# Genome evolution in filamentous plant pathogens: why bigger can be better

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Abstract | Many species of fungi and oomycetes are plant pathogens of great economic importance. Over the past 7 years, the genomes of more than 30 of these filamentous plant pathogens have been sequenced, revealing remarkable diversity in genome size and architecture. Whereas the genomes of many parasites and bacterial symbionts have been reduced over time, the genomes of several lineages of filamentous plant pathogens have been shaped by repeat-driven expansions. In these lineages, the genes encoding proteins involved in host interactions are frequently polymorphic and reside within repeat-rich regions of the genome. Here, we review the properties of these adaptable genome regions and the mechanisms underlying their plasticity, and we illustrate cases in which genome plasticity has contributed to the emergence of new virulence traits. We also discuss how genome expansions may have had an impact on the co-evolutionary conflict between these filamentous plant pathogens and their hosts.

Effector
A pathogenic molecule that alters host cell structure and function, thereby facilitating infection and/or triggering defence responses.

Clade
A group of organisms that has evolved from a common ancestor.

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Numerous eukaryotes, notably filamentous micro-organisms such as fungi and oomycetes (BOX 1), have evolved the ability to colonize and grow within plant tissues. These organisms can have positive effects on plant growth, but often cause disease in natural and agricultural plant communities. Indeed, filamentous plant pathogens, such as the rust fungi and members of the oomycete genus *Phytophthora*, cause major diseases of crop plants and pose a clear and present threat to global food security[1]. Over the past 7 years, the genomes of more than 30 plant pathogenic fungi and oomycetes have been sequenced. Co-evolutionary conflicts with host plants have shaped the genomes of these filamentous plant pathogens into diverse architectures, but some common features can be noted in phylogenetically unrelated species.

It is widely accepted that parasites and bacterial symbionts tend to evolve smaller and more compact genomes than their free-living relatives[2–5]. In some cases, the shrinkage is extreme; for example, the genome of one intracellular bacterial symbiont is less than 300 kb in length[5]. By contrast, whole-genome sequencing has revealed that several species of filamentous plant pathogen exhibit a trend towards the proliferation of repetitive DNA and increased genome size (FIG. 1). In eukaryotes, although the haploid genome size (termed the 'C-value' as it is constant in a given species[6]) usually correlates with the number of genes in the smallest genomes, this correlation weakens as genome size increases[7,8]. In many filamentous plant pathogens, non-coding DNA is associated with plastic, rapidly evolving regions that harbour virulence genes, notably effector genes that modulate plant cell processes and enable pathogenic infection[9–13]. These expanded genomes are likely to represent examples of evolutionary trade–offs, as the cost of maintaining the extra DNA is counterbalanced by the functional advantages it confers.

Here, we review the genomic features of filamentous plant pathogens, with a particular focus on genome structure and plasticity. We also discuss the mechanisms and constraints that have driven the evolution of these genomes, and how they affect virulence.

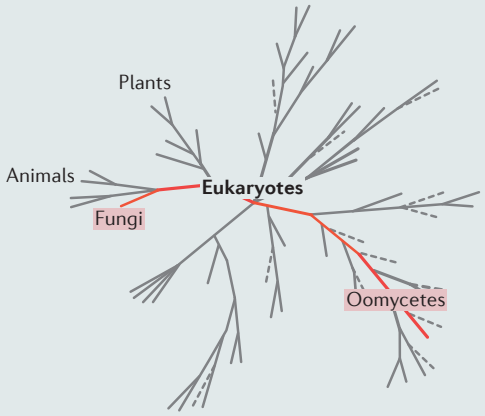
## Genome size variation

Plant pathogenic fungi and oomycetes show extreme variability in genome size. Among the ~30 filamentous plant pathogens with sequenced genomes (FIG. 1), there is a nearly 15-fold variation between the ~19–21 Mb genomes of the smut fungi *Ustilago maydis* and *Sporisorium reilianum* and the ~220–280 Mb genomes of species in the clade containing *Phytophthora infestans*, the pathogen responsible for the Irish potato famine[11,14–16].

The genomes of several, but not all, filamentous plant pathogens are larger than those of their free-living relatives and represent remarkable cases of genome

### Box 1 | **Oomycetes are not fungi**

Organisms from two distinct lineages of eukaryotes are grouped under the general term 'filamentous plant pathogens' (see the figure): the true fungi and the oomycetes (belonging to the stramenopiles)[119,120]. Fungi and oomycetes both grow as filamentous hyphae but their lineages diverged before the split of fungi from plants and animals. These eukaryotic microorganisms present important biological differences. Oomycete cell walls mainly consist of β-glucan and cellulose rather than chitin as in the fungi[121]. Oomycete hyphae are rarely septated, unlike those of fungi. The oomycetes are diploid in the vegetative stage, whereas the fungi are typically haploid. The oomycetes are related to photosynthetic brown algae and diatoms and are thought to have evolved from phototrophic ancestors[33,120,122], although this hypothesis has been disputed by some authors[123]. Although the oomycetes probably emerged in marine environments, more than 60% of the species are pathogenic on plants and have evolved plant pathogenicity independently of other eukaryotic pathogens[120,124]. Within the oomycetes, the ability to infect plants has evolved at least three times[120]. The most notorious oomycetes are members of the genus *Phytophthora* (which means 'plant killer' in Greek). They cause a wide array of diseases on a multitude of plants in both managed and natural environments[125]. The genus includes the causative agent of the Irish potato famine, *Phytophthora infestans*, which continues to cause costly epidemics in modern agriculture[126–128]. Unlike most *Phytophthora* species, *P. infestans* generally infects upper plant parts and is adapted to aerial dispersal through the production of caducous sporangia, which easily break off at maturity. Among others, these features contribute to making *P. infestans* a pathogen with a high evolutionary potential that can rapidly evolve to overcome plant resistance[128,129].




expansion[11,12,17]. The largest genomes sequenced so far in each of the two major filamentous pathogen lineages, the fungi and the stramenopiles, are those of plant pathogens. Powdery mildews have the largest genomes in the ascomycete fungi, reaching an estimated 160 Mb for *Golovinomyces orontii*[12]. Rust fungi have the largest genomes among the basidiomycete fungi, with *Melampsora larici-populina* and *Puccinia graminis* f. sp. *tritici* both having genomes that are larger than 89 Mb[17,18]. Several oomycete pathogens, which are unrelated to the fungi and belong to the stramenopiles, have genomes reaching or exceeding 100 Mb, including the obligate biotrophic pathogen downy mildew (*Hyaloperonospora arabidopsidis*; ~100 Mb[19]), and species in the *P. infestans* clade (~240 Mb[11,16]). By comparison, the genomes of non-pathogenic filamentous fungi sequenced to date, such as *Aspergillus oryzae* (37 Mb[20]), *Neurospora crassa* (41 Mb[21]) and *Schizophyllum commune* (38.5 Mb[22]), are around 40 Mb in size, and diatoms, the closest non-pathogenic relatives of oomycetes, have genomes that range in size from 27 Mb (*Phaeodactylum tricornutum*[23]) to 56 Mb (*Aureococcus anophagefferens*[24]). However, although for the filamentous plant pathogens there is a trend towards larger genomes in phylogenetically unrelated lineages, some filamentous plant pathogens have comparatively small genomes owing to intron loss (for example, *U. maydis*; 21 Mb[14]), gene loss (for example, *Albugo laibachii*; 37 Mb[25]) or reduced transposon content (for example, *Sclerotinia sclerotiorum*; 38 Mb[26]).

