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Fusarium Pathogenomics

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Fusarium, genome evolution, horizontal gene transfer, mycotoxins, pathogenicity, pathogenomics

Abstract

Fusarium is a genus of filamentous fungi that contains many agronomically important plant pathogens, mycotoxin producers, and opportunistic human pathogens. Comparative analyses have revealed that the *Fusarium* genome is compartmentalized into regions responsible for primary metabolism and reproduction (core genome), and pathogen virulence, host specialization, and possibly other functions (adaptive genome). Genes involved in virulence and host specialization are located on pathogenicity chromosomes within strains pathogenic to tomato (*Fusarium oxysporum* f. sp. *lycopersici*) and pea (*Fusarium* '*solani*' f. sp. *pisi*). The experimental transfer of pathogenicity chromosomes from *F. oxysporum* f. sp. *lycopersici* into a nonpathogen transformed the latter into a tomato pathogen. Thus, horizontal transfer may explain the polyphyletic origins of host specificity within the genus. Additional genome-scale comparative and functional studies are needed to elucidate the evolution and diversity of pathogenicity mechanisms, which may help inform novel disease management strategies against fusarial pathogens.

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Contents

FUSARIUM SPECIES ARE PHYLOGENETICALLY AND ECOLOGICALLY DIVERSE AND ECONOMICALLY IMPORTANT

Fusarium is a cosmopolitan genus of filamentous ascomycete fungi (Sordariomycetes: Hypocreales: Nectriaceae) that includes many toxin-producing plant pathogens of agricultural importance. Collectively, *Fusarium* diseases include wilts, blights, rots, and cankers of many horticultural, field, ornamental, and forest crops in both agricultural and natural ecosystems. Fusaria also produce a diverse array of toxic secondary metabolites (mycotoxins), such as trichothecenes and fumonisins (reviewed in 85), that can contaminate agricultural products, making them unsuitable for food or feed. Trichothecenes can also act as virulence factors in plant diseases (7, 22, 23, 39, 62). Although opportunistic *Fusarium* infections (fusarioses) of humans and other animals are relatively rare, they typically show broad resistance to antifungal drugs (2). Fusaria are disproportionately associated with fungal infections of the cornea (30). Owing to their importance to agriculture and medicine, dedicated web-accessible databases have been developed for their identification using portions of phylogenetically informative genes to conduct BLAST queries (29, 57, 59).

Fusaria: *Fusarium* spp.

Mycotoxins: toxic secondary metabolites produced by fungi

Hemibiotrophic pathogens: pathogens that incorporate both biotrophic and necrotrophic infection strategies

Biotrophic pathogens: pathogens that colonize living plant tissue and obtain nutrients from living host cells

Necrotrophic pathogens: pathogens that kill host cells and obtain nutrients from dead cells

A comprehensive phylogenetic analysis resolved a monophyletic group that encompasses all economically important *Fusarium* species (56). All but 9 of the species within this clade resolve into 20 monophyletic species complexes that consist of as many as 60 species (**Figure 1**). At least seven alternative generic names based on sexual stages are linked to *Fusarium* (e.g., *Gibberella*, *Nectria*, and *Neocosmospora*). To avoid confusion, only the name *Fusarium* should be used (28).

The *Fusarium* time-calibrated phylogeny (**Figure 1**) provides a valuable blueprint for future studies directed at elucidating the phylogenomic diversity of *Fusarium*. The genus includes close to 300 phylogenetically diagnosable species, the majority of which lack formal names (28). Time calibration of the phylogeny suggests that *Fusarium* originated ~91.3 Mya, indicating that its diversification coincided with that of flowering plants (72). Most early-diverging *Fusarium* lineages are associated with woody plants as parasites, endophytes, or saprophytes (56), reflecting the hypothesized dominance of a woody habit for early-diverging, terrestrial angiosperms (73).

Some *Fusarium* species produce meiotic (sexual) spores and as many as three types of mitotic (asexual) spores. However, not all spore types are known to be produced by all species (**Figure 2**), and fewer than 20% of fusaria have a known sexual cycle. As phytopathogens, fusaria employ a broad range of infection strategies. Most can be loosely classified as hemibiotrophs, because infection initially resembles that of a pathogen that relies on a living host (biotrophic), but eventually transitions to killing and consuming host cells (necrotrophic). *Fusarium* diseases

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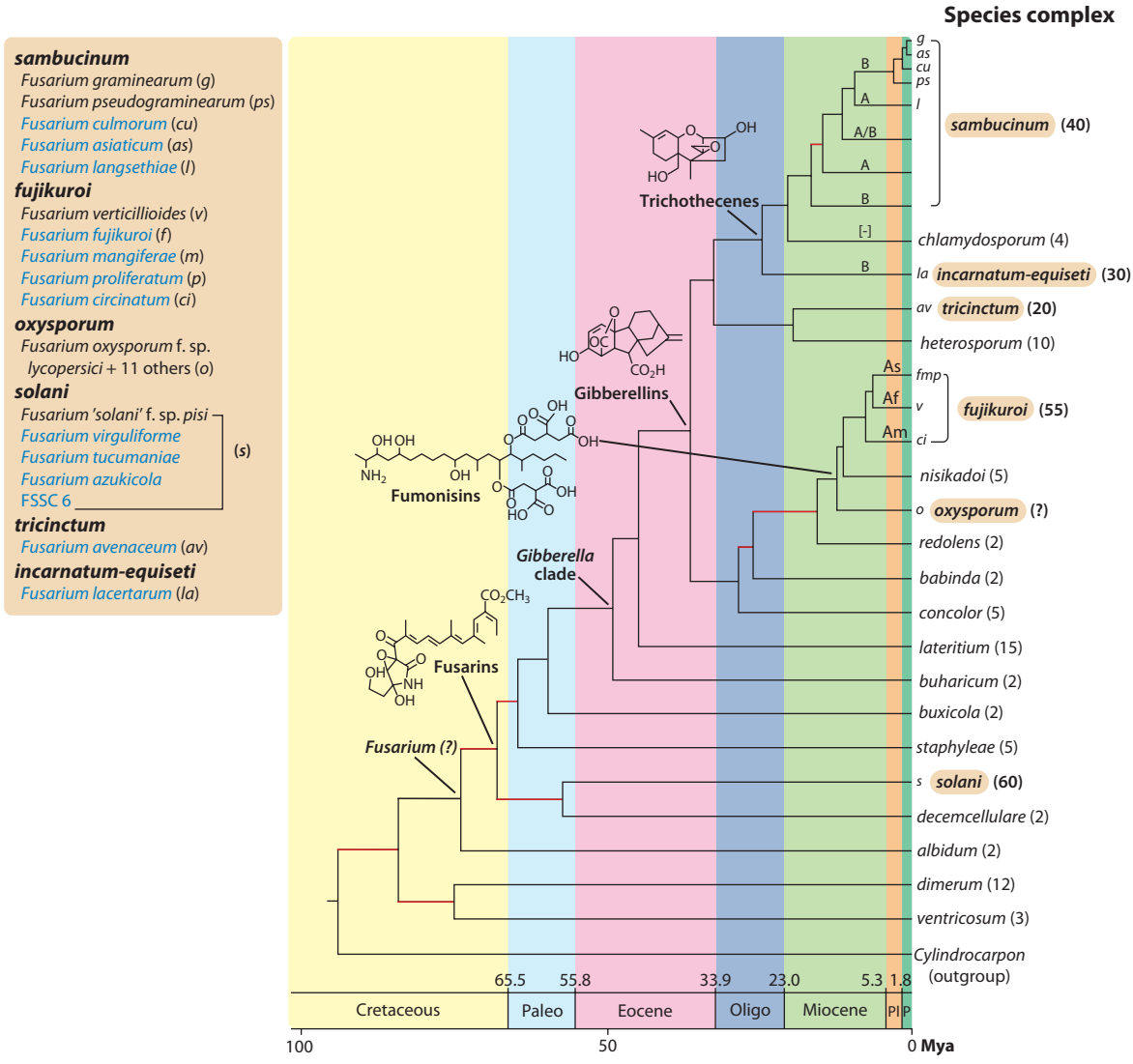

