

# Population genomics reveals historical and ongoing recombination in the *Fusarium oxysporum* species complex

A.R. McTaggart<sup>1\*</sup>, T.Y. James<sup>2</sup>, R.G. Shivas<sup>3</sup>, A. Drenth<sup>1</sup>, B.D. Wingfield<sup>4</sup>, B.A. Summerell<sup>5</sup>, and T.A. Duong<sup>4</sup>

<sup>1</sup>Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Ecosciences Precinct, Dutton Park, 4102, Queensland, Australia; <sup>2</sup>Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI, 48109, USA; <sup>3</sup>Centre for Crop Health, Institute for Life Sciences and the Environment, University of Southern Queensland, Toowoomba, 4350, Australia; <sup>4</sup>Department of Biochemistry, Genetics and Microbiology, Tree Protection Co-operative Programme (TPCP), Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa; <sup>5</sup>Australian Institute of Botanical Science, Royal Botanic Gardens & Domain Trust, Sydney, Australia

\*Correspondence: A.R. McTaggart, [alistair.mctaggart@gmail.com](mailto:alistair.mctaggart@gmail.com)

**Abstract:** The *Fusarium oxysporum* species complex (FOSC) is a group of closely related plant pathogens long-considered strictly clonal, as sexual stages have never been recorded. Several studies have questioned whether recombination occurs in FOSC, and if it occurs its nature and frequency are unknown. We analysed 410 assembled genomes to answer whether FOSC diversified by occasional sexual reproduction interspersed with numerous cycles of asexual reproduction akin to a model of predominant clonal evolution (PCE). We tested the hypothesis that sexual reproduction occurred in the evolutionary history of FOSC by examining the distribution of idiomorphs at the mating locus, phylogenetic conflict and independent measures of recombination from genome-wide SNPs and genes. A phylogenomic dataset of 40 single copy orthologs was used to define structure *a priori* within FOSC based on genealogical concordance. Recombination within FOSC was tested using the pairwise homoplasy index and divergence ages were estimated by molecular dating. We called SNPs from assembled genomes using a k-mer approach and tested for significant linkage disequilibrium as an indication of PCE. We clone-corrected and tested whether SNPs were randomly associated as an indication of recombination. Our analyses provide evidence for sexual or parasexual reproduction within, but not between, clades of FOSC that diversified from a most recent common ancestor about 500 000 years ago. There was no evidence of substructure based on geography or host that might indicate how clades diversified. Competing evolutionary hypotheses for FOSC are discussed in the context of our results.

**Key words:** Ascomycota, Clonal reproduction, Index of association, Phylogenomic networks, Phylogenomics, Population genomics, Sexual reproduction, Taxonomic boundaries.

Available online 17 December 2021; <https://doi.org/10.1016/j.simyco.2021.100132>.

## INTRODUCTION

Fungi possess different reproductive strategies to increase their survival. Most fungi can reproduce mitotically, which facilitates rapid proliferation and maintains successful genotypes. Many fungi intersperse extended bouts of asexual reproduction with occasional sexual reproduction (Tsai *et al.* 2008, Tibayrenc & Ayala 2012, Taylor *et al.* 2015, Nieuwenhuis & James 2016) resulting in life cycles with many more asexual than sexual generations, termed predominant clonal evolution (PCE) (Tibayrenc & Ayala 2021).

The PCE model assumes clonal evolution is dominant and genetic recombination is rare, but not absent. Clonality is evidenced by statistically significant linkage disequilibrium and the presence of identical or near identical multilocus genotypes over space and time (Tibayrenc & Ayala 2012, Tibayrenc & Ayala 2014, Tibayrenc & Ayala 2017). Very few, if any, fungal species have forgone sexuality entirely, and most exchange genetic material through sexual and/or parasexual reproduction, hybridisation and/or horizontal gene transfer (Taylor *et al.* 2015, Nieuwenhuis & James 2016, Steenkamp *et al.* 2018). The occurrence of genetic recombination events is not always clear in fungal plant pathogens, especially where asexual stages dominate the life cycle (Taylor *et al.* 2015, Drenth *et al.* 2019).

Virulent fungal genotypes benefit from asexual recombination, which preserves favourable combinations of alleles over multiple generations, and avoids recombinational load. Asexual populations in general evolve through mutation and non-recombinant sharing of DNA (for example, Ma *et al.* 2010). Asexual populations may show low levels of recombination through inbreeding, *i.e.* haploid selfing (Billiard *et al.* 2012), with no or scarce evidence of sexual recombination (Tibayrenc & Ayala 2012, Tibayrenc & Ayala 2017). Geographic isolation, disruption of life cycles, or other mechanisms that structure populations, may restrain recombination. Evidence for reduced recombination in fungal populations is not evidence for the absence of sex in fungal populations.

The *Fusarium oxysporum* species complex (FOSC) is a ubiquitous and cosmopolitan group of fungi (Summerell 2019). FOSC includes economically and medically important pathogens (Dean *et al.* 2012) and mycotoxigenic fungi (Munkvold *et al.* 2019) that impact agriculture, horticulture, and human and animal health (O'Donnell *et al.* 2004, O'Donnell *et al.* 2016).

FOSC has been cited as an example in *Fungi* of evolution by restrained recombination (Tibayrenc & Ayala 2012). FOSC were thought to have evolved as independent clonal lineages (Gordon & Martyn 1997, Koenig *et al.* 1997). More recently, competing taxonomic hypotheses have treated FOSC as either a complex

of more than 10 closely-related, asexual taxa (Lombard *et al.* 2019, Maryani *et al.* 2019), or fewer than five phylogenetic groups (Laurence *et al.* 2014, Brankovics *et al.* 2017, Achari *et al.* 2020). Each of these hypotheses depends on gene selection, taxon sampling and species criteria.

Studies on genetic diversity and plasticity indicate that FOSC is comprised of clonal populations that have exchanged DNA through homologous recombination of mitochondrial genomes and horizontal sharing of nuclear chromosomes. O'Donnell *et al.* (2004) showed that opposite mating types were present in populations of FOSC in the United States and questioned whether MAT genes were functional. Ma *et al.* (2010) showed that *F. oxysporum* shared accessory chromosomes through horizontal chromosome transfer. Laurence *et al.* (2015) and van Dam & Rep (2017) used phylogenetic incongruence of genes and mobile elements to demonstrate the natural occurrence of horizontal gene transfer in FOSC. Vlaardingerbroek *et al.* (2016) found horizontal transfer of chromosomes in FOSC as well as homologous recombination among the core chromosomes. Brankovics *et al.* (2017) and Achari *et al.* (2020) concluded there was evidence of recombination in mitochondria of FOSC.

We used 410 publicly available genomes of FOSC to resolve the current ambiguity around speciation in FOSC. Phylogenomics and population genomics were used to test the hypothesis that *F. oxysporum* diversified by sexual reproduction under a model of predominant clonal evolution. Evidence of sexual reproduction was sought from (i) near-equal frequencies of different MAT-idiomorphs among phylogroups of FOSC, (ii) phylogenetic incongruence and reticulation of single-copy orthologs, (iii) random associations of SNPs in clone-corrected data, and (iv) past activity of repeat-induced point mutation (RIP) as a signature of meiosis. Evidence of PCE was sought from statistically significant linkage disequilibrium in non-clone-corrected data. The occurrence, contribution and mechanisms of sexual recombination in *Fusarium* is fundamental to understanding their diversity and a key component in developing strategies for disease management.

## METHODS

### Genome sampling and annotation

We downloaded 490 nucleotide assemblies of FOSC publicly available on GenBank (including genomes from these studies: Ma *et al.* 2010, Thatcher *et al.* 2012, Guo *et al.* 2014, Kitts *et al.* 2016, Pu *et al.* 2016, van Dam *et al.* 2016, Williams *et al.* 2016, Singh *et al.* 2017, van Dam & Rep 2017, Armitage *et al.* 2018, Ayhan *et al.* 2018, Lv *et al.* 2018, Urbaniak *et al.* 2018, Asai *et al.* 2019, Gebru *et al.* 2019, Henry *et al.* 2019, Seo *et al.* 2019, Taylor *et al.* 2019, Urbaniak *et al.* 2019, Achari *et al.* 2020, Batson *et al.* 2020, Fokkens *et al.* 2020, Henry *et al.* 2020, Hudson *et al.* 2020, Kanapin *et al.* 2020, Khayi *et al.* 2020, Kim *et al.* 2020, Krasnov *et al.* 2020, Li *et al.* 2020, Srivastava *et al.* 2020, Thangavelu *et al.* 2020, Wang *et al.* 2020, Yu *et al.* 2020, Zhang *et al.* 2020, Henry *et al.* 2021, Chang & Cook unpublished). Genes were predicted in Augustus using a model of *F. graminearum* (Stanke & Morgenstern 2005). Seqtk (available at: <https://github.com/lh3/seqtk>) was used to exclude

assemblies that had fewer than 3700 genes longer than 600 amino acids; 410 assemblies met our criteria for inclusion (Table S1).