Powdery mildews, poplar and wheat rusts and *H. arabidopsidis* are obligate biotrophic pathogens that can only proliferate in their host plants, whereas *P. infestans* and its sister species are hemibiotrophs that require a biotrophic infection phase before a necrotrophic phase. A biotrophic infection style and a tendency to specifically infect one or a few host plant species are common features of the above-mentioned pathogens, which have the largest genomes among the filamentous plant pathogens (FIG. 1). A trend towards genome expansion has also been noted in symbiotic fungi. The black truffle fungus, *Tuber melanosporum*, which forms an ectomycorrhizal association with plant roots, has a genome of ~125 Mb with only ~7,500 protein-coding genes[27]. Nonetheless, it is important to note that some host-specific biotrophs, such as the smut fungi *U. maydis* (21 Mb) and *S. reilianum* (19 Mb), have reduced genomes[14,15].

Host domestication can favour the emergence of asexual pathogen lineages[28,29]. This switch in the mode of reproduction may have had an impact on the evolution of pathogen genome size, for example through polyploidy. However, in the case of the potato blight lineage (*Phytophthora* clade 1c), genome expansion clearly predates domestication because the sister species of *P. infestans*, which are found exclusively on wild host plants, have expanded genomes in the order of 220–280 Mb in length (FIG. 1). The impact of sex on the evolution of genome size remains a matter of debate[30,31].

Gene content also varies considerably between species and does not always correlate with genome size (FIG. 1). For example, despite having a genome that is approximately sixfold larger than that of *U. maydis*, *Blumeria graminis* has nearly 1,000 fewer genes[12,14]. Typically, the expansion of filamentous plant pathogen genomes can be largely accounted for by a proliferation of repetitive DNA, particularly transposable elements (TEs)[9,11–13,17,19]. Several species have genomes with an extremely high proportion of repetitive DNA, reaching 74% in *P. infestans* and ~65% in the fungi *B. graminis* and *Leptosphaeria maculans* (FIG. 1). These pathogens are either obligate biotrophs or hemibiotrophs, suggesting that high DNA repeat content is associated with the presence of a biotrophic phase during host infection.

Variation in genome size can be accompanied by lineage-specific expansions and contractions of specific gene families[9,11,12,17,32–36] (FIG. 1). Several families of virulence-related genes that are expanded in fungal plant pathogens relative to their non-pathogenic relatives have been identified[34]. The expanded genes mainly encode lytic enzymes and putative transporters, two functional classes of proteins that have been implicated in the evolution of fungal plant parasitism. More recently[17], it was shown that 26% of the predicted protein families of the wheat stem rust *P. graminis* f. sp. *tritici* and the poplar leaf rust *M. larici-populina* are lineage-specific. These proteins are diverse and include oligopeptide membrane transporters, copper/zinc superoxide dismutases, glycosyl hydrolases, lipases, peptidases,

Biotrophic
A pathogen that requires living host cells to complete its life cycle.

Necrotrophic
A pathogen that kills host cells and colonizes the dead tissue.

