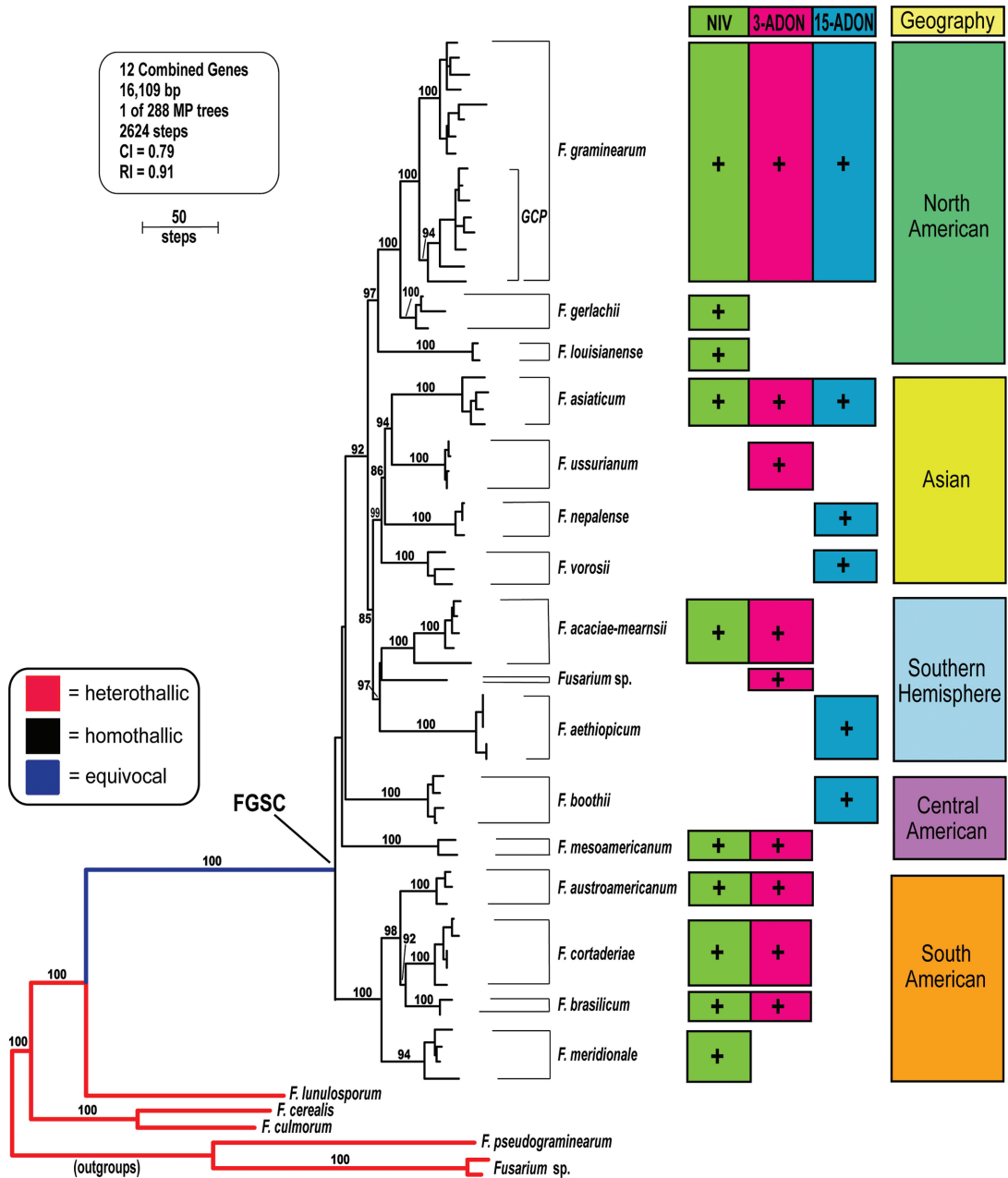


Fig. 1. Multilocus molecular phylogeny modified from Fig. 1 in Sarver et al.'s paper⁸⁾ of B-type trichothecene toxin-producing fusaria inferred from portions of 12 genes comprising 16.1 kb of DNA sequence data. The phylogram was rooted on sequences of *F. pseudograminearum* and *Fusarium* sp. NRRL 29298 and 29380. The phylogram is one of 288 equally most-parsimonious trees inferred from the combined data set. Numbers above nodes represent maximum parsimony and maximum likelihood bootstrap support based on 1,000 pseudoreplicates of the data. The five basal-most species lineages are self-sterile or heterothallic. The 16 species within the *F. graminearum* species complex (FGSC) are self-fertile or homothallic. B-type trichothecene toxin chemotype (i.e., NIV, 3ADON, 15ADON) and putative geographic origin is mapped on the FGSC phylogeny. CI, consistency index; GCP, Gulf Coast population of *F. graminearum*; RI, retention index.

Table 2. Conidial morphology^a of members of the *Fusarium graminearum* species complex (FGSC)^b and related heterothallic B clade species.

Species	Width of 5-septate conidia (mean value in μm) ^a	Longitudinal axis of conidia	Narrow apical beak (+ / -)	Upper and lower half of conidia	Widest region of conidia
<i>Fusarium graminearum</i> species complex (FGSC):					
<i>F. austroamericanum</i>	<4.5	typically straight	+ / -	asymmetric	mid-region
<i>F. boothii</i>	<4.5	gradually curved	+	mostly symmetric	mid-region
<i>F. meridionale</i>	<4.5	gradually curved	+	mostly symmetric	mid-region
<i>F. mesoamericanum</i>	4 – 4.5	typically straight	-	asymmetric	above mid-region
<i>F. louisianense</i>	4 – 4.5	gradually curved	-	asymmetric	mid-region
<i>F. acaciae-mearnsii</i>	4.5 – 5	gradually curved	+	asymmetric	below mid-region ^c
<i>F. brasilicum</i>	4.5 – 5	straight or gradually curved	+	asymmetric	below mid-region
<i>F. cortaderiae</i>	4.5 – 5	straight or gradually curved	+	asymmetric	below mid-region
<i>F. gerlachii</i>	4.5 – 5	gradually curved	+	asymmetric	mid-region