### Identification of orthologs and MAT idiomorphs

We used OrthoFinder v. 1.0.6 (Emms & Kelly 2019) with a Diamond search (Buchfink *et al.* 2015) to identify single copy orthologs in annotated genomes of *F. oxysporum*. MAT genes annotated from KT876066 (MAT1-1-1) and KT883580 (MAT1-2-1) were searched for in orthogroup outputs from OrthoFinder, and PopART (Leigh & Bryant 2015) was used to visualise networks of haplotypes.

### Phylogenetic analyses and tests for recombination with single copy orthologs

We used phylogenetic concordance of 40 single copy orthologs (48 868 amino acids) identified by OrthoFinder to assign structure *a posteriori* from 410 genomes of FOSC and test for recombination among and within the assigned groups. Analysed genes occurred on 10 of 11 core chromosomes in a chromosome-level assembly of *F. oxysporum* (Table S2, Fokkens *et al.* 2020). Single copy orthologs were aligned using default settings with MUSCLE (Edgar 2004), trimmed using ClipKIT (Steenwyk *et al.* 2020) and concatenated with FASconCAT-G (Kück & Longo 2014). The most likely tree was searched in IQ-TREE v. 2 (Minh *et al.* 2020b) with a model test for each partition (command -spp -m TEST), 10 000 ultrafast bootstraps (Minh *et al.* 2013), and genealogical concordance factors calculated from gene trees for each locus and applied to the concatenated topology (Minh *et al.* 2020a). We considered a group well-supported if it was recovered by at least eight out of 40 single copy orthologs (20 % genealogical concordance factor, from here referred to as phylogroups).

We visualised the discordance of gene tree topologies using DensiTree v. 2.2.5 (Bouckaert 2010). We visualised putative recombination events from the aligned, single-copy orthologs as a neighbour net in SplitsTree v. 4.14.8, and tested recombination by calculating the pairwise homoplasy index (PHI) for the entire dataset and each phylogroup (Huson & Klopper 2005).

### Dating analyses with subsampled genomes and loci

The divergence ages of phylogroups in *F. oxysporum* were estimated to determine whether reproduction has occurred on a long or short geological time scale. We subsampled the phylogroups to reduce potential tree space, including 118 of the 410 genomes, and two outgroup taxa, *F. fujikuroi* and *F. verticillioides*. We used 11 of the 40 single-copy orthologs (11 999 amino acids) that were congruent with the concatenated topology, which was constrained for divergence analyses in BEAST v. 2.5.2 (Bouckaert *et al.* 2019). The most recent common ancestor of *F. oxysporum* was calibrated with a mean age of 5 million years, and a log normal distribution sampled ages between 0.198–26.5 mya (median distribution 2.29 mya). The sampling age covers time estimated for a clock-like rate of speciation

(Hedges *et al.* 2015), and has a wide sampling space based on age estimates for *Fusarium* in studies of *Sordariomycetes* (van der Nest *et al.* 2015).

## Identification of SNPs and tests for recombination

We used kSNP v. 3.1.2 (Gardner *et al.* 2015) with strict settings to identify SNPs across all genomes and in each of the phylogroups. kSNP used kmers to search for SNPs at sites present in coding regions annotated by Augustus (mean size of 24.4 million base pairs) of all genomes (min\_frac = 1.0) over 101 homologous base pairs for the entire dataset (k = 101) and 31 homologous base pairs in each phylogroup (k = 31). These settings ensured each SNP site was homologous in all genomes, with either 101 or 31 flanking base pairs.

We visualised evolutionary relationships using SNP data from across the whole dataset and in each phylogroup using SplitsTree. The index of association (Brown *et al.* 1980) implemented in the R package *poppr* (Kamvar *et al.* 2014, R Core Team 2014) was used to test for evidence of clonality in phylogroups (where significant linkage disequilibrium is expected due to linkage among loci) and whether there was evidence of recombination in clone-corrected SNP data. We pruned SNP loci that were under linkage disequilibrium using PLINK (Chang *et al.* 2015), with a window size set to the number of SNPs, and a strict  $r^2$  threshold of 0.999. Haploid VCF files were imported into R with *vcfR* (Knaus & Grünwald 2017), and the *bitwise.ia* and *samp.ia* functions in *poppr* were used to determine the standardised index of association (*rbarD*) as a measure of linkage disequilibrium (Agapow and Burt, 2001). Genomes were clone-corrected based on their genetic distance across 40 loci using SplitsTree with a cut-off of <0.00002.

## Analyses of repeat-induced point mutation

We searched for evidence of past RIP activity in genome assemblies with the highest N50 value from each phylogroup as an indication of meiosis in FOSC. We used the REPET pipeline (available at: <http://urgi.versailles.inra.fr/index.php/urgi/Tools/REPET> Bao & Eddy 2002, Quesneville *et al.* 2003, Edgar & Myers 2005, Flutre *et al.* 2011) to identify and annotate TE families, then searched the most dominant retrotransposon family for RIP-like mutations. We aligned TE sequences using MAFFT (Katoh & Standley 2013) and used RIPCAL (Hane & Oliver 2008) to quantify RIP-like mutations using the alignment-based method and majority consensus options. Only TE copies that were longer than half of the total alignment length were considered in the analyses.

## Application of taxonomic names to phylogroups

We applied taxonomic names based on a phylogenetic species hypothesis of the *TEF* and *RPB2* genes, aligned to the dataset of Lombard *et al.* (2019). The most likely tree was searched for in IQ-TREE v. 2 (Minh *et al.* 2020b) with a model test for each

partition (command `-spp -m TEST`), and 10 000 UltraFast Bootstraps and aLRT values calculated for each node.