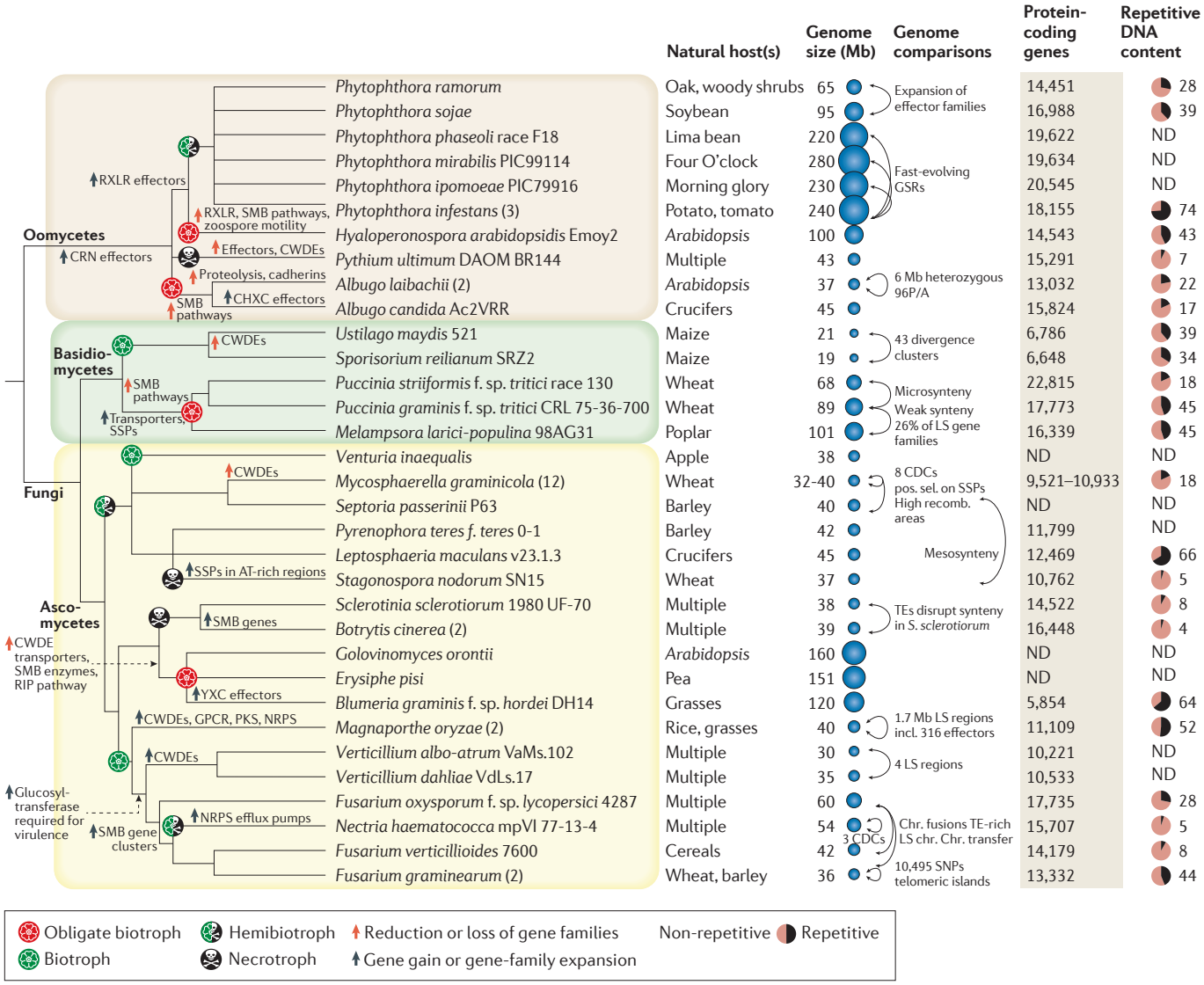



Figure 1 | **Main features of sequenced filamentous plant pathogen genomes.** Filamentous eukaryotic plant pathogens belong to either the fungi or the stramenopiles (oomycetes). The representative phylogeny depicts filamentous plant pathogens with sequenced genomes and has been generated using Interactive Tree Of Life (iTOL) with National Center for Biotechnology Information (NCBI) taxonomy identifiers (branch lengths are arbitrary). Isolate identifier, or the number of isolates sequenced, is indicated next to the species name. Pathogen lifestyles and major variations in gene families are indicated along the tree branches. The principal host plants, genome size, main insights gained from genome comparisons, number of predicted protein-coding genes and repetitive DNA content (as a percentage of genome size) are shown next to the tree branches. The reference citations for the full genome sequences for each pathogen list are included in the main text, with the exceptions of *Pythium ultimum*[138], *Venturia inaequalis*[139] and *Stagonospora nodorum*[140]. CDC, conditionally dispensable chromosome; chr., chromosome; CNV, copy number variation; CRN, Crinkler; CWDE, cell wall-degrading enzyme; GPCR, G protein-coupled receptor; GSR, gene-sparse region; incl., including; LS, lineage-specific; ND, not determined; NRPS, non-ribosomal peptide synthetase; P/A, presence/absence; PKS, polyketide synthase; pos. sel., positive selection; rec., recombination; RIP, repeat-induced point; SMB, secondary metabolites biosynthesis; SNPs, single-nucleotide polymorphisms; SSP, small secreted protein; TE, transposable element.

kinases and transcription factors. In addition, several gene families encoding small secreted proteins that are upregulated during infection were determined to be specific to one of the two species of rust fungi, and are robust candidate effectors[17,37,38]. Conversely, the genomes of several biotrophic pathogens lack, or have a reduced complement of, particular gene classes. The smut fungus *U. maydis* has a reduced set of genes encoding plant cell wall hydrolytic enzymes, presumably as an adaptation to its biotrophic lifestyle[14]. Obligate biotrophic pathogens, including powdery mildews, rust fungi, downy mildews and *Albugo* spp., lack genes encoding particular classes of plant cell wall hydrolases and various metabolic processes, such as nitrate and proteins involved in sulphate assimilation[12,14,17,19,25,39] (FIG. 1). These losses are likely to be convergent adaptations to obligate plant

parasitism, given that they have occurred in several unrelated lineages within the fungi and the oomycetes. Nonetheless, in spite of the gene losses, the genomes of these obligate pathogens are usually larger than the genomes of their non-pathogenic relatives owing to an excess of non-coding DNA.

## Genome architecture

Filamentous plant pathogen genomes are typically rich in non-coding DNA and display an irregular architecture, with an uneven distribution of genes and repetitive elements across and between chromosomes[9–13,15,16,32,33,40–43]. Repeat-rich genomic regions frequently coincide with synteny breakpoints[10–13], having evolved at accelerated rates compared with the rest of the genome[16]. Such regions tend to harbour genes that are implicated in virulence and host adaptation, such as effector genes (BOX 2). These properties are reminiscent of bacterial pathogenicity islands[44,45] but can take various forms in filamentous plant pathogens. Here, we discuss the different types of genomic regions that harbour virulence and effector genes (FIG. 2).

***Gene clusters.*** In plant pathogenic fungi, genes encoding metabolic enzymes often occur in co-expressed clusters[46,47]. Classical examples include gene clusters for secondary metabolite pathways that mediate the synthesis of host-selective toxins, such as HC-toxin, victorin, T-toxin, AK-toxin and sirodesmin, which are associated with the virulence of necrotrophic plant pathogenic fungi[46]. Clusters of genes encoding secreted proteins are less frequent but have been described in the smut fungi. In the maize pathogen *U. maydis*, genes encoding secreted proteins occur in clusters of 3–26 co-regulated genes[14] (FIG. 2a). The deletion of several such gene clusters in *U. maydis* resulted in altered virulence on maize[14,15]. Several of these clusters show low sequence conservation in otherwise well-conserved syntenic regions between *U. maydis* and its close relative *S. reilianum*, indicating that they have rapidly evolved in this lineage of maize pathogens[15]. This suggests that, even though the genomes of the smut fungi are not globally expanded relative to those of other fungi, local plastic regions that harbour clusters of effector genes do occur.

***Gene-sparse regions.*** Several filamentous plant pathogens have compartmentalized genomes with a discontinuous distribution of gene density owing to the occurrence of repeat-rich, gene-sparse regions (also known as transposon islands) (FIG. 2b). This trend is particularly accentuated in the genomes of the oomycete genus *Phytophthora*, and is most extreme in the *P. infestans* genome, in which the gene-dense and gene-sparse regions can be distinguished based on the length of the flanking intergenic regions[11,16,33,48]. Nearly 2,000 gene-sparse regions, typically containing fewer than 10 genes, have been identified in the *P. infestans* genome[11,16,48]. Unlike the *L. maculans* isochore-like regions described below[13], *Phytophthora* gene-sparse regions do not differ in GC content from the remainder of the genome (FIG. 2b). This is consistent with the previous observation that GC content and codon bias is similar in *Phytophthora* genes and retrotransposons, leading Jiang and Govers[49] to propose that this is due to an ancient invasion of *Phytophthora* genomes by TEs that predated the divergence of several species.

