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Comparative genomics reveals mobile pathogenicity chromosomes in Fusarium


Fusarium species are among the most important phytopathogenic and toxigenic fungi. To understand the molecular underpinnings of pathogenicity in the genus Fusarium, we compared the genomes of three phenotypically diverse species: Fusarium graminearum, Fusarium verticillioides and Fusarium oxysporum f. sp. lycopersici. Our analysis revealed lineage-specific (LS) genomic regions in F. oxysporum that include four entire chromosomes and account for more than one-quarter of the genome. LS regions are rich in transposons and genes with distinct evolutionary profiles but related to pathogenicity, indicative of horizontal acquisition. Experimentally, we demonstrate the transfer of two LS chromosomes between strains of F. oxysporum, converting a non-pathogenic strain into a pathogen. Transfer of LS chromosomes between otherwise genetically isolated strains explains the polyphyletic origin of host specificity and the emergence of new pathogenic lineages in F. oxysporum. These findings put the evolution of fungal pathogenicity into a new perspective.

Results

Genome organization and gene clusters. We sequenced Fv strain 7600 and Foli strain 4287 (Methods, Supplementary Table 1) using a whole-genome shotgun approach and assembled the sequence using Arachne (Table 1, ref. 6). Chromosome level ordering of the scaffolds was achieved by anchoring the assemblies either to a genetic map for Fv (ref. 7), or an optical map for Foli (Supplementary Information A and Supplementary Table 2). We predicted Fol and Fv genes and re-annotated a new assembly of the Fg genome using a combination of manual and automated annotation (Supplementary Information B).

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The Fol genome (60 megabases) is about 44% larger than that of its most closely related species, Fv (42 Mb), and 65% larger than that of Fg (36 Mb), resulting in a greater number of protein-encoding genes in Fol (Table 1).

The relatedness of the three Fusarium genomes enabled the generation of large-scale unambiguous alignments (Supplementary Figs 1–3) and the determination of orthologous gene sets with high confidence (Methods, Supplementary Information C). On average, Fol and Fv orthologues display 91% nucleotide sequence identity, and both have 85% identity with Fg counterparts (Supplementary Fig. 4). Over 9,000 conserved syntenic orthologues were identified among the three genomes. Compared to other ascomycete genomes, these three-species orthologues are enriched for predicted transcription factors ($P = 2.6 \times 10^{-8}$), lytic enzymes ($P = 0.001$), and transmembrane transporters ($P = 7 \times 10^{-9}$) (Supplementary Information C and Supplementary Tables 3–8), in agreement with results reported for the Fg genome.

Fusarium species produce diverse secondary metabolites, including mycotoxins that exhibit toxicity to humans and other mammals. In the three genomes, we identified a total of 46 secondary metabolite biosynthesis (SMB) gene clusters. Microarray analyses confirmed the co-expression of genes in 14 of 18 Fg and 10 of 16 Fv SMB gene clusters. Ten out of the 14 Fg and eight out of the 10 Fv co-expressed SMB gene clusters are novel (Supplementary Information D, Supplementary Fig. 5 and Supplementary Table 9, and online materials), emphasizing the potential impact of uncharacterized secondary metabolites on fungal biology.

**Lineage-specific chromosomes and pathogenicity.** The genome assembly of Fol has 15 chromosomes, the Fv assembly 11 and the Fg assembly only four (Table 1). The smaller number of chromosomes in Fg is the result of chromosome fusion relative to Fv and Fo, and fusion sites in Fg match previously described high diversity regions (Supplementary Fig. 3, ref. 5). Global comparison among the three Fusarium genomes shows that the increased genomic territory in Fol is due to additional, unique sequences that reside mostly in extra chromosomes. Syntenic regions in Fol cover approximately 80% of the Fg and more than 90% of the Fv genome (Supplementary Information E and Supplementary Table 10), referred to as the ‘core’ of the genomes. Except for telomere-proximal regions, all 11 mapped chromosomes in the Fv assembly (41.1 Mb) correspond to 11 of the 15 chromosomes in Fol (41.8 Mb). The co-linear order of genes between Fol and Fv has been maintained within these chromosomes, except for one chromosomal translocation event and a few local rearrangements (Fig. 2a).