## RESULTS

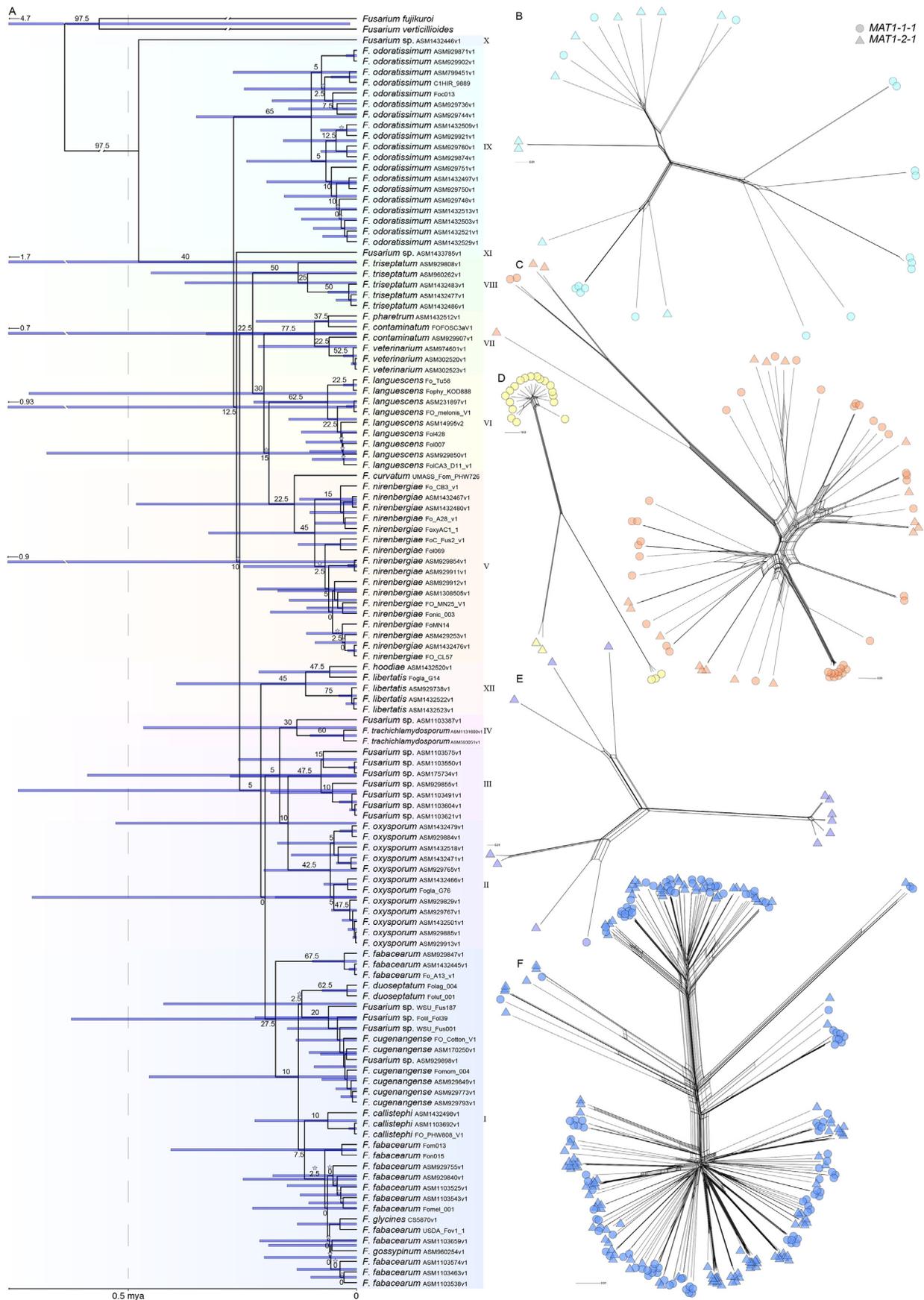
### Analyses of single copy orthologs

Genealogical concordance (20 % of 40 loci) from a maximum likelihood analysis (Fig. S1) recovered 12 phylogroups, congruent with relationships from a neighbour net analysis in SplitsTree (Fig. 2). Phylogenetic analyses and PHI tests provided evidence of recombination within *F. oxysporum* (Table 1). Single copy orthologs were not concordant in phylogenetic analyses based on genealogical concordance factors (Figs 1, S1) and visualisation of incongruence between topologies in DensiTree (Fig. S2), which indicates either incomplete lineage sorting of the 40 selected genes, or recombination between selected genomes. The reticulate neighbour net for the entire dataset was evidence of recombination, with homoplasy and incomplete lineage sorting as alternative explanations of reticulation. PHI test values < 0.05 supported recombination across the entire dataset (PHI = 0.0) as well as within most phylogroups, with the exceptions of phylogroups 7, 8 and 12. Phylogroups 2, 3 and 6 had less support for recombination based on their higher PHI scores than phylogroups 1, 5 and 9.

The BEAST analyses recovered a mean age for the most recent common ancestor of FOSC as 382 000 years ago (maximum sampled age 1.47 million years ago), with sampled ages calibrated to a mean of 5 million years (Fig. 1). All phylogroups were sampled with a mean age lower than 200 000 years, with the maximum sampled ages of phylogroup 1 the oldest at 547 000 years ago (mean 142 000).

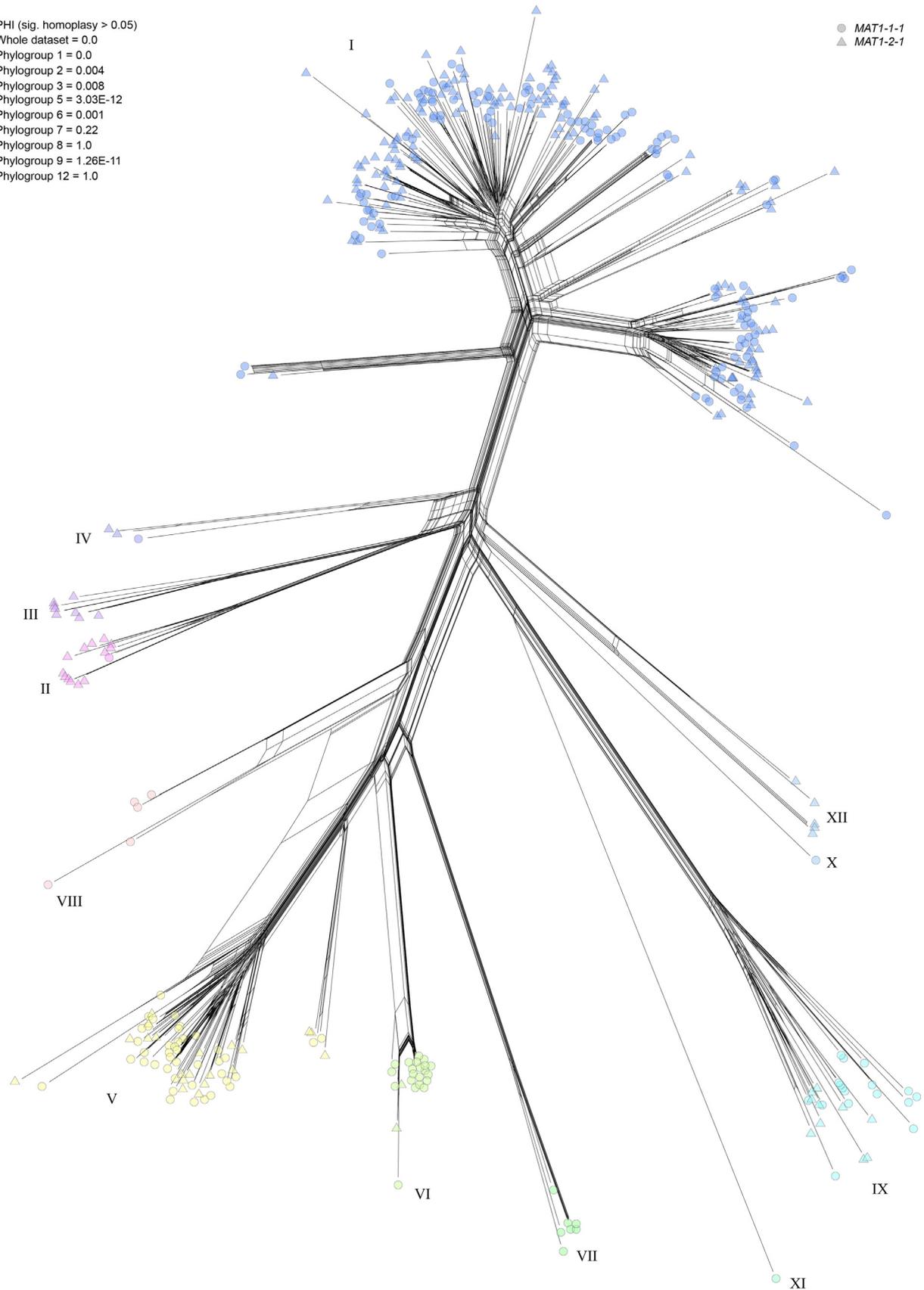
### MAT idiomorphs

The frequencies of *MAT1-1-1* (52 %) and *MAT1-2-1* (48 %) across the entire dataset were not significantly different from a 1:1 ratio (Table 1). Phylogroups with PHI test values <0.05 had similar frequencies of *MAT1-1-1* and *MAT1-2-1*, which supports recombination in those groups, *i.e.* phylogroup 1 with *MAT1-1-1* (46 %) and *MAT1-2-1* (54 %), phylogroup 5 with *MAT1-1-1* (69 %) and *MAT1-2-1* (31 %), and phylogroup 9 with *MAT1-1-1* (63 %) and *MAT1-2-1* (37 %). Phylogroups with lower support for recombination based on high PHI test values had a high frequency of one copy of a MAT locus, *e.g.* phylogroup 2 with *MAT1-1-1* (8 %) and *MAT1-2-1* (92 %), phylogroup 3 with *MAT1-1-1* (0 %) and *MAT1-2-1* (100 %), phylogroup 6 with *MAT1-1-1* (91 %) and *MAT1-2-1* (9 %), phylogroup 7 with *MAT1-1-1* (100 %) and *MAT1-2-1* (0 %), phylogroup 8 with *MAT1-1-1* (100 %) and *MAT1-2-1* (0 %), and phylogroup 12 with *MAT1-1-1* (0 %) and *MAT1-2-1* (100 %). Haplotypes of *MAT1-2-1* were admixed within phylogroups, and sequences of *MAT1-1-1* and *MAT1-2-1* varied in phylogroups, although *MAT1-2-1* showed more sequence conservation (Fig. 3). *MAT1-1-1* had greater variability and fewer shared genotypes in different phylogroups than *MAT1-2-1*.