***Isochore-like regions.*** The genome of *L. maculans*, a dothideomycete fungus and a pathogen of *Brassica* spp., has an unusual bipartite structure with alternating blocks that differ sharply in GC content[13] (FIG. 2c). A total of 216 AT-rich heterochromatin-like blocks ranging in length from 13 kb to 325 kb were identified[13]; these areas resemble the so-called isochore regions that have been described in mammals and other vertebrates[50]. In *L. maculans*, the isochore-like regions are populated with TEs and are almost devoid of coding sequences, with only 148 out of 12,469 genes occurring in these areas[13]. Rouxel *et al.*[13] proposed that isochore-like regions emerged in the *L. maculans* lineage following a relatively recent invasion of the genome by a few families of TEs.

***Subtelomeric regions.*** Effector genes in plant pathogenic fungi are sometimes located near telomeres, which tend to evolve at higher rates than the rest of the genome[10,51,52] (FIG. 2d). A classic example is the *Magnaporthe oryzae* effector gene *Avr-Pita*, which was originally described to reside in a highly unstable region only 48 bp from the telomeric repeats on chromosome 3 (REF. 51). In a recent study[53], it was shown that *Avr-Pita* has undergone multiple translocations in *M. oryzae* and related species, often ending up in retrotransposon-rich subtelomeric regions. The genomic location of *Avr-Pita* is reminiscent of the subtelomeric position of antigenic variation genes of animal parasites, such as *Trypanosoma brucei* and *Plasmodium falciparum*[54,55].

***Conditionally dispensable chromosomes.*** Several plant pathogenic fungi, including *Nectria haematococca*, *Alternaria alternata*, *Fusarium oxysporum* and

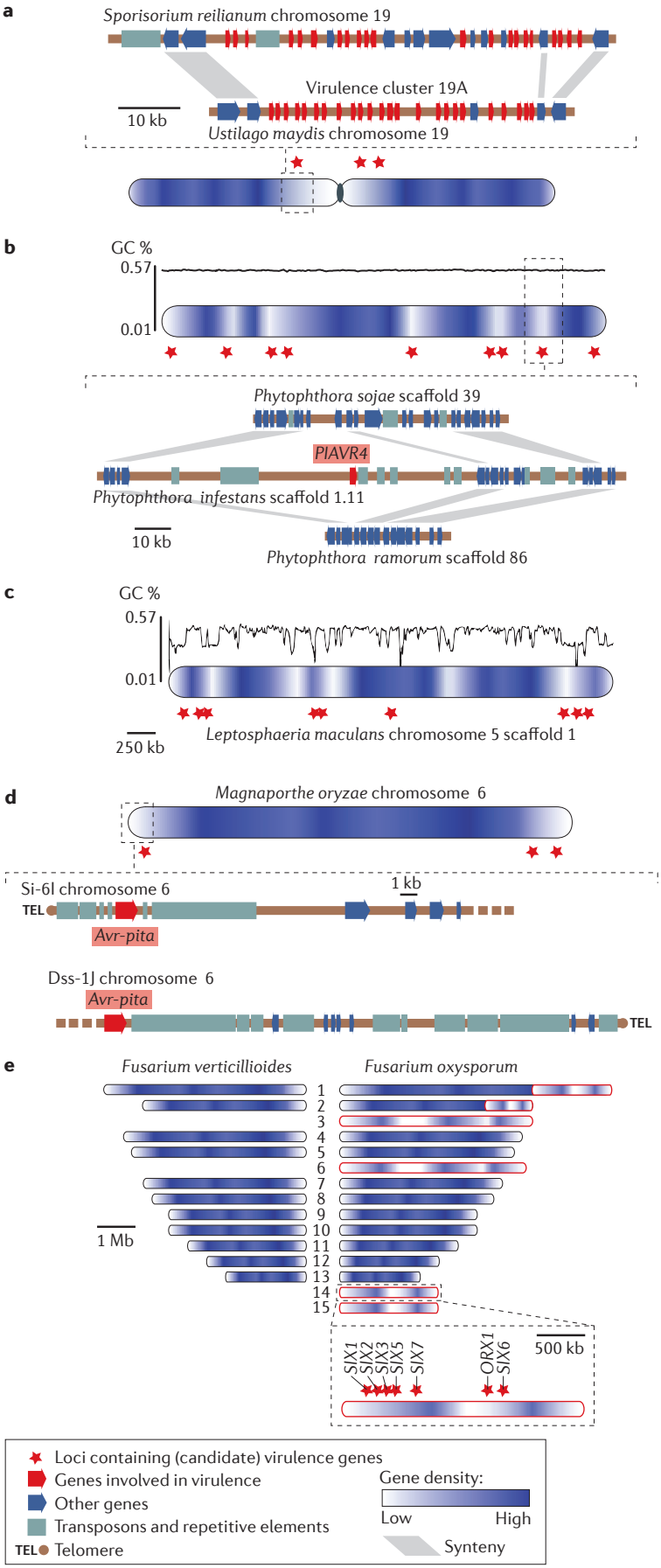
Host-selective toxins
Molecules produced by necrotrophic plant pathogens that trigger cell death in particular plant genotypes.

Isochore
A genome block of homogeneous GC content that differs from the remainder of the genome.

Heterochromatin
A tightly packed form of DNA that affects gene expression and can be modulated by epigenetic imprinting.

Box 2 | **RXLR effectors, when good genes become a liability**

Plant-associated organisms secrete proteins and other molecules, collectively known as effectors, to modulate host physiology and enable colonization of plant tissue[130]. Filamentous pathogen effectors target different sites in the plant tissue. Some effectors function in the apoplast, whereas others translocate inside the plant cytoplasm[131–133]. The largest class of oomycete host-translocated effectors is formed by the RXLR effectors, with more than 300 members in *Phytophthora ramorum* and *Phytophthora sojae*[33] and ~550 genes in the *Phytophthora infestans* genome[11]. RXLR effectors contain a host-translocation domain, defined by the conserved Arg-X-Leu-Arg (RXLR) motif, at their amino terminus and variable carboxy-terminal effector domains[131] (FIG. 4). Some RXLR effectors display conflicting activities; they contribute to virulence, for instance by suppressing host immunity, but can 'trip the wire' and activate immunity on plant genotypes that carry matching immune receptors[130,134–136]. In these cases, the effectors are said to have an 'avirulence' activity. As a consequence, loss-of-function mutations in effector genes with avirulence activity can confer a dramatic increase in fitness on plants that carry the corresponding resistance (*R*) gene. All *P. infestans* effectors with avirulence activity identified so far belong to the RXLR family[101]. Similarly to many other plant pathogen avirulence effectors, RXLR effectors can evolve to circumvent recognition by plant *R* genes while maintaining their virulence activity[137]. How RXLR effectors suppress and activate immunity is a current and active research topic.