<i>Fusarium</i> sp. NRRL34461 ^c	4.5 – 5	gradually curved	+	asymmetric	above mid-region ^c
<i>F. nepalense</i>	4.5 – 5	gradually curved	+	asymmetric	above mid-region
<i>F. graminearum</i> (Gulf Coast) ^d	4.5 – 5	gradually curved	+ ^d	asymmetric	above mid-region
<i>F. graminearum</i>	4.5 – 5	gradually curved	- ^d	asymmetric	above mid-region
<i>F. asiaticum</i>	4.5 – 5	gradually curved	-	asymmetric	above mid-region
<i>F. aethiopicum</i>	$\approx$ 5	gradually curved	-	asymmetric	above mid-region
<i>F. vorosii</i>	>5	straight or gradually curved	+ / -	asymmetric	above mid-region
<i>F. ussuriatum</i>	>5	curved	+	symmetric	above mid-region
Species closely related to the FGSC:					
<i>F. lunulosporum</i>	<4.5	curved	+	symmetric	mid-region
<i>F. pseudograminearum</i>	4 – 4.5	curved	+	symmetric	mid-region
<i>F. cerealis</i>	>5	curved	+	symmetric	mid-region
<i>F. culmorum</i>	>5	curved	-	symmetric	mid-region

^a Examination of conidial morphology of the species was made on strains cultured on SNA under continuous black light.^b When using the combined conidial characters, the following six species and four species groups could be distinguished within the FGSC: *F. austroamericanum*, *F. mesoamericanum*, *F. louisianense*, *F. acaciae-mearnsii*, *F. gerlachii*, *F. ussuriatum*, *F. boothii* + *F. meridionale*, *F. brasilicum* + *F. cortaderiae*, *Fusarium* sp. NRRL 34461 + *F. nepalense* + *F. graminearum* (Gulf Coast), *F. graminearum* + *F. asiaticum* + *F. aethiopicum* + *F. vorosii*. These morphological groupings are not reflecting and corresponding to the phylogenetic relationships of the species.^c A single strain of *Fusarium* sp. NRRL 34461, forming asymmetric conidia widest above mid-region and previously considered as a unique strain of *F. acaciae-mearnsii*⁽⁶⁾ appears to represent a phylogenetically distinct species⁽⁸⁾.^d Strains of the divergent Gulf Coast population of *F. graminearum* are unique in that they produced conidia with a narrow apical beak.