The unique sequences of Fol are a substantial fraction (40%) of the Fol assembly, designated as Fol lineage-specific (Fol LS) regions, to distinguish them from the conserved core genome. The Fol LS regions include four entire chromosomes (chromosomes 3, 6, 14 and 15), parts of chromosome 1 and 2 (scaffold 27 and scaffold 31, respectively), and most of the small scaffolds not anchored to the optical map (Fig. 2b). In total, the Fol LS regions encompass 19 Mb, accounting for nearly all of the larger genome size of Fol.

Notably, the LS regions contain more than 74% of the identifiable transposable elements (TEs) in the Fol genome, including 95% of all DNA transposons (Fig. 2b, Supplementary Fig. 6 and Supplementary Table 11). In contrast to the low content of repetitive sequence and...
The minimal amount of TEs in the Fv and Fg genomes (Table 1 and Supplementary Table 11), about 28% of the entire Fol genome was identified as repetitive sequence (Methods), including many retro-elements (copia-like and gypsy-like LTR retrotransposons, LINEs (long interspersed nuclear elements) and SINEs (short interspersed nuclear elements) and DNA transposons (Tc1-mariner, hAT-like, Mutator-like, and MITEs) (Supplementary Information E.3), as well as several large segmental duplications. Many of the TEs are full-length and present as highly similar copies. Particularly well represented DNA transposon classes in Fol are pogo, hAT-like elements and MITEs (in total approximately 550, 200 and 350 copies, respectively). In addition, there are one intra-chromosomal and two inter-chromosomal segmental duplications, totalling approximately 7 Mb and resulting in three- or even fourfold duplications of some regions (Fig. 2c). Overall, these regions share 99% sequence identity (Supplementary Fig. 7), indicating recent duplication events.

Only 20% of the predicted genes in the Fol LS regions could be functionally classified on the basis of homology to known proteins. These genes are significantly enriched ($P < 0.0001$) for the functional categories ‘secreted effectors and virulence factors’, ‘transcription factors’, and ‘proteins involved in signal transduction’, but are deficient in genes for house-keeping functions (Supplementary Information E and Supplementary Tables 12–18). Among the genes with a predicted function related to pathogenicity were known effector proteins (see below) as well as necrosis and ethylene-inducing peptides and a variety of secreted enzymes predicted to degrade or modify plant or fungal cell walls (Supplementary information E and Supplementary Tables 14, 15). Notably, many of these enzymes are expressed during early stages of tomato root infection (Supplementary Tables 15, 16 and Supplementary Fig. 8). The expansion of genes for lipid metabolism and lipid-derived secondary messengers in Fol LS regions indicates an important role for lipid signalling in fungal pathogenicity (Supplementary Fig. 9 and Supplementary Tables 13, 17). A family of transcription factor sequences related to FTF1, a gene transcribed specifically during early stages of infection of F. oxysporum f. sp. phaseoli (Supplementary Information E and Supplementary Table 4; ref. 10) is also expanded.

The recently published genome of F. solani, a more diverged species, enabled us to extend comparative analysis to a larger evolutionary framework (Fig. 1). Whereas the ‘core’ genomes are well conserved among all four sequenced Fusarium species, the Fol LS regions are also absent in Fs (Supplementary Fig. 2). Additionally, Fv has three LS chromosomes distinct from the genome core and the Fol LS regions. In conclusion, each of the four Fusarium species blocks; row ii displays the syntenic mapping of Fv chromosomes, with one major translocation between chr 4/chr 12 in Fol and chr 4/chr 8 in Fv; row iii represents the density of transposable elements calculated with a 10 kb window. LS chromosomes include four entire chromosomes (chr 3, chr 6, chr 14 and chr 15) and parts of chromosome 1 and 2 (scaffold 27, scaffold 31), which lack similarity to syntenic chromosomes in Fv but are enriched for TEs. c, Two of the four Fol LS chromosomes showing the inter- (green) and intra- (yellow) chromosomal segmental duplications. The three traces below are density distribution of TEs (blue lines), secreted protein genes (green lines) and lipid metabolism related genes (red line). Chr, chromosomes; Un, unmapped.

