Evolution and genome architecture in fungal plant pathogens

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Abstract | The fungal kingdom comprises some of the most devastating plant pathogens. Sequencing the genomes of fungal pathogens has shown a remarkable variability in genome size and architecture. Population genomic data enable us to understand the mechanisms and the history of changes in genome size and adaptive evolution in plant pathogens. Although transposable elements predominantly have negative effects on their host, fungal pathogens provide prominent examples of advantageous associations between rapidly evolving transposable elements and virulence genes that cause variation in virulence phenotypes. By providing homogeneous environments at large regional scales, managed ecosystems, such as modern agriculture, can be conducive for the rapid evolution and dispersal of pathogens. In this Review, we summarize key examples from fungal plant pathogen genomics and discuss evolutionary processes in pathogenic fungi in the context of molecular evolution, population genomics and agriculture.

Biotrophs
Pathogens that manipulate host defences and obtain their nutrients from living plant cells through specialized 'feeding' structures or hyphae formed intracellularly in the host.

Necrotrophs
Pathogens that kill host cells by secreting toxins and enzymes to obtain nutrients for growth and reproduction.

Hemibiotrophs
Pathogens that undergo a longer latent or biotrophic phase followed by a switch to necrotrophic growth.

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Infectious diseases have an enormous economic impact on crop production worldwide. Among the most devastating plant pathogens are fungi that use plant tissues for their reproduction and dispersal[1]. Fungal plant pathogens exhibit remarkable genomic and reproductive plasticity, and are represented in different phyla throughout the fungal tree of life (FIG. 1). This widespread distribution suggests that there have been numerous transitions from non-pathogenic to pathogenic lifestyles and vice versa[2]. Fungi can infect and colonize all plant tissues and have evolved different strategies to feed on plant-derived substrates. Broadly speaking, plant pathogens are defined as biotrophs[3], necrotrophs[4,5] and hemibiotrophs (TABLE 1).

Plant pathogens colonize their host by escaping host defences (induced and non-induced), which include physical and chemical barriers, and specific resistance proteins that target pathogen-produced molecules[6,7]. To avoid recognition by the host immune system and to suppress host defences, fungi secrete 'effectors' that act in the apoplastic space or the symplastic space[3]. Some effectors are translocated to cellular compartments (for example, chloroplasts or nuclei), where they manipulate host transcription to favour pathogen invasion[8,9]. Other effectors manipulate host defences by interfering with plant metabolism[10]. To counteract pathogen invasion, plants produce resistance proteins; these proteins are activated by effector-mediated signals and are responsible for signal-transduction events that activate plant defences and block pathogen growth[7]. Ultimately, the outcome of infection is determined by the ability of the pathogen to overcome the immune system of the host and the ability of the plant to block pathogen invasion[7].

In natural ecosystems, the antagonistic interaction between plants and their pathogens drives a co-evolutionary dynamic in which plants evolve to recognize pathogens, and pathogens evolve to avoid plant defence systems[11–14]. These co-evolutionary processes shape genetic variation at the genomic and population levels in both plants and pathogens. However, in managed ecosystems, crops evolve through artificial (rather than natural) selection, in which agriculturally desired traits are favoured. Therefore, pathogens may be confronted with the replacement of host resistance factors in each new growing season. Moreover, instant changes in the genetic make-up of host populations can occur over regional and continental scales, at which new crop cultivars are distributed. The complete replacement of host genotypes and resistance factors over large geographical areas selects for the recurrent replacement of virulence phenotypes, the fast and efficient spread of virulent pathogens (FIG. 2).

We still lack systematic comparisons of pathogens on wild and cultivated plants that assess whether agriculture selects for rapid evolution in fungal pathogens. However, comparative genomic analyses of plant and animal pathogens have revealed that fungi have the capacity to rapidly evolve and adapt to new environmental conditions[15–19]. In fungal plant pathogens, transposable elements and genome plasticity have been described as

important drivers of rapid evolution[20–22], which challenges the development of sustainable management strategies for plant production and protection.

In this Review, we summarize key examples from fungal plant pathogen genomics and discuss evolutionary processes in pathogenic fungi in the light of molecular evolution, population genetics and agriculture. In addition, we consider how the field can move forward, considering the roles of genome plasticity and population genetics parameters in pathogen evolution.

Pathogen evolution and agriculture

Managed and natural ecosystems represent different environments for the propagation and evolution of pathogens. The host population in an agricultural field consists of densely, but uniformly (both in space and time), distributed genetically identical individuals. Moreover, cultivars of agricultural crops are genetically highly similar and mainly differ at a few loci that have been targets for selective crop breeding. By contrast, the host population in a natural ecosystem consists of genetically diverse individuals that are heterogeneously distributed in space and time.

Arabidopsis thaliana has been a valuable model for studying the co-evolutionary dynamics of plants and pathogens in natural ecosystems. Resistance genes in *A. thaliana* represent some of the most variable genes in the genome, which suggests that pathogens select for genetic variation at interacting loci[23,24]. The effect of ecological variation in wild host–pathogen systems has furthermore been extensively studied in two model pathosystems: flax–rust *Linum marginale–Melampsora lini* and *Plantago*–mildew *Plantago lanceolata–Podosphaera plantaginis*[12,25,26]. These studies have shown that both biotic (biological) and abiotic (environmental) factors contribute to the metapopulation dynamics of pathogens on wild plants. However, in both pathosystems, a main determinant of genetic variation and disease prevalence is antagonistic host–pathogen co-evolution. Consequently, metapopulation dynamics of both pathogens in a natural ecosystem are characterized by recurrent population extinction and re-colonization events, which lead to population bottlenecks and the severe loss of genetic variation at local scales[25,26].

How do these dynamics compare with the dynamics in managed ecosystems that have uniform host distributions and in which a complete change in the genetic make-up of the host population can occur from one year to the next? The introduction of new host resistance genes in managed ecosystems can also trigger the local extinction of pathogen populations and give rise to the rapid emergence of new populations that are genetically distinct from the ancestral populations. Population genetics analyses (on the basis of temporal and spatial sampling) of the poplar rust fungus *Melampsora larici-populina* revealed substantial changes in the structure of the pathogen population, which coincided with the breakdown of a major poplar resistance gene[27]. A few virulent *M. larici-populina* isolates gave rise to this new population, which rapidly expanded and replaced the ancestral rust population on poplar trees in France within 20 years[27].

Existing pathogen populations may also rapidly evolve to overcome new host resistances. A study of the pathogen *Leptosphaeria maculans* 'brassicae', which causes blackleg disease in oilseed rape, showed how fast a new plant resistance gene can be overcome by positive selection of a mechanism that increases local genetic diversity[28]. Changes in the frequency of effector alleles in this pathogen were monitored in response to a new oilseed rape resistance gene, *RLM7*. The product of this gene, RLM7, recognizes the effector gene product AvrLm7, which is a virulence factor expressed by *L. maculans* 'brassicae' during infection. The introduction of *RLM7* caused a rapid allelic diversification of *AVRLM7,* which included mutations that prevent recognition by RLM7. Over four years, the frequency of virulent *AVRLM7 L. maculans* 'brassicae' isolates increased by 40%[28]. *AVRLM7* is found in a repeat-rich genomic compartment that is characterized by an increased mutation rate; this promoted the rapid escape of host resistances[29]. Repeat-rich genome compartments are found in numerous other fungal pathogen genomes and will be described in detail below.

The rapid evolution of crop pathogens is also promoted by agricultural trade that distributes genetically identical crops between continents[30]. Not only are agricultural products moved around but also pathogens are introduced to new continents, and species that would otherwise be separated by continents come into contact with each other. The consequences of agricultural trade are recently exemplified by the emergence of the wheat blast pathogen *Magnaporthe oryzae* (also known as *Pyricularia oryzae*) in Bangladesh[31,32]. Population genomics and phylogenetic analyses have shown that the *M. oryzae* lineage that caused the outbreak in Bangladesh originated from South America and was probably introduced through infected wheat material imported from Brazil[31]. The global movement of pathogens with crop or forestry products can also promote the emergence of new hybrid pathogens by mixing native and introduced pathogen species (for example, the emergence of Dutch elm disease). The mixing of geographically separated lineages of *Ophiostoma novo-ulmi* and *Ophiostoma ulmi* through trade gave rise to new virulent pathogens, which greatly affected the European population of elm trees[33,34].

In short, agricultural ecosystems, including managed forest systems, represent environments that are highly conducive for the rapid evolution and dispersal of fungal plant pathogens[35]. Managed ecosystems can promote large and stable populations of pathogens that can respond to rapid changes in host resistance factors by evolving new virulence traits[35]. Understanding the evolution mechanisms of plant pathogens in managed and natural ecosystems should help researchers to develop more diverse selection environments, which could slow down rates of evolution in crop pathogens[35].

Host–pathogen co-evolution

The host is the strongest driver of evolution in a pathogen, as successful infection is required for reproduction and dispersal. Therefore, infection-related genes are

Effectors
Pathogen-produced molecules that are secreted during infection to manipulate host defences and facilitate pathogen invasion.

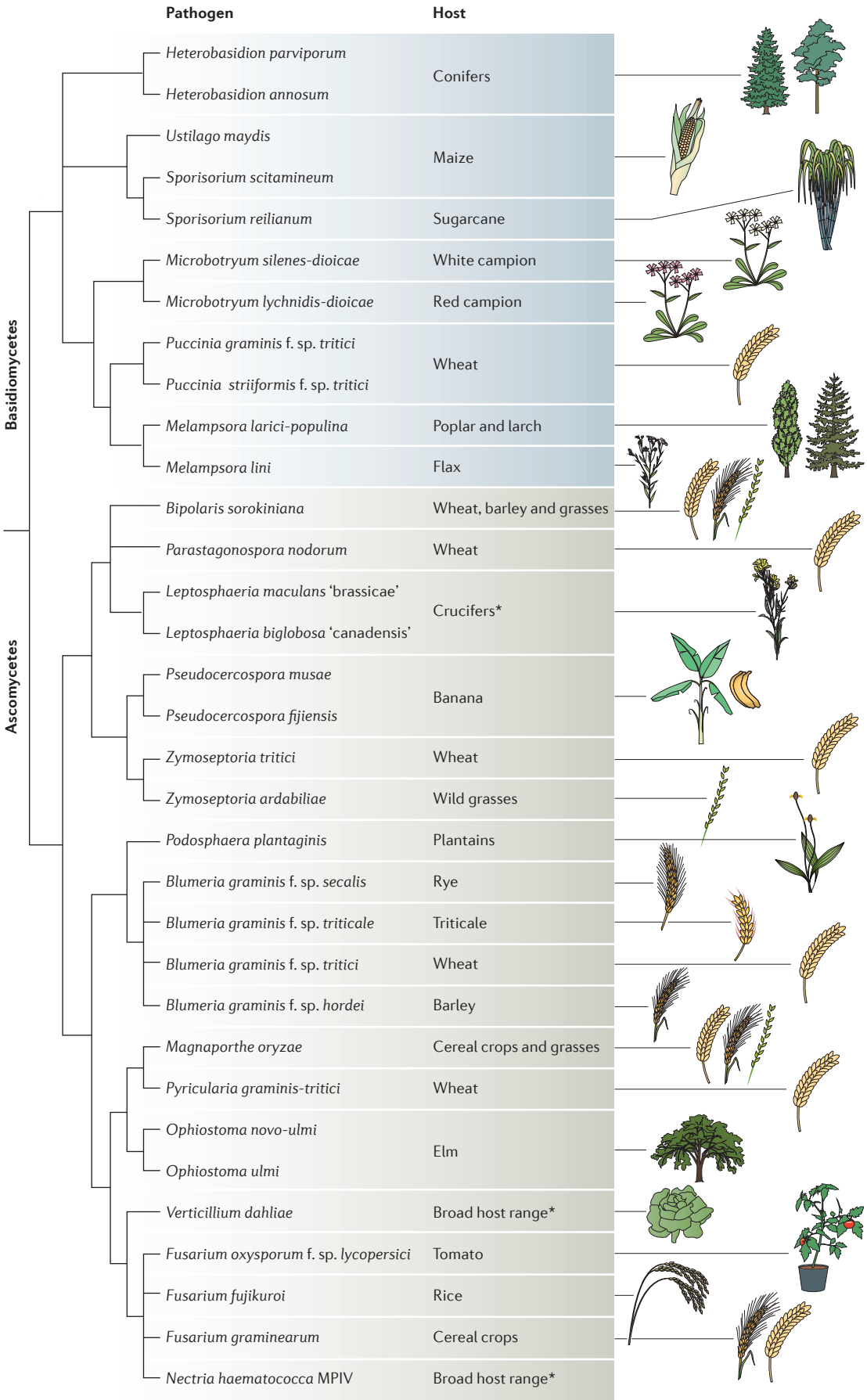
Apoplastic space
Space outside of the plant plasma membrane.