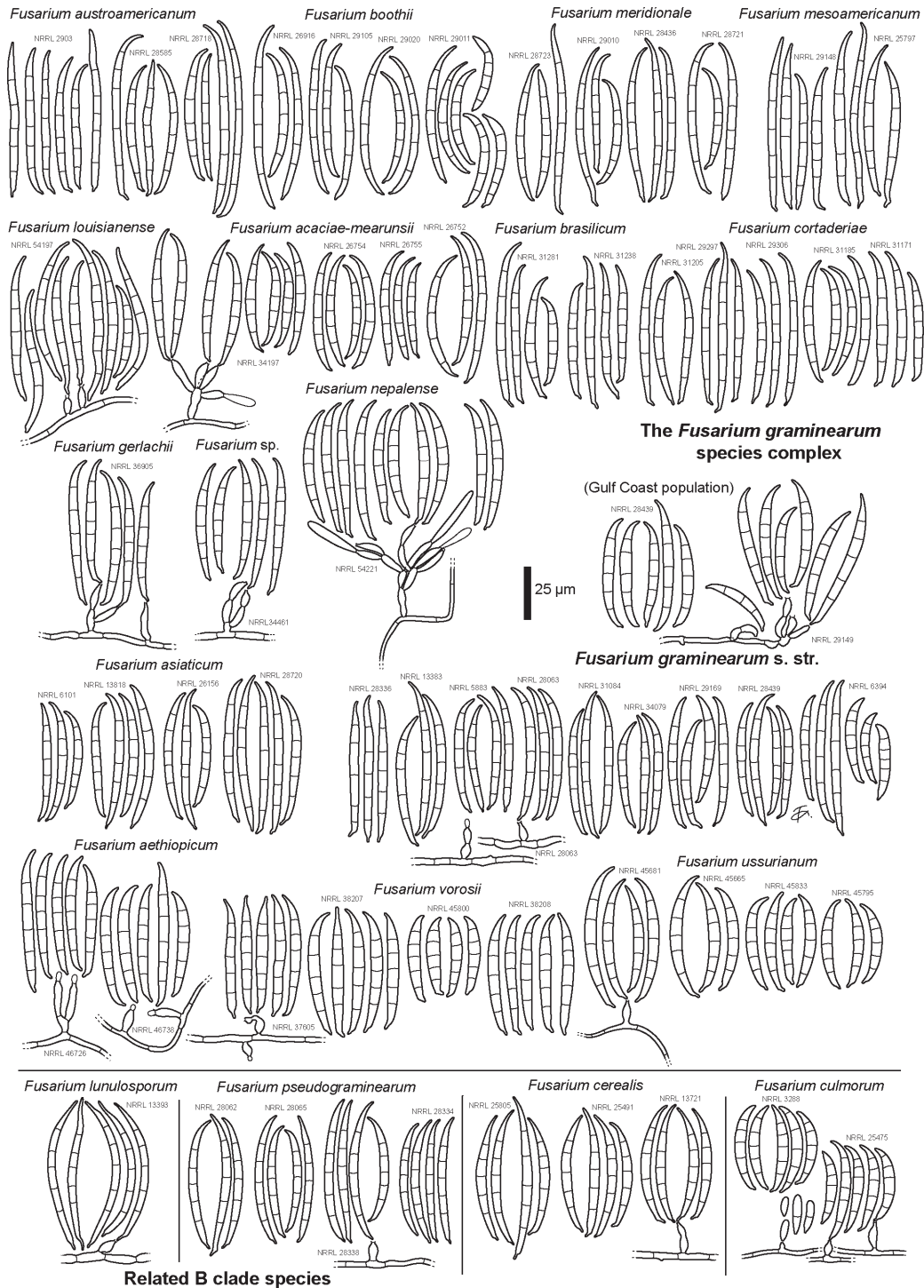


Fig. 2. Comparative morphology of 5-septate conidia of 16 self-fertile or homothallic FGSC species cultured on SNA under continuous black light together with representative isolates of four closely related self-sterile or heterothallic species, *F. cerealis*, *F. culmorum*, *F. lunulosporum* and *F. pseudograminearum*.