Figure 1

Chronogram showing the age estimates for the origin and evolutionary diversification of the 20 *Fusarium* species complexes (modified with permission from Reference 56). Numbers in parentheses to the right of each species complex represent a conservative estimate of the number of phylogenetically distinct species within each of these lineages; the question mark by the *F. oxysporum* species complex indicates species-level structure requires further study. Italicized lowercase letters at the tips of the chronogram identify species in the six highlighted species complexes whose whole genome sequences have been released or are in production (listed in legend in the upper-left-hand corner). Black and blue font is used to distinguish species whose genomes are publicly available from those that are in production, respectively. The question mark by *Fusarium* indicates that its circumscription might include the *dimerum* and *ventricosum* complexes; however, the internode supporting their inclusion in *Fusarium* received ≤70% parsimony and likelihood bootstrap support (unresolved internodes are in red). By contrast, all internodes in black, including the most recent common ancestor of the *albidum* complex, were strongly supported by parsimony, likelihood, and Bayesian analyses. The inferred evolutionary origin of three mycotoxins and the gibberellin phytohormones in separate epochs is mapped on the chronogram, which is scaled in millions of years before present. A, B, and [−] within the clade of trichothecene toxin-producing fusaria indicate the production of type A trichothecenes, type B trichothecenes, and no trichothecenes, respectively. Three biogeographically structured clades within the *fujikuroi* complex are identified as follows: As, Asian; Af, African; Am, American. Abbreviations: Paleo, Paleocene; Oligo, Oligocene; Pl, Pliocene; P, Pleistocene.

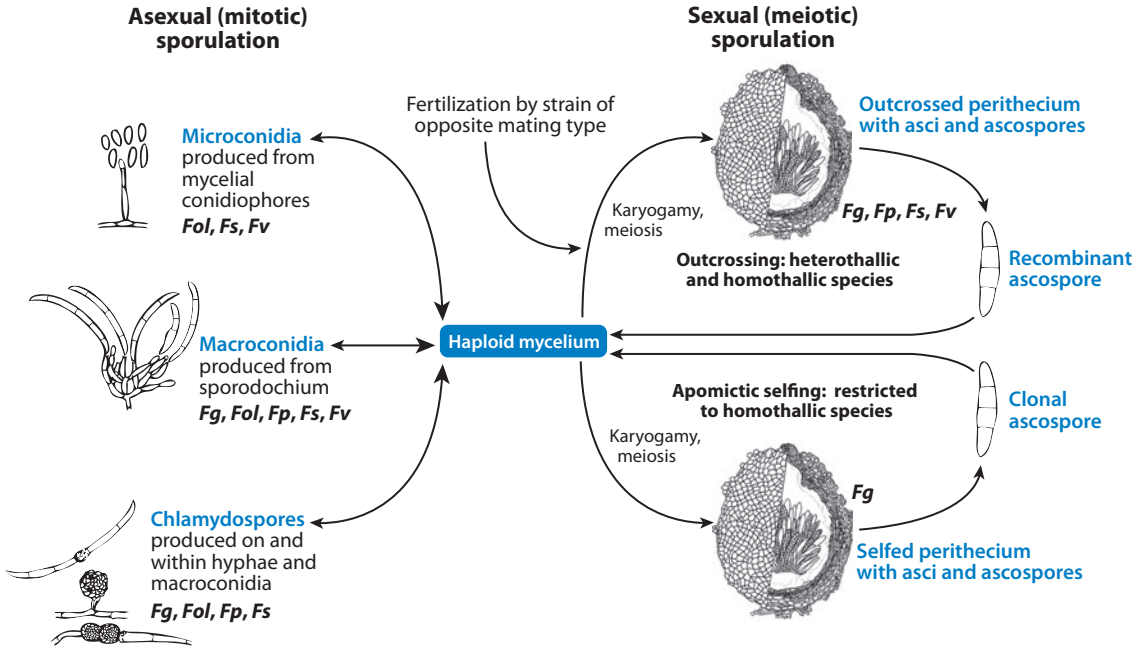

Figure 2

Generalized life cycle of *Fusarium*. The organism grows as a haploid colony of hyphae, except for brief dikaryotic (each cell containing two parental haploid nuclei) and diploid stages preceding meiosis and the production of haploid, sexually produced spores (ascospores). Ascospores are produced in groups of eight in a sac (ascus) contained within a flask-shaped structure (perithecium). Homothallic species are capable of self-fertilization, producing clonal ascospore progeny (apomixis); heterothallic species are self-sterile. Three major forms of mitotic (asexual) spores may be produced, depending on the species. (*Top left*) Small asexual spores (microconidia) are produced in the mycelium from simple spore-forming structures (conidiophores). (*Middle left*) Long, canoe-shaped, septate spores (macroconidia) are produced in cushion-shaped aggregations of conidiophores called sporodochia and/or on conidiophores in the aerial mycelium. (*Bottom left*) Thick-walled resistant spores (chlamydospores) are produced within or on hyphae or macroconidia. Species with complete genome sequences possessing each spore stage are indicated. Abbreviations: *Fg*, *F. graminearum; Fol*, *F. oxysporum* f. sp. *lycopersici*; *Fp*, *F. pseudograminearum*; *Fs*, *F. 'solani'* f. sp. *pisi*; *Fv*, *F. verticillioides*. Drawings reproduced with permission from References 3 and 4.

may initiate in roots from soilborne inoculum or in above-ground plant parts via air or water. *F. oxysporum*, for example, initially penetrates roots asymptomatically; subsequently it colonizes vascular tissue and triggers massive wilting, necrosis, and chlorosis of aerial plant parts. In contrast, *F. graminearum*, the major cause of *Fusarium* head blight of cereals worldwide, produces limited necrosis. It infects floral tissues during anthesis and spreads into uninfected flowers through the central axis of the inflorescence, eventually damaging kernels and contaminating them with toxins.