Symplastic space
Space on the inner side of the plant plasma membrane.

Cultivars
Varieties of crop plants of the same species that have distinct phenotypes and genotypes. Distinct cultivars are obtained by plant breeding, whereby desired traits are selected and propagated.

Transposable elements
Genetic elements that move from one location in the genome of their host to another. Transposable elements are also known as transposons.

Metapopulation dynamics
Local fluctuations in the (actual as well as effective) population size of spatially separated populations that belong to the same species.



Non-synonymous mutations
Nucleotide changes in coding sequences that alter the amino acid sequence in the translated proteins.

Synonymous mutations
Nucleotide changes in coding sequences that alter the codons, but not the amino acid sequence, in translated proteins.

Trench warfare
A model that predicts constant diversity in a host–pathogen system, due to the maintenance of multiple alleles at a co-evolving locus by positive diversifying selection or balancing selection.

'Arms race' evolution
The co-evolution of host and pathogen alleles, which results in the recurrent fixation of advantageous alleles at a co-evolving locus. The fixation of advantageous alleles is mediated by positive directional selection.

Tajimas D
A statistical test parameter that is used in population genetics and DNA sequence analyses. Tajima's test is used to identify sequences that do not fit the neutral theory model, by which the fate of any mutation is determined by genetic drift.

Divergence patterns
The distribution of substitutions varies along the genome, as different parts of the genome evolve by processes and by different rates. The underlying pattern of divergence can be investigated to unravel the history of mutational events and the effect of selection versus neutral processes on the sequence evolution.

under strong selection pressure in pathogen genomes. Some of these genes encode conserved proteins that are important for *in planta* signalling, invasive growth and the formation of specific infection structures. Others are rapidly evolving virulence-related genes, the products of which include effectors that directly interact with plant defence proteins.

Genomic signatures of selection can be used to identify traits that are involved in host–pathogen interactions. This is because genes or loci that are subjected to strong positive or negative selection pressure can be identified by the distribution of polymorphisms (variable sites in species) and substitutions (variable sites between species), and the proportions of non-synonymous mutations and synonymous mutations in coding sequences[36]. The co-evolution of effectors and resistance genes can result in two different scenarios that are distinguished as 'trench warfare' or 'arms race' evolution[37] (FIG. 2). Both types of selection favour genetic variation, but the genomic signatures are markedly different. Several different statistical tests have been applied to search genomes for signatures of selection in plant–pathogen systems. Statistical test parameters such as Tajimas D explore the site frequency spectra of polymorphisms[38], whereas other methods contrast the patterns of intraspecific polymorphisms and interspecific divergence patterns. For example, the McDonald–Kreitmann test detects signals of selection by comparing non-synonymous and synonymous divergence to non-synonymous and synonymous polymorphisms[15].

Under the trench-warfare scenario, a diverse set of alleles are maintained at the co-evolving locus, a phenomenon that is referred to as balancing selection[39] (FIG. 2a). This may occur in heterogeneous environments (such as natural ecosystems), in which natural selection favours different alleles in distinct metapopulations (populations of the same species, which are spatially separated) that have specialized to distinct local ecological conditions. The genomic signature of balancing selection is an excess of polymorphisms at intermediate (balanced) frequencies in the population (FIG. 2b,c). Genetic variation of resistance genes in natural plant populations is, to a large extent, maintained by balancing selection[40]. An experimentally validated example is the disease resistance gene *RPP13* in *A. thaliana*. RPP13 interacts with an avirulence protein produced by the oomycete pathogen *Hyaloperonospora parasitica*. Allelic variation in *RPP13* is maintained by balancing selection imposed by polymorphic avirulence alleles in the pathogen population[23].

Arms race evolution drives the continuous replacement of alleles (selective sweep) in the pathogen population in response to the continuous emergence of new resistance alleles in the host[39] (FIG. 2d). The genomic signature of a selective sweep is a local depletion of genetic variation in the genomic region that surrounds the locus under selection (FIG. 2e,f). During a selective sweep, neighbouring polymorphisms will also become fixed, which results in strong linkage disequilibrium across the regions. Genetic variation at the locus is re-introduced over time through recombination and by new mutations; however, the selected locus can be identified by having a different pattern of genetic variation compared with neutrally evolving loci. In particular, the selected locus will contain an excess of low-frequency alleles that can distinguish the locus from neighbouring regions. Genome-wide maps of selective sweeps were generated for the two anther smut species, *Microbotryum lychnidis-dioicae* and *Microbotryum silenes-dioicae*, using estimates of nucleotide variation, frequency-spectra of polymorphisms and statistical genetics[41]. The analyses revealed recent signatures of selective sweeps in putative pathogenicity related genes that are probably important in the host specialization of pathogenic *Microbotryum* spp.

Antagonistic host–pathogen co-evolution requires the maintenance and generation of genetic variation in both partners. The dynamic of resistance genes and virulence genes involves a combination of trench-warfare and arms race evolution. In managed ecosystems, the dynamic of arms races occurs at a much faster pace than in natural ecosystems, selecting specifically for rapid evolution in crop pathogens. Furthermore, trench-warfare evolution is probably less important in pathogens of crop plants, owing to the uniform composition and distribution in time and space of host individuals. Through the comparative analyses of genome structures and compositions, we have gained detailed insight into the genomics of adaptive evolution in several fungal pathogens and the role that genome plasticity has in evolutionary changes. Below, we summarize particular characteristics of fungal plant pathogen genomes and discuss these in the light of adaptation.

Genomic variation of fungal pathogens

Genes that are located in genomic environments with high mutation and/or recombination rates can evolve faster through the increased generation of novel mutations that selection can act on[39]. Effector genes that are located in transposable element-rich regions (which have higher mutations rates) will accumulate more mutations, some of which may be beneficial non-synonymous changes that confer a fitness advantage. Natural selection can thereby indirectly select for an association of effector genes with rapidly evolving transposable element-rich regions. Genome-based studies of plant pathogenic fungi have indeed shown that virulence-related genes are typically located in transposable element-rich genomic compartments or on specific chromosomes that have higher rates of sequence evolution[29,42–44] (FIG. 3; TABLE 1). These regions include accessory or lineage-specific chromosomes[43,45–47], AT-rich isochores[29], transposon-rich 'islands' (REF. 44), and clusters of tandem duplicated genes[48]. The increased rates of evolution in particular regions of pathogen genomes have given rise to the term 'two-speed genomes' (REF. 49).

◀ Figure 1 | **Fungal plant pathogens with diverse lifestyles and hosts.** This figure shows the phylogenetic relationships between the major groups of fungal plant pathogens that belong to the two fungal phyla Ascomycetes and Basidiomycetes, and their respective host plants (right hand side). *In the case of a broad host range, the host plant of a reference isolate that was used for genome sequencing is depicted. The phylogenetic tree was generated using phyloT, based on NCBI taxonomy and visualized using interactive Tree of Life (iTOL)[137]. f. sp., formae speciales.

Table 1 | **Genome and lifestyle characteristics of different fungal plant pathogens**

Species (isolate)	Isolate-specific host	Species-specific host	Lifestyle	Reproduction	Genome size (Mb)*	% Repetitive sequence*	Genome features linked to pathogenicity‡	Refs
Sporisorium scitamineum (Sscl8)	Sugar cane	Sugar cane	Biotroph	Sexual	20	6.7	Repeat-rich gene clusters that encode effector candidates	48
Ustilago maydis (521)	Maize	Maize	Biotroph	Sexual	20	6.7	Repeat-rich gene clusters that encode effector candidates	48, 66
Microbotryum lychnidis-dioicae (p1A1 Lamole)	Red campion	Red campion	Biotroph	Sexual	33	14	Repeat-rich gene clusters that encode effector candidates	103, 104
Melampsora larici-populina (98AG31)	Poplar and larch	Poplar and larch	Biotroph	Sexual and asexual	101	45	ND#	73
Puccinia graminis f. sp. *tritici* (CDL75-36–700-3, race SCCL)	Wheat	Wheat and barley	Biotroph	Sexual and asexual	89	45	Highly polymorphic effector candidates	73
Zymoseptoria tritici (IPO323)	Wheat	Wheat	Hemi-biotroph	Sexual and asexual	40	• 18.6 (genome mean) • 16.6 (core) • 33.6 (accessory)	• Orphan regions are enriched in *in planta*-expressed genes • Possible function of accessory chromosomes in virulence	45, 57, 58
Leptosphaeria biglobosa 'canadensis' (J154)	Mustard	Crucifers	Necrotroph	Sexual and asexual	32	3.9	ND#	71
Leptosphaeria maculans 'brassicae' (v23.1.3)	Oilseed rape	Crucifers	Hemi-biotroph	Sexual and asexual	45	35.5 (99.8% of all repeats located in AT-isochores)	• Enrichment of effector candidates and chromatin-mediated effector candidate regulation in AT isochores • Conditionally dispensable chromosome contains avirulence-encoding gene	29, 60
Blumeria graminis f. sp. *tritici* (96224)	Wheat	Various	Biotroph	Sexual and asexual	180	90	Presence and/or absence of polymorphisms of candidate effectors	140, 141
Blumeria graminis f. sp. *hordei* (DH14)	Barley	Various	Biotroph	Sexual and asexual	120	64	Repeat-rich accessory regions that encode infection-specific transcribed genes	142
Magnaporthe oryzae (70–15)	Rice	Various crops and wild grasses	Hemi-biotroph	Sexual and asexual	41	10	Highly polymorphic effector candidates and translocations of effector genes	22, 143
Ophiostoma novo-ulmi (H327)	Elm	Elm	Necrotroph	Sexual and asexual	32	3.4	ND#	144
Verticillium dahliae (VdLs17)	Lettuce	Various	Necrotroph	Asexual	37	12	Enrichment of *in planta*-expressed effector candidates in LS# regions	16, 44
Fusarium solani/*Nectria haematococca* MPVI (77-13-4)	Pea	Various	Hemi-biotroph	Sexual and asexual	54	• < 5 (core) • >10–25 (supernumerary)	LS chromosomes confer host specificity and virulence	46
Fusarium graminearum (PH-1)	Wheat	Wheat and barley	Hemi-biotroph	Sexual and asexual	36	<3	Enrichment of *in planta*-expressed and species-specific genes in regions of high SNP# density	145
Fusarium oxysporum f. sp. *lycopersici* (4287)	Tomato	Various	Hemi-biotroph	Asexual	60	28 (~74% of TEs# located on LS# chromosomes)	LS chromosomes confer host specificity and virulence	43

LS, lineage-specific; ND, no data; TEs, transposable elements. *Genome size and repeat content refer to the respective reference isolate. Isolate-specific hosts refer to the host plant from which the reference isolate was collected. In some cases, other isolates of the same species infect other hosts. ‡Genome characteristics have been inferred from comparative genomics analyses.

Distinct regions in pathogen genomes evolve at different rates, and the rapidly evolving compartments of plant pathogen genomes (FIG. 3) can also be seen as regions in one extreme of a continuum of evolutionary rates. Below, we describe particular characteristics of rapidly evolving pathogen genomes.

Accessory chromosomes. Accessory chromosomes show non-Mendelian inheritance and are present in some, but not all, individuals in a population (FIGS 3,4). In the field of fungal genetics and phytopathology, accessory chromosomes have drawn a lot of attention as they can encode specific virulence determinants. Accessory chromosomes are typically enriched with repetitive DNA and have a lower gene density than the core genome. Moreover, accessory chromosomes encode genes that have higher incidences of non-synonymous substitutions, which indicates a lower efficacy of selection in removing non-adaptive mutations in coding sequences on these chromosomes[50]. They are found in different eukaryotic taxa, including plants, animals and fungi, and are referred to by different names, such as B-chromosomes, supernumerary chromosomes or lineage-specific chromosomes[51]. In this Review, we will use the terms accessory and lineage-specific chromosomes.

The first characterization of virulence traits encoded by an accessory chromosome was in the pathogen *Nectria haematococca* (anamorph *Fusarium solani)*[52]. *N. haematococca* isolates that infect peas have an accessory chromosome that encodes cluster of pea pathogenicity (PEP) genes that are necessary for virulence in pea plants[53]. Isolates that do not have the chromosome are avirulent on peas but can infect other hosts. This indicates that the accessory chromosomes have a role in defining the host range of *N. haematococca* isolates. A related species, *Fusarium oxysporum*, has similar accessory chromosomes, which are termed lineage-specific chromosomes, as they distinguish the specific host of the pathogen[43]. These lineage-specific chromosomes also encode effectors and effector-inducing transcription factors that are important for pathogenicity[54]. Notably, there is evidence that lineage-specific chromosomes can move horizontally and mediate the exchange of host specificities between *F. oxysporum* lineages. This is a potential mechanism that has evolved to compensate for the lack of sexual reproduction in this pathogen[43].