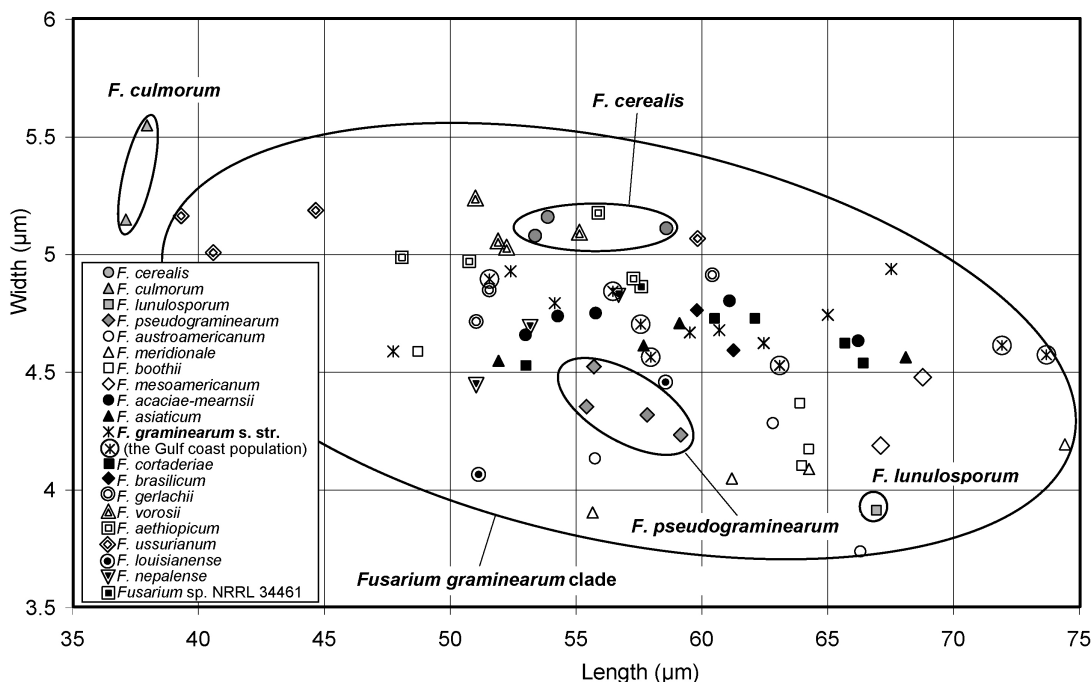


Fig. 3. Scatter diagram reporting mean length and width of 5-septate conidia of the 16 FGSC species cultured on SNA under continuous black light together with representative strains of four closely related species, *F. cerealis*, *F. culmorum*, *F. lunulosporum* and *F. pseudograminearum*. Plots are based on mean size of 50 randomly selected 5-septate conidia.

Of the three species that comprise a putative Asian clade, *F. asiaticum* O'Donnell et al. has been found to be the most important FHB pathogen in East Asian countries, i.e. Japan, Korea and China associated with rice production. While it has been established that the ranges of *F. graminearum* and *F. asiaticum* overlap on the Japanese islands of Hokkaido, Honshu and Shikoku, these two species form a latitudinal cline in which the former species predominates in the North whereas the latter species is found exclusively on the southern island of Kyushu³⁰). A third FGSC species in Japan, *F. vorosii* B. Tóth et al., appears to be adapted to northern climates given that it has only been reported from the northern-most island of Hokkaido in Japan, Hungary, and the Far East of the Russian Federation⁷). Excluding *F. graminearum*, which accounts for close to 100% of FHB throughout North America, the minor FGSC species within the United States also appear to be regionally distributed with *F. gerlachii* T. Aoki et al.⁶) in the north and *F. louisianense* Gale et al. and the Gulf Coast population of *F. graminearum* in the south^{6,31}). Our survey of FHB in the U.S. also detected *F. boothii* O'Donnell et al. on corn in Texas, *F. mesoamericanum* T. Aoki et al. on grape ivy in Pennsylvania, *F. meridionale* T. Aoki et al. and *F. asiaticum* on wheat in Pennsylvania; the latter species was also recovered from rice in Louisiana (Table 3). We hypothesize that the latter four FGSC species were introduced into the U.S. inadvertently in association with agriculturally and horticulturally important plants.