*Mycosphaerella graminicola*, have conditionally dispensable chromosomes (CDCs) that differ from the remainder of the genome in several structural features, such as the number of repeats, gene density and GC content[40,56–58] (FIG. 2e). CDCs often carry virulence and effector genes, but they are accessory chromosomes: their loss does not affect fungus viability, and they only persist in a population if they confer an adaptive advantage[57]. In the pea pathogen *N. haematococca*, virulence genes such as *PDA*, which encodes the phytoalexin-detoxifying enzyme pisatin demethylase, occur on a 1.6-Mb chromosome that can be lost during meiosis[32,58]. In the tomato pathogen *A. alternata*, CDCs carry genes involved in the production of the host-specific AAL-toxin[56]. In the broad host-range pathogen *F. oxysporum*, CDCs carry all known effector genes (called secreted in xylem (*SIX*) genes) and horizontal transfer of two CDCs converted a non-pathogenic *F. oxysporum* strain into a pathogen[40]. The domesticated wheat pathogen *M. graminicola* has eight CDCs, ranging in size from 0.39 Mb to 0.77 Mb[41,57]. However, unlike CDCs in other fungal pathogens, *M. graminicola* CDCs are not particularly enriched in genes encoding secreted proteins or effectors, and the degree to which they carry virulence genes remains to be determined[41,59].

## Mechanisms driving genome structure

In the previous section, we described the diverse repeat-rich and effector-rich regions that delineate distinct and remarkably plastic regions in the genomes of filamentous plant pathogens. Here, we summarize some of the mechanisms that drive genetic diversity in these genomes, as illustrated in FIG. 3.

Figure 2 | **Genome niches housing effector genes in filamentous plant pathogens. a** | Gene clusters. The example depicts rapidly diverging virulence gene clusters on chromosome 19 of the smut fungi *Sporisorium reilianum* and *Ustilago maydis* (cluster 19A), which occupy retroelement-rich pericentromeric regions. The grey lines mark regions sharing at least 57% similarity between the two species[15]. **b** | Gene-sparse regions. Tens to hundreds of these regions, 10–1,000 kb long, are scattered throughout the genomes of the oomycetes *Phytophthora* spp., and they do not show bias in GC content. The avirulence effector gene *PiAvr4* resides in a ~100-kb-long gene-sparse region in the *Phytophthora infestans* genome, which is greatly expanded relative to that of *Phytophthora sojae* and of *Phytophthora ramorum*[11]. **c** | Isochore-like regions. The genome of the blackleg fungus *Leptosphaeria maculans* has tens of AT-rich isochore-like regions, 10–350 kb long, scattered along each GC-equilibrated chromosome. Small secreted proteins (including validated and candidate virulence genes) are enriched in the AT-rich isochore regions[13]. **d** | Subtelomeric regions. The example depicts the *Magnaporthe oryzae* effector gene *Avr-Pita*, which lies in very close proximity to telomere-like repeats, in isolates Si-6I and Dss-1J[53]. These regions are typically 0.03–500 kb long. **e** | Conditionally dispensable chromosomes (CDCs). The four CDCs of *Fusarium oxysporum*, which are missing in *Fusarium verticillioides*, are depicted[40]. Translocations that are unique to *F. oxysporum* are also shown. One CDC is enriched in effector (*SIX*) and secreted protein genes (including validated and candidate virulence genes).

***DNA point mutations.*** The rates of nucleotide substitution vary among species and strains and also locally along chromosomes[60]. More than 10,000 single-nucleotide polymorphisms (SNPs) were identified in a comparison between two strains of *Fusarium graminearum*[10]. Regions of very high SNP density were found near telomeres and discrete AT-rich chromosome regions[10]. In other cases, variations in SNP frequency across the genomes were not detected but local biases in the ratio of synonymous to non-synonymous SNPs were observed and interpreted as a signature of selection[16,25,29,59]. In one striking example, Stuckenbrock *et al.*[59] reported reduced synonymous substitution rates (dS) and increased non-synonymous substitution rates (dN) in *M. graminicola* CDCs. Similarly, Raffaele *et al.*[16] observed a higher frequency of genes with elevated dN/dS ratios in the gene-sparse regions of the *P. infestans* genome than in the gene-dense regions, showing that these regions have evolved significantly faster than the rest of the genome.