The genome of the reference isolate IPO323 of the wheat pathogen *Zymoseptoria tritici* has eight accessory chromosomes, which are up to 1 Mb in size and can be lost and generated *de novo* during meiosis and mitosis[45,55,56] (FIG. 4). Accessory chromosomes are found in several species in the *Zymoseptoria* genus, which suggests that they represent an ancestral dynamic trait that predates existing species diversification[15]. The accessory chromosomes of *Z. tritici* are not enriched in effector genes; however, quantitative trait locus mapping and detailed phenotyping of progeny with and without different accessory chromosomes have revealed that accessory chromosomes have a role during *in planta* disease development[57]. Strains that lacked particular chromosomes showed statistically significant increases in several virulence traits. Interestingly, preliminary data suggest that the effect of the accessory chromosomes in *Z. tritici* is dependent on host genotype, which indicates an effect of balancing selection on the maintenance of these chromosomes over long evolutionary times[15] (M. Habig and E.H.S., unpublished observations).

Transposable element-rich genome compartments. The genomes of several pathogenic fungi (pathogens of wild and cultivated hosts), include genome compartments that exist as accessory 'islands' on core chromosomes (FIG. 3). These regions can span several hundred kilobases and can encode virulence determinants[16,43,44,58,59]. Accessory compartments often resemble accessory chromosomes, as they are gene-sparse, enriched in transposable elements, and are highly variable in sequence composition and structure. In *L. maculans* 'brassicae' and *Z. tritici*, transposable element-rich genome compartments are also characterized by epigenetic modifications that further associate with distinct patterns of transcription and accumulation of mutations[60–62] (BOX 1; FIG. 3). The source of distinct epigenetic modifications may derive from the ability of repetitive DNA to induce particular epigenetic changes[63,64] (BOX 1).

Transposable element-rich genome compartments may be generated by structural rearrangements or they may evolve in regions with suppressed recombination[65]. Single-molecule real-time (SMRT) sequencing data have enabled more detailed analyses of transposable element-rich genome compartments in pathogenic fungi. In the wilt pathogen *Verticillium dahliae* and in *Z. tritici*, accessory genome compartments originate from structural rearrangements and unfaithful DNA repair across repeated sequences[44,58]. The discovery of active transposable elements in accessory compartments in *V. dahliae* further supports a role for transposable elements in shaping these genomic regions[44,58]. In *Z. tritici*, a transposable element-rich compartment on chromosome 7 has similar sequence composition (gene density, gene annotation and GC content) and histone modifications as the accessory chromosomes of the pathogen, and may be an ancestral accessory chromosome that has fused with a core chromosome (FIG. 3c).

Pathogen genomes that have low transposable element content can also contain rapidly evolving genomic regions that favour effector evolution. The genomes of the three smut fungi *Ustilago maydis*, *Sporisorium scitamineum* and *Sporisorium reilianum*[48,66,67] have a low transposable element content (7%); however, the few transposable elements that are present are associated with virulence gene clusters[66] (TABLE 1). These gene clusters show signatures of accelerated evolution and gene diversification that are driven by a high rate of tandem gene duplications, which are facilitated by the repetitive DNA[48].

A local accumulation of transposable elements may occur in regions in which recombination is suppressed; for example, in non-recombining mating-type loci of heterothallic fungi[65]. Thus far, few studies have addressed recombination rates across genome compartments of

Selective sweep
An increase in the frequency of an advantageous allele (and closely linked chromosomal segments) that is caused by positive selection. Sweeps initially decrease genetic variation and subsequently lead to a local excess of rare alleles (homozygosity excess) as new unique mutations accumulate.

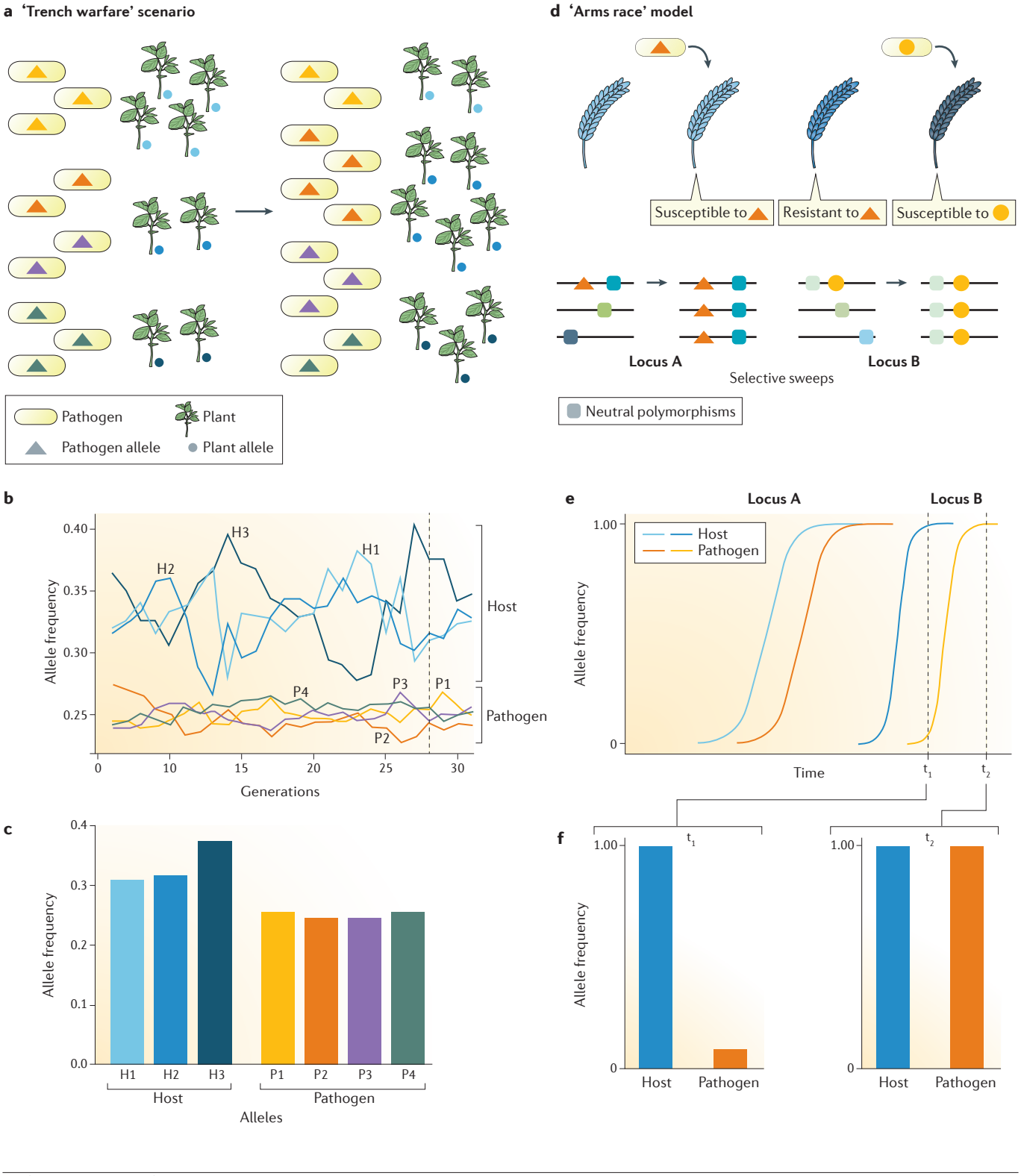
Linkage disequilibrium
The non-random association of alleles at different loci.

Accessory chromosomes
Chromosomes that are not present in all isolates of the same pathogenic species. Such chromosomes often encode determinants of host specificity.

Heterothallic
In heterothallic species, two individuals that have opposite mating types are required for sexual reproduction. By contrast, homothallic organisms have both mating types in one thallus.

pathogenic fungi, and the correlation between recombination and transposable element content has been poorly addressed. However, recombination seems to be significantly reduced in the accessory chromosomes of *Z. tritici*, which supports a role for recombination in shaping the genome architecture of fungal pathogens, including the initial emergence and maintenance of transposable element-rich compartments[68].

Genome architecture and plasticity. The genomes of several hundred fungal plant pathogens have been sequenced, which has provided a key resource for the analysis of genome architecture. An important discovery was that the genome architecture, notably the genome size and transposable element content, can vary greatly between closely related species. A detailed comparison of 18 genomes of species in the class Dothideomycetes



Mesosynteny
A term that refers to the conservation of gene content on chromosomes, but a variation in the gene order and orientation. This is a phenomenon that is, thus far, particularly described in dothideomycete fungi.

Effective population size
(N_e). The approximate number of breeding individuals that produce offspring that live to reproductive age. This number influences the rate of loss of genetic variation, the efficiency of natural selection, and the accumulation of beneficial and deleterious mutations. It is frequently much smaller than the number of individuals in a population.

(including the genomes of several prominent plant pathogens, including *Alternaria brassicicola*, *Pyrenophora tritici-repentis*, *Z. tritici* and *Cladosporium fulvum*) revealed a particular phenomenon described as mesosynteny[69]. Mesosynteny refers to the conservation of gene content in chromosomes, but variation in gene order and orientation[70]. Thus, species with mesosynteny have an excess of intra-chromosomal rearrangements. The underlying mechanisms that are responsible for this phenomenon are poorly understood, but they may relate to the specific transposition mechanisms of genomic elements that are abundant in species in the Dothideomycetes.

Another remarkable discovery among species in the Dothideomycetes is the large variation in genome sizes. Species in the *L. maculans–Leptosphaeria biglobosa* species complex are pathogens of cruciferous plants. *L. maculans* 'brassicae' is the only member that has a high transposable element content (35%)[71]. By contrast, the genomes of four other members of the *L. maculans–L. biglobosa* species complex contain less than 4% repeat sequences, which indicates a recent invasion of transposable elements in *L. maculans* 'brassicae' (REFS 29,71). Similar examples of lineage-specific expansions of transposable element families in related fungal plant pathogens include *Pseudocercospora* spp. in the Sigatoka disease complex of the banana plant[72]. Genome size variation and the distribution of different transposable element families between the three species *Pseudocercospora musae* (83 Mb), *Pseudocercospora eumusae* (54 Mb) and *Pseudocercospora fijiensis* (74 Mb), suggest that lineage-specific transposable element expansions have an important role during species divergence.

Transposable element invasions in fungal genomes can result in the expansion of certain gene families and also substantial changes in genome architecture, which includes chromosome numbers[43,73]. For example, differences in genome organization are present among plant pathogens in the genus *Fusarium*[43,74]. *Fusarium graminearum* and *F. oxysporum* represent two closely related virulent pathogens that have distinct lifestyles and markedly different genome architectures. The genome of *F. oxysporum* (60 Mb) comprises 20% repetitive DNA and 15 chromosomes, and includes lineage-specific chromosomes[43]. By contrast, the genome of *F. graminearum* comprises only 3% repetitive DNA and a total of four chromosomes.

In summary, pathogenic fungi exhibit high levels of genome plasticity and variable genome architectures. The variation that is observed between related species is mainly due to differences in transposable element containment. Although transposable elements predominantly have negative effects on their host genome, fungal pathogens provide remarkable examples of the advantageous association between transposable elements and rapidly evolving virulence determinants. Below, we consider mechanisms that can cause the observed differences in genome architecture and transposable element content among related species.

Population genetics and evolution

The evolution of gene variants in a population is governed by a set of evolutionary forces. Essentially, genetic variation is the product of mutations and recombination that contribute to variation in the genome (BOX 2). The fate of any given mutation or allele in the genome is determined by natural selection and genetic drift (stochastic events), and the relative contribution of these processes is determined by the effective population size (N_e) of the species. In terms of evolution, the N_e is more relevant than the actual number of individuals in a population. N_e expresses the degree to which gene frequencies are transmitted across generations. Thus, a large population can behave genetically similar to a small population if only a minor fraction contributes to the next generation[75]. Notably, asexual species generally have a lower N_e than sexually reproducing species, as offspring are essentially copies of their parents. In populations that have a large N_e, the effect of genetic drift is relatively small compared with populations that have a small N_e. Two important points to consider here are that the N_e of an organism can vary over time and, consequently, the importance of genetic drift in genome evolution can also vary over time.