Host specificity varies among *Fusarium* species. *F. verticillioides* causes ear rot mostly in maize and sorghum, but it can infect many other plants. Members of the *F. oxysporum* species complex are capable of causing wilt diseases in over one hundred agronomically important plant species. However, individual *F. oxysporum* isolates often exhibit a high degree of host specificity; isolates that are pathogenic on the same host are grouped into the same *forma specialis* (e.g., *F. oxysporum* f. sp. *lycopersici* for tomato pathogens). In several cases, *F. oxysporum formae speciales* consist of multiple, independent lineages that evolved polyphyletically through convergent evolution (6, 54). All human pathogenic fusaria produce microconidial stages, and many produce biofilms on plumbing surfaces (70).

***Forma specialis* (f. sp.):** artificial taxonomic grouping based on ability to cause disease on a specific plant host

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Table 1 List of published *Fusarium* genomes

Species[a]	Genes	Strain	Scaffolds	Size (Mb)	Chromosomes[b]	Platform	NCBI[c]	Reference
Fusarium verticillioides	14,179	7600	36	42	11	Sanger	AAIM	50
Fusarium circinatum	15,713	FSP34	3457	44	Unknown	454	–	84
Fusarium fujikuroi	14,017	B14	338	44	12	Illumina	–	42
Fusarium fujikuroi	14,813	IMI58289	12	43.9	12	Pyro-sequencing	–	88
Fusarium oxysporum f. sp. *lycopersici*	17,735	4287	113	61	15	Sanger	AAXH	50
Fusarium oxysporum 5176	17,817	5176	3395	55	Unknown	454	AFQF	77
Fusarium graminearum	13,332	PH-1	31	36	4	Sanger	AACM	19
Fusarium pseudograminearum	12,488	CS3096	655	37	Unknown	Illumina	AFNW	27
Fusarium '*solani*' f. sp. *pisi*	15,707	77-13-4	209	51	17	Sanger	ACJF	17

[a]The pea pathogen *Fusarium* '*solani*' f. sp. *pisi* belongs to an undescribed species in the *F. solani* species complex. It is often referred to as *Nectria haematococca* mating population VI (17).
[b]Twelve chromosomes were detected in *F. verticillioides* via electrophoresis and a corresponding genetic map (86), but the genome assembly lacked the smallest chromosome (50).
[c]Designation in the NCBI GenBank genome database.

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This review summarizes the major discoveries contributed by genomic analyses of *Fusarium*, with a focus on plant pathogenicity and production of toxins and other secondary metabolites. A key theme is the finding that a *Fusarium* genome is compartmentalized into core and adaptive regions that encode functions associated mostly with primary growth versus adaptation to specific niches (e.g., virulence on specific hosts, growth in specific environments). This genome compartmentalization should enable functional studies focused on the development of improved means for controlling *Fusarium* diseases and toxin contamination.

EVOLUTION OF GENOME SIZE: THE ROLE OF HORIZONTAL TRANSFER

The five completely sequenced *Fusarium* genomes (*F. graminearum*, *F. oxysporum* f. sp. *lycopersici*, *F. pseudograminearum*, *F.* '*solani*' f. sp. *pisi*, and *F. verticillioides*) (**Table 1**), which also have mostly completed genetic and physical maps available, present an excellent opportunity to explore the evolution of genome size and content within the genus (17, 19, 27, 50, 84). These five species diverged roughly near the Cretaceous-Tertiary boundary, about 65 Mya (**Figure 1**). Their genomes vary greatly in size and repeat content (**Figure 3**), from the 36-Mb genome of *F. graminearum*, to the 61-Mb *F. oxysporum* f. sp. *lycopersici* genome. An obvious trend is exploring whether there has been a tendency toward an increase or decrease in genome size. The *F.* '*solani*' f. sp. *pisi* genome, the first of the five species to diverge from their common ancestor (**Figure 1**), has a genome size of 51 Mb. Roughly 30 million years later in the late Eocene, the ancestor of the clade containing *F. oxysporum* f. sp. *lycopersici* and *F. verticillioides* diverged from the ancestor of the clade containing *F. graminearum*. *F. oxysporum* f. sp. *lycopersici* has a genome size of 61 Mb, which is the largest fusarial genome sequenced so far. In contrast, *F. verticillioides* has

Compartmentalized genomes: genomes within which basic cellular function (core genome) and host specialization and pathogen virulence (adaptive genome) are physically separated