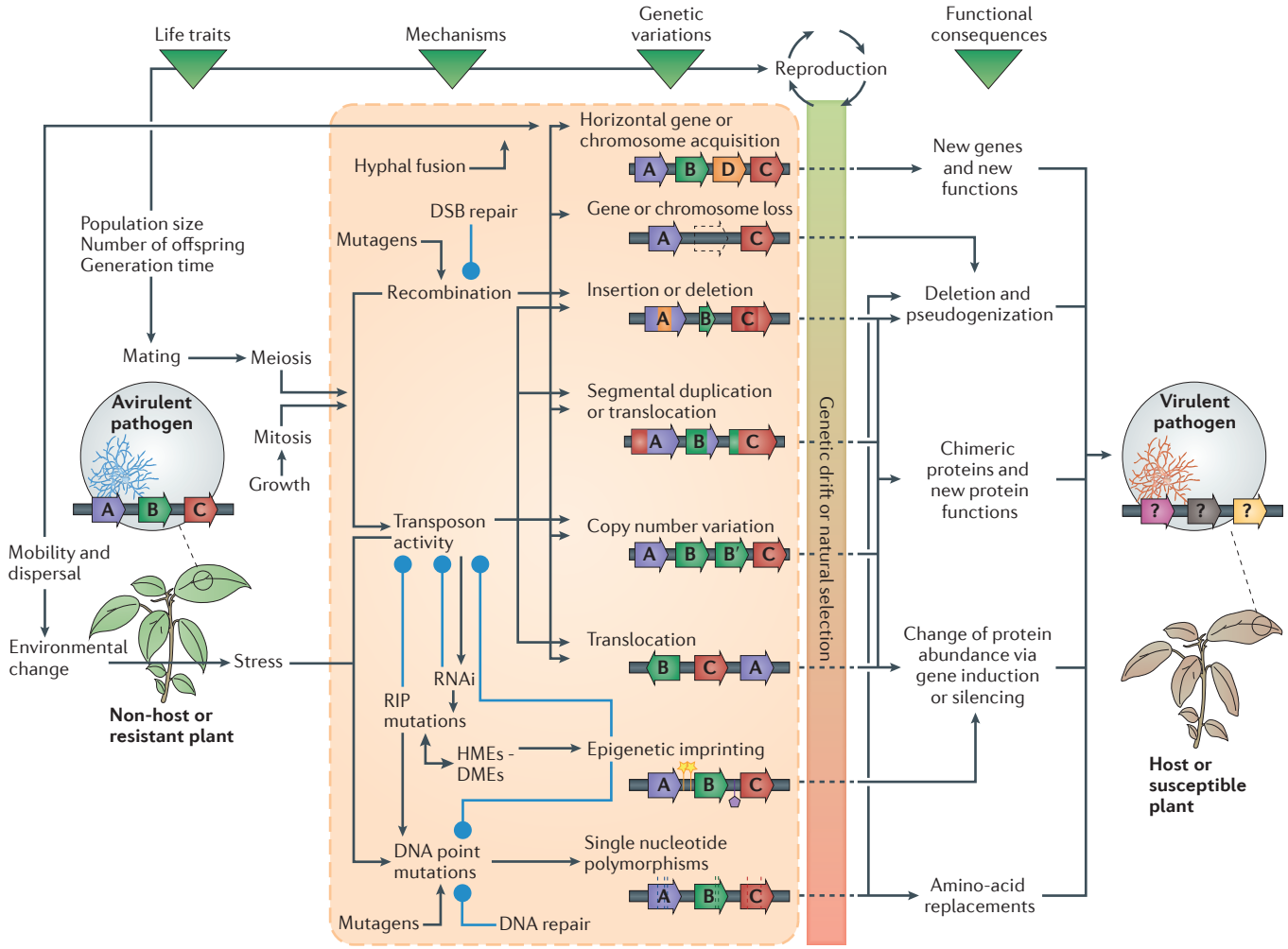



Figure 3 | **Evolution of virulence in filamentous plant pathogens.** The figure illustrates the life traits and mechanisms that shape the structure of filamentous pathogen genomes, and their genetic and functional consequences. Causative relationships are shown with black arrows, whereas inhibitory relations are shown in blue. Some connections were omitted for clarity. Mating, growth, and environment-induced stress are among the processes that lead to genetic variation. They are influenced by life traits such as population size, number of offspring, generation time and mode of dispersal. These processes trigger, more or less directly, a range of mechanisms that shape genome structure and expression, including DNA point mutations, repeat-induced point (RIP) mutations, epigenetic imprinting, recombination, transposon activity and horizontal gene or chromosome transfer. Eight major types of genetic variations arise from these mechanisms: horizontal gene or chromosome acquisition; gene or chromosome loss; small insertions and deletions; domain shuffling by segmental duplication or translocation; gene copy number variation; gene translocation; epigenetic imprinting; and single-nucleotide polymorphisms. Evolutionary forces will then modulate the frequency of these variants in the population via genetic drift or natural selection. The functional consequences can be classified as: acquisition of new functions via newly acquired genes; gene deletion and pseudogenization; formation of chimeric proteins leading to new protein functions; change in protein abundance via changes in gene expression patterns; and amino-acid replacements in protein sequences. In many cases, these genetic variations increase the fitness of the pathogen via enhanced virulence. DME, DNA methylation enzyme; DSB, double-strand break; HME, histone methylation enzyme; RNAi, RNA interference.

Conditionally dispensable chromosomes
(CDCs). Accessory chromosomes that are not required for basic growth but can confer advantages under certain conditions.

Single-nucleotide polymorphisms
(SNPs). DNA sequence polymorphisms involving the replacement of one nucleotide by another.

Positive selection has had a marked impact on the evolution of filamentous plant pathogen genes, particularly effector genes (BOX 2). The intragenic distribution of positively selected sites is not random in the RXLR class of effector genes of oomycetes, which have a distinct modular structure (FIG. 4). The C-terminal domains, which are responsible for the effector biochemical activity, show a much higher frequency of sites under positive selection compared with the amino-terminal domains, which function in secretion and host translocation[61]. Functionally important and polymorphic residues tend to be on the surface of RXLR proteins, further supporting the view that many non-synonymous substitutions in RXLR effectors have been subject to positive selection[62–64] (FIG. 4a).

***RIP mutations.*** Repeat-induced point (RIP) mutations are formed in a process during which duplicated sequences are mutated from CpA to TpA and from TpG to TpA in the dikaryotic cells that are formed during meiosis in most ascomycete fungi[65,66]. RIP mutations typically end up inactivating genes by producing stop codons. By an unknown mechanism, RIP mutations can leak to regions flanking the repeated sequence, therefore potentially affecting proximal single-copy genes and resulting in new functional alleles with premature stop codons or non-synonymous substitutions[67,68]. The higher mutation rate associated with the RIP process could accelerate the evolution of effector genes that populate repeat-rich regions in *Fusarium* spp.[67]. RIP mutations can be inferred bioinformatically and have been observed in several plant-pathogenic ascomycetes (for example, *F. graminearum*), in which they probably account for the reduced repeat content and low number of paralogous genes[10]. RIP mutations have also affected the TEs of *L. maculans* and have probably generated the decayed long terminal repeat retrotransposons that populate the AT-rich isochores in this species[13]. Interestingly, the expanded genomes of powdery mildew fungi, which are obligate biotrophic ascomycetes, lack the RIP mutation machinery, suggesting that the absence of RIP mutations may have contributed to genome expansion in this lineage[12]. RIP has not been reported in oomycetes to date and has been found only in the Pucciniomycotina subphylum of the basidiomycetes so far[69].

***Recombination.*** DNA recombination is another process that shaped the genomes of filamentous plant pathogens. It can arise from the action of mutagens or can be genetically determined[70] (FIG. 3). The genomes of several ascomycete fungi display conserved gene blocks within homologous chromosomes, but with randomized gene order and orientation[71]. This phenomenon, known as mesosynteny, is thought to arise from a high frequency of recombination, particularly inversions[71]. As discussed earlier, the *F. graminearum* genome displays discrete regions of very high sequence diversity proximal to the subtelomeric repeats[10]. Discrete regions of elevated SNP density are also found along *F. graminearum* chromosomes. These intra-chromosomal variable regions were proposed to have arisen from telomeric fusion of chromosomes, a process implicating homologous or non-homologous recombination[10].

The oomycete Crinkler (CRN) proteins form a particularly diverse family of host-translocated effectors[11,72,73]. CRN proteins are modular proteins with N-terminal secretion signals and translocation domains (FIG. 4c). The genes encoding CRN proteins exhibit a high rate of domain swapping and chimaera formation, probably resulting from non-allelic homologous recombination[11]. Remarkably, a highly conserved HVLVXXP motif marks the junction between the N-terminal targeting domains and the highly diverse carboxy-terminal domains, which encode the effector biochemical activity. This suggests that a recombination process that targets this site, specifically between the second and third base in the proline codon (CC↓X), generated the chimeric CRN genes (FIG. 4c).