Figure 2 | Whole genome comparison between Fv and Fol. a, Argo® dotplot of pair-wise MEGABLAST alignment (1 x 10^-15) between Fv and Fol showing chromosome correspondences between the two genomes in the black dashed boxes. The vertical blue lines illustrate the chromosomal translocations, and the red dashed horizontal boxes highlight the Fol LS chromosomes. b, Global view of syntenic alignments between Fol and Fv and the distribution of transposable elements. Fol linkage groups are shown as the reference, and the length of the light grey background for each linkage group is defined by the Fol optical map. For each chromosome, row i represents the genomic scaffolds positioned on the optical linkage groups separated by scaffold breaks. Scaffold numbers for Fol are given above the
carries a core genome with a high level of synteny whereas Fol and Fs each have LS chromosomes that are distinct with regard to repetitive sequences and genes related to host–pathogen interactions.

**Origin of LS regions.** Three possible explanations for the origin of LS regions in the Fol genome were considered: (1) Fol LS regions were present in the last common ancestor of the four Fusarium species but were then selectively and independently lost in Fv, Fg and Fs lineages during vertical transmission; (2) LS regions arose from the core genome by duplication and divergence within the Fol lineage; and (3) LS regions were acquired by horizontal transfer. To distinguish among these hypotheses, we compared the sequence characteristics of the genes in the Fol LS regions to those of genes in Fusarium core regions and genes in other filamentous fungi. If Fol LS genes have clear orthologues in the other Fusarium species, or paralogues in the core region of Fol, this would favour the vertical transmission or duplication with divergence hypotheses, respectively. We found that, whereas 90% of the Fol genes in the core regions have homologues in the other two Fusarium genomes, about 50% of the genes on Fol LS regions lack homologues in either Fv or Fg (1 × 10⁻²⁸). Furthermore, there is less sequence divergence between Fol and Fv orthologues in core regions compared to Fol and Fg orthologues (Fig. 3a), consistent with the species phylogeny. In contrast, the LS genes that have homologues in the other Fusarium species are roughly equally distant from both Fv and Fg genes (Fig. 3b), indicating that the phylogenetic history of the LS genes differs from genes in the core region of the genome.

Both codon usage tables and codon adjustment index (CAI) analysis indicate that the LS-encoding genes exhibit distinct codon usage (Supplementary Information E.5, Supplementary Fig. 10 and Supplementary Table 19) compared to the conserved genes and the non-pathogenic F. oxysporum isolates (Supplementary Fig. 14), indicating that Fol LS chromosomes may be present in strains causing the tomato xylem system and at least two of these, Six1 (Avr3) and Six3 (Avr2), are involved in virulence functions. Interestingly, the genes for these proteins, as well as a gene for an in planta-secreted oxoreductase (ORX1), are located on chromosome 14, one of the Fol LS chromosomes. These genes are all conserved in strains causing tomato wilt, but are generally not present in other strains. The genome data enabled the identification of the genes for three additional small in planta-secreted proteins on chromosome 14, named non-pathogenic F. oxysporum isolates (Supplementary Fig. 14), indicating that Fol LS chromosomes may also be specifically involved in pathogenic adaptation.

**Transfer of Fol pathogenicity chromosomes.** It is well documented that small proteins are secreted during Fol colonizing the tomato xylem system and at least two of these, Six1 (Avr3) and Six3 (Avr2), are involved in virulence functions. Interestingly, the genes for these proteins, as well as a gene for an in planta-secreted oxoreductase (ORX1), are located on chromosome 14, one of the Fol LS chromosomes. These genes are all conserved in strains causing tomato wilt, but are generally not present in other strains. The genome data enabled the identification of the genes for three additional small in planta-secreted proteins on chromosome 14, named...
SIX5, SIX6 and SIX7 (Supplementary Table 22) based on mass spectrometry data obtained previously\(^{20}\). Together these seven genes can be used as markers to identify each of the three supercontigs (SC 22, 36 and 51) localized to chromosome 14 (Supplementary Table 23 and Supplementary Fig. 15).