**Fig. 1.** A. BEAST time-tree estimate with ages sampled from 11 concatenated, protein-coding genes, calibrated to a mean age of 5 million years at the most recent common ancestor of the *Fusarium oxysporum* species complex. The BEAST search was constrained to a maximum likelihood topology from 40 concatenated, protein-coding genes in IQ-TREE, from which genealogical concordance factors are provided above nodes. Blue stars indicate nodes that were not supported by UltraFast Bootstraps (<95%, 10 000 replicates). Taxon names are based on a phylogenetic species hypothesis shown in Fig. S5. B–E. SplitsTree neighbour networks based on SNPs called in each phylogroup. B. Phylogroup 9 (471 669 SNPs), C. Phylogroup 5 (625 191 SNPs), D. Phylogroup 6 (70319 SNPs), E. Phylogroup 2 (197 928 SNPs), F. Phylogroup 1 (1 630 980 SNPs).

PHI (sig. homoplasy > 0.05)  
 Whole dataset = 0.0  
 Phylogroup 1 = 0.0  
 Phylogroup 2 = 0.004  
 Phylogroup 3 = 0.008  
 Phylogroup 5 = 3.03E-12  
 Phylogroup 6 = 0.001  
 Phylogroup 7 = 0.22  
 Phylogroup 8 = 1.0  
 Phylogroup 9 = 1.26E-11  
 Phylogroup 12 = 1.0



**Fig. 2.** SplitsTree neighbour network based on 40 concatenated genes. Tests for the pairwise homoplasy index calculated in SplitsTree are provided for the entire dataset and alignments of individual phylogroups. Genotypes are coloured by phylogroup. SplitsTree networks show all putative evolutionary relationships between tips and reticulation is an indication of recombination.

**Table 1.** Summary of datasets, distribution of MAT idiomorphs, dating and phylogenetic analyses in all phylogroups.

Phylogroup	Genomes	Taxonomic names	MAT1-1	MAT1-2	$\chi^2$	P-value	PHI	Mean age at MRCA	Distribution	Monophyletic TEFIRPB2
1	256	<i>F. fabacearum</i> , <i>F. gossypinum</i> , <i>F. glycines</i> , <i>F. cugenangense</i> , <i>F. elaeidis</i> , <i>F. callistephi</i> , <i>F. carminascens</i> , <i>F. duoseptatum</i> , <i>F. tardichlamydosporum</i>	119	137	1.266	0.2606	0	142 000	Australia, China, Ethiopia, Israel, Greece, Japan, Netherlands, Russia, Spain, Taiwan, UK, USA	Paraphyletic with Phylo4
2	13	<i>F. oxysporum</i>	1	12	9.308	0.0023	0.004	46 000	Australia, Italy, Spain	Yes
3	8	NA	0	8	8	0.0047	0.008	62 000	Australia, Ethiopia, India	Yes
4	3	NA	1	2	0.333	0.5637	NA	23 000	Ethiopia, India, Japan	No
5	61	<i>F. nirenbergiae</i> , <i>F. curvatum</i>	42	19	8.672	0.0032	3.03E-12	109 000	Australia, Canada, China, France, Greece, Morocco, Netherlands, South Korea, Space, Spain, Switzerland, UK, USA	No
6	23	<i>F. languescens</i>	21	2	15.696	0.0001	0.001	51 000	Australia, France, India, Netherlands, Spain, USA	Yes
7	7	<i>F. contaminatum</i> , <i>F. veterinarianum</i> , <i>F. pharetrum</i>	7	0	7	0.0082	0.22	74 000	Australia, Space, Ukraine, USA	Yes
8	5	<i>F. triseptatum</i>	5	0	5	0.0253	1.0	102 000	Australia, Spain, USA	Yes
9	27	<i>F. odoratissimum</i>	17	10	1.286	0.2568	1.25E-11	79 000	Australia, China, India, Malaysia, UK	Yes
10	1	NA	1	0	NA	NA	NA	NA	USA	NA
11	1	NA	1	0	NA	NA	NA	NA	Spain	NA
12	5	<i>F. libertatis</i>	0	5	5	0.0253	1.0	89 000	Australia	No
All	410	NA	215	195	0.976	0.3233	0	382 000	NA	NA

Taxonomic names based on phylogenetic species concept in Fig. S5.

$\chi^2$  = Chi-square value relative to the expected 1:1 ratio from a random mating population.

P-value = Two-tailed P-value obtained from Chi-square test.

PHI = Pairwise Homoplasmy Index over 40 loci (sig. < 0.05).

NA = not applicable.

MRCA = Most recent common ancestor (calibrated to 5 million years at MRCA of *F. oxysporum*).

## Analyses of SNP data

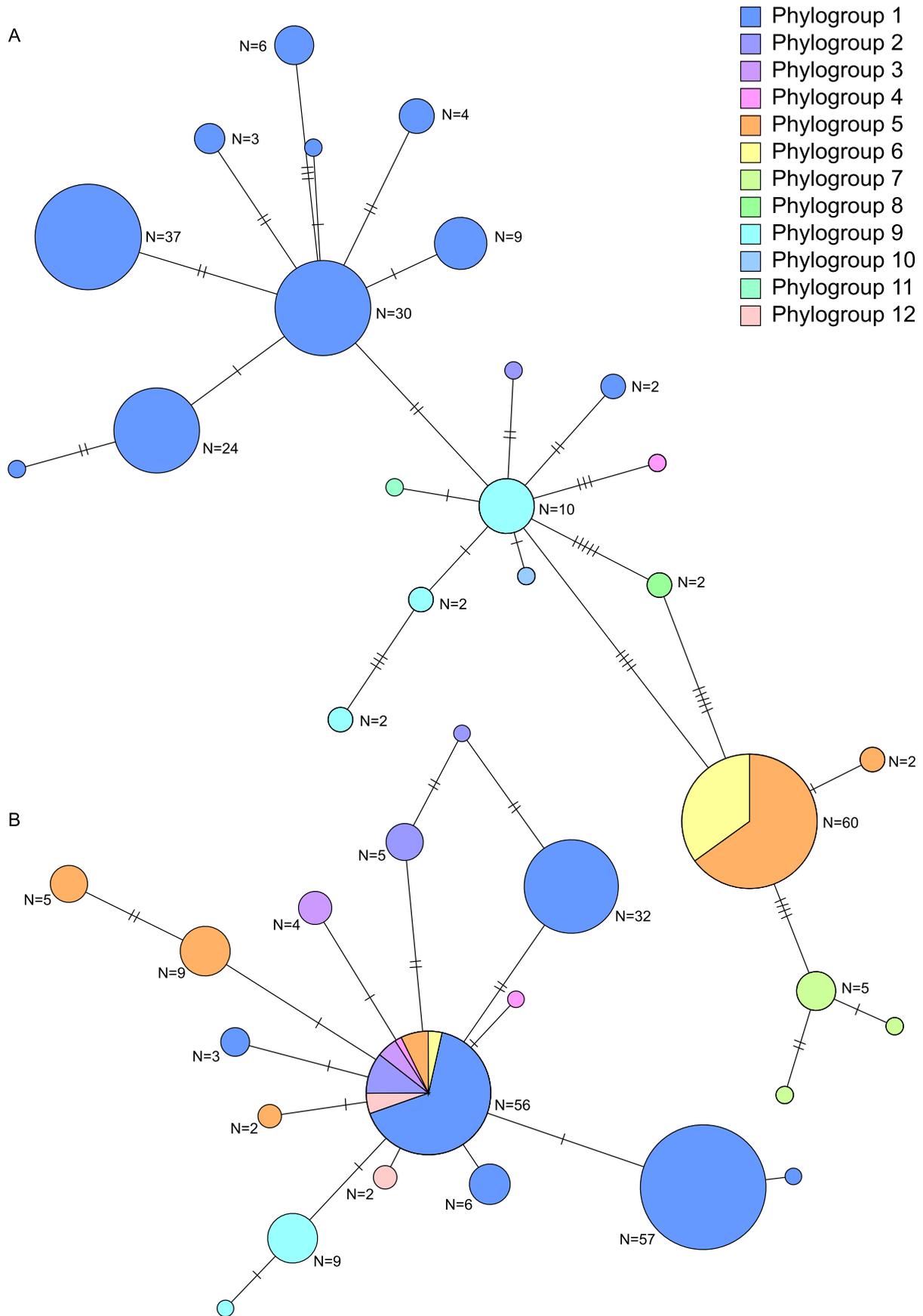
Up to 56 % of called SNPs were filtered from phylogroups based on their  $r^2$  pairwise frequencies across all loci (Table 2). The rbarD values, which approach zero in randomly recombining populations, were high (>0.01) for all phylogroups in non-clone-corrected data (Table 2). The non-clone-corrected rbarD values in phylogroups 1, 5 and 9 were an order of magnitude smaller than other phylogroups and suggested incomplete linkage between sites and recombination. Clone-corrected datasets reduced the standardised index of association by an order of magnitude, which supported recombination in all examined phylogroups except 6 and 8. rbarD values did not change by an order of magnitude when phylogroup 9 and the entire dataset