***TE activity.*** TEs generate a local genome environment that favours chromosomal rearrangements, deletions and duplications, as well as greater sequence diversity[74–76] (FIG. 3). This can be mediated by increased recombination, as probably occurred in the disruption of gene collinearity in the TE-rich regions of *Phytophthora* genomes[11,33], as discussed above (FIG. 2). In addition, TEs, particularly retrotransposons, can directly contribute to genetic innovation by segmental retroduplication, transduction and genome insertion of an mRNA, sometimes including the flanking sequences[77]. Such processes appear to have occurred frequently in the expanded ~120 Mb genome of the barley powdery mildew *Blumeria graminis* f. sp. *hordei*[12]. This genome has experienced a massive proliferation of retrotransposons associated with loss of RIP, which has probably contributed to the extensive gene losses, expansions and reshuffles. A positive correlation between recombination rates and the distribution of TEs in clusters that break synteny between *M. oryzae* and other fungi has been noted[78], indicating increased rates of structural variation in TE-rich regions (see below for more on the effect of TEs)[78]. Finally, TEs can also insert within genes, therefore directly disrupting gene activity[76].

***Epigenetic regulatory processes.*** Epigenetic processes result in transcriptional or post-transcriptional modification of gene expression without the alteration of DNA sequences[79,80]. Silencing of endogenous genes by overexpression of transgenes has been reported in oomycetes, including in *P. infestans* and *Phytophthora sojae*[81–83]. In these species, gene silencing is thought to act at the transcriptional level, can be reversible and is transmitted independently of the transgenes[82,83]. Genes encoding the major components of the RNA interference (RNAi) pathway, namely Dicer-like, Argonaute, RNA-directed RNA polymerase and histone deacetylase proteins, have been identified in the *P. infestans* genome[84]. Judelson *et al.*[83] and van West *et al.*[82] proposed that chromatin remodelling triggers gene silencing in *P. infestans* and that limited spread of heterochromatin can affect neighbouring genes at the target locus. In the smut fungus

Mesosynteny
Conservation of gene content but not gene order or orientation.

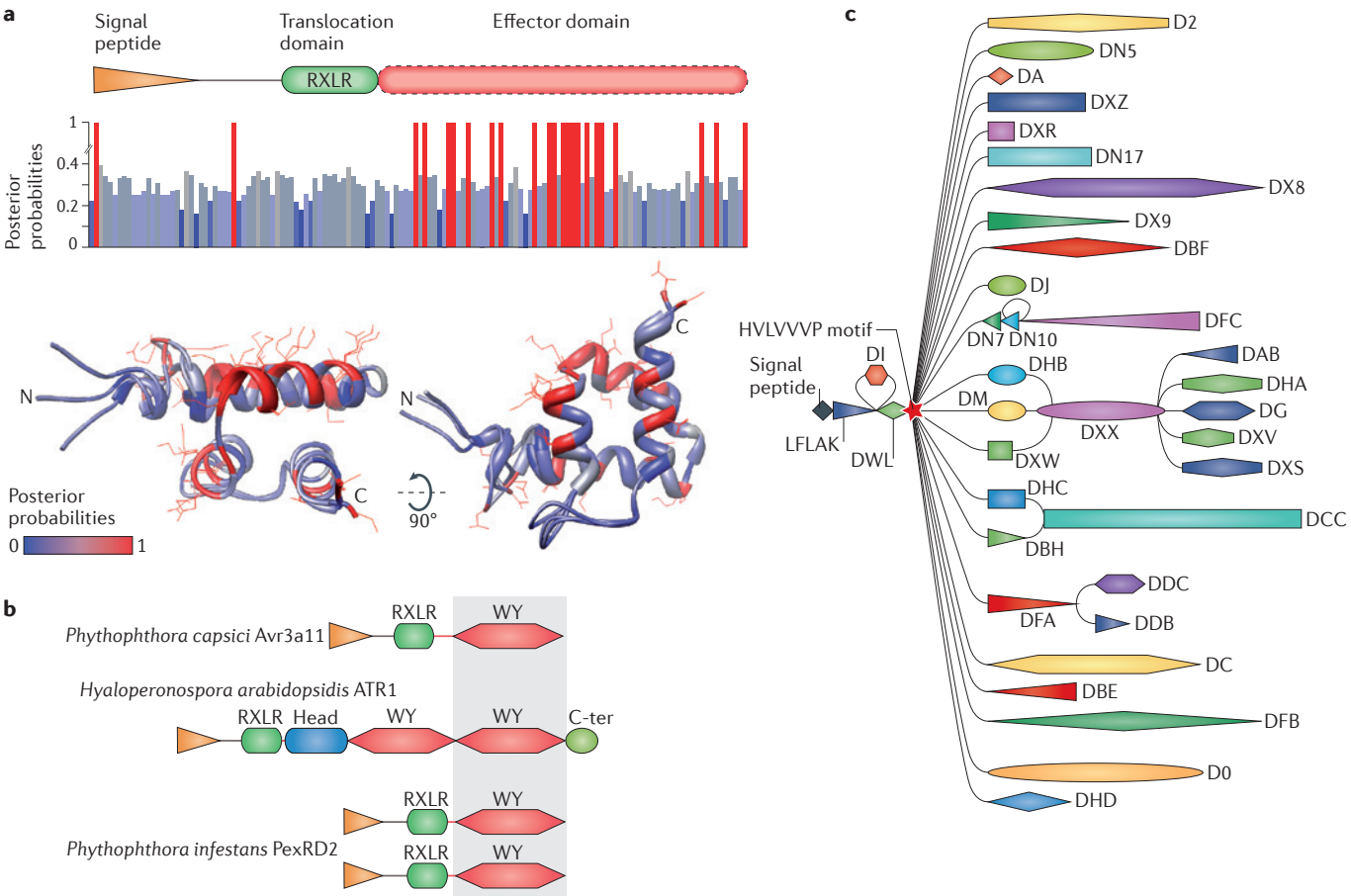



Figure 4 | **Modular filamentous plant pathogen effectors: oomycete RXLR and CRN families. a** | Members of the Avr3a family of oomycete RXLR effectors, *Phytophthora sojae* Avh1b and Avr1-b[141,142], are depicted. These effectors are composed of a signal peptide, a translocation domain featuring the RXLR conserved motif, and a carboxy-terminal effector domain. The C-terminal domains of RXLR effectors are targeted by positive selection. When mapped onto protein structures, positively selected residues are distributed at the periphery of the four-helix bundle core, with side chains facing toward the solvent. The structures of Avh1b and Avr1-b were modelled based on alignment with *Phytophthora capsici* Avr3a4 and the 3 models were superimposed (REF. 63). **b** | Adaptable sequence modules in the C-terminal domain of RXLR effectors. The α-helix bundle WY domain[62] can occur as a single unit, as in the *Phytophthora capsici* Avr3a family, as a duplicated module, as in *Hyaloperonospora arabidopsidis* ATR1 (REF. 64), or in association with other protein domains. An additional level of modularity exists at the level of mature proteins that can associate into multimers, as in the case of *Phytophthora infestans* PexRD2 that forms homodimers *in vitro* and *in vivo*[62]. **c** | The Crinkler (CRN) effector proteins have mosaic domains that have been shuffled by recombination. The amino-terminal region (containing the signal peptide, LFLAK, DI and DWL domains) mediates translocation inside plant cells and is found linked to a wide range of C-terminal domains[11].