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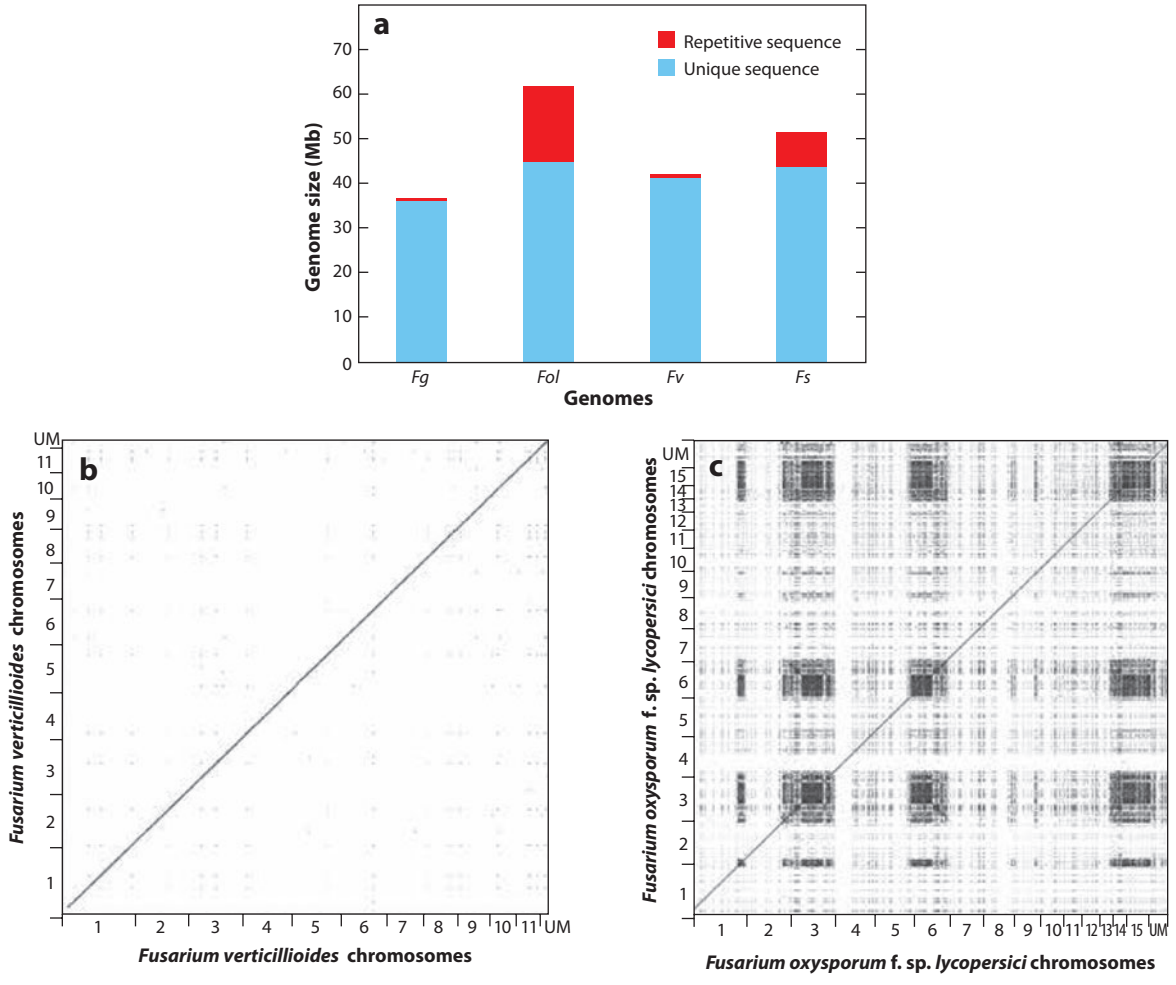

Figure 3

Fusarium genomes differ in repeat content. (*a*) Repeat sequences were detected by searching genome sequences against themselves using CrossMatch, filtering for alignments longer than 200 bp with greater than 60% sequence similarity. Self-alignments of (*b*) *F. verticillioides* and (*c*) *F. oxysporum* f. sp. *lycopersici* using PatternHunter (49) with e-score cutoff of 1e-10 to compute local-alignment anchors.

a genome size of 42 Mb and is therefore closer in size to the 36-Mb genome of *F. graminearum*, even though *F. verticillioides* is more closely related to *F. oxysporum* f. sp. *lycopersici*, with which it last shared a common ancestor roughly 10–11 Mya. Except for the highly syntenic 36-Mb *F. graminearum* and 37-Mb *F. pseudograminearum*, which diverged from a common ancestor relatively recently (roughly 3.4 Mya), no phylogenetic correlation with a trend in size or reduction can be seen among these five genomes. Assuming that this prediction holds true, despite the small sample size, then what might be an explanation for increases or decreases in genome size among these species?

Mechanisms that could decrease genome size include unequal crossover that induces localized deletions of individual or multiple genes, or other types of chromosomal rearrangement that could potentially induce large-scale deletions. By contrast, mechanisms that could increase genome size include gene duplication, whole or partial genome duplication (45), hybridization between

species (55), and/or horizontal transfer. In contrast, localized and large-scale deletions, as well as counterbalancing gene duplications, are certain to have affected *Fusarium* genome evolution. But to what extent has horizontal transfer influenced the evolution of fusarial genomes? The evidence suggests that it played an important role, as discussed below.

In general, two types of integration are possible when considering the acquisition of foreign genes (i.e., xenologs). Xenologs may be acquired through the transfer of an entire plasmid or chromosome, or they may be integrated into the chromosomal complement of a species. On the basis of studies to date, it appears that the former type may be more common in *Fusarium* (17, 50). Furthermore, chromosomes most likely to contain horizontally acquired genes are supernumerary or conditionally dispensable chromosomes. These are usually small (<2 Mb) chromosomes and are not required for survival under standard conditions. Supernumerary chromosomes share certain features that are conducive to, or perhaps result from, horizontal transfer: (*a*) a lack of housekeeping genes involved in primary metabolism, (*b*) a highly biased level of G+C content (relative to the normal chromosomal complement), (*c*) a lack of synteny with related species, and (*d*) the presence of a large number of transposable elements. An excellent example is chromosome 14 in the *F.* '*solani*' f. sp. *pisi* genome, which harbors the *PEP* gene cluster (76). This gene cluster is reported to have a G+C content very different from the rest of the *F.* '*solani*' f. sp. *pisi* genome. In addition, its presence in the genomes of closely related species is variable; although the presence or absence of a gene or gene cluster is not necessarily indicative of horizontal transfer (66), it certainly can result from it. While they do not fully account for the larger size (51 Mb) and greater number of predicted genes (15,707) in *F.* '*solani*' f. sp. *pisi*, supernumerary chromosomes have contributed significantly to genome evolution in this species.

Supernumerary chromosomes provide clear evidence for genomic compartmentalization at both structural and functional levels by essentially providing a de facto division of a genome into two components: a core component that encodes functions necessary for growth and survival and an accessory component (18) that encodes pathogenicity (or virulence) factors and secondary metabolites. Akagi et al. (1) predicted that transfer of supernumerary chromosomes could provide a mechanism for the evolution of pathogenicity (32). Their hypothesis was confirmed experimentally when pathogenicity on tomato (*Solanum lycopersicum*) was introduced through the in vitro transfer of supernumerary chromosomes between strains of *F. oxysporum* (50). In addition to horizontal chromosome transfer, forces involving transposition and other events involving repetitive sequences are bound to play important roles in the horizontal transfer of adaptive functions (65).