were clone-corrected, which may indicate near-clones were not removed with the criteria for clone-correction.

SplitsTree relationships based on SNP loci are shown for the entire dataset (Fig. S5) and recombinant phylogroups (Fig. 1). The neighbour net based on SNP data across the entire dataset was congruent with evolutionary hypotheses obtained with the 40 single-copy orthologs (compare Figs 2 and S3).

## Analyses of RIP

RIPCAL analyses of the dominant retrotransposon families from a genome of each of the phylogroups indicated RIP activity in all phylogroups of FOSC (Fig. S4). The most frequent RIP type was CpT > TpT mutation followed by CpA > TpA mutation.



**Fig. 3.** Haplotype networks from alignments of (A) *MAT1-1-1* and (B) *MAT1-2-1*. Haplotypes are coloured by whether they are present in different phylogroups.

**Table 2.** Tests for linkage disequilibrium based on the standardised index of association, which approaches zero in recombining populations.

Phylogroup	Non-clone-corrected					Clone-corrected				
	Genomes	No. called SNPs <sup>1</sup>	LD corrected SNPs <sup>2</sup>	% SNPs removed under LD	rbarD <sup>3</sup>	Genomes	No. called SNPs <sup>1</sup>	LD corrected SNPs <sup>2</sup>	% SNPs removed under LD	rbarD <sup>3</sup>
1	256	1 630 980	714 955	56	0.014	61	802 433	413 923	48	0.002
2	13	197 928	140 357	29	0.111	9	183 604	136 284	26	0.057
3	8	150 897	102 449	32	0.155	4	120 558	95 211	21	0.018
4	3	99 029	93 548	6	0.245	NA	NA	NA	NA	NA
5	61	625 191	352 735	44	0.025	28	401 258	238 426	41	0.008
6	23	70 319	54 140	23	0.455	9	63 419	54 160	15	0.316
7	7	141 561	120 891	15	0.228	4	136 590	119 677	12	0.004
8	5	117 541	100 285	15	0.326	4	114 316	99 302	13	0.220
9	27	471 669	310 211	34	0.026	17	440 908	296 782	33	0.021
10	1	NA	NA	NA	NA	NA	NA	NA	NA	NA
11	1	NA	NA	NA	NA	NA	NA	NA	NA	NA
12	5	144 481	124 829	14	0.337	3	132 108	119 599	9	0.021
All	410	4 664 221	4 628 213	1	0.034 <sup>4</sup>	136	748 225	377 645	50	0.038 <sup>4</sup>

<sup>1</sup> Number of SNPs from a k-mer search (k = 31 for phylogroups, k = 101 for all) of coding sequences within each phylogroup using kSNP.

<sup>2</sup> SNPs were filtered based on an  $r^2$  cutoff of 0.9999 calculated in PLINK.

<sup>3</sup> Calculated from bitwise.ia across LD corrected SNPs in poppr.

<sup>4</sup> Calculated from samp.ia with 1 000 repeats of 1 000 SNPs in poppr.

## Application of taxonomic names

Based on a concatenated phylogeny of *TEF* and *RPB2*, the phylogroups were monophyletic, with the exception of phylogroups 4 and 12 (Table 1, Fig. S5). Species names can be applied to phylogroups 2 (*F. oxysporum*), 6 (*F. languescens*), 8 (*F. triseptatum*), 9 (*F. odoratissimum*) and 12 (*F. libertatis*). Phylogroup 5 included two monophyletic groups and two named species (*F. nirenbergiae*, *F. curvatum*). Phylogroup 7 was monophyletic with three named species (*F. contaminatum*, *F. pharetrum* and *F. veterinarium*), of which *F. veterinarium* applies to species isolated from the international space station and humans. Phylogroup 1 contained nine names applied by Lombard *et al.* (2019), and was similarly rich in names below species rank (Table S1). The *TEF* and *RPB2* genes had congruent topologies and supported a hypothesis of restrained recombination in lineages of *F. oxysporum*.

## DISCUSSION

We have shown using gene and SNP data from 410 genomes that FOSC fits a model of predominant clonal evolution. The extant diversity of FOSC is the product of long-term sexual or parasexual reproduction, as demonstrated by (i) equal frequencies of mating-type idiomorphs within phylogroups, (ii) low phylogenetic concordance between 40 orthologous genes, (iii) significant recombination based on PHI, (iv) standardised indices of association that were evidence of recombination in clone-corrected datasets, and (v) past activity of RIP in TEs from all phylogroups. Asexual reproduction is frequent, evidenced by multiple near clones in all phylogroups and high rbarD values in non-clone-corrected phylogroups. We estimated the recent common ancestor for all lineages of *F. oxysporum* existed

approximately 500 000 years ago. Independent lineages have likely diversified within the last 200 000 years, with episodic recombination within, but not between, restrained phylogroups.

FOSC shows signatures of restrained recombination, as evidenced by clustering in phylogenetic groups of house-keeping genes (shared multilocus genotypes in *TEF* and *RPB2*) and SNP sites under linkage disequilibrium in all phylogroups (Tibayrenc & Ayala 2012). Extant phylogroups of FOSC evolved from recombinant ancestors without subsequent outcrossing among sister lineages, and the diverse haplotype network of *MAT1-1-1* suggests that mating is restricted within phylogroups. Based on the divergence age estimates of monophyletic phylogroups, and patterns of reticulation and edge lengths in SplitsTree analyses, phylogroups have not exchanged core genes by recombination since they shared a most recent common ancestor. We interpreted central reticulation in SplitsTree analyses as recombination, although incomplete lineage sorting and homoplasy are alternative explanations.

RIP is active during sexual reproduction (Hane *et al.* 2015), and has supported sexual reproduction in other *Fungi*, including taxa hypothesized to be clonal (Ikeda *et al.* 2002, Braumann *et al.* 2008, Crouch *et al.* 2008, Ropars *et al.* 2012). The activity of RIP in all phylogroups supported meiosis in FOSC, although signatures of RIP are not evidence of recent recombination. Hane *et al.* (2015) consider sexual reproduction the most likely explanation of RIP, and outlined possible alternatives to explain RIP in clonal species, namely loss of sexual reproduction, non-meiotic RIP and horizontal transfer from a meiotic donor.

Taylor *et al.* (2015) used *Cryptococcus gattii* as a case study (see Engelthaler *et al.* 2014) to show that clones under restrained recombination warranted names to improve communication between researchers. Clones under restrained recombination may be short-lived in an evolutionary sense (Drenth *et al.* 2019), but as agricultural pathogens they have long-lasting

impact to humans. The assignment of (species) names to pathogens that have become successful clones under predominant clonal evolution can be accommodated under the current nomenclatural code.