Table 3. FGSC species geographic distribution, known hosts, and B-type trichothecene mycotoxin potential.

FGSC species	Countries of occurrence	Known hosts	Trichothecene chemotype ^a		
			NIV	3ADON	15ADON
<i>Fusarium acaciae-mearnsii</i> O'Donnell et al.	Australia, South Africa	black wattle (<i>Acacia mearnsii</i>), soil	+	+	
<i>Fusarium aethiopicum</i> O'Donnell et al.	Ethiopia	wheat			+
<i>Fusarium asiaticum</i> O'Donnell et al.	Asia (China, Nepal, Japan, Korea), Brazil, USA	barley, wheat, corn, oat, rice	+	+	+
<i>Fusarium austroamericanum</i> T. Aoki et al.	South America (Brazil, Venezuela)	herbaceous vine, corn, unknown host	+	+	
<i>Fusarium boothii</i> O'Donnell et al.	South Africa, Mexico, Guatemala, Nepal, Korea, USA	corn			+
<i>Fusarium brasiliicum</i> T. Aoki et al.	Brazil	barley, oat	+	+	
<i>Fusarium cortaderiae</i> O'Donnell et al.	South America (Argentina, Brazil), Oceania (Australia, New Zealand)	pampas grass (<i>Cortaderia selloana</i>), corn, carnation, barley, wheat, soil	+	+	
<i>Fusarium gerlachii</i> T. Aoki et al.	USA	wheat, giant cane (<i>Arundo donax</i>)	+		
<i>Fusarium graminearum</i> Schwabe	North America, South America, Europe, Asia (Japan, China, Korea), South Africa	corn, wheat, millet, various cereals, leather leaf fern (<i>Rumohra adiantiformis</i>)	+	+	+
<i>Fusarium louisianense</i> Gale et al.	USA	wheat	+		
<i>Fusarium meridionale</i> T. Aoki et al.	South America (Brazil), Central America (Guatemala), South Africa, Australia, New Caledonia, Nepal, Korea, USA	orange twig, corn, barley stem, wheat, soil	+		
<i>Fusarium mesoamericanum</i> T. Aoki et al.	Central America (Honduras), USA	banana, grape ivy (<i>Parthenocissus tricuspidata</i>)	+	+	
<i>Fusarium nepalense</i> T. Aoki et al.	Nepal	rice			+
<i>Fusarium ussuriianum</i> T. Aoki et al.	Far East of the Russia Federation	wheat, oat		+	
<i>Fusarium vorosii</i> B. Tóth et al.	Japan (Hokkaido), Hungary	wheat		+	+
<i>Fusarium</i> sp. NRRL 34461	South Africa	soil			

^a NIV: nivalenol and acetylated derivatives; 3ADON: deoxynivalenol and 3-acetyldeoxynivalenol; 15ADON: deoxynivalenol and 15-acetyldeoxynivalenol.

Mycotoxin production and evolution of the trichothecene gene cluster within the *Fusarium graminearum* species complex

Species within the FGSC produce trichothecene mycotoxins and estrogenic compounds that can contaminate cereals, rendering them unsuitable for consumption by human and other animals. Trichothecenes are also phytotoxic and act as virulence factors on sensitive host plants³²⁾. The trichothecenes produced by diverse fusaria are classified as either A-type or B-type depending on the absence or presence of a keto group at the C-8 position of the trichothecene ring^{33, 34)}. Species within the FGSC produce B-type trichothecenes such as deoxynivalenol (DON, also known as vomitoxin), nivalenol (NIV), and their acetylated derivatives. Many genes involved in trichothecene biosynthesis have been identified based on biochemical and genetic investigations of *Fusarium sporotrichioides* Sherb. (an A-type trichothecene producer) and the FGSC species, *F. asiaticum* and *F. graminearum*^{35, 36)}. All known trichothecene genes are localized within a gene cluster, with the exception of a 3-*O*-acetyltransferase (*TRI101*)³⁷⁾ and *TRI1* and *TRI16*³⁸⁾. Three trichothecene chemotypes, i.e., strain-specific profiles of trichothecenes, have been found within the B clade of trichothecene producing fusaria: (1) nivalenol and acetylated derivatives (NIV chemotype), (2) deoxynivalenol and 3-acetyldeoxynivalenol (3ADON chemotype), and (3) deoxynivalenol and 15-acetyldeoxynivalenol (15ADON chemotype)³⁾. NIV vs. DON B-type trichothecene chemotype differences are determined by *TRI3*^{39, 40)}, whereas differences in *TRI3*⁴¹⁾ and *TRI8* are responsible for the 3ADON and 15ADON dichotomy^{35, 42)}.