Evolution with and without sexual reproduction. The reproductive modes of fungi range from predominantly clonal to obligate out-crossing (FIG. 4; TABLE 1). The advantages of clonal reproduction are that co-adapted allele combinations are maintained in the population and that fit genotypes can be rapidly propagated[76]. By contrast, sexual reproduction can enable a rapid response to environmental changes by increasing the efficacy by which natural selection can fix and combine advantageous mutations[77]. In some species, sexual mating is directly linked to pathogenicity and therefore constitutes an important step in life cycles. For example,

◀ Figure 2 | **Models of host–pathogen co-evolution. a–c** | The 'trench warfare' scenario. **a** | The trench warfare scenario can occur in heterogeneous environments, such as those present in natural ecosystems, in which local adaptation leads to a diversity of effector and resistance alleles in the host–pathogen populations that are maintained over evolutionary time. **b** | Positive diversifying selection (balancing selection) maintains multiple alleles in the host (H1, H2 and H3) and the pathogen (P1, P2, P3 and P4) populations over evolutionary time. The vertical line shows a random time point. **c** | Inference of allele frequencies at a given generation shows an excess of alleles at intermediate frequencies in the host and pathogen populations. **d–f** | 'Arms race' model. **d** | The arms race model describes the recurrent fixation of alleles at co-evolving loci in a host–pathogen system and reflects the dynamics in agricultural ecosystems. The interactions between effectors and resistance gene products determine the outcome of pathogen infection; pathogens that have effector alleles that enable successful invasion select for new resistance alleles in the host. A new resistance allele confers a fitness advantage and is selected for by positive directional selection. The allele will reach fixation when all individuals in the host population have the respective resistance allele. A new virulence allele that confers susceptibility in the host population is strongly selected for and is fixed in the pathogen population. This cycle continues ad infinitum. Positive selection of a highly advantageous allele can result in a selective sweep (local depletion of genomic variation), as the allele is fixed in the population. **e** | In agricultural ecosystems, new alleles in the host population can be introduced very rapidly (blue lines), which drives the recurrent fixation of alleles in pathogen populations (orange and yellow lines) by positive directional selection. The vertical lines show a random time point. **f** | At the start of a new season (t_1), a crop that has a new resistance allele (blue) is introduced. In the beginning, the frequency of the respective virulence allele (orange) in the pathogen population is low, but rapidly increases until it reaches fixation (t_2).

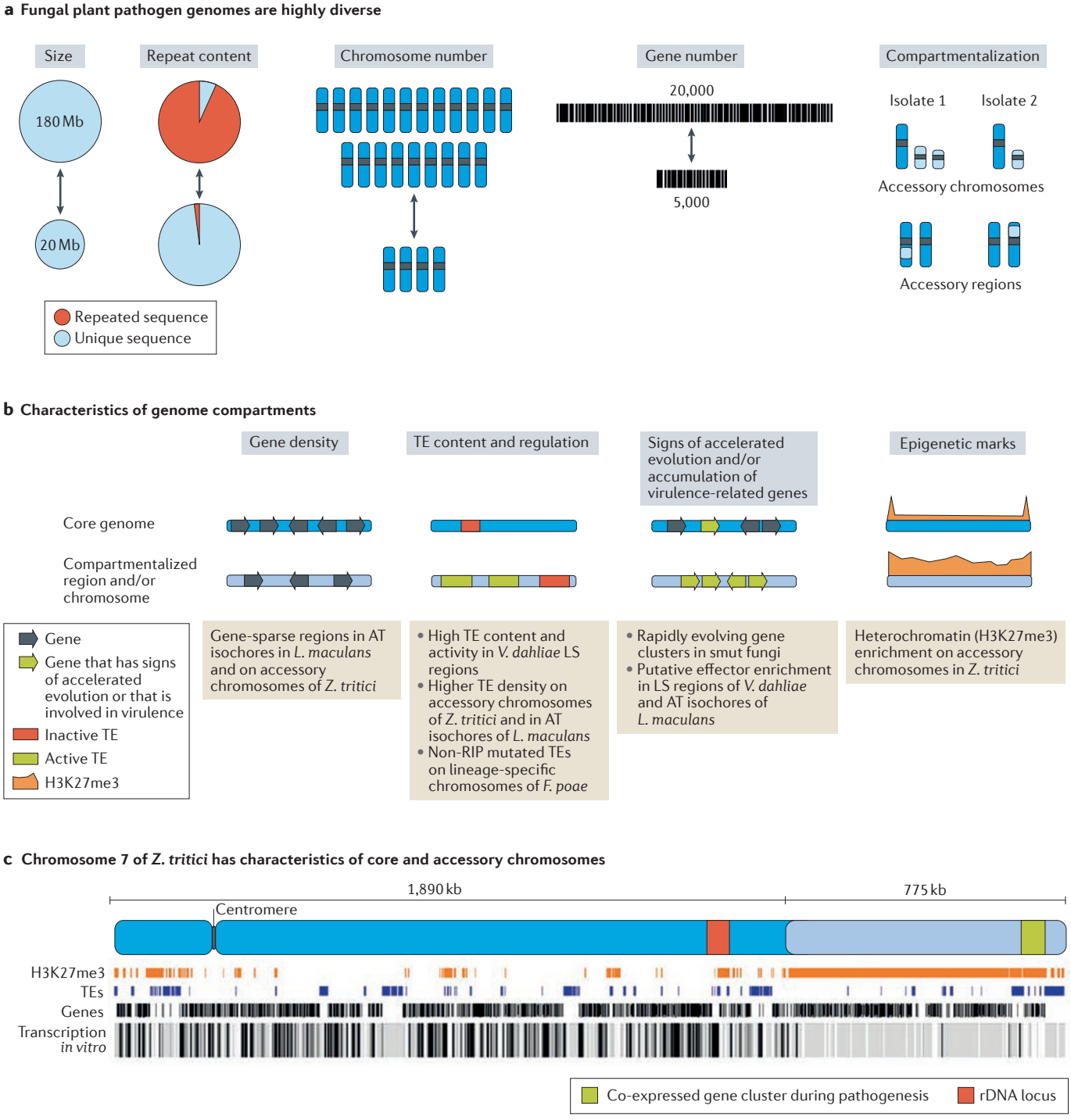


Figure 3 | **Characteristics of fungal plant pathogen genomes. a** | The genome architecture of fungal plant pathogens is highly diverse. Genomes vary in total size, repeat content and gene number. The genome organization can vary from few chromosomes to many, and can include accessory chromosomes and particular genome compartments. **b** | Specific characteristics that distinguish genome compartments from the core genome include lower gene density, higher transposable element content, higher rates of evolution and distinct epigenetic marks[16,44,138,139]. **c** | Chromosome 7 of *Zymoseptoria tritici* has characteristics of both core and accessory chromosomes[61]. The right arm is enriched with the heterochromatin-associated histone mark H3K27me3 and is poorly transcribed[61]. A ribosomal DNA (rDNA) locus close to the start of the accessory region may be involved in the fusion of an accessory chromosome to a core chromosome. A gene cluster that is potentially involved in virulence and the production of secondary metabolites is transcribed at the right arm of the chromosome in the accessory-like region[62]. Active transcription of the accessory chromosome region must be mediated by chromatin remodelling during infection. *F. poae*, *Fusarium poae*; *L. maculans*, *Leptosphaeria maculans*; LS, lineage-specific; RIP, repeat-induced point; TE, transposable element; *V. dahliae*, *Verticillium dahliae*. Figure adapted from REF. 61.

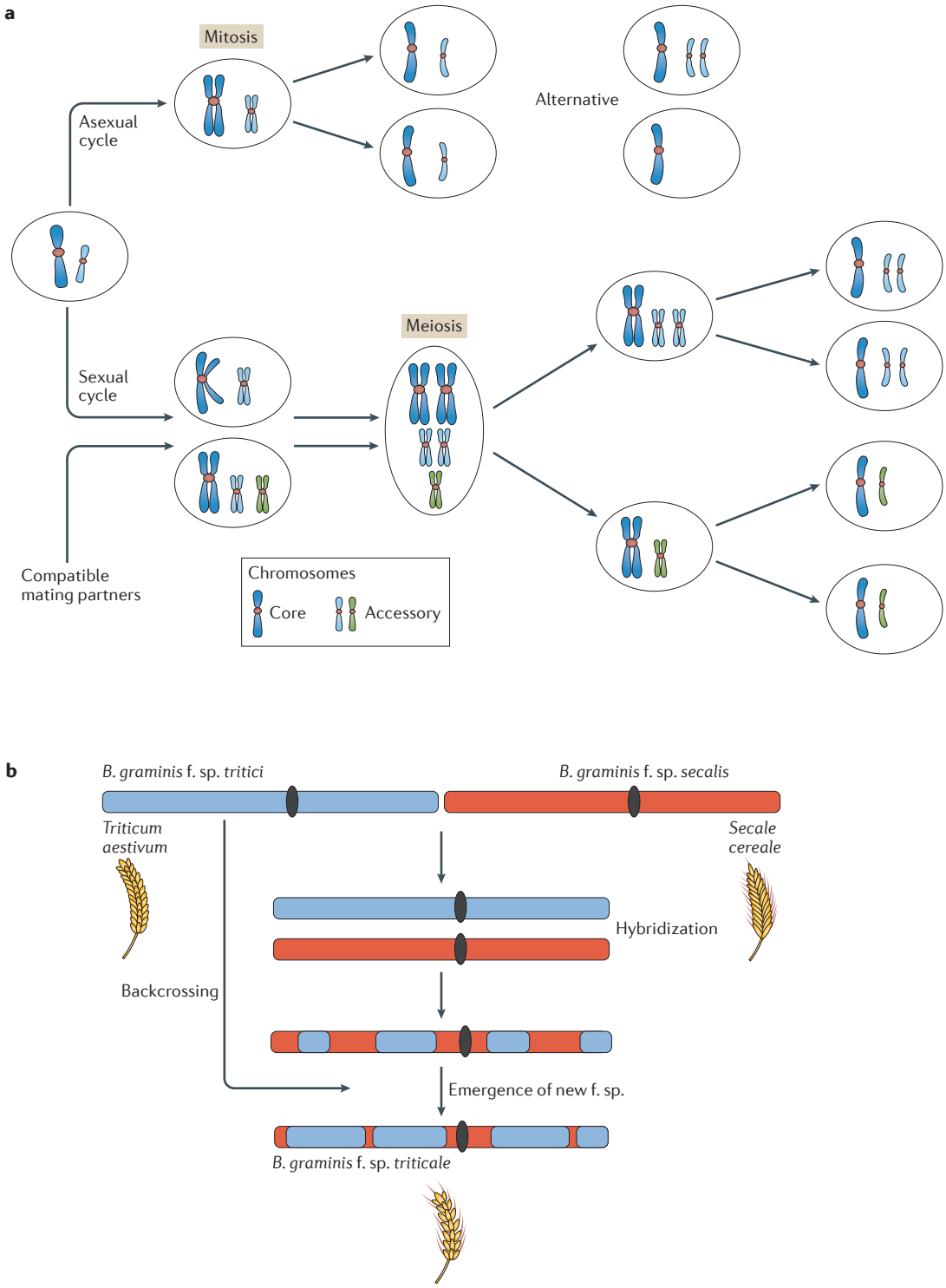


Figure 4 | **Accessory chromosome dynamics and hybridization in pathogenic fungi. a** | The reproductive mode of fungal pathogens ranges from strictly clonal to obligate out-crossing. Many species have mixed reproductive systems and their life cycles include both asexual and sexual stages. Accessory chromosomes, which are found in several pathogenic fungal species, were shown to be lost during both mitosis and meiosis, resulting in aneuploid progeny strains[55]. The underlying mechanisms that are responsible for chromosome instability are unknown. A possible cause for chromosome loss during mitosis could be errors during the segregation of sister chromatids (alternative scenario during mitosis). **b** | Hybridization of two *Blumeria graminis* formae speciales (f. sp.), *B. graminis* f. sp. *tritici* (blue) and *B. graminis* f. sp. *secalis* (red), led to the emergence of a new pathogen, *B. graminis* f. sp. *triticale*, which infects the hybrid crop triticale; a hybrid of wheat and rye[17]. After the initial hybridization, the triticale-infecting hybrid backcrossed with *B. graminis* f. sp. *tritici*. This example illustrates an extraordinarily fast adaptation to a new host that is mediated by a combination of parental virulence factors necessary for host infection.

Sexual spores
Spores that originate from sexual crossings that differ morphologically from asexual spores.

Muller's ratchet
The irreversible accumulation of deleterious mutations in organisms that reproduce asexually.

the dikaryotic filamentous structures that are required for the invasive growth of smut fungi, such as *U. maydis*, are only formed through sexual mating; therefore, cells that are unable to mate are avirulent[78]. In addition, mating can be important for the production of sexual spores, such as ascospores, basidiospores and zygospores, as dispersal propagules and for long-time survival in the environment. Below, we consider the importance of sex in the context of genome evolution.