Maryani *et al.* (2019) and Lombard *et al.* (2019) proposed taxonomic hypotheses that reflected restrained recombination in FOSC. Our phylogenomic and population genomic analyses partially support their taxonomies. We found congruent taxonomic groups (phylogroups) with sexual reproduction within these groups, but not between them, demonstrated by reticulation within but not among phylogroups in network analyses. Phylogroups 1 and 5 contain multiple species names. Networks based on over 600 000 SNPs revealed distinct clusters within phylogroups 1 and 5, despite recent evidence of recombination across them. We did not find population substructure based on host or geography in any of the phylogroups. Nor did we find pathogenicity on different hosts as a reliable indicator for taxonomic identification.

Successful asexual clones (genotypes) of FOSC are often collected during plant disease outbreaks. The sampling in our study may be biased by the collection of *F. oxysporum* during plant disease outbreaks, which results in under-sampling of non-pathogenic lineages. Recombination was most evident in phylogroups that were well-sampled, either based on a PHI test, or with rbarD values that approached zero in clone-corrected and non-clone-corrected datasets (e.g. phylogroups 1 and 5). In the more highly sampled phylogroups we detected near-equal frequencies of both mating types, a result that is most congruent with sexual reproduction. We hypothesise that with increased sampling, recombination will be supported in all lineages and that both mating types will be found in all lineages. The implication is that capacity for sexual/parasexual reproduction is maintained, despite predominant clonal evolution, as it is required for long-term adaptability of the species.

Our criteria to include single copy genes and SNPs that were present in all genomes analysed likely excluded loci on accessory chromosomes, which may be horizontally transferred between individuals without subsequent meiosis. A limitation of our study is that we did not test whether horizontal chromosome transfer, without recombination, occurs among phylogroups of FOSC.

Physical evidence of sexual recombination (ascomata) in FOSC has never been found. Leslie & Summerell (2006) reported structures that resembled sclerotia in cultures of *F. oxysporum* and hypothesised these were protoperithecia, similar in morphology to those of the *F. fujikuroi* species complex. Attempts to cross these isolates and produce sexual structures have been unsuccessful (Visser *et al.* 2005, and Summerell unpublished data). Parasexual reproduction is a competing hypothesis with sexual reproduction to explain recombination in FOSC in the absence of a meiotic stage, however, parasexual reproduction in nature may be hard to witness, may occur on host taxa that are less commonly investigated, and is a less parsimonious explanation for the occurrence of equal mating type frequencies.

O'Donnell *et al.* (2004) outlined other hypotheses competing with sexual reproduction and explained the presence of two mating loci in populations of FOSC as a remnant of past sexual reproduction and/or that the MAT genes have evolved a different function and no longer regulated mating. Tibayrenc & Ayala (2014) proposed that restrained recombination is a ubiquitous evolutionary strategy used to avoid recombinational load or

break-up of favourable multilocus allele combinations, and is another valid hypothesis to explain a lack of recombination.

Asexual reproduction has evolutionary advantages for successful genotypes in the short-term, whereas long-term clonal genotypes decline via the accumulation of deleterious mutations (McDonald *et al.* 2016). Infrequent sexual/parasexual reproduction may lead to skewed mating type frequencies, as seen in *MAT1-1-1* of FOSC, further reducing the potential for sexual reproduction. Despite fitting a model of predominant clonal evolution and the short-term success of clones, FOSC has maintained the capacity for sexual/parasexual reproduction. The signature of recombination in FOSC is clear and may guide species-rank taxonomic hypotheses for these fungi.

## DATA AVAILABILITY

All data and commands are available at [https://drive.google.com/drive/folders/17EifliuLLR-1wx\\_hXK0d-UvNc4X5XhB7?usp=sharing](https://drive.google.com/drive/folders/17EifliuLLR-1wx_hXK0d-UvNc4X5XhB7?usp=sharing)

## ACKNOWLEDGEMENTS

ARM acknowledges the University of Queensland Development Fellowships (UQFEL1718905) and support from the Department of the Environment and Energy under the Australian Biological Resources Study (grant number RG18-43). TAD and BDW acknowledge the Tree Protection Co-operative Programme (TPCP), the National Research Foundation of South Africa and the DST-NRF Centre of Excellence in Tree Health Biotechnology (CTHB). We thank Doug Cook for preliminary access to genomic data and comments that helped improve the manuscript. We thank two anonymous reviewers for their improvements to the final manuscript.

## APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.simyco.2021.100132>.

## REFERENCES

- Achari SR, Kaur J, Dinh Q, *et al.* (2020). Phylogenetic relationship between Australian *Fusarium oxysporum* isolates and resolving the species complex using the multispecies coalescent model. *BMC Genomics* **21**: 248.
- Agapow PM, Burt A (2001). Indices of multilocus linkage disequilibrium. *Molecular Ecology Notes* **1**: 101–102.
- Armitage AD, Taylor A, Sobczyk MK, *et al.* (2018). Characterisation of pathogen-specific regions and novel effector candidates in *Fusarium oxysporum* f. sp. *cepae*. *Scientific Reports* **8**: 13530.
- Asai S, Ayukawa Y, Gan P, *et al.* (2019). High-quality draft genome sequence of *Fusarium oxysporum* f. sp. *cubense* strain 160527, a causal agent of Panama disease. *Microbiology Resource Announcements* **8**: e00654-19.
- Ayhan DH, López-Díaz C, Di Pietro A, *et al.* (2018). Improved assembly of reference genome *Fusarium oxysporum* f. sp. *lycopersici* strain Fo4287. *Microbiology Resource Announcements* **7**: e00910–e00918.
- Bao Z, Eddy SR (2002). Automated de novo identification of repeat sequence families in sequenced genomes. *Genome Research* **12**: 1269–1276.
- Batson AM, Fokkens L, Rep M, *et al.* (2020). Putative effector genes distinguish two pathogenicity groups of *Fusarium oxysporum* f. sp. *spinaciae*. *Molecular Plant-Microbe Interactions* **34**: 141–156.
- Billiard S, LoPez-Villavicencio M, Hood ME, *et al.* (2012). Sex, outcrossing and mating types: unsolved questions in fungi and beyond. *Journal of Evolutionary Biology* **25**: 1020–1038.
- Bouckaert R, Vaughan TG, Barido-Sottani J, *et al.* (2019). BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. *PLoS Computational Biology* **15**: e1006650.

