PNAS COMMENTARY

Population genomics reveals meiotic recombination in *Fusarium oxysporum*

Like Fokkens[a] and Martijn Rep[b,1]

Because of Muller's rachet—the slow but inevitable accumulation of mutations that reduce fitness—clonal evolution is a hazardous strategy but one that seems to be commonly adopted by fungal pathogens. Many pathogens appear to arise through "epidemic clonality": as rapidly spreading clonal lineages from a background recombining population (1). In these predominantly asexual populations, recombination is limited due to structural variation and mutation accumulation, or only occurs between highly similar individuals due to niche formation and population structuring (restrained recombination) (1). Over time, the ability to reproduce sexually may be lost altogether. Extensive genome reshuffling, horizontal gene transfer, and hybridization facilitate adaptation in clonally evolving pathogens (2). Although these processes may help delay extinction, they are probably not enough to escape Muller's ratchet (3). For this reason, clonally evolving lineages, also known as "imperfect fungi," are generally considered evolutionary dead ends (4).

Given its impact on the evolutionary prospects of fungal pathogens, it is important to know whether sexual recombination can occur in pathogen populations. However, recognizing the sexual capacity of fungal species has been proven difficult (5). Many fungal species produce sexual structures only under specific circumstances, e.g., on special growth media (e.g., ref. 6). If these circumstances are unknown, the occurrence of recombination can only be inferred from population genetic analyses, including the distribution of the mating type loci, phylogenetic clustering, and linkage disequilibrium decay (LD decay). Fayyaz et al. have now applied population genomics on a unique dataset of whole-genome sequence data of around one hundred strains of the important fungal pathogen *Fusarium oxysporum*, isolated from chickpea in Ethiopia, and have found evidence for recent recombination (7).

F. oxysporum has drawn much attention due to its potential to cause disease in many crops and ornamental plant species (8). Importantly however, most strains do not seem to be particularly pathogenic: *F. oxysporum* is commonly found inside plants without causing symptoms and is also isolated from soil (9). Disease-causing strains of this species complex are grouped into so-called *formae speciales* based on host-specificity, and most of these *formae speciales* consist of more than one clonal line of polyphyletic origin (8). Like many microbes, the genome of *F. oxysporum* can be divided into conserved core and conditionally dispensable accessory regions, which include entire chromosomes. New pathogenic lineages seem to arise from horizontal transfer of particular accessory chromosomes that contain a combination of genes encoding effectors and other proteins that enable infection of a particular host (10). This can be described with a variation on the epidemic clonality model, in which not clonal lineages but clonal pathogenicity chromosomes explore and occupy new niches.

Because of the clonal nature of pathogenic strains and because sexual reproduction has not been observed in nature or in the lab, *F. oxysporum* has long been presumed to be largely or completely asexual (11). Two different mating type genes have been identified (12). Individual strains only contain one of the two mating type genes, indicating that if sexual reproduction does occur, *F. oxysporum* is heterothallic. This may have important consequences: If, for example, horizontal acquisition of a chromosome drives a lineage into a specific niche, finding a mate in that niche may be problematic. This could result in predominantly clonal evolution, even when the capability of sexual recombination is maintained (12). However, if *F. oxysporum* as a species would be generally asexual, one would, due to genetic drift, expect a skewed distribution toward one of the mating loci, while in fact several studies, including the one by Fayyaz et al., report more or less equal frequencies of the two mating type loci (7, 13, 14). No sexual structures have been observed for *F. oxysporum* although Fourie et al. report protoperithecium-like structures that were formed after coincubating banana-infecting strains with different mating type loci (13).

In addition to reports on mating type genes, several studies that involved multiple loci concluded that recombination takes place in *F. oxysporum* populations. For example, in 1999, more than a decade before the first *F. oxysporum* genome became available, Taylor et al. suggested that at least some *F. oxysporum* populations are recombining, based on an analysis of restriction fragment length polymorphism (RFLP) data (4). Recombination leads to discordance between individual gene genealogies, and Laurence et al. applied this principle to 36 strains from uncultivated areas in Australia and sequences of eight genes to delineate two phylogenetic species in the *F. oxysporum* species complex, suggesting that within these cryptic species, recombination takes place (9). A similar approach revealed recombination within *F. oxysporum* mitochondrial genomes (15).