A pattern that is shared among most sexually reproducing species is that meiotic recombination events are unevenly distributed along chromosomes. In many species, fine-scale variation in the recombination rate is due to the presence of recombination hotspots, at which crossover events tend to concentrate. In *Z. tritici*, variation in recombination rates has been studied using both experimental mating and population genomic analyses[68,79]. These studies demonstrate exceptionally high rates of recombination (~60 cM per Mb) and a high abundance of intragenic recombination hotspots that may facilitate the rapid fixation of advantageous mutations. Preliminary data from a comparative study of *Z. tritici* and its sister species *Zymoseptoria ardabiliae* show that in *Z. tritici* the recombination rate varies across and between chromosomes, with smaller core chromosomes having a higher rate of recombination. Fine-scale variation in recombination rate is mainly explained by the distribution of recombination hotspots that frequently colocalize with genes, although these data require validation. The recombination landscapes of *Z. tritici* and its sister species *Z. ardabiliae* show some conservation, whereas recombination hotspot positions are not conserved[79] and even vary between independent crosses of *Z. tritici*[68]. The consequence of such a dynamic recombination landscape is that orthologous genes can be exposed to markedly different rates of recombination and therefore evolve at varying rates in closely related species[41]. High recombination rates and recombination hotspots in, or near, coding sequences are also found in other species, such as *M. oryzae*, *F. graminearum* and *Microbotryum violaceum*[41,80,81], which suggests that high recombination rates may be a more general mechanism to accelerate gene evolution in pathogenic fungi.

Clonal populations are sometimes considered to be 'evolutionarily impaired', because of their inability to recombine advantageous mutations that occur at independent loci in the genome. In addition, non-advantageous and deleterious mutations may accumulate in the genome in an irreversible manner, a phenomenon that is termed 'Muller's ratchet' (REF. 82). If these are the consequences of clonal propagation, why are asexual species common and successful among fungal plant pathogens?

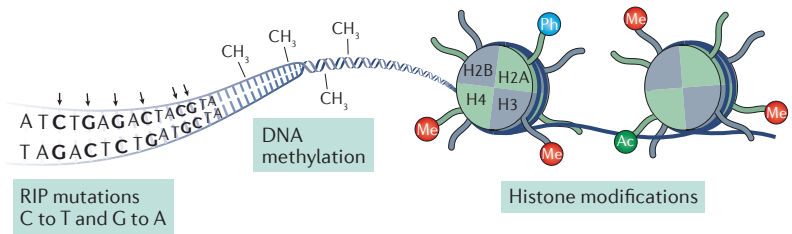
Studies are required to understand the long-term evolutionary processes in asexual fungal pathogens. However, the association between transposable elements and effector genes suggests that high mutation rates in repetitive genome compartments support effector innovation and adaptation, as shown in clonal populations of the rice blast pathogen *M. oryzae* and the *Fusarium* wilt pathogen *F. oxysporum*[29,42,43]. The complete fitness effects of mutations that are mediated by transposable elements have not been assessed in fungal pathogens. However, transposable elements also cause non-adaptive and deleterious changes that reduce the fitness of the host. Population genomic data of well-assembled fungal genomes and measures of mutational fitness effects are required to address the effects of transposable elements on adaptive evolution.

Another factor to consider is the time span of asexual reproduction[83]. Evidence of rare or cryptic sex has been documented in asexual species, such as *V. dahliae* and *F. oxysporum*, by analyses of multilocus genotypes, mating-type frequencies and the conservation of meiosis-related genes[84,85]. Furthermore, unisexual mating, in which meiotic basidiospores are produced from the fusion of mitotically produced nuclei, has been documented in the otherwise heterothallic human pathogen

Box 1 | Silencing of transposable elements in compartmentalized regions

In sexually reproducing populations, transposable elements can be transmitted during meiosis. Asexual lineages have a lower probability of acquiring new transposable elements; however, once invaded, they are more susceptible to their expansion and deleterious effects[126]. Different mechanisms to prevent and control the spread of transposable elements in fungal genomes have been described, which include DNA methylation, the production of small RNAs that are involved in quelling and meiotic silencing[127], and repeat-induced point (RIP) mutation[93] (see the figure). RIP mutation is a process that mutates duplicated DNA sequences in the genome through homology recognition[128,129]. Signatures of RIP mutation are C to T and G to A mutations, which have been described in several genomes of fungal plant pathogens, including both ascomycete and basidiomycete species[29,130,131]. Experimental evidence for active RIP mutation has been shown in *Leptosphaeria maculans*[132] but is still lacking in other fungal pathogen species. Interestingly, studies of histone and DNA methylation and RIP mutation in fungal plant pathogens have revealed a correlated non-random distribution of heterochromatin, DNA methylation and RIP mutations[61,133,134].

Highly expressed effector genes have, in many cases, been shown to associate with transposable element-rich compartments. Mechanisms that enable gene transcription in the vicinity of transposable elements are therefore crucial in many plant pathogen genomes. Epigenetic modifications have been shown to have an important role in the maintenance of genome compartments and effector expression. In the genus *Zymoseptoria*, lineage-specific or accessory chromosomes show enrichment for the 'facultative' heterochromatic histone methylation mark H3K27me3 (REFS 61,135,136). Furthermore, in *Fusarium graminearum* and *Fusarium fujikuroi*, H3K27me3 has a crucial role in the regulation of genes that are related to pathogenicity[135,136]. In *Z. tritici*, H3K27me3 is significantly enriched on the accessory chromosomes but is only observed in the subtelomeric regions (the DNA immediately neighbouring the telomeres) of the core chromosomes[61]. Thus, genes on the accessory chromosomes may be regulated by chromatin modifications, similarly to genes related to pathogenicity in *Fusarium* spp. In *in vitro* experiments on *Leptosphaeria maculans* 'brassicae', another histone mark (H3K9me3) contributed to the regulation of effector genes embedded in repeat-rich DNA[60]. Marked upregulation of effector genes during plant infection suggests that dynamic chromatin modifications are associated with the pathogenic lifestyle of *L. maculans* 'brassicae'. Furthermore, in *L. maculans* 'brassicae', RIP mutation can have an important role in silencing transposable elements and in effector evolution[29].



Box 2 | **Determinants of genome evolution: genetic drift and selection**

Genetic diversity results from mutation (including point mutations, deletions, insertions and duplications) and recombination (including chromosome crossover and heterozygote gene conversion during meiosis), which contribute to allelic variation within and among chromosomes. The fitness effect of a mutation can be classified into three categories: first, mutations can be selectively neutral, thus there is no fitness effect for individuals that have the particular mutation; second, mutations can be deleterious and reduce the fertility or survival of an individual; and, third, mutations can be advantageous and increase the fitness of an individual. The three categories comprise a continuum of fitness effects that range from completely deleterious (individuals that have the mutations either die or are infertile) to highly adaptive (individuals that have the mutations are among the only individuals that can reproduce in the population).

The fate of neutral mutations is determined purely by chance; a population genetics process termed 'genetic drift'. As biological populations have finite sizes, a mutation that evolves by genetic drift will, over time, either be lost or become spread and ultimately fixed in the population. This process is determined by the demography of the population, its size and its variation in time (for example, population growth or bottleneck) and space (also known as population structure)[99].

Conversely, the fate of non-neutral mutations depends on their fitness, which is their relative selective advantage or disadvantage (selection coefficient '*s*'). Positively and negatively selected mutations are also affected by genetic drift, which can counteract the effect of selection. Mutations that have negative effects can therefore spread in the population by chance, whereas positively selected mutations might be lost. The relative importance of these two forces is determined by the effective population size (N_e) and the selection coefficient of mutations. In particular, genetic drift will dominate when $s<1/4N_e$ (REF. 75).

Cryptococcus neoformans[86]. These findings suggest that recombination, even if rare or occurring as unisexual or parasexual reproduction, may counteract the effect of Muller's ratchet and contribute to adaptive evolution in species that are otherwise predominantly clonal.

Interestingly, sexual reproduction has been lost or decreased in several important crop pathogens that have evolved from sexual progenitors on wild hosts[87–89]. The loss of sexual reproduction in the rice blast pathogen *M. oryzae* and the rust fungus *Puccinia striiformis* formae speciales (f. sp.) *tritici* coincided with the emergence of clonal linages that successfully achieved global spread on agricultural hosts[87,90]. However, in Asia, the population genetics structure of these species is characterized by sexual recombination and high levels of genetic variation[91,92]. As Asia is the point of origin for both pathogens, the sexual populations probably reflect the ancestral population biology of both species. To what extent the clonality of many crop pathogens is a product of agricultural environments remains unclear and can only be elucidated by further population genetics studies of pathogens of wild plants.

The origin of transposable element invasions and expansions. Transposable elements are mobile genetic elements that replicate by invading the genome of a host cell. Most transposable elements exert negative effects, as they replicate and can be inserted into functionally important DNA, which can be damaging to the host organism. Fungi have evolved a wide range of genome defence mechanisms to silence transposable elements, including DNA methylation, heterochromatin, repeat-induced point (RIP) mutation and small RNAs[93] (BOX 1). Interestingly, these mechanisms exhibit variability between closely related species, which suggests another layer of variation in genome evolution and variation in the co-evolution of transposable elements and the host cell[94].

Closely related species can vary considerably in the composition and abundance of transposable element families. The composition of these families can vary from the lineage-specific dynamics of ancestral transposable elements or from lineage-specific introductions of transposable elements through infection and horizontal transfer (for example, endogenous retroviruses)[95,96]. In addition, expansions of transposable elements may be caused by different conditions. At the mechanistic level, transposable elements may be activated by various environmental stresses[97], or they may expand when genome defences become inactivated or less efficient[94]. In terms of population genetics, transposable element expansions can occur during prolonged asexual reproduction, as deleterious mutations, such as an increase in the copy number of transposable elements, accumulate in the genome in the absence of sexual recombination[98]. An increase in the strength of genetic drift, which can occur in organisms that have low N_e, may also result in the expansion of transposable elements[99].

Correlation analyses of genome sizes and N_e using data from many different taxa have shown that multicellular organisms with large genomes generally have smaller N_e and contain more transposable elements than those with small genomes[75]. A model that was proposed to explain these correlations posits that a new mutation (for example, the insertion or transposition of a transposable element that has a selective disadvantage, with a selection coefficient (s) <0) can be fixed by genetic drift despite being under negative selection pressure if the product $N_e \times s$ is small[99]. This can be explained by the reduced efficacy of selection and the increased effect of genetic drift in organisms that have a small N_e (BOX 2). Therefore, the invasion and proliferation of transposable elements in pathogens may have occurred during periods in which the N_e was small. A small N_e can be the product of a population bottleneck or of asexual reproduction; features that are both frequently observed in fungal plant pathogens[87,89,90,100,101].

Across the genome, the strength of genetic drift and selection may vary. This difference primarily results from variation in recombination across the genome. Just as asexual species may accumulate more transposable elements, regions in the genome that recombine less may also accumulate more transposable elements and non-adaptive mutations as a consequence of stronger genetic drift and a lower efficacy of selection. A model to illustrate this phenomenon is provided by dimorphic mating-type chromosomes that carry genes that are responsible for mating compatibility[65,102]. Dimorphic mating chromosomes and mating-type loci are characterized by a suppression of recombination, which has fundamental consequences for the evolution of these chromosomes. For example, in *Neurospora tetrasperma*, genes that are encoded by the *mat a* and *mat A* chromosomes have an excess of allele-specific non-synonymous codon substitutions; this is consistent with less efficient negative selection and linkage disequilibrium in these non-recombination regions[102]. Furthermore,

Parasexual
A process whereby genetic material is exchanged between fused hyphae or cells without meiosis. Parasexuality enables the organism to recombine its genome and generate new genotypes in the absence of sexual mating.

Selection coefficient
The average proportional reduction in fitness of one genotype relative to another owing to selection (designated by '*s*').

in the smut fungus *M. lychnidis-dioicae,* mating-type chromosomes are also characterized by an accumulation of non-synonymous substitutions, and an excess of repetitive DNA and structural rearrangements[65,103,104]. These features resemble the evolutionary patterns in transposable element-rich compartments, including accessory chromosomes and repeat islands, and illustrate how the suppression of recombination may support the emergence and maintenance of rapidly evolving genome compartments.

In summary, genome analyses have shown that compartmentalized genomes in many fungal plant pathogens originate from transposable element invasions and expansions. Variation in population genetics parameters (including natural selection and genetic drift) can explain the different quantitative and qualitative distributions of transposable elements in fungal species. In particular, population bottlenecks that result in low N_e and prolonged clonal reproduction may favour the invasion and expansion of transposable elements in pathogen genomes. Last, it has also been proposed that transposable element invasion in the genomes of fungal plant pathogens could involve selection beyond the species level, with species that have higher levels of transposable elements being less likely to become extinct[49]. However, the evolutionary relevance of such 'clade -selection' is debated[105].