- Bouckaert RR (2010). DensiTree: making sense of sets of phylogenetic trees. *Bioinformatics* **26**: 1372–1373.
- Brankovics B, van Dam P, Rep M, et al. (2017). Mitochondrial genomes reveal recombination in the presumed asexual *Fusarium oxysporum* species complex. *BMC Genomics* **18**: 735.
- Braumann IM, van den Berg M, Kempken F (2008). Repeat induced point mutation in two asexual fungi, *Aspergillus niger* and *Penicillium chrysogenum*. *Current Genetics* **53**: 287–297.
- Brown AHD, Feldman MW, Nevo E (1980). Multilocus structure of natural populations of *Hordeum spontaneum*. *Genetics* **96**: 523–536.
- Buchfink B, Xie C, Huson DH (2015). Fast and sensitive protein alignment using DIAMOND. *Nature Methods* **12**: 59–60.
- Chang CC, Chow CC, Tellier LCAM, et al. (2015). Second-generation PLINK: rising to the challenge of larger and richer datasets. *GigaScience* **4**: s13742-015-0047-8.
- Crouch JA, Glasheen BM, Giunta MA, et al. (2008). The evolution of transposon repeat-induced point mutation in the genome of *Colletotrichum cereale*: reconciling sex, recombination and homoplasmy in an "asexual" pathogen. *Fungal Genetics and Biology* **45**: 190–206.
- Dean R, Van Kan JAL, Pretorius ZA, et al. (2012). The Top 10 fungal pathogens in molecular plant pathology. *Molecular Plant Pathology* **13**: 414–430.
- Drenth A, McTaggart AR, Wingfield BD (2019). Fungal clones win the battle, but recombination wins the war. *IMA Fungus* **10**: 18.
- Edgar RC (2004). MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics* **5**: 113.
- Edgar RC, Myers EW (2005). PILER: identification and classification of genomic repeats. *Bioinformatics* **21**: i152–i158.
- Emms DM, Kelly S (2019). OrthoFinder: phylogenetic orthology inference for comparative genomics. *Genome Biology* **20**: 238.
- Engelthaler DM, Hicks ND, Gillece JD, et al. (2014). *Cryptococcus gattii* in North American Pacific Northwest: whole-population genome analysis provides insights into species evolution and dispersal. *mBio* **5**: e01464-14.
- Flutre T, Duprat E, Feuillet C, et al. (2011). Considering transposable element diversification in de novo annotation approaches. *PLoS ONE* **6**: e16526.
- Fokkens L, Guo L, Dora S, et al. (2020). A chromosome-scale genome assembly for the *Fusarium oxysporum* strain Fo5176 to establish a model *Arabidopsis*-fungal pathosystem. *G3 Genes|Genomes|Genetics* **10**: 3549–3555.
- Gardner SN, Slezak T, Hall BG (2015). kSNP3.0: SNP detection and phylogenetic analysis of genomes without genome alignment or reference genome. *Bioinformatics* **31**: 2877–2878.
- Gebru ST, Mammel MK, Gangiredla J, et al. (2019). Draft genome sequences of 12 isolates from 3 *Fusarium* species recovered from moldy peanuts. *Microbiology Resource Announcements* **8**: e01642-18.
- Gordon TR, Martyn RD (1997). The evolutionary biology of *Fusarium oxysporum*. *Annual Review of Phytopathology* **35**: 111–128.
- Guo L, Han L, Yang L, et al. (2014). Genome and transcriptome analysis of the fungal pathogen *Fusarium oxysporum f. sp. cubense* causing banana vascular wilt disease. *PLoS ONE* **9**: e95543.
- Hane JK, Oliver RP (2008). RIPCAL: a tool for alignment-based analysis of repeat-induced point mutations in fungal genomic sequences. *BMC Bioinformatics* **9**: 478.
- Hane JK, Williams AH, Taranto AP, et al. (2015). Repeat-Induced Point Mutation: A fungal-specific, endogenous mutagenesis process. In: *Genetic transformation systems in fungi Volume 2* (van den Berg MA, Maruthachalam K, eds). Springer International Publishing, Denmark: 55–68.
- Hedges SB, Marin J, Suleski M, et al. (2015). Tree of life reveals clock-like speciation and diversification. *Molecular Biology and Evolution* **32**: 835–845.
- Henry P, Kaur S, Pham QAT, et al. (2020). Genomic differences between the new *Fusarium oxysporum f. sp. apii* (Foa) race 4 on celery, the less virulent Foa races 2 and 3, and the avirulent on celery *f. sp. coriandrii*. *BMC Genomics* **21**: 730.
- Henry PM, Pincot DDA, Jenner BN, et al. (2021). Horizontal chromosome transfer and independent evolution drive diversification in *Fusarium oxysporum f. sp. fragariae*. *New Phytologist* **230**: 327–340.
- Henry PM, Stueven M, Li S, et al. (2019). Genome sequence of a California isolate of *Fusarium oxysporum f. sp. lycopersici* Race 3, a fungus causing wilt disease on tomato. *Microbiology Resource Announcements* **8**: e01713–e01718.
- Hudson O, Hudson D, Ji P, et al. (2020). Draft genome sequences of three *Fusarium oxysporum f. sp. niveum* isolates used in designing markers for race differentiation. *Microbiology Resource Announcements* **9**: e01004–e01020.
- Huson DH, Kloepper TH (2005). Computing recombination networks from binary sequences. *Bioinformatics* **21**: ii159–ii165.
- Ikeda K-i, Nakayashiki H, Kataoka T, et al. (2002). Repeat-induced point mutation (RIP) in *Magnaporthe grisea*: implications for its sexual cycle in the natural field context. *Molecular Microbiology* **45**: 1355–1364.
- Kamvar ZN, Tabima JF, Grünwald NJ (2014). Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. *PeerJ* **2**: e281.
- Kanapin A, Samsonova A, Rozhmina T, et al. (2020). The Genome Sequence of Five Highly Pathogenic Isolates of *Fusarium oxysporum f. sp. lini*. *Molecular Plant-Microbe Interactions* **33**: 1112–1115. <https://doi.org/10.1094/MPMI-05-20-0130-SC>.
- Katoh K, Standley DM (2013). MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* **30**: 772–780.
- Khayi S, Khoulassa S, Gaboun F, et al. (2020). Draft genome sequence of *Fusarium oxysporum f. sp. albedinis* strain Foa 133, the causal agent of Bayoud disease on date palm. *Microbiology Resource Announcements* **9**: e00462-20.
- Kim H-S, Lohmar JM, Busman M, et al. (2020). Identification and distribution of gene clusters required for synthesis of sphingolipid metabolism inhibitors in diverse species of the filamentous fungus *Fusarium*. *BMC Genomics* **21**: 510.
- Kitts PA, Church DM, Thibaud-Nissen F, et al. (2016). Assembly: a resource for assembled genomes at NCBI. *Nucleic Acids Research* **44**: D73–D80.
- Knaus BJ, Grünwald NJ (2017). vcfR: a package to manipulate and visualize variant call format data in R. *Molecular Ecology Resources* **17**: 44–53.
- Koenig RL, Ploetz RC, Kistler HC (1997). *Fusarium oxysporum f. sp. cubense* consists of a small number of divergent and globally distributed clonal lineages. *Phytopathology* **87**: 915–923.
- Krasnov GS, Pushkova EN, Novakovskiy RO, et al. (2020). High-quality genome assembly of *Fusarium oxysporum f. sp. lini*. *Frontiers in Genetics* **11**: 959.
- Kück P, Longo GC (2014). FASconCAT-G: extensive functions for multiple sequence alignment preparations concerning phylogenetic studies. *Frontiers in Zoology* **11**: 81.
- Laurence MH, Summerell BA, Burgess LW, et al. (2014). Genealogical concordance phylogenetic species recognition in the *Fusarium oxysporum* species complex. *Fungal Biology* **118**: 374–384.
- Laurence MH, Summerell BA, Liew ECY (2015). *Fusarium oxysporum f. sp. canariensis*: evidence for horizontal gene transfer of putative pathogenicity genes. *Plant Pathology* **64**: 1068–1075.
- Leigh JW, Bryant D (2015). popart: full-feature software for haplotype network construction. *Methods in Ecology and Evolution* **6**: 1110–1116.
- Leslie JF, Summerell BA (2006). *The Fusarium Laboratory Manual*. Blackwell Publishing Professional, USA.
- Li J, Fokkens L, van Dam P, et al. (2020). Related mobile pathogenicity chromosomes in *Fusarium oxysporum* determine host range on cucurbits. *Molecular Plant Pathology* **21**: 761–776.
- Lombard L, Sandoval-Denis M, Lamprecht SC, et al. (2019). Epitypification of *Fusarium oxysporum* - clearing the taxonomic chaos. *Persoonia* **43**: 1–47.
- Lv H, Yang Y, Liu X, et al. (2018). Draft genome sequence of FGL03-6, a race 1 strain of *Fusarium oxysporum f. sp. conglutinans*, the causal agent of cabbage *Fusarium* wilt. *Genome Announcements* **6**: e00191-18.
- Ma L-J, van der Does HC, Borkovich KA, et al. (2010). Comparative genomics reveals mobile pathogenicity chromosomes in *Fusarium*. *Nature* **464**: 367–373.
- Maryani N, Lombard L, Poerba YS, et al. (2019). Phylogeny and genetic diversity of the banana *Fusarium* wilt pathogen *Fusarium oxysporum f. sp. cubense* in the Indonesian centre of origin. *Studies in Mycology* **92**: 155–194.
- McDonald MJ, Rice DP, Desai MM (2016). Sex speeds adaptation by altering the dynamics of molecular evolution. *Nature* **531**: 233–236.
- Minh BQ, Hahn MW, Lanfear R (2020a). New methods to calculate concordance factors for phylogenomic datasets. *Molecular Biology and Evolution* **37**: 2727–2733.
- Minh BQ, Nguyen MAT, von Haeseler A (2013). Ultrafast approximation for phylogenetic bootstrap. *Molecular Biology and Evolution* **30**: 1188–1195.
- Minh BQ, Schmidt HA, Chernomor O, et al. (2020b). IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution* **37**: 1530–1534.
- Munkvold GP, Arias S, Taschl I, et al. (2019). Chapter 9 – Mycotoxins in Corn: Occurrence, Impacts, and Management. In: *Corn* (Serna-Saldivar SO, ed), Third Edition. AACC International Press, England: 235–287.