In view of the combined experimental findings and computational evidence, we proposed that LS chromosome 14 could be responsible for pathogenicity of Fol towards tomato, and that its mobility between strains could explain its presence in tomato wilt pathogens, comprising several clonal lineages polyphyletic within the Fo species complex, but absent in other lineages\(^{24}\). To test these hypotheses, we investigated whether chromosome 14 could be transferred and whether the transfer would shift pathogenicity between different strains of Fo, using the genes for in planta-secreting proteins on chromosome 14 as markers. Fo007, a strain that is able to cause tomato wilt, was co-incubated with a non-pathogenic isolate (Fom) or banana (Foc), respectively. A gene conferring resistance against zeocin (BLE) was inserted close to SIX1 as a marker to select for transfer of chromosome 14 from the donor strain into Fo-47, Fom or Foc. The receiving strains were transformed with a hygromycin resistance gene (HYG), inserted randomly into the genome; three independent hygromycin resistant transformants per recipient strain were selected. Microconidia of the different strains were isolated and mixed in a 1:1 ratio on agar plates. Spores emerging on these plates after 6–8 days of incubation were selected for resistance to both zeocin and hygromycin. Double drug-resistant colonies were recovered with Fom and Fo-47, but not using Foc as the recipient, at a frequency of roughly 0.1 to 10 per million spores (Supplementary Table 24).

Pathogenicity assays demonstrated that double drug-resistant strains derived from co-inoculating Fo007 with Fo-47, referred to as Fo-47\(^+\), had gained the ability to infect tomato to various degrees (Fig. 4a, b). In contrast, none of the double drug-resistant strains derived from co-incubating Fo007 with Fom were able to infect tomato. All Fo-47\(^+\) strains contained large portions of Fol chromosome 14 as demonstrated by PCR amplification of the seven gene markers (Fig. 4c, Supplementary Fig. 15 and Supplementary Information F). The parental strains, as well as the sequenced strain Fol4287, each have distinct karyotypes. This enabled us to determine with chromosome electrophoresis whether the entire chromosome 14 of Fo007 was transferred into Fo-47\(^+\) strains. All Fo-47\(^+\) strains had the same karyotype as Fo-47, except for the presence of one or two additional small chromosomes (Fig. 4d). The chromosome present in all Fo-47\(^+\) strains (Fig. 4d, arrow number 1) was confirmed to be chromosome 14 from Fo007 based on its size and a Southern hybridization using a SIX6 probe (Fig. 4e). Interestingly, two double drug-resistant strains (Fo-47\(^+\) 1C and Fo-47\(^+\) 2A in Fig. 4a), which caused the highest level of disease (Fig. 4a, b), have a second extra chromosome, corresponding in size to the smallest chromosome in the donor strain Fo007 (Fig. 4d, arrow number 2).

To rigorously assess whether additional genetic material other than chromosome 14 may have been transferred from Fo007 into Fo-47\(^+\) strains, we developed PCR primers for amplification of 29 chromosome-specific markers from Fo007 but not Fo-47. These markers (on average two for each chromosome) were used to screen Fo-47\(^+\) strains for the presence of Fo007-derived genomic regions (Supplementary information F.4 and Supplementary Fig. 16). All Fo-47\(^+\) strains were shown to have the chromosome 14 markers (Supplementary Fig. 17), but not Fo007 markers located on any core chromosome, confirming that core chromosomes were not transferred. Interestingly, the two Fo-47\(^+\) strains (1C and 2A) that have the second small chromosome and caused more disease symptoms were also positive for an additional Fo007 marker (Supplementary Fig. 17), associated with a large duplicated LS region in Fol4287: scaffold 18 (1.3 Mb on chromosome 3) and scaffold 21 (1.0 Mb on chromosome 6) (Fig. 2c). The presence of most or all of the sequence of scaffold 18/21 in strains 1C and 2A was confirmed with an additional nine primer pairs for genetic markers scattered over this region (data not shown, see Supplementary Tables 25a, b for primer sequences) (Fig. 4d).

Taken together, we conclude that pathogenicity of Fo-47\(^+\) strains towards tomato can be specifically attributed to the acquisition of Fol chromosome 14.
chromosome 14, which contains all known genes for small in planta-secreted proteins. In addition, genes on other LS chromosomes may further enhance virulence as demonstrated by the two strains containing the additional LS chromosome from Fol007. We did not find a double drug-resistant strain with a tagged chromosome of Fo-47 in the Fol007 background. Also, a randomly tagged transformant of Fol007 did not render any double drug-resistant colonies when co-incubated with Fo-47 (data not shown). This indicates that transfer between strains may be restricted to certain chromosomes, perhaps determined by various factors, including size and TE content of the chromosome. Their propensity for transfer is supported by the fact that the smallest LS chromosome in Fol007 moved to Fo-47 without being selected for drug resistance in two out of nine cases.