Heterokaryons and heterozygosity. The examples above build on the genome analyses of haploid genome sequences. Indeed, the nuclei and hyphae of many fungal pathogens are haploid, except during transient diploid stages that form during sexual life cycles. However, many ascomycete and basidiomycete fungi have heterokaryotic life stages, in which genetically distinct nuclei coexist within the same cell; this provides another layer of genetic variation. Heterokaryons may arise through mating or hyphal fusion of homokaryotic individuals, and represent a more complex association of the two parental genomes relative to diploidy[106]. Interestingly, experimental mating of genetically distinct homokaryons of the pathogenic white rot fungus *Heterobasidion parviporum* showed that nuclear ratios in heterokaryons frequently deviate from a 1:1 ratio and that the environment can affect the frequencies of nuclei[107]. The underlying nuclear ratio in *H. parviporum* heterokaryons affects gene expression and growth rate, and also potentially affects virulence, as shown in heterokaryons of *Heterobasidion annosum*, which is the causative agent of root and butt rot in conifers[108].

Another interesting example can be found in the life cycle of agriculturally important rust fungi that have complex life cycles that often involve two hosts and the production of several types of spore, including haploid pycniospores (n), dikaryotic aeciospores ($n+n$) and diploid ($2n$) teliospores. The wheat stripe rust fungus *P. striiformis* f. sp. *tritici* infects wheat by dikaryotic aeciospores produced on the alternative host *Berberis vulgaris*. During infection of wheat, the pathogen propagates asexually to produce dikaryotic urediniospores or diploid teliospores that function as inoculum for new infections on wheat and *B. vulgaris*, respectively[88]. Genome sequencing and assembly of the diploid asexual stage identified regions of exceptionally high heterozygosity[109]. How this is advantageous for pathogenicity is unclear; however, the asexual form of *P. striiformis* f. sp. *tritici* may benefit from variation in heterozygous genomic regions. The genetic and functional dissection of the dikaryotic, diploid and haploid stages of rust fungi life cycles are required to understand the importance of nuclear variability in virulence.

Hybridization, introgression and horizontal gene transfer. Hybridization refers to the fusion or mating of non-conspecific individuals[110]. Recurrent backcrossing between hybrids and parental species is referred to as introgression, and both hybridization and introgression are thought to be important for the evolution of fungal plant pathogens. Both can occur when intersterility is incomplete, which enables sexual mating or cellular fusion between non-conspecific lineages. Under the appropriate conditions, the outcome of introgression or hybridization can be successful, which may generate new lineages or new adaptive traits in existing species. Hybridization can involve both sexual and asexual interactions. Homoploid hybrids may emerge from sexual mating, which results in hybrids that have the same chromosome number as the parental species[111]. By contrast, in heteroploid hybrids, both parental genomes are entirely or partially maintained, which results in aneuploid or polyploid hybrids[112,113].

Phylogenetic studies and comparative genome analyses have provided several examples of hybrid fungal plant pathogens that have originated from interspecific mating[110,114]. Hybrids can propagate as new distinct lineages if they are fertile, can compete with coexisting parental species and may have adaptive traits that enable them to colonize a new host. In some cases, hybrids can represent transient stages that backcross with the parental species and function as a 'bridge' for the transfer of genetic material from one species to the other through 'introgressive hybridization' (REFS 110,115) (FIG. 4). This is typically the outcome if the fitness of the hybrid is inferior to the parental species. However, hybrid genetic elements may persist and increase in frequency if they confer a selective advantage.

An interesting example of hybridization is provided by a recent population genomics study of the cereal mildew pathogen *Blumeria graminis*. This revealed an unusual distribution of SNPs in *B. graminis* f. sp. *triticale*[17], a lineage that infects triticale (a hybrid of wheat and rye) (FIG. 4). The genome of *B. graminis* f. sp. *triticale* comprises a mosaic of *B. graminis* f. sp. *tritici* and *B. graminis* f. sp. *secalis* genomic segments with only a small number of private polymorphisms (not present in *B. graminis* f. sp. *tritici* and *B. graminis* f. sp. *secalis*), which is consistent with a recent hybridization event[17]. The results from this study suggested that after initial hybridization (between *B. graminis* f. sp. *tritici* and *B. graminis* f. sp. *secalis*) the triticale-infecting hybrid backcrossed a few times with the parental *B. graminis* f. sp. *tritici*. The recent emergence of *B. graminis* f. sp. *triticale* (within the past 30 years) shows the rapid adaptation of a hybrid fungal plant pathogen to a new hybrid host.

Heterokaryons
Cells that contain two or more genetically distinct nuclei.

Introgressive hybridization
The transfer of genes from one species to another through hybridization followed by backcrossing with the parental species.

With the availability of genome sequences from many different taxa, there is increasing evidence of multiple gene transfers between species and even between kingdoms[116]. Notably, horizontal gene transfer has been identified between fungal plant pathogens. For example, *Parastagonospora nodorum* and *Pyrenophora tritici-repentis* are wheat pathogens, and both have an almost identical effector gene that is involved in host susceptibility, known as ToxA[117–119]. Comparative genome analyses and population genetics analyses of the *TOXA* allele revealed that it was inserted in the *P. tritici-repentis* genome through horizontal gene transfer from *P. nodorum*[117]. In this case, the transfer probably occurred either through hyphal fusion or from a wheat-associated vector, such as a virus or bacterium. Interestingly, *TOXA* was identified in the genome of a third wheat pathogen, *Bipolaris sorokiniana*[120], and thus is an example of a gene that is readily taken up by different wheat pathogens to induce host susceptibility[119]. Studies have revealed similar signatures of horizontal gene transfer in the genomes of other fungal plant pathogens. The abundance of phylogenetic 'outlier' genes suggests that the acquisition of foreign DNA from other fungi, bacteria or plants can contribute to genome and phenotype evolution[121–123]. However, evidence for mechanisms of horizontal gene transfer in fungi is sparse and further studies are necessary to validate the importance of this process in genome evolution[43].

Conclusions and outlook

So far, our research on the genomes of fungal plant pathogens has been descriptive and has focused on the diversity of mechanisms that promote the rapid evolution of pathogenicity related traits. However, the underlying population genetics processes that have been important in shaping genome architecture in pathogens are still poorly understood; understanding them will require improved models for the inference of mutational and recombination processes. These should incorporate the particularities of fungal pathogen genomes, including high and variable mutation and recombination rates, marked variation in transposable element content and genome compartmentalization.

Given the discovery of substantial levels of genome plasticity, fungal plant pathogens currently function as model organisms to address fundamental questions in evolutionary biology beyond plant pathology, such as the importance of sexual reproduction, the evolution of gene organization, and chromosomal stability and integrity[44,48,58]. This understanding may also have relevance to cancer research. Cancer cells are known to accumulate a wide range of somatic mutations, including whole chromosome duplications and losses, and structural rearrangements. Fungal pathogens may, with their highly dynamic genomes, function as new models in the research of cancer cell evolution[124,125].

Finally, agriculture can markedly affect demographic fluctuations of crop-associated pathogens by providing homogeneous environments, which may be conducive for the evolution and dispersal of pathogens. We need to improve our understanding of the effect of environmental conditions on the population genetics structure of plant pathogens. Changes in population genetics parameters over time may have a crucial role in shaping genome architecture, including advantageous associations between transposable elements and rapidly evolving effector genes.

1. Fisher, M., Henk, D. & Briggs, C. Emerging fungal threats to animal, plant and ecosystem health. *Nature* **484**, 186–194 (2012).
2. James, T. Y. *et al.* Reconstructing the early evolution of Fungi using a six-gene phylogeny. *Nature* **443**, 818–822 (2006).
3. Lo Presti, L. *et al.* Fungal effectors and plant susceptibility. *Annu. Rev. Plant Biol.* **66**, 513–545 (2015).
4. Wolpert, T. J., Dunkle, L. D. & Ciuffetti, L. M. Host-selective toxins and avirulence determinants: what's in a name? *Annu. Rev. Phytopathol.* **40**, 251–285 (2002).
5. Friesen, T. L., Faris, J. D., Solomon, P. S. & Oliver, R. P. Host-specific toxins: effectors of necrotrophic pathogenicity. *Cell. Microbiol.* **10**, 1421–1428 (2008).
6. Javelle, M., Vernoud, V., Rogowsky, P. M. & Ingram, G. C. Epidermis: the formation and functions of a fundamental plant tissue. *New Phytol.* **189**, 17–39 (2011).
7. Jones, J. D. & Dangl, J. L. The plant immune system. *Nature* **444**, 323–329 (2006).
8. Weiberg, A. *et al.* Fungal small RNAs suppress plant immunity by hijacking host RNA interference pathways. *Science* **342**, 118–123 (2013).
9. Manning, V. A. & Ciuffetti, L. M. Localization of Ptr ToxA produced by *Pyrenophora tritici-repentis* reveals protein import into wheat mesophyll cells. *Plant Cell* **17**, 3203–3212 (2005).
10. Djamei, A. *et al.* Metabolic priming by a secreted fungal effector. *Nature* **478**, 395–398 (2011).
11. Bergelson, J., Kreitman, M., Stahl, E. A. & Tian, D. Evolutionary dynamics of plant *R*-genes. *Science* **292**, 2281–2285 (2001).
12. Barrett, L. G. *et al.* Diversity and evolution of effector loci in natural populations of the plant pathogen *Melampsora lini*. *Mol. Biol. Evol.* **26**, 2499–2513 (2009).
13. Dodds, P. N. *et al.* Direct protein interaction underlies gene-for-gene specificity and coevolution of the flax resistance genes and flax rust avirulence genes. *Proc. Natl Acad. Sci. USA* **103**, 8888–8893 (2006).
14. Kanzaki, H. *et al.* Arms race co-evolution of *Magnaporthe oryzae AVR-Pik* and rice *Pik* genes driven by their physical interactions. *Plant J.* **72**, 894–907 (2012).
15. Stukenbrock, E. H. *et al.* The making of a new pathogen: Insights from comparative population genomics of the domesticated wheat pathogen *Mycosphaerella graminicola* and its wild sister species. *Genome Res.* **21**, 2157–2166 (2011).
16. de Jonge, R. *et al.* Extensive chromosomal reshuffling drives evolution of virulence in an asexual pathogen. *Genome Res.* **23**, 1271–1282 (2013).
17. Menardo, F. *et al.* Hybridization of powdery mildew strains gives rise to pathogens on novel agricultural crop species. *Nat. Genet.* **48**, 201–205 (2016). **This study provides an example of rapid adaptation to new host plants. The hybridization of two *Blumeria graminis* formae speciales resulted in the emergence of a new pathogen that infects a hybrid crop of wheat and rye.**
18. Selmecki, A., Forche, A. & Berman, J. Genomic plasticity of the human fungal pathogen *Candida albicans*. *Eukaryot. Cell* **9**, 991–1008 (2010).
19. Farrer, R. A. *et al.* Multiple emergences of genetically diverse amphibian-infecting chytrids include a globalized hypervirulent recombinant lineage. *Proc. Natl Acad. Sci. USA* **108**, 18732–18736 (2011).
20. Raffaele, S. & Kamoun, S. Genome evolution in filamentous plant pathogens: why bigger can be better. *Nat. Rev. Microbiol.* **10**, 417–430 (2012).
21. Seidl, M. F. & Thomma, B. P. Sex or no sex: evolutionary adaptation occurs regardless. *Bioessays* **36**, 335–345 (2014).
22. Galazka, J. M. & Freitag, M. Variability of chromosome structure in pathogenic fungi — of 'ends and odds'. *Curr. Opin. Microbiol.* **20**, 19–26 (2014).
23. Allen, R. L. *et al.* Host–parasite coevolutionary conflict between *Arabidopsis* and Downy Mildew. *Science* **306**, 1957–1960 (2004).
24. Cao, J. *et al.* Whole-genome sequencing of multiple *Arabidopsis thaliana* populations. *Nat. Genet.* **43**, 956–963 (2011).
25. Thrall, P. H. *et al.* Rapid genetic change underpins antagonistic coevolution in a natural host–pathogen metapopulation. *Ecol. Lett.* **15**, 425–435 (2012).
26. Laine, A.-L., Burdon, J. J., Nemri, A. & Thrall, P. H. Host ecotype generates evolutionary and epidemiological divergence across a pathogen metapopulation. *Proc. R. Soc. B Biol. Sci.* **281**, 20140522 (2014).
27. Persoons, A. *et al.* The escalatory Red Queen: population extinction and replacement following arms-race dynamics in poplar rust. *Mol. Ecol.* **26**, 1902–1918 (2016).
28. Daverdin, G. *et al.* Genome structure and reproductive behaviour influence the evolutionary potential of a fungal phytopathogen. *PLoS Pathog.* **8**, e1003020 (2012).
29. Rouxel, T. *et al.* Effector diversification within compartments of the *Leptosphaeria maculans* genome affected by Repeat-Induced Point mutations. *Nat. Commun.* **2**, 202 (2011).
30. Brown, J. K. & Hovmøller, M. S. Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease. *Science* **297**, 537–541 (2002).
31. Islam, M. T. *et al.* Emergence of wheat blast in Bangladesh was caused by a South American lineage of *Magnaporthe oryzae*. *BMC Biol.* **14**, 84 (2016).
32. Castroagudin, V. L. *et al.* Wheat blast disease caused by *Pyricularia graminis-tritici* sp. nov. Preprint at *bioRxiv* http://dx.doi.org/10.1101/051151 (2016).