- Nieuwenhuis BPS, James TY (2016). The frequency of sex in fungi. *Philosophical Transactions of the Royal Society B: Biological Sciences* **371**: 20150540.
- O'Donnell K, Sutton DA, Rinaldi MG, et al. (2004). Genetic diversity of human pathogenic members of the *Fusarium oxysporum* complex inferred from multilocus DNA sequence data and amplified fragment length polymorphism analyses: evidence for the recent dispersion of a geographically widespread clonal lineage and nosocomial origin. *Journal of Clinical Microbiology* **42**: 5109–5120.
- O'Donnell K, Sutton DA, Wiederhold N, et al. (2016). Veterinary Fusarioses within the United States. *Journal of Clinical Microbiology* **54**: 2813–2819.
- Pu Z, Ino Y, Kimura Y, et al. (2016). Changes in the proteome of xylem sap in *Brassica oleracea* in response to *Fusarium oxysporum* stress. *Frontiers in Plant Science* **7**: 31.
- R Core Team (2014). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Quesneville H, Nouaud D, Anxolabéhère D (2003). Detection of new transposable element families in *Drosophila melanogaster* and *Anopheles gambiae* genomes. *Journal of Molecular Evolution* **57**: S50–S59.
- Ropars J, Dupont J, Fontanillas E, et al. (2012). Sex in cheese: evidence for sexuality in the fungus *Penicillium roqueforti*. *PLoS ONE* **7**: e49665.
- Seo S, Pokhrel A, Coleman JJ (2019). The genome sequence of five genotypes of *Fusarium oxysporum* f. sp. *vasinfectum*: A resource for studies on *Fusarium* wilt of cotton. *Molecular Plant-Microbe Interactions* **33**: 138–140.
- Singh NK, Blachowicz A, Romsdahl J, et al. (2017). Draft genome sequences of several fungal strains selected for exposure to microgravity at the International Space Station. *Genome Announcements* **5**: e01602–e01616.
- Srivastava SK, Zeller KA, Sobieraj JH, et al. (2020). Genome resources of four distinct pathogenic races within *Fusarium oxysporum* f. sp. *vasinfectum* that cause vascular wilt disease of cotton. *Phytopathology* **111**: 593–596.
- Stanke M, Morgenstern B (2005). AUGUSTUS: a web server for gene prediction in eukaryotes that allows user-defined constraints. *Nucleic Acids Research* **33**: W465–467.
- Steenkamp ET, Wingfield MJ, McTaggart AR, et al. (2018). Fungal species and their boundaries matter – Definitions, mechanisms and practical implications. *Fungal Biology Reviews* **32**: 104–116.
- Steenwyk JL, Buida TJ, Li Y, et al. (2020). ClipKIT: A multiple sequence alignment trimming software for accurate phylogenomic inference. *PLoS Biology* **18**: e3001007.
- Summerell BA (2019). Resolving *Fusarium*: current status of the genus. *Annual Review of Phytopathology* **57**: 323–339.
- Taylor A, Armitage AD, Handy C, et al. (2019). Basal rot of *Narcissus*: understanding pathogenicity in *Fusarium oxysporum* f. sp. *narcissi*. *Frontiers in Microbiology* **10**: 2905.
- Taylor JW, Hann-Soden C, Branco S, et al. (2015). Clonal reproduction in fungi. *Proceedings of the National Academy of Sciences of the USA* **112**: 8901–8908.
- Thangavelu R, Edwin Raj E, Pushpakanth P, et al. (2020). Draft genome of *Fusarium oxysporum* f. sp. *cubense* strain tropical race-4 infecting cavendish (AAA) group of banana in India. *Plant Disease* **105**: 481–483.
- Thatcher LF, Gardiner DM, Kazan K, et al. (2012). A highly conserved effector in *Fusarium oxysporum* is required for full virulence on *Arabidopsis*. *Molecular Plant Microbe Interactions* **25**: 180–190.
- Tibayrenc M, Ayala FJ (2012). Reproductive clonality of pathogens: a perspective on pathogenic viruses, bacteria, fungi, and parasitic protozoa. *Proceedings of the National Academy of Sciences of the USA* **109**: E3305–3313.
- Tibayrenc M, Ayala FJ (2014). *Cryptosporidium*, *Giardia*, *Cryptococcus*, *Pneumocystis* genetic variability: cryptic biological species or clonal near-clades? *PLoS Pathogens* **10**: e1003908.
- Tibayrenc M, Ayala FJ (2017). Is predominant clonal evolution a common evolutionary adaptation to parasitism in pathogenic parasitic Protozoa, Fungi, bacteria, and viruses? *Advances in Parasitology* **97**: 243–325.
- Tibayrenc M, Ayala FJ (2021). Models in parasite and pathogen evolution: Genomic analysis reveals predominant clonality and progressive evolution at all evolutionary scales in parasitic protozoa, yeasts and bacteria. *Advances in Parasitology* **111**: 75–117.
- Tsai IJ, Bensasson D, Burt A, et al. (2008). Population genomics of the wild yeast *Saccharomyces paradoxus*: Quantifying the life cycle. *Proceedings of the National Academy of Sciences of the USA* **105**: 4957.
- van Dam P, Fokkens L, Schmidt SM, et al. (2016). Effector profiles distinguish *formae speciales* of *Fusarium oxysporum*. *Environmental Microbiology* **18**: 4087–4102.
- van Dam P, Rep M (2017). The distribution of miniature impala elements and SIX genes in the *Fusarium* genus is suggestive of horizontal gene transfer. *Journal of Molecular Evolution* **85**: 14–25.
- van der Nest MA, Steenkamp ET, McTaggart AR, et al. (2015). Saprophytic and pathogenic fungi in the *Ceratocystidaceae* differ in their ability to metabolize plant-derived sucrose. *BMC Evolutionary Biology* **15**: 273.
- Urbaniak C, van Dam P, Zaborin A, et al. (2019). Genomic characterization and virulence potential of two *Fusarium oxysporum* isolates cultured from the International Space Station. *mSystems* **4**: e00345-18.
- Urbaniak C, Massa G, Hummerick M, et al. (2018). Draft genome sequences of two *Fusarium oxysporum* isolates cultured from infected *Zinnia hybrida* plants grown on the International Space Station. *Genome Announcements* **6**: e00326-18.
- Visser M, Gordon TR, Wingfield BD, et al. (2005). *Chapter 2: Mating type genes and the reproductive potential of Fusarium oxysporum f. sp. cubense*. PhD Dissertation. Department of Genetics, University of Pretoria, South Africa.
- Vlaardingerbroek I, Beerens B, Rose L, et al. (2016). Exchange of core chromosomes and horizontal transfer of lineage-specific chromosomes in *Fusarium oxysporum*. *Environmental Microbiology* **18**: 3702–3713.
- Wang B, Yu H, Jia Y, et al. (2020). Chromosome-scale genome assembly of *Fusarium oxysporum* strain Fo47, a fungal endophyte and biocontrol agent. *Molecular Plant-Microbe Interactions* **33**: 1108–1111.
- Williams AH, Sharma M, Thatcher LF, et al. (2016). Comparative genomics and prediction of conditionally dispensable sequences in legume-infecting *Fusarium oxysporum formae speciales* facilitates identification of candidate effectors. *BMC Genomics* **17**: 191.
- Yu H, Ayhan DH, Diener AC, et al. (2020). Genome sequence of *Fusarium oxysporum* f. sp. *matthiolae*, a *Brassicaceae* pathogen. *Molecular Plant-Microbe Interactions* **33**: 569–572.
- Zhang Y, Yang H, Turra D, et al. (2020). The genome of opportunistic fungal pathogen *Fusarium oxysporum* carries a unique set of lineage-specific chromosomes. *Communications Biology* **3**: 50.