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

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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.

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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

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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 3 700 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 & Kloepper 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 *RPB*2 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*.

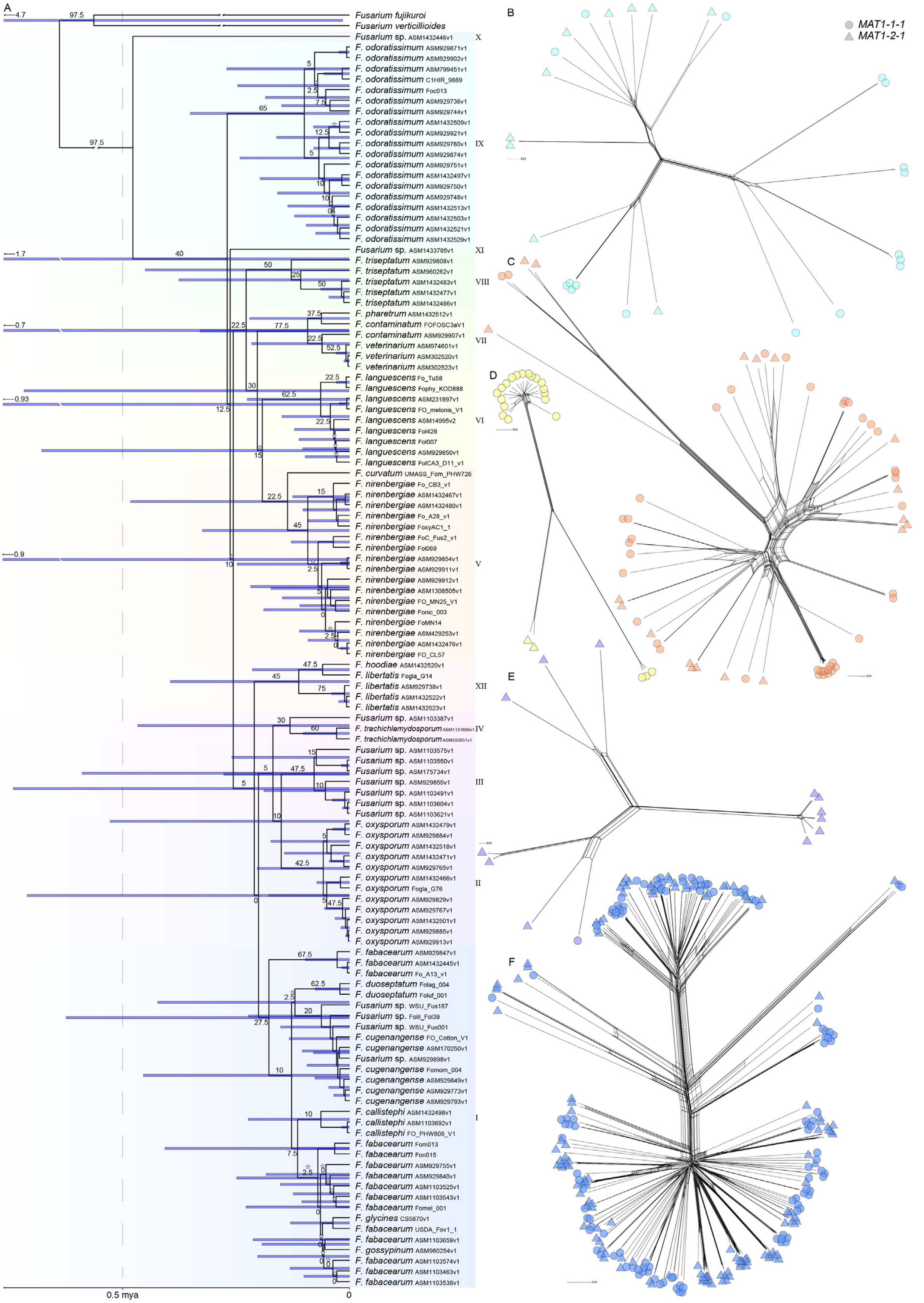

Fig. 1. A. BEAST time-tree estimate with ages sampled from 11 concatenated, protein-coding genes, calibrated to a mean age of 5 million years at the most recent common ancestor of the *Fusarium oxysporum* species complex. The BEAST search was constrained to a maximum likelihood topology from 40 concatenated, 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).