PEP: pea pathogenicity gene cluster

NRPS: nonribosomal peptide synthetase

PKS: polyketide synthase

TS: terpene synthase

EVOLUTION AND DIVERSIFICATION OF SECONDARY METABOLITES

Secondary metabolites are low-molecular-weight organic molecules that are not essential for normal growth but may provide a selective advantage in some environments. Fungal secondary metabolites are diverse in structure and biological activity. Some are pigments, others are toxic to plants and/or animals, some regulate plant growth or have pharmaceutical properties (e.g., antibiotics), and others, including some *Fusarium* metabolites (e.g., enniatins and trichothecenes), contribute to plant pathogenesis (11, 20, 34, 51). The majority of fungal secondary metabolites are synthesized via the activities of nonribosomal peptide synthetases (NRPSs), polyketide synthases (PKSs), or terpene synthases (TSs). These enzymes catalyze condensation or rearrangement of structurally simple molecules to form more complex structures: nonribosomal peptides, polyketides, or terpenes. These chemical products typically undergo multiple enzymatic modifications to form biologically active secondary metabolites. In *Fusarium* and many other fungi, genes encoding successive enzymes in a secondary metabolite biosynthetic pathway are usually physically

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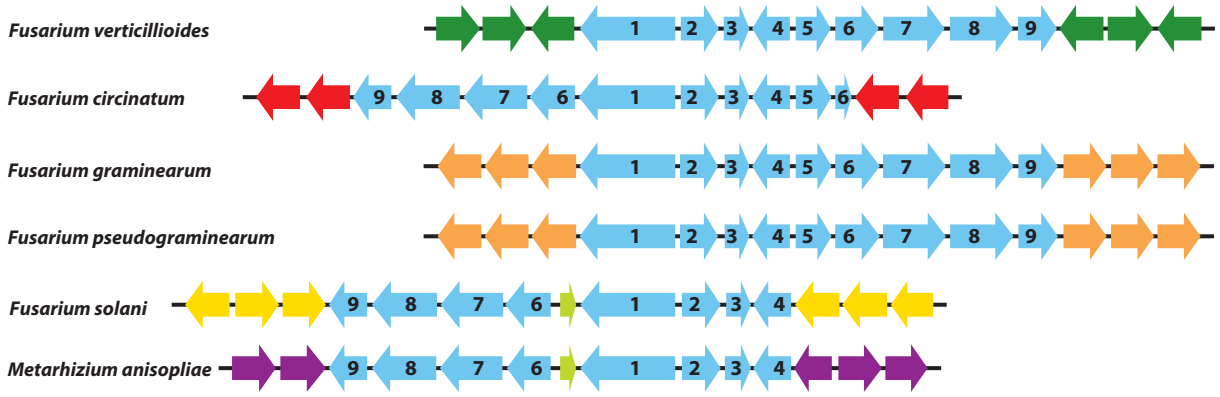

Figure 4

Comparison of fusarin biosynthetic gene (*FUS*) clusters and flanking regions retrieved from genome sequences of five *Fusarium* species and *Metarhizium anisopliae*, a distantly related (Hypocreales: Clavicipitaceae) insect pathogen that produces the fusarin analogs NG-391 and NG-393 (46). Cluster genes *FUS1* through *FUS9* are shown as blue arrows numbered 1 through 9. The *FUS5* homolog is absent in *F. solani* and *M. anisopliae*. There are two different arrangements of genes within the cluster: the arrangement in *F. verticillioides*, *F. graminearum*, and *F. pseudograminearum*, and the arrangement in *F. circinatum*, *F. solani*, and *M. anisopliae*. Genes flanking clusters are shown as red, orange, yellow, dark green, and purple arrows; flanking genes represented by the same color in two species are closely related homologs. Light green arrows represent a gene inserted into the FUS cluster of *F. solani* and *M. anisopliae*. The *F. circinatum* cluster is presented as described in Reference 84.

clustered (**Figure 4**). In addition to enzymes that form and tailor the parent compounds, secondary metabolite biosynthetic gene clusters can encode transporters that move the metabolites across membranes and pathway-specific transcription factors that activate expression of genes in a cluster.

Comparative genome analyses have revealed that *Fusarium* and other filamentous fungi have the genetic potential to produce many more secondary metabolites than previously thought. Prior to completion of the first *Fusarium* genome sequence, the genus as a whole was reported to produce ~40 structurally distinct families of secondary metabolites, although some families, such as fumonisins and trichothecenes, consist of tens of different analogs (15, 16, 80). Despite this metabolic diversity within the genus, individual species and isolates were reported to produce relatively few metabolites. For example, reports indicated that *F. graminearum* produced eight secondary metabolite families: aurofusarin, butenolide, chlamydosporol, culmorin, cyclonerodiol, fusarins, trichothecenes, and zearalenones (21). Analysis of the *F. graminearum* genome sequence, however, surprisingly identified 16 PKSs, 19 NRPSs, and 8 TSs, revealing that a single species has the genetic potential to produce about the same number of secondary metabolites as previously reported for the entire genus.

Analysis of genome sequences of multiple *Fusarium* species, as well as PCR and Southern blot surveys, indicates marked differences in the distribution of secondary metabolite biosynthetic genes and therefore differences in the genetic potential of species to produce secondary metabolites (12, 33, 50, 56). Some genes, such as those required for the production of fumonisins, gibberellins, and trichothecenes, have a limited distribution, occurring in only one or a few species complexes, whereas others occur in a wide range of species. For example, the PKS gene *PGL1*, which is required for production of a blackish perithecial pigment and a family of reddish mycelial pigments (fusarubins), was present in all fusaria examined in multiple studies (56, 61, 75). By contrast, some *Fusarium* secondary metabolite gene clusters exhibit a discontinuous distribution that does not necessarily correlate with phylogenetic relationships of species. For example, the narrowly

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distributed fumonisin and gibberellin gene clusters are present in some but not all species of the *F. fujikuroi* and *F. oxysporum* species complexes (11, 63), and fusarin biosynthetic genes, which are widely distributed in *Fusarium* (**Figure 4**), are absent in all *F. oxysporum* isolates that have been examined (56).

ROLE OF HORIZONTAL GENE TRANSFER IN THE EVOLUTION OF SECONDARY METABOLISM

The relatively large numbers of NRPS, PKS, and TS genes present per *Fusarium* genome beg the question, How many of these types of genes are present in the genus overall and how many classes of secondary metabolite does the genus produce? Existing genomic data do not provide a clear answer to this question, but instead indicate the potential diversity of secondary metabolite biosynthetic genes in *Fusarium*. Comparisons of five *Fusarium* genomes that span much of the phylogenetic diversity within the genus revealed that there are a similar number of NRPS and PKS genes per genome: 13 to 16 of each type of synthase gene per genome (12, 33, 50). Within each species, however, some of the genes are functional orthologs of genes in other species, and not surprisingly, more closely related species have more PKS and NRPS genes in common than more distantly related species. Collectively, the five genomes have 35 and 31 nonorthologous PKS and NRPS genes, respectively (12, 33), indicating that the five species have the collective potential to produce 35 and 31 distinct families of polyketide and nonribosomal peptide-derived secondary metabolites. Analysis of more genome sequences is therefore required for an accurate estimation of the numbers of nonorthologous NRPS, PKS, and TS genes in *Fusarium*, and this in turn will provide further insight into its potential metabolic diversity. Given the numbers of NRPS, PKS, and TS genes in the five genome sequences that have been analyzed in detail, it is likely that substantial numbers of additional *Fusarium* secondary metabolite gene clusters remain to be identified. Although understanding the role of each of these in the biology of the respective species will be extremely challenging, functional and comparative genomic approaches have and will continue to transform our understanding of their toxigenic potential.