33. Brasier, C. M. Rapid evolution of introduced plant pathogens via interspecific hybridization. *Bioscience* **51**, 123 (2001).
34. Brasier, C. M. & Kirk, S. A. Rapid emergence of hybrids between the two subspecies of *Ophiostoma novo-ulmi* with a high level of pathogenic fitness. *Plant Pathol.* **59**, 186–199 (2010).
35. McDonald, B. A. & Stukenbrock, E. H. Rapid emergence of pathogens in agro-ecosystems: global threats to agricultural sustainability and food security. *Phil. Trans. R. Soc. B* **371**, 20160026 (2016).
36. Stukenbrock, E. H. Evolution, selection and isolation: a genomic view of speciation in fungal plant pathogens. *New Phytol.* **199**, 895–907 (2013).
37. Brown, J. K. & Tellier, A. Plant–parasite coevolution: bridging the gap between genetics and ecology. *Annu. Rev. Phytopathol.* **49**, 345–367 (2011).
38. Hörger, A. C. *et al.* Balancing selection at the tomato *RCR3* guardee gene family maintains variation in strength of pathogen defense. *PLoS Genet.* **8**, e1002813 (2012).
39. Tellier, A., Moreno-Gámez, S. & Stephan, W. Speed of adaptation and genomic footprints of host–parasite coevolution under arms race and trench warfare dynamics. *Evolution.* **68**, 2211–2224 (2014).
40. Van der Hoorn, R. A., De Wit, P. J. & Joosten, M. H. Balancing selection favors guarding resistance proteins. *Trends Plant Sci.* **7**, 67–71 (2002).
41. Badouin, H. *et al.* Widespread selective sweeps throughout the genome of model plant pathogenic fungi and identification of effector candidates. *Mol. Ecol.* **26**, 2041–2062 (2016).
42. Chuma, I. *et al.* Multiple translocation of the *AVR-Pita* effector gene among chromosomes of the rice blast fungus *Magnaporthe oryzae* and related species. *PLoS Pathog.* **7**, e1002147 (2011).
43. Ma, L.-J. *et al.* Comparative genomics reveals mobile pathogenicity chromosomes in *Fusarium*. *Nature* **464**, 367–373 (2010).
44. Faino, L. *et al.* Transposons passively and actively contribute to evolution of the two-speed genome of a fungal pathogen. *Genome Res.* **26**, 1091–1100 (2016).
This study describes the importance of transposable elements in the formation of lineage-specific regions and their differential regulation in distinct genome compartments in *V. dahliae*.
45. Goodwin, S. B. *et al.* Finished genome of the fungal wheat pathogen *Mycosphaerella graminicola* reveals dispensome structure, chromosome plasticity, and stealth pathogenesis. *PLoS Genet.* **7**, e1002070 (2011).
46. Coleman, J. J. *et al.* The genome of *Nectria haematococca*: contribution of supernumerary chromosomes to gene expansion. *PLoS Genet.* **5**, e1000618 (2009).
47. Williams, A. H. *et al.* Comparative genomics and prediction of conditionally dispensable sequences in legume-infecting *Fusarium oxysporum formae speciales* facilitates identification of candidate effectors. *BMC Genomics* **17**, 191 (2016).
48. Dutheil, J. Y. *et al.* A tale of genome compartmentalization: the evolution of virulence clusters in smut fungi. *Genome Biol. Evol.* **8**, 681–704 (2016).
This study provides details of the evolution of virulence-associated gene clusters in *S. scitamineum* that are driven by tandem gene duplication and transposable elements.
49. Dong, S., Raffaele, S. & Kamoun, S. The two-speed genomes of filamentous pathogens: waltz with plants. *Curr. Opin. Genet. Dev.* **35**, 57–65 (2015).
50. Stukenbrock, E. H. *et al.* Whole-genome and chromosome evolution associated with host adaptation and speciation of the wheat pathogen *Mycosphaerella graminicola*. *PLoS Genet.* **6**, e1001189 (2010).
51. Houben, A., Banaei-Moghaddam, A. M., Klemme, S. & Timmis, J. N. Evolution and biology of supernumerary B chromosomes. *Cell. Mol. Life Sci.* **71**, 467–478 (2014).
52. Miao, V. P., Covert, S. F. & VanEtten, H. D. A fungal gene for antibiotic resistance on a dispensable ('B') chromosome. *Science* **254**, 1773 (1991).
53. Temporini, E. D. & VanEtten, H. D. Distribution of the pea pathogenicity (*PEP*) genes in the fungus *Nectria haematococca* mating population VI. *Curr. Genet.* **41**, 107–114 (2002).
54. van der Does, H. C. *et al.* Transcription factors encoded on core and accessory chromosomes of *Fusarium oxysporum* induce expression of effector genes. *PLoS Genet.* **12**, e1006401 (2016).
55. Wittenberg, A. H. J. *et al.* Meiosis drives extraordinary genome plasticity in the haploid fungal plant pathogen *Mycosphaerella graminicola*. *PLoS ONE* **4**, e5863 (2009).
56. Croll, D., Zala, M. & McDonald, B. A. Breakage–fusion–bridge cycles and large insertions contribute to the rapid evolution of accessory chromosomes in a fungal pathogen. *PLoS Genet.* **9**, e1003567 (2013).
57. Stewart, E. I. *et al.* Quantitative trait locus mapping reveals complex genetic architecture of quantitative virulence in the wheat pathogen *Zymoseptoria tritici*. *Mol. Plant Pathol.* http://dx.doi.org/10.1111/mpp.12515 (2017).
58. Plissonneau, C. & Stürchler, A. The evolution of orphan regions in genomes of a fungal pathogen of wheat. *mBio* **7**, e01231-16 (2016).
In this study, comparison of the genome structure of two *Z. tritici* isolates identifies large chromosomal inversions and losses and/or gains of transposable element clusters, which highlights intraspecies genome diversity.
59. Chiara, M. *et al.* Genome sequencing of multiple isolates highlights subtelomeric genomic diversity within *Fusarium fujikuroi*. *Genome Biol. Evol.* **7**, 3062–3069 (2015).
60. Soyer, J. L. *et al.* Epigenetic control of effector gene expression in the plant pathogenic fungus *Leptosphaeria maculans*. *PLoS Genet.* **10**, e1004227 (2014).
Effectors in *L. maculans* are located in AT-rich genome compartments. In this study, their regulation is shown to be mediated by the presence and absence of heterochromatin during infection.
61. Schotanus, K. *et al.* Histone modifications rather than the novel regional centromeres of *Zymoseptoria tritici* distinguish core and accessory chromosomes. *Epigenetics Chromatin* **8**, 1 (2015).
In this study, Genome-wide comparison of histone methylation marks reveals distinct patterns of the facultative heterochromatin mark H3K27me3 on core and accessory chromosomes.
62. Kellner, R. *et al.* Expression profiling of the wheat pathogen *Zymoseptoria tritici* reveals genomic patterns of transcription and host-specific regulatory programs. *Genome Biol. Evol.* **6**, 1353–1365 (2014).
63. Miao, V. P., Freitag, M. & Selker, E. U. Short TpA-rich segments of the ζ-η region induce DNA methylation in *Neurospora crassa*. *J. Mol. Biol.* **300**, 249–273 (2000).
64. Tamaru, H. & Selker, E. U. Synthesis of signals for *de novo* DNA methylation in *Neurospora crassa*. *Mol. Cell. Biol.* **23**, 2379–2394 (2003).
65. Fontanillas, E. *et al.* Degeneration of the non-recombining regions in the mating-type chromosomes of the anther-smut fungi. *Mol. Biol. Evol.* **32**, 928–943 (2014).
66. Kämper, J. *et al.* Insights from the genome of the biotrophic fungal plant pathogen *Ustilago maydis*. *Nature* **444**, 97–101 (2006).
67. Schirawski, J. *et al.* Pathogenicity determinants in smut fungi revealed by genome comparison. *Science* **330**, 1546–1548 (2010).
68. Croll, D., Lendenmann, M. H., Stewart, E. & McDonald, B. A. The impact of recombination hotspots on genome evolution of a fungal plant pathogen. *Genetics* **201**, 1213–1228 (2015).
69. Ohm, R. A. *et al.* Diverse lifestyles and strategies of plant pathogenesis encoded in the genomes of eighteen *Dothideomycetes fungi*. *PLoS Pathog.* **8**, e1003037 (2012).
70. Hane, J. K. *et al.* A novel mode of chromosomal evolution peculiar to filamentous *Ascomycete fungi*. *Genome Biol.* **12**, 1 (2011).
71. Grandaubert, J. *et al.* Transposable element-assisted evolution and adaptation to host plant within the *Leptosphaeria maculans–Leptosphaeria biglobosa* species complex of fungal pathogens. *BMC Genomics* **15**, 891 (2014).
This study shows that the expansion of transposable elements in one member of the *L. maculans–L. biglobosa* species complex correlates to the evolution of pathogenicity in this species.
72. Chang, T.-C. *et al.* Comparative genomics of the sigatoka disease complex on banana suggests a link between parallel evolutionary changes in *Pseudocercospora fijiensis* and *Pseudocercospora eumusae* and increased virulence on the banana host. *PLoS Genet.* **12**, e1005904 (2016).
73. Duplessis, S. *et al.* Obligate biotrophy features unraveled by the genomic analysis of rust fungi. *Proc. Natl Acad. Sci. USA* **108**, 9166–9171 (2011).
74. Niehaus, E.-M. *et al.* Comparative 'omics' of the *Fusarium fujikuroi* species complex highlights differences in genetic potential and metabolite synthesis. *Genome Biol. Evol.* **8**, 3574 (2016).
75. Lynch, M. & Walsh, B. *The origins of genome architecture* (Sinauer Associates Sunderland, 2007).
76. McDonald, B. A. & Linde, C. Pathogen population genetics, evolutionary potential, and durable resistance. *Annu. Rev. Phytopathol.* **40**, 349–379 (2002).
77. Marais, G. & Charlesworth, B. Genome evolution: recombination speeds up adaptive evolution. *Curr. Biol.* **13**, R68–R70 (2003).
78. Feldbrügge, M., Kämper, J., Steinberg, G. & Kahmann, R. Regulation of mating and pathogenic development in *Ustilago maydis*. *Curr. Opin. Microbiol.* **7**, 666–672 (2004).
79. Stukenbrock, E. H. & Dutheil, J. Y. Comparison of fine-scale recombination maps in fungal plant pathogens reveals dynamic recombination landscapes and intragenic hotspots. Preprint at *bioRxiv* https://dx.doi.org/10.1101/158907 (2017).
80. Zheng, Y. *et al.* Development of microsatellite markers and construction of genetic map in rice blast pathogen *Magnaporthe grisea*. *Fungal Genet. Biol.* **45**, 1340–1347 (2008).
81. Talas, F. & McDonald, B. A. Genome-wide analysis of *Fusarium graminearum* field populations reveals hotspots of recombination. *BMC Genomics* **16**, 996 (2015).
This study provides details of the genomic structure of *Fusarium graminearum* field populations, which reveals a high degree of sexual recombination and gene flow that enables rapid adaptation to changing environments.
82. Muller, H. J. Some genetic aspects of sex. *Am. Nat.* **66**, 118–138 (1932).
83. Taylor, J. W., Jacobson, D. J. & Fisher, M. C. The evolution of asexual fungi: reproduction, speciation and classification. *Annu. Rev. Phytopathol.* **37**, 197–246 (1999).
84. Milgroom, M. G., del Mar Jiménez-Gasco, M., García, C. O., Drott, M. T. & Jiménez-Díaz, R. M. Recombination between clonal lineages of the asexual fungus *Verticillium dahliae* detected by genotyping by sequencing. *PLoS ONE* **9**, e106740 (2014).
85. Short, D. P. G., Gurung, S., Hu, X., Inderbitzin, P. & Subbarao, K. V. Maintenance of sex-related genes and the co-occurrence of both mating types in *Verticillium dahliae*. *PLoS ONE* **9**, e112145 (2014).
86. Ni, M. *et al.* Unisexual and heterosexual meiotic reproduction generate aneuploidy and phenotypic diversity *de novo* in the yeast *Cryptococcus neoformans*. *PLoS Biol.* **11**, e1001653 (2013).
87. Couch, B. C. *et al.* Origins of host-specific populations of the blast pathogen *Magnaporthe oryzae* in crop domestication with subsequent expansion of pandemic clones on rice and weeds of rice. *Genetics* **170**, 613–630 (2005).
88. Jin, Y., Szarbo, L. J. & Carson, M. Century-old mystery of *Puccinia striiformis* life history solved with the identification of *Berberis* as an alternate host. *Phytopathology* **100**, 432–435 (2010).
89. Xhaard, C. *et al.* The genetic structure of the plant pathogenic fungus *Melampsora larici-populina* on its wild host is extensively impacted by host domestication. *Mol. Ecol.* **20**, 2739–2755 (2011).
90. Ali, S. *et al.* Origin, migration routes and worldwide population genetic structure of the wheat yellow rust pathogen *Puccinia striiformis* f.sp. *tritici*. *PLoS Pathog.* **10**, e1003903 (2014).
91. Saleh, D. *et al.* Sex at the origin: an Asian population of the rice blast fungus *Magnaporthe oryzae* reproduces sexually. *Mol. Ecol.* **21**, 1330–1344 (2012).
92. Ali, S., Leconte, M., Walker, A. S., Enjalbert, J. & Vallavieille-Pope, C. Reduction in the sex ability of worldwide clonal populations of *Puccinia striiformis* f.sp *tritici*. *Fungal Genet. Biol.* **47**, 828–838 (2010).
93. Smith, K. M. *et al.* Epigenetics of filamentous fungi. *Rev. Cell Biol. Mol. Med.* http://dx.doi.org/10.1002/3527600906.mcb.201100035 (2012).
94. Dhillon, B., Cavaletto, J. R., Wood, K. V. & Goodwin, S. B. Accidental amplification and inactivation of a methyltransferase gene eliminates cytosine methylation in *Mycosphaerella graminicola*. *Genetics* **186**, 67–77 (2010).