Although phylogenetic analysis of the individual and the combined 12 gene data set robustly supports the recognition of 16 phylogenetically distinct species under GCPSR, phylogenies inferred from eight trichothecene cluster genes did not track with species phylogeny^{5, 8, 26)}. Discordance between the trichothecene gene trees and the species phylogeny appears to be due to: (1) nonphylogenetic sorting of ancestral polymorphism into descendant species (i.e., transspecies polymorphism), and (2) maintenance of trans-specific polymorphism by a novel form of balancing selection acting on chemotype differences within the trichothecene mycotoxin gene cluster^{26, 35)}. It is worth noting that phylogenetic relationships and species limits within each of the three trichothecene chemotype clades, i.e., the NIV, 3ADON, or 15ADON, are largely consistent with the species phylogeny²⁶⁾. Because genes within the trichothecene toxin gene cluster are evolving under strong balancing selection, they cannot be used to infer evolutionary relationships among and species limits within the B clade of *Fusarium*. This finding adds to a growing number of studies that have reported discordance between gene trees and species trees within *Fusarium*. Other examples of phylogenetically discordant genealogies include *TRI1* and *TRI16* within the *F. sambucinum* species complex³⁸⁾, highly divergent intragenomic ITS2 rDNA types within the *Gibberella fujikuroi* species complex and related fusaria⁴³⁾, divergent amino adipate reductase (*lys2*) paralogs or xenologs within the *F. oxysporum* and *F. solani* species complexes⁴⁴⁾, and discordant sterol C-14 reductase (*erg-3*) gene genealogies within the *F. solani* species complex⁴⁵⁾. Taken together, these findings illustrate the importance of multilocus GCPSR-based studies in inferring robust species phylogenies.

Conclusion

Our GCPSR-based analyses of the *F. graminearum* species complex (FGSC) over the past decade

support the recognition of at least of 16 phylogenetically distinct species. However, morphological species recognition (MSR) based on conidial characters together with growth rate and colony morphology only resolved 6 species and 3 species groups within the FGSC. The latter finding is important because it alerts end users that MSR cannot be used to accurately identify most of the species within the FGSC. The morphological simplicity and overlapping conidial characters is consistent with diversification time estimates that suggest the FGSC's evolutionary origin and radiation occurred relatively recently in the late Pliocene and Pleistocene over the past 2.7 million years⁴⁶⁾. Phylogenetic analyses also revealed that the evolutionary history of most of the trichothecene biosynthesis genes was discordant with the species phylogeny. We attribute this discordance to genes on either end of the cluster evolving under strong balancing selection, as reflected in the maintenance of trans-specific polymorphism that predates the evolutionary diversification of the B clade. Thus, not all genes within the FGSC and their closely related heterothallic ancestors track with the species phylogeny.

Our current understandings of the global distribution of FHB pathogens and their toxin potential, including evidence for species clines in Asia and North America, have been significantly advanced by a validated multilocus genotyping (MLGT) assay for species determination and toxin chemotype prediction⁴⁷⁾. The putative species clines and significant biogeographic structure within the FGSC are consistent with widespread allopatric speciation. Because global trade in plants and plant products could easily result in the accidental geographic transposition of FHB pathogens into non-indigenous areas, ongoing molecular surveillance is essential to support agricultural biosecurity by obtaining a detailed, up-to-date picture of FHB species distributions and their toxin potential worldwide.

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