The presence of 31 NRPS and 35 PKS genes in the collective genomes of *F. graminearum*, *F. oxysporum* f. sp. *lycopersici*, *F. pseudograminearum*, *F.* '*solani*' f. sp. *pisi*, and *F. verticillioides* raises another question, How did so many secondary metabolite synthase genes arrive in *Fusarium* if individual species have only 13 to 16 of each synthase? One possibility is that synthase genes were inherited vertically from a common ancestor. In such a scenario, the ancestral *Fusarium* would have had many more NRPS and PKS genes than extant species, and there would have been extensive loss of the genes during evolution of the genus. An alternative hypothesis is that some secondary metabolite biosynthetic genes clusters evolved from preexisting genes by duplication followed by functional divergence of the resulting paralogs. However, of the 35 PKS genes in four *Fusarium* genomes that have been examined in detail, only two are paralogous: the *F. oxysporum* f. sp. *lycopersici* genes FOXG_14850 and FOXG_15886 (12, 33). With the exception of the functional orthologs in some species, most *Fusarium* PKS genes are more closely related to PKS genes in other fungal genera than to other PKS genes in *Fusarium* (8, 12, 47). This, combined with the low frequency of paralogous PKS genes, suggests that gene duplication followed by divergence has contributed relatively little to metabolic diversity in *Fusarium* since it diverged from other fungi. A third explanation for the metabolic diversity of *Fusarium* is that biosynthetic gene clusters were acquired by horizontal gene transfer. Evidence in support of horizontal gene transfer includes presence of homologs of the fumonisin and gibberellin biosynthetic gene clusters, once thought to be unique to *Fusarium*, in the distantly related fungi *Aspergillus niger* (Eurotiomycetes) and *Sphaceloma manihoticola* (Dothideomycetes), respectively (10, 25, 44).

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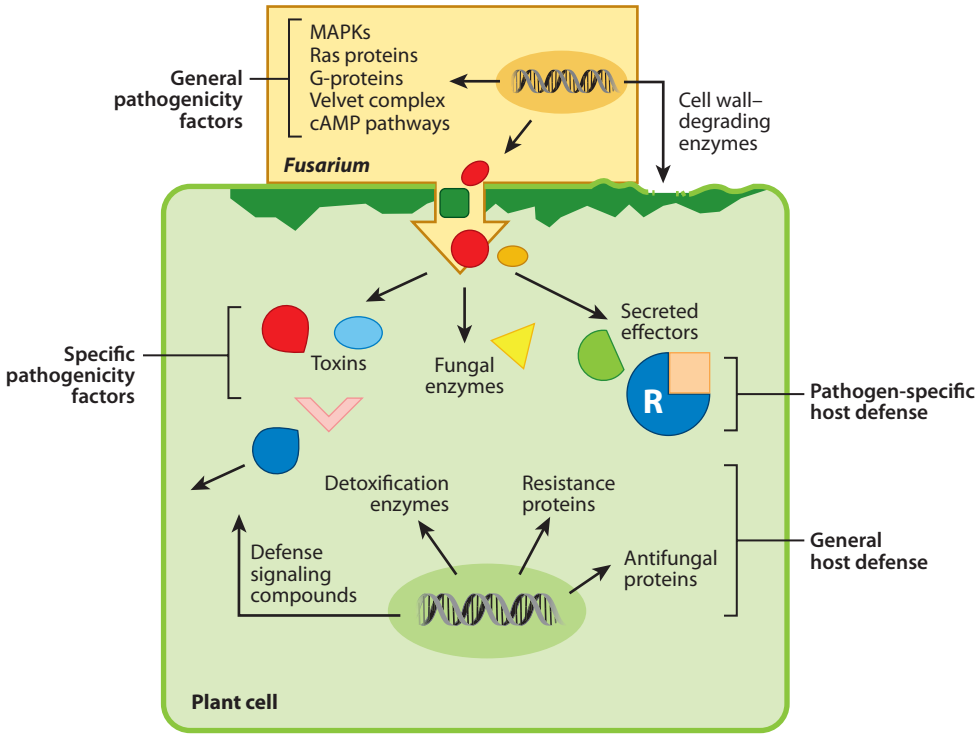

Figure 5

Fusarium pathogenicity and host defense mechanisms. *Fusarium* pathogens use both general and pathogen-specific pathogenicity mechanisms to invade their hosts. General pathogenicity factors may include components of cellular signaling pathways, which are often required for proper development, and fungal enzymes used for degradation of the plant cell wall. Specific virulence factors are employed by one or a few related *Fusarium* species and may include host-specific toxins and secreted effectors. Counter-defense mechanisms used by the host plant can also be both general (e.g., production of antifungal proteins and activation of defense signaling pathways) and pathogen specific (e.g., detoxification of pathogen-specific toxins and specific recognition of pathogen effectors by plant resistance gene products) (60).

Moreover, there is phylogenetic evidence that the gene cluster responsible for production of the pigment bikaverin was transferred from *Fusarium* to the gray mold fungus *Botrytis cinerea* (14).

EVOLUTIONARY PATHOGENOMICS

The forward and reverse genetic strategies employed to identify virulence/pathogenicity genes in *Fusarium* spp. are summarized in review articles (43, 53, 82). Two classes of pathogenicity genes are generalized here: basic pathogenicity genes, which are shared by *Fusarium* and other pathogenic fungi; and specialized pathogenicity genes, which in most cases are specific to individual *Fusarium* spp. on specific hosts.

Basic pathogenicity genes (**Figure 5**) encode essential components of pathways involved in sensing of exogenous or endogenous signals, such as the genes encoding various components of mitogen-activated protein kinase (MAPK) signaling pathways (24, 35, 79), Ras proteins (small GTPases) (9), G-protein signaling components and their downstream pathways (40, 58), components of the velvet (*LaeA/VeA/VelB*) complex (48, 83), and cAMP pathways (26). Mutations in

these genes often affect the fitness and pathogenicity of mutants. The pleiotropic effects observed in many of the fungal mutants modified for these factors can complicate efforts to understand the exact nature of the pathogen virulence mechanisms controlled by these pathways.