95. Levin, H. L. & Moran, J. V. Dynamic interactions between transposable elements and their hosts. *Nat. Rev. Genet.* **12**, 615–627 (2011).
96. Daboussi, M.-J. & Capy, P. Transposable elements in filamentous fungi. *Annu. Rev. Microbiol.* **57**, 275–299 (2003).
97. Capy, P., Gasperi, G., Biémont, C. & Bazin, C. Stress and transposable elements: co-evolution or useful parasites? *Heredity (Edinb.)* **85**, 101–106 (2000).
98. Dolgin, E. S. & Charlesworth, B. The fate of transposable elements in asexual populations. *Genetics* **174**, 817–827 (2006).
99. Lynch, M. & Conery, J. S. The origins of genome complexity. *Science* **302**, 1401–1404 (2003).
100. Halkett, F. *et al.* Genetic discontinuities and disequilibria in recently established populations of the plant pathogenic fungus *Mycosphaerella fijiensis*. *Mol. Ecol.* **19**, 3909–3923 (2010).
101. Munkacsi, A. B., Stoxen, S. & May, G. *Ustilago maydis* populations tracked maize through domestication and cultivation in the Americas. *Proc. R. Soc. B Biol. Sci.* **275**, 1037–1046 (2008).
102. Whittle, C. A. & Johannesson, H. Evidence of the accumulation of allele-specific non-synonymous substitutions in the young region of recombination suppression within the mating-type chromosomes of *Neurospora tetrasperma*. *Heredity (Edinb.)* **107**, 305–314 (2011).
103. Badouin, H. *et al.* Chaos of rearrangements in the mating-type chromosomes of the anther-smut fungus *Microbotryum lychnidis-dioicae*. *Genetics* **200**, 1275–1284 (2015).
104. Perlin, M. H. *et al.* Sex and parasites: genomic and transcriptomic analysis of *Microbotryum lychnidis-dioicae*, the biotrophic and plant-castrating anther smut fungus. *BMC Genomics* **16**, 1 (2015).
105. Okasha, S. *Evolution and the Levels of Selection*. (Oxford Univ. Press, 2006).
106. Anderson, J. B. & Kohn, L. M. *in Sex in Fungi: Molecular Determination and Evolutionary Implications.* (eds Heitman J. *et al.*) 333–348 (ASM Press, 2007).
107. James, T. Y., Stenlid, J., Olson, Å. & Johannesson, H. Evolutionary significance of imbalanced nuclear ratios within heterokaryons of the Basidiomycete fungus *Heterobasidion parviporum*. *Evolution.* **62**, 2279–2296 (2008).
108. Olson, A. & Stenlid, J. Plant pathogens: mitochondrial control of fungal hybrid virulence. *Nature* **411**, 438 (2001).
109. Zheng, W. *et al.* High genome heterozygosity and endemic genetic recombination in the wheat stripe rust fungus. *Nat. Commun.* **4**, 2673 (2013).
110. Stukenbrock, E. H. The role of hybridization in the evolution and emergence of new fungal plant pathogens. *Phytopathology* **106**, 104–112 (2016).
111. Stukenbrock, E. H., Christiansen, F. B., Hansen, T. T., Dutheil, J. Y. & Schierup, M. H. Fusion of two divergent fungal individuals led to the recent emergence of a unique widespread pathogen species. *Proc. Natl Acad. Sci. USA* **109**, 10954–10959 (2012).
112. Inderbitzin, P., Davis, R. M., Bostock, R. M. & Subbarao, K. V. The ascomycete *Verticillium longisporum* is a hybrid and a plant pathogen with an expanded host range. *PLoS ONE* **6**, e18260 (2011).
113. Shoji, J.-Y., Charlton, N. D., Yi, M., Young, C. A. & Craven, K. D. Vegetative hyphal fusion and subsequent nuclear behavior in *Epichloë* grass endophytes. *PLoS ONE* **10**, e0121875 (2015).
114. Schardl, C. & Craven, K. Interspecific hybridization in plant-associated fungi and oomycetes: a review. *Mol. Ecol.* **12**, 2861–2873 (2003).
115. Baack, E. J. & Rieseberg, L. H. A genomic view of introgression and hybrid speciation. *Curr. Opin. Genet. Dev.* **17**, 513–518 (2007).
116. Richards, T. A. *et al.* Phylogenomic analysis demonstrates a pattern of rare and ancient horizontal gene transfer between plants and fungi. *Plant Cell* **21**, 1897–1911 (2009).
117. Friesen, T. L. *et al.* Emergence of a new disease as a result of interspecific virulence gene transfer. *Nat. Genet.* **38**, 953–956 (2006).
118. Liu, Z. *et al.* The Tsn1–ToxA interaction in the wheat–*Stagonospora nodorum* pathosystem parallels that of the wheat–tan spot system. *Genome* **49**, 1265–1273 (2006).
119. Faris, J. D. *et al.* A unique wheat disease resistance-like gene governs effector-triggered susceptibility to necrotrophic pathogens. *Proc. Natl Acad. Sci. USA* **107**, 13544–13549 (2010).
120. Mcdonald, M. C., Ahren, D., Simpfendorfer, S., Milgate, A. & Solomon, P. S. The discovery of the virulence gene *ToxA* in the wheat and barley pathogen *Bipolaris sorokiniana*. *Mol. Plant Pathol.* http://dx.doi.org/10.1111/mpp.12535 (2017).
This study shows that horizontal gene transfer of ToxA confers host-specific virulence across pathogens that infect wheat.
121. Nikolaidis, N., Doran, N. & Cosgrove, D. J. Plant expansins in bacteria and fungi: evolution by horizontal gene transfer and independent domain fusion. *Mol. Biol. Evol.* **31**, 376–386 (2014).
122. de Jonge, R. *et al.* Tomato immune receptor Ve1 recognizes effector of multiple fungal pathogens uncovered by genome and RNA sequencing. *Proc. Natl Acad. Sci. USA* **109**, 5110–5115 (2012).
123. Gardiner, D. M. *et al.* Comparative pathogenomics reveals horizontally acquired novel virulence genes in fungi infecting cereal hosts. *PLoS Pathog.* **8**, e1002952 (2012).
124. Burrell, R. A., McGranahan, N., Bartek, J. & Swanton, C. The causes and consequences of genetic heterogeneity in cancer evolution. *Nature* **501**, 338–345 (2013).
125. Garsed, D. W. *et al.* The architecture and evolution of cancer neochromosomes. *Cancer Cell* **26**, 653–667 (2014).
126. Wright, S. & Finnegan, D. Genome evolution: sex and the transposable element. *Curr. Biol.* **11**, R296–R299 (2001).
127. Dang, Y., Yang, Q., Xue, Z. & Liu, Y. RNA interference in fungi: pathways, functions, and applications. *Eukaryot. Cell* **10**, 1148–1155 (2011).
128. Cambareri, E. B., Jensen, B. C., Schabtach, E. & Selker, E. U. Repeat-induced G-C to A-T mutations in *Neurospora*. *Science* **244**, 1571–1575 (1989).
129. Gladyshev, E. & Kleckner, N. Direct recognition of homology between double helices of DNA in *Neurospora crassa*. *Nat. Commun.* **5**, 3509 (2014).
130. Laurie, J. D. *et al.* Genome comparison of barley and maize smut fungi reveals targeted loss of RNA silencing components and species-specific presence of transposable elements. *Plant Cell* **24**, 1733–1745 (2012).
This study details a genome comparison of three closely related smut fungi that reveals transposable element expansion in *Ustilago hordei* and the loss of RNAi components in *U. maydis*.
131. Hood, M. E., Katawczik, M. & Giraud, T. Repeat-induced point mutation and the population structure of transposable elements in *Microbotryum violaceum*. *Genetics* **170**, 1081–1089 (2005).
132. Idnurm, A. & Howlett, B. J. Analysis of loss of pathogenicity mutants reveals that repeat-induced point mutations can occur in the Dothideomycete *Leptosphaeria maculans*. *Fungal Genet. Biol.* **39**, 31–37 (2003).
133. Zemach, A., McDaniel, I. E., Silva, P. & Zilberman, D. Genome-wide evolutionary analysis of eukaryotic DNA methylation. *Science* **328**, 916–919 (2010).
134. Galagan, J. E. & Selker, E. U. RIP: The evolutionary cost of genome defense. *Trends Genet.* **20**, 417–423 (2004).
135. Studt, L. *et al.* Knock-down of the methyltransferase Kmt6 relieves H3K27me3 and results in induction of cryptic and otherwise silent secondary metabolite gene clusters in *Fusarium fujikuroi*. *Environ. Microbiol.* **18**, 4037–4054 (2016).
136. Connolly, L. R., Smith, K. M. & Freitag, M. The *Fusarium graminearum* histone H3 K27 methyltransferase KMT6 regulates development and expression of secondary metabolite gene clusters. *PLoS Genet.* **9**, e1003916 (2013).
137. Letunic, I. & Bork, P. Interactive tree of life (iTOL): an online tool for phylogenetic tree display and annotation. *Bioinformatics* **23**, 127–128 (2007).
138. Grandaubert, J., Bhattacharyya, A. & Stukenbrock, E. H. RNA-seq based gene annotation and comparative genomics of four fungal grass pathogens in the genus *Zymoseptoria* identify novel orphan genes and species-specific invasions of transposable elements. *G3 (Bethesda)* **5**, 1323–1333 (2015).
139. Vanheule, A. *et al.* Living apart together: crosstalk between the core and supernumerary genomes in a fungal plant pathogen. *BMC Genomics* **17**, 670 (2016).
140. Spanu, P. D. *et al.* Genome expansion and gene loss in powdery mildew fungi reveal tradeoffs in extreme parasitism. *Science* **330**, 1543–1546 (2010).
141. Wicker, T. *et al.* The wheat powdery mildew genome shows the unique evolution of an obligate biotroph. *Nat. Genet.* **45**, 1092–1096 (2013).
142. Hacquard, S. *et al.* Mosaic genome structure of the barley powdery mildew pathogen and conservation of transcriptional programs in divergent hosts. *Proc. Natl Acad. Sci. USA* **110**, E2219–E2228 (2013).
143. Dean, R. A. *et al.* The genome sequence of the rice blast fungus *Magnaporthe grisea*. *Nature* **434**, 980–986 (2005).
144. Forgetta, V. *et al.* Sequencing of the Dutch elm disease fungus genome using the Roche/454 GS-FLX Titanium System in a comparison of multiple genomics core facilities. *J. Biomol. Tech.* **24**, 39–49 (2013).
145. Cuomo, C. A. *et al.* The *Fusarium graminearum* genome reveals a link between localized polymorphism and pathogen specialization. *Science* **317**, 1400–1402 (2007).

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