Discussion

Comparison of Fusarium genomes revealed a remarkable genome organization and dynamics of the assexual species Fol. This tomato pathogen contains four unique chromosomes making up more than one-quarter of its genome. Sequence characteristics of the genes in the LS regions indicate a distinct evolutionary origin of these regions. Experimentally, we have demonstrated the transfer of entire LS chromosome(s) through simple co-incubation between two otherwise genetically isolated members of Fol. The relative ease by which new tomato pathogenic genotypes are generated supports the hypothesis that such transfer between Fo strains may have occurred in nature and has a direct impact on our understanding of the evolving nature of fungal pathogens. Although rare, horizontal gene transfer has been documented in other eukaryotes, including metazoans. However, spontaneous horizontal transfer of such a large portion of a genome and the direct demonstration of associated transfer of host-specific pathogenicity has not been previously reported.

Horizontal transfer of host specificity factors between otherwise distant and genetically isolated lineages of Fo may explain the apparent polyphyletic origins of host specialization and the rapid emergence of new pathogenic lineages in otherwise distinct and incompatible genetic backgrounds. Fol LS regions are enriched for genes related to host–pathogen interactions. The mobilization of these chromosomes could, in a single event, transfer an entire suite of genes required for host compatibility to a new genetic lineage. If the recipient lineage were able to co-opt genes with different co-evolutionary histories, such a transfer could increase the overall incidence of disease in the host by introducing pathogenicity in a genetic background pre-adapted to a local environment. Such knowledge of the mechanisms underpinning rapid path adaptation will affect the development of strategies for disease management in agricultural settings.

METHODS SUMMARY

Generation of genome sequencing and assembly. The whole genome shotgun (WGS) assemblies of Fo (8 × coverage) and Fol (6.8 × coverage) were generated using Sanger sequencing technology and assembled using Arachne. Physical maps were created by anchoring the assemblies to the Fo genetic linkage map and to the Fol optical map, respectively.

Defining hierarchical synteny. Local-assignment anchors were detected using PatternHunter (1 X 10^5) (ref. 29). Contiguous sets of anchors with conserved order and orientation were chained together within 10 kb distance and filtered to ensure that no block overlaps another block by more than 90% of its length.

Identification of repetitive sequences. Repeats were detected by searching the genome sequence against itself using CrossMatch (≥ 200 bp and ≥ 60% sequence similarity). Full-length TEs were annotated using a combination of computational predictions and manual inspection. Large segmental duplications were identified using Map Aligner.

Characterization of proteomes. Orthologous genes were determined based on BLASTP and pair-wise syntenic alignments (SI). The blast score ratio tests were used to compare relatedness of proteins among three genomes. The EMBOSS tool ‘cup’ (http://emboss.sourceforge.net/) was used to calculate codon usage frequencies. Gene Ontology terms were assigned using Blast2GO software (BLASTP 1 X 10^6 and tested for enrichment using Fisher’s exact test, corrected for multiple testing). A combination of homology search and manual inspection was used to characterize gene families. Potentially secreted proteins were identified using SignalP (http://www.cbs.dtu.dk/services/SignalP/) after removing trans-membrane/mitochondrial proteins based with TMHMM (http://www.cbs.dtu.dk/services/TMHMM/), Phobius (except in the first 50 amino acids), and TargetP (RC score 1 or 2) predictions. Small cysteine-rich secreted proteins were defined as secreted proteins that are less than 200 amino acids in length and contain at least 4% cysteine residues. GPI (glycosylphosphatidylinositol)-anchor proteins were identified by the GPI-anchor attachment signal among the predicted secreted proteins using a custom PERL script.

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**Supplementary Information** is linked to the online version of the paper at www.nature.com/nature.

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**Author Information** All sequence reads can be downloaded from the NCBI trace repository. The assemblies of Fv and Fol have been deposited at GenBank under the project accessions AAIM02000000 and AAXH01000000. Detailed information can be accessed through the Broad Fusarium comparative website: http://www.broad.mit.edu/annotation/genome/fusarium_group.3/MultiHome.html. Reprints and permissions information is available at www.nature.com/reprints. This paper is distributed under the terms of the Creative Commons Attribution-Non-Commercial-Share Alike licence, and is freely available to all readers at www.nature.com/nature. The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to H.C.K. (hckist@umn.edu) or M.R. (m.rep@uva.nl).