Specialized pathogenicity genes are directly involved in host-pathogen interactions. Among *Fusarium* pathogens, *F. oxysporum* f. sp. *lycopersici* has established a gene-for-gene interaction with its tomato host. Virulence factors, such as *SIX* (secreted in xylem) genes (37), play significant roles in determining host specificity. For instance, *AVR1* (=*SIX4*), *AVR2* (=*SIX3*) and *AVR3* (=*SIX1*) are the avirulence genes that interact with the tomato resistance genes *I-1* (*Immunity-1*), *I-2*, and *I-3*, respectively (36, 64). *SIX* genes are located on the *F. oxysporum* f. sp. *lycopersici* pathogenicity chromosomes discussed above, and it is proposed that the loss of *AVR1* enabled *F. oxysporum* f. sp. *lycopersici* race 1 strains to escape *I-1*-mediated disease resistance (36). Although extracellular effectors such as *SIX* genes were thought to be uncommon in *Fusarium* (43), reanalyses of the *F. graminearum* genome suggested the presence of hundreds of small secreted proteins, including possible effector molecules such as small, cysteine-rich, internal amino acid repeat-containing proteins (13). Not all of the candidate effectors are essential for species-specific fungus-plant interactions, as more than half of the putative effectors have homologs in other *Fusarium* species and some share homologs in a broad array of fungi and oomycetes (13).

Pathogenicity chromosomes: chromosomes that harbor virulence factors

***SIX*:** secreted in xylem

Other specialized virulence factors that act in a host- or pathogen-specific manner include mycotoxins. Trichothecene mycotoxins produced by certain *Fusarium* species, for instance, can promote virulence toward wheat (*Triticum* spp.) and maize (*Zea mays*) (7, 39, 62) but not barley (*Hordeum vulgare*) (41). Trichothecenes probably act via inhibition of protein translation and show elicitor-like activities that stimulate plant defense and promote plant cell death (23). Gibberellins produced by *F. fujikuroi*, the causal agent of bakanae disease of rice (*Oryza* spp.), cause characteristic developmental alterations in the host during disease development. Interestingly, surveys of other members of the *F. fujikuroi* species complex identified the complete gibberellin gene cluster in nearly every species, but gibberellins were detected only in *F. fujikuroi*, *F. sacchari*, and *F. konzum* (78).

Although many genes are involved in host-pathogen interactions, direct evidence of pathogenicity is hard to prove. For instance, all *Fusarium* genomes encode a suite of cell wall–degrading and other hydrolytic enzymes presumed to be deployed during infection to gain access to nutrition. Perhaps due to the redundancy of these degrading enzymes or the adaptability of the pathogens to utilize different nutrient sources, very few genes of this class have been directly connected to pathogenicity. One notable exception is the secreted lipase FGL1, which contributes to *F. graminearum* virulence on wheat, barley, and maize (39, 81). Furthermore, overexpression of the *FGL1* gene partially restored virulence of a nonpathogenic mitogen-activated kinase mutant (*Δgpmk1*) on wheat (67).

COMPARATIVE GENOMICS OF REPRODUCTIVE STRATEGIES

The available complete genome sequences in *Fusarium* represent much of the known spectrum of diversity in reproductive strategies in the genus (**Figure 2**), presenting excellent opportunities to elucidate their underlying developmental genetics. Studies of these processes have fallen into two categories: large-scale knockout projects of gene families and expression studies.

Genome-based studies of asexual sporulation in *Fusarium* remain relatively limited. Analysis of gene expression during conidium development and germination has been performed in several species, including *F. graminearum* and *F. oxysporum* f. sp. *lycopersici* using Affymetrix GeneChips (69) and expressed sequence tags (38), respectively. Comparative next-generation transcriptomic studies of asexual spore development and function, particularly regarding comparisons among

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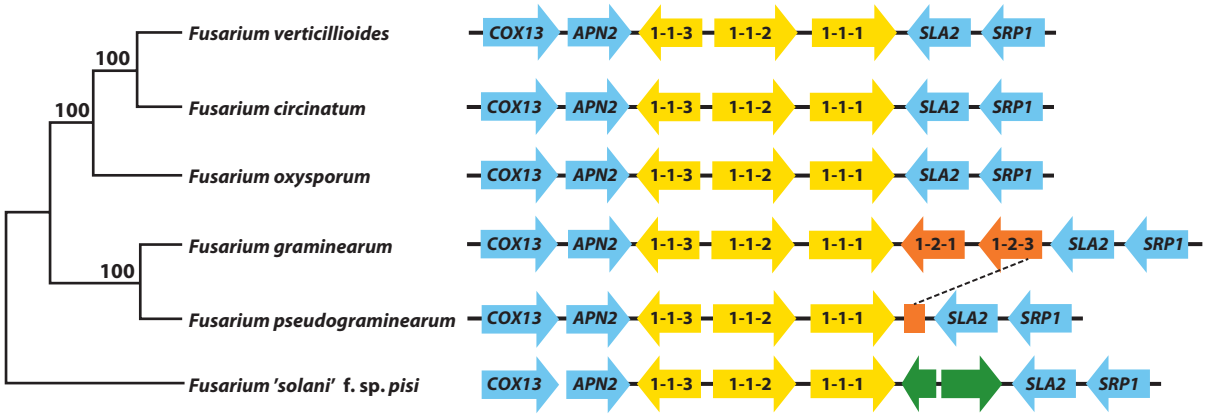

Figure 6

Comparison of the mating-type locus in *Fusarium* genomes. The cladogram on the left shows the phylogenetic relationships inferred from maximum parsimony analysis of exon sequences of genes in the *MAT1-1* idiomorph. *MAT1-1* idiomorph genes (*MAT1-1-1*, *MAT1-1-2*, and *MAT1-1-3*) are depicted as yellow arrows; *MAT1-2* idiomorph genes (*MAT1-2-1*, *MAT1-2-3*) are depicted as orange arrows; genes that flank the *MAT* locus are depicted as blue arrows; flanking genes unique to *F.* '*solani*' f. sp. *pisi* are depicted as green arrows. *MAT1-1* and *MAT1-2* idiomorphs are present at the *MAT* locus in the homothallic species *F. graminearum*. The genome sequences of all the heterothallic species shown possess the *MAT1-1* idiomorph. Strains of these species that are of the opposite mating type possess the *MAT1-2* idiomorph; that is, they possess only the two *MAT1-2* genes (*MAT1-2-1*, and *MAT1-2-2*) located between *APN2* and *SLA2*.

the major mitotic spore types, are lacking. Only 4 transcription factors were found to affect conidiation alone, whereas 13 were specific to sexual development (74). This difference may reflect the relative complexity of meiosis and fruiting body development compared with conidiation.