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Sampling bias may impact detection of recombination (16). Recently, McTaggart et al. queried a global collection of 410 complete genomes of *F. oxysporum* with different host preferences for a variety of signatures of recombination. They concluded that sexual or parasexual reproduction has frequently taken place within phylogenetic species, but that most current lineages appear to evolve clonally (14). However, the authors also note that recombination was easier to detect in well-sampled clades. The observed clonality could thus also be (partially) explained by biases in sampling and in selecting isolates for whole-genome sequencing: skewed toward pathogenic isolates and typically not including many isolates from the same region (3). Fayyaz et al. zoom in on genomes from isolates that have been sampled from the same country—Ethiopia and from the same plant species—chickpea (7). All isolates had been sampled from symptomatic chickpea plants, but pathogenicity tests on a subset of 21 isolates revealed that around half of the isolates tested showed little or no virulence toward chickpea, and may thus be considered endophytes.

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Most of the isolates used in the study of Fayyaz et al. belong to a single phylogenetic species. To further differentiate within this species, they identify 10 genotype clusters. For all clusters except one, all members within a cluster carry the same mating type gene. About one third of the isolates that belong to this phylogenetic species are not grouped into one of the clusters but appear to have a mixed haplotype, suggesting that extensive recombination occurred between clusters, which is confirmed by patterns of LD decay in SNPs that are shared between groups. Interestingly, four of the clusters show clear signatures of recent recombination based on structure analyses and LD decay of group-specific SNPs. Remaining questions are where and when recombination happens in nature and whether it takes place sexually or parasexually. Fayyaz et al. observe that meiosis-related genes are present in *F. oxysporum* and that recombination is more frequent toward the telomeres, which suggests sexual recombination. However, the same may hold for a type of parasexual recombination called parameiosis, in which unstable diploid nuclei in heterokaryotic mycelium undergo mitotic recombination and haploidization (17). In *Candida albicans*, recombination during parameiosis depends on at least two meiosis-specific genes (18). A similar process may occur in *F. oxysporum*, which would explain the recombination pattern observed after protoplast fusion of strains from the same clonal lineage and with the same mating type, in which most markers showed 1:1 segregation (19).

Another interesting finding by Fayyaz et al. is that the core genomes in the isolates investigated are genetically more isolated than the accessory genomes. Moreover, core genome diversity is not structured by geography, but the diversity of the accessory genome is. Both observations can be explained by horizontal transfer of accessory chromosomes. The final question we have is whether core genome recombination and horizontal chromosome transfer are two different outcomes of one process or are entirely different mechanistically. A similarity is the (likely) requirement for transient diploidy (20). A clear difference is that in all cases of experimental horizontal chromosome transfer, no core genome recombination took place between the parental strains (10) with one exception in which a single core chromosome had recombined (20). Regardless of exact underlying mechanisms, it is evident from the work of Fayyaz et al. and others that (para)sexual recombination should be considered as a factor influencing pathogen diversity. More extensive sampling, including from soil and nonhost plants in the same region or field as symptomatic hosts, will help to further assess the relative contributions of the genetic processes that underlie the emergence and spread of Fusarium wilt.