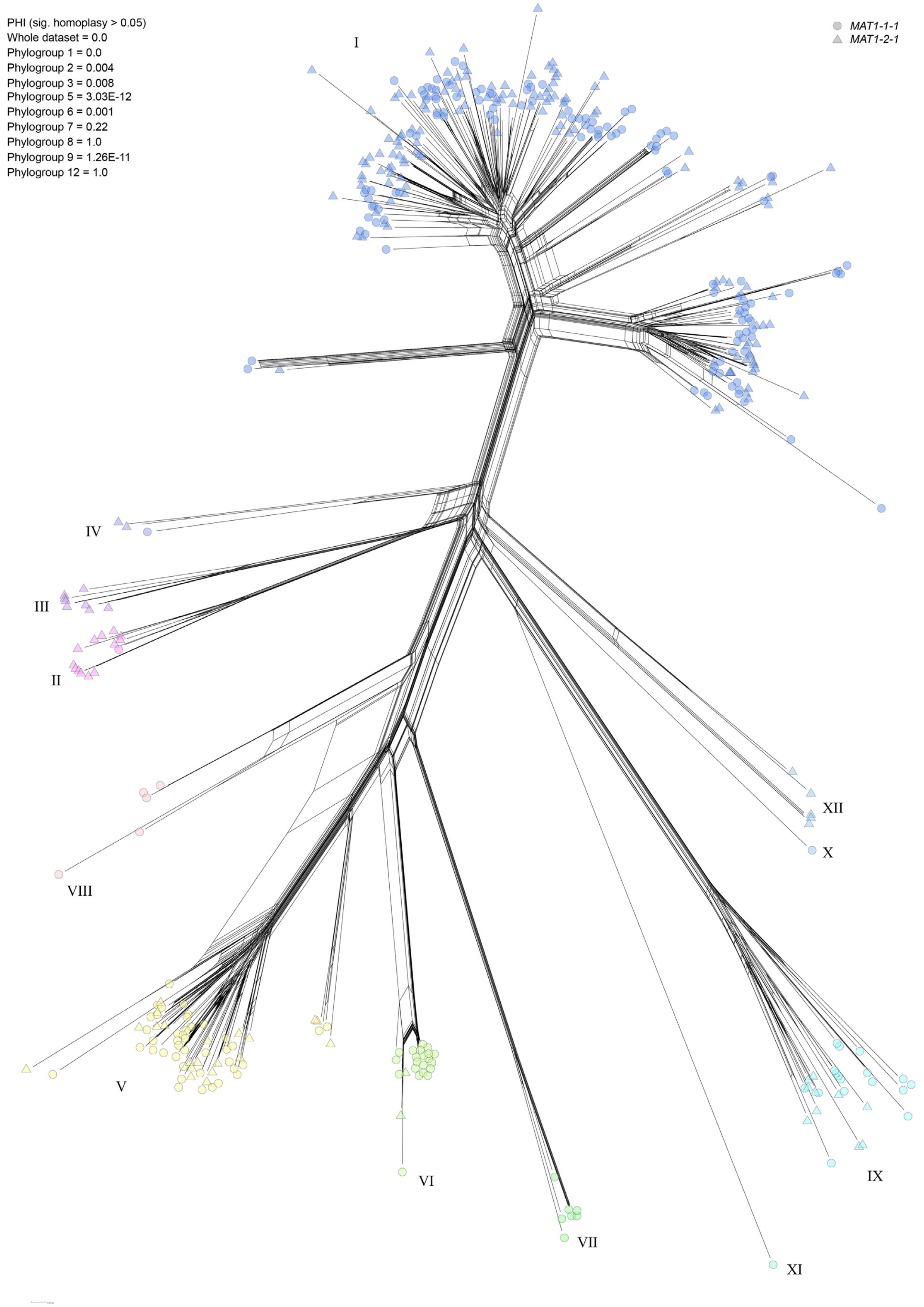

Fig. 2. SplitsTree neighbour network based on 40 concatenated genes. Tests for the pairwise homoplasy index calculated in SplitsTree are provided for the entire dataset and alignments of individual phylogroups. Genotypes are coloured by phylogroup. SplitsTree networks show all putative evolutionary relationships 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*	χ^2	P-value	PHI	Mean age at MRCA	Distribution	Monophyletic *TEF*/*RPB2*
1	256	*F. fabacearum*, *F. gossypinum*, *F. glycines*, *F. cugenangense*, *F. elaeidis*, *F. callistephi*, *F. carminascens*, *F. duoseptatum*, *F. tardichlamydosporum*	119	137	1.266	0.2606	0	142 000	Australia, China, Ethiopia, Greece, Israel, Japan, Netherlands, Russia, Spain, Taiwan, UK, USA	Paraphyletic with Phylo4
2	13	*F. oxysporum*	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	*F. nirenbergiae*, *F. curvatum*	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	*F. languescens*	21	2	15.696	0.0001	0.001	51 000	Australia, France, India, Netherlands, Spain, USA	Yes
7	7	*F. contaminatum*, *F. veterinarium*, *F. pharetrum*	7	0	7	0.0082	0.22	74 000	Australia, Space, Ukraine, USA	Yes
8	5	*F. triseptatum*	5	0	5	0.0253	1.0	102 000	Australia, Spain, USA	Yes
9	27	*F. odoratissimum*	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	*F. libertatis*	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.