More attention has been given to sexual sporulation. Of the five species with fully sequenced genomes, sexual cycles are known for all species except *F. oxysporum*. Typical of Ascomycota, mating compatibility is determined by a single locus (*MAT1*) in *Fusarium*. In heterothallic species, individual isolates possess either *MAT1-1* or *MAT1-2* genes (idiomorphs) (52) at *MAT1*, and mating isolates must be of different idiomorphs; in the homothallic *F. graminearum*, both *MAT1-1* and *MAT1-2* genes occupy the *MAT1* locus (87) (**Figure 6**). *F. oxysporum* isolates possess either *MAT1-1* or *MAT1-2* mating-type idiomorphs, and through heterologous expression in other fusaria, they have been shown to be functional (5, 87). This finding suggests that a cryptic sexual cycle may be present within the *F. oxysporum* species complex. Coincidentally, the four heterothallic species for which complete genome sequences are available are from *MAT1-1* isolates. Phylogenetic analysis of *MAT1-1* exons resolves the same phylogenetic relationships as those inferred from other genes within the core genome (56) (**Figures 1** and **6**).

Transcriptomic studies of sexual sporulation have focused primarily on *F. graminearum*. In addition to its importance in promoting genetic variation, the ascospore stage is essential in the disease cycle of *F. graminearum* (68). *F. graminearum* overwinters on crop stubble and produces ascospores in the spring when its hosts are in full flower. Ascospores are forcibly ejected into the air column, which facilitates their dispersal to susceptible hosts. A functional analysis of all predicted transcription factors in the genome of *F. graminearum* was performed (74), covering 657 genes and investigating 17 phenotypes, including perithecium formation and function. Surprisingly, of 170 transcription factor deletion mutants that exhibited clear mutant phenotypes, 105 showed defects in sexual development: 44 did not initiate the sexual cycle, 4 were enhanced in perithecium production, 34 initiated perithecium production but did not produce normal ascospores, and

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23 produced normal ascospores but fewer perithecia. This study demonstrates that regulation of the sexual cycle is complex.

Gene expression studies comparing perithecium development in the homothallic *F. graminearum* versus the heterothallic *F. verticillioides* indicate that we have only scratched the surface in understanding the genetics underlying the sexual cycle. A study using the Affymetrix *Fusarium* GeneChip across five stages of development revealed approximately 2,000 genes that were expressed uniquely in sexual development (31). A comparative study of gene expression across these same developmental stages in *F. graminearum* and *F. verticillioides* revealed that more than half of the genes expressed during sexual development encode unclassified proteins (71). Taken together, these results suggest that the genetic underpinnings of the sexual cycle, possibly involving the activity of a large portion of the genome, remain mostly uncharacterized. Comparisons of gene expression in sexual development among *Fusarium* species, as well as with other fungal genera, will surely yield important information on the genes involved and clarify the evolutionary processes that generated the diverse array of morphologies and life-history strategies seen in the Ascomycota.

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SUMMARY POINTS

1. Comparative analyses have revealed that *Fusarium* genomes are compartmentalized into regions responsible for essential functions (core genome) and for host specialization and pathogen virulence (adaptive/accessory genome).
2. Horizontal inheritance of supernumerary chromosomes enriched for host-specific virulence may have played a major role in the polyphyletic distribution of host specificity within the *F. oxysporum* species complex and the rapid emergence of novel pathogens.
3. Comparative genome analyses suggest that *Fusarium* has the genetic potential to produce many more secondary metabolites than previously indicated by chemical analyses.
4. Multiple evolutionary processes, including vertical inheritance, horizontal gene transfer, gene duplication, and gene deletion, could have given rise to the current distribution of secondary metabolite biosynthetic gene clusters and production in *Fusarium*.
5. Owing to the often minor contributions of individual genes to pathogenicity and their tendency to have pleiotropic effects, assigning definitive roles in virulence can be a challenging task.
6. Deletion of genes with roles in the production of asexual and sexual spore types tends to have pleiotropic effects. Transcriptomic studies indicate that a large number of genes are differentially expressed in the sexual cycle.

FUTURE ISSUES

1. Comparative phylogenomic studies that sample the full diversity of *Fusarium* are needed to elucidate the origin and diversity of toxic secondary metabolites and to identify phylogenetically informative genes. Genomics will aid the discovery of additional informative genes needed to develop a highly resolved phylogenetic framework for *Fusarium*, resolve species boundaries, and develop robust molecular diagnostics in support of agricultural biosecurity.

2. Fully characterizing the phylogenetic distribution of horizontal chromosome transfer within *Fusarium* should help elucidate what genetic mechanism(s) mediates the integration, regulation, and long-term maintenance of accessory and core genomes.
3. There is a need to develop novel forward genetic approaches directed at discovering and overexpressing genes that detoxify mycotoxins without disrupting normal plant development. This approach provides a vehicle for developing cultivars with broad-based resistance to diverse toxin-associated fusarial diseases.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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50. Confirms lateral transfer of pathogenicity chromosomes experimentally and computationally and suggests *forma specialis* polyphyly may be explained by widespread horizontal chromosome transfer within the *F. oxysporum* species complex.

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56. Provides the first robust phylogeny for *Fusarium*, giving a blueprint for future comparative phylogenomics within the genus.

59. Develops internet resource to support archiving and utilization of fusarial genomes together with genome browsing and analysis.

60. Shows that UDP-glycosyltransferase (DOGT1) from *Arabidopsis thaliana* detoxified 3- and 15-acetyl deoxynivalenol (DON), but not nivalenol.

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RELATED RESOURCES

Fusarium 'solani' f. sp. *pisi* (*'Nectria haematococca'*) Genome Browser. **http://genome.jgi-psf.org/Necha2/Necha2.home.html**

Fusarium Comparative Database. **http://www.broadinstitute.org/**

Fusarium Comparative Genomics Platform. **http://www.Fusariumdb.org/index.php**

Fusarium MLST Database. **http://www.cbs.knaw.nl/Fusarium/**

Fusarium-ID. **http://isolate.fusariumdb.org/**

MIPS *Fusarium graminearum* Genome Database. **http://mips.helmholtz-muenchen.de/genre/proj/FGDB/**

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Errata

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