1. M. Tibayrenc, F. J. Ayala, Reproductive clonality of pathogens: A perspective on pathogenic viruses, bacteria, fungi, and parasitic protozoa. *Proc. Natl. Acad. Sci. U.S.A.* **109**, E3305–E3313 (2012).
2. S. de Vries, E. H. Stukenbrock, L. E. Rose, Rapid evolution in plant–microbe interactions – an evolutionary genomics perspective. *New Phytol.* **226**, 1256–1262 (2020).
3. A. Drenth, A. R. McTaggart, B. D. Wingfield, Fungal clones win the battle, but recombination wins the war. *IMA Fungus* **10** (2019). https://doi.org/10.1186/s43008-019-0020-8. PMID: 32647622; PMCID: PMC7325676.
4. J. W. Taylor, D. J. Jacobson, M. C. Fisher, THE EVOLUTION OF ASEXUAL FUNGI: Reproduction, speciation and classification. *Annu. Rev. Phytopathol.* **37**, 197–246 (1999).
5. P. S. Dyer, U. Kück, Sex and the imperfect fungi. *Microbiol. Spectr.* **5** (2017). https://doi.org/10.1128/microbiolspec.FUNK-0043-2017. PMID: 28597816.
6. C. M. O'Gorman, H. T. Fuller, P. S. Dyer, Discovery of a sexual cycle in the opportunistic fungal pathogen *Aspergillus fumigatus*. *Nature* **457**, 471–474 (2009).
7. A. Fayyaz *et al.*, Hiding in plain sight: Genome-wide recombination and a dynamic accessory genome drive diversity in *Fusarium oxysporum* f. sp. *ciceris*. *Proc. Natl. Acad. Sci. U.S.A.* **120**, e2220570120 (2023).
8. C. B. Michielse, M. Rep, Pathogen profile update: *Fusarium oxysporum*. *Mol. Plant Pathol.* **10**, 311–324 (2009).
9. M. H. Laurence, B. A. Summerell, L. W. Burgess, E. C. Y. Liew, Genealogical concordance phylogenetic species recognition in the *Fusarium oxysporum* species complex. *Fungal Biol.* **118**, 374–384 (2014).
10. J. Li, L. Fokkens, P. van Dam, M. Rep, Related mobile pathogenicity chromosomes in *Fusarium oxysporum* determine host range on cucurbits. *Mol. Plant Pathol.* **21**, 761–776 (2020).
11. H. C. Kistler, Genetic diversity in the plant-pathogenic fungus *Fusarium oxysporum*. *Phytopathology* **87**, 474–479 (1997).
12. T. Arie *et al.*, Mating-type genes from asexual phytopathogenic ascomycetes *Fusarium oxysporum* and *Alternaria alternata*. *Mol. Plant Microbe Interact.* **13**, 1330–1339 (2000).
13. G. Fourie, E. T. Steenkamp, T. R. Gordon, A. Viljoen, Evolutionary relationships among the *Fusarium oxysporum* f. sp. cubense vegetative compatibility groups. *Appl. Environ. Microbiol.* **75**, 4770–4781 (2009).
14. A. R. McTaggart *et al.*, Population genomics reveals historical and ongoing recombination in the *Fusarium oxysporum* species complex. *Stud. Mycol.* **99**, 100132 (2021).
15. B. Brankovics *et al.*, Mitochondrial genomes reveal recombination in the presumed asexual *Fusarium oxysporum* species complex. *BMC Genomics* **18**, 735 (2017).
16. J. W. Taylor, C. Hann-Soden, S. Branco, I. Sylvain, C. E. Ellison, Clonal reproduction in fungi. *Proc. Natl. Acad. Sci. U.S.A.* **112**, 8901–8908 (2015).
17. P. A. Sybuia *et al.*, Limitation of nitrogen source facilitated the production of nonmeiotic recombinants in *Aspergillus nidulans*. *J. Basic Microbiol.* **60**, 380–385 (2020).
18. M. Z. Anderson, G. J. Thomson, M. P. Hirakawa, R. J. Bennett, A 'parameiosis' drives depolyploidization and homologous recombination in *Candida albicans*. *Nat. Commun.* **10**, 4388 (2019).
19. H. A. S. Teunissen, M. Rep, P. M. Houterman, B. J. C. Cornelissen, M. A. Haring, Construction of a mitotic linkage map of *Fusarium oxysporum* based on Foxy-AFLPs. *Mol. Genet. Genomics* **269**, 215–226 (2003).
20. I. Vlaardingerbroek *et al.*, Exchange of core chromosomes and horizontal transfer of lineage-specific chromosomes in *Fusarium oxysporum*. *Environ. Microbiol.* **18**, 3702–3713 (2016).