χ^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 Homoplasy 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.

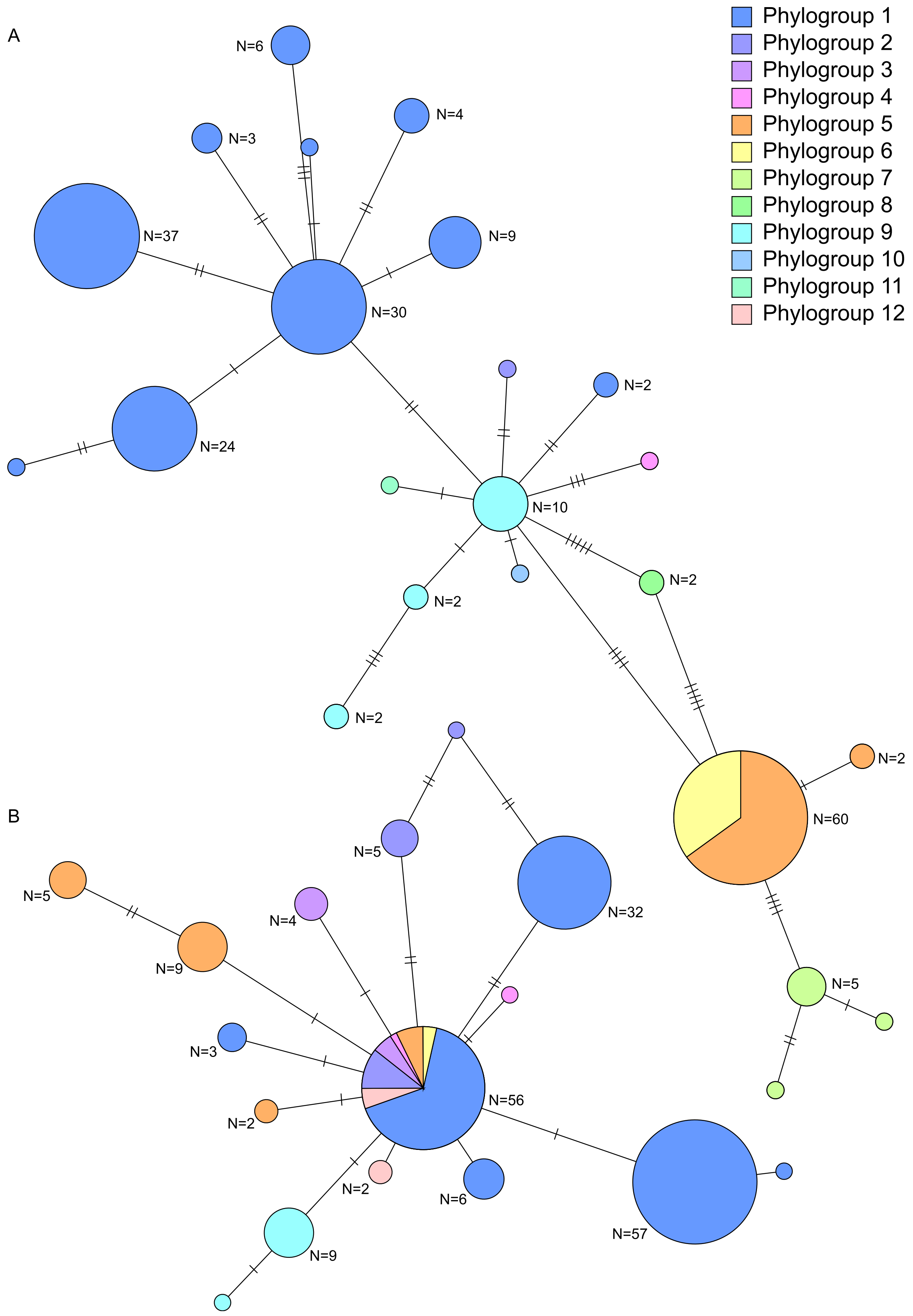

Fig. 3. Haplotype networks from alignments of (**A**) *MAT1-1-1* and (**B**) *MAT1-2-1*. Haplotypes are coloured by whether they are present in different phylogroups.

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**[1]	**LD corrected SNPs**[2]	**% SNPs removed under LD**	**rbarD**[3]	**Genomes**	**No. called SNPs**[1]	**LD corrected SNPs**[2]	**% SNPs removed under LD**	**rbarD**[3]
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[4]	136	748 225	377 645	50	0.038[4]

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

[2] SNPs were filtered based on an r^2 cutoff of 0.9999 calculated in PLINK.

[3] Calculated from bitwise.ia across LD corrected SNPs in *poppr.*

[4] 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 *RPB*2, 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 *RPB*2 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

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APPENDIX A. SUPPLEMENTARY DATA

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

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