*S. reilianum*, the RNAi machinery does not directly contribute to virulence, but its loss in the closely related *U. maydis* might have affected the rate of local gene duplication[15].

Epigenetic silencing frequently inactivates TEs and neighbouring genes[79,85]. In *P. infestans*, a short interspersed retrotransposable element (SINE) was shown to be targeted by the RNAi machinery[86]. Therefore, genes, such as effector genes, that reside in repeat-rich regions located proximal to TEs could be affected by heterochromatin spread and may exhibit a higher frequency of gene silencing than genes in repeat-poor regions of the genome. The extent to which heterochromatin diffusion drives phenotypic diversity in oomycetes remains to be determined.

***Horizontal gene and chromosome transfer.*** Horizontal gene transfer (HGT), the non-sexual transfer of genetic material between organisms, is well established as a major evolutionary process in bacteria and archaea, enabling, for example, bacterial pathogens to acquire new virulence functions[87]. However, the importance of HGT in the evolution of fungi and oomycetes is only starting to be fully appreciated. Hyphal fusion (anastomosis) and transduction by viruses are among the cellular mechanisms that facilitate HGT between filamentous pathogens[88,89]. HGT is known to occur between conspecific fungi through chromosomal translocation[90] or chromosome non-disjunction. Non-disjunction is observed during meiosis[57] and hyphal anastomosis in parasexual cycles[91]. Entire accessory chromosomes, such as an

*A. alternata* 1.0-Mb chromosome[56] and the *F. oxysporum* chromosome 14 (REF. 40), can also be transferred horizontally following hyphal anastomosis.

## The effect of genome plasticity on virulence

Filamentous plant pathogens are engaged in antagonistic co-evolutionary conflicts with their hosts that have left dramatic marks in their genomes. In this section, we describe how the mechanisms of genome evolution described above can affect the fitness of these pathogens on their hosts. We illustrate this with examples selected from an ever-growing list that documents how the mechanisms underlying genetic and genomic plasticity result in altered virulence.

***Deletion and pseudogenization.*** Loss-of-function mutations in an avirulence effector gene of a plant pathogen enable evasion of recognition by the matching plant immune receptor, therefore resulting in virulence and increased fitness in particular host genotypes (BOX 2). Pseudogenization and the deletion of avirulence effector genes are common in virulent races of filamentous plant pathogens. The virulence of *L. maculans* in *Brassica napus* (canola) plants carrying the genes resistance to *L. maculans* 1 (*RLM1*) and *RLM6* has evolved rapidly in the field[92,93]. Almost all isolates virulent in *RLM1*-positive plants and about half of the *RLM6*-virulent isolates carry deletions of the regions spanning the corresponding *AvrLm1* and *AvrLm6* avirulence effector genes, respectively[92,93]. The high retrotransposon content in the AT-rich isochore-like regions that harbour the effector genes in *L. maculans* may have increased the frequency of recombination events, leading to the deletions[92]. *AvrLm6* is a single-copy gene located proximal to degenerated TEs. RIP mutation-derived premature stop codon mutations, which probably leaked from adjacent inactivated TEs, have been detected in *AvrLm6* alleles[68,93]. Therefore, the RIP mutation process appears to have facilitated the emergence of non-functional effector alleles, resulting in *L. maculans* strains that evade recognition by the RLM6 immune receptor[68,93]. However, the frequency of RIP mutation-induced premature stop alleles is much lower than the frequency of deletions. In the *B. napus* cultivars found in Australia, all of the *L. maculans AvrLm6* RIP mutation haplotypes have a single phylogenetic origin, indicating that RIP mutation-derived pseudogenization occurred only once in this pathogen population[68].

In *M. oryzae* strains that are virulent in rice plants carrying the immune receptor Pi-ta, the *Avr-Pita* avirulence effector gene is typically deleted or pseudogenized by frameshift mutations[51,94]. However, in some strains, the *Avr-Pita* gene is disrupted by insertions of the DNA transposon *Pot3* in the promoter or the coding sequence[95,96]. Similarly, an isolate of *M. oryzae* virulent on rice plants carrying the *Pi33* resistance gene has a 1.9 kb MINE retrotransposon inserted in the last exon of the *ACE1* avirulence gene[97].

In *P. infestans* and *P. sojae*, deletions and pseudogenization events through nonsense and premature termination mutations have been identified in RXLR effector genes with avirulence activity[98–101]. For example, *P. infestans* strains virulent in potatoes expressing the *R4* resistance gene carry deleted or non-functional alleles of the RXLR effector Avr4 (REF. 98). *P. infestans Avr4* is the only gene in a 100-kb-long repeat-rich expanded region in the *P. infestans* genome (FIG. 2b), a position that could contribute to its genomic instability[11,98].

***Gene silencing.*** In plant pathogenic oomycetes, transcriptional silencing of effector genes is another mechanism by which avirulent strains have evolved to overcome resistant hosts[101,102]. The *P. sojae* RXLR effector genes *Avr1a* and *Avr3a* are silenced in some of the pathogen strains that are virulent in soybean plants expressing the *RPS1A* and *RPS3A* genes, respectively[99]. *P. sojae Avr1a* and *Avr3a* silencing is associated with extensive copy number variation (CNV) at these loci[99]. However, whether the CNV polymorphisms have an impact on the transcriptional regulation of these effector genes has not yet been elucidated. Epigenetic regulation may have also contributed to host adaptation in the *P. infestans* lineage. It has been reported[16] that several genes involved in epigenetic processes occupy the gene-sparse regions of the *P. infestans* genome and display significant polymorphisms between host-specific sister species in the *P. infestans* lineage. Also, components of the small interfering RNA and histone modification pathways are differentially regulated during plant infection by *P. infestans*, indicating that modulation of epigenetic processes may play a part in host adaptation[84].

***Amino acid replacements.*** Plant pathogen effectors can display conflicting activities; for example, they contribute to virulence by suppressing host immunity in susceptible plants but can 'trip the wire' and activate plant immunity in resistant host genotypes (BOX 2). As a consequence, some effectors carry mutations that enable them to circumvent recognition by the plant while maintaining their virulence activity. One example is the cysteine-rich apoplastic effector Avr4 of *Cladosporium fulvum*, which binds chitin and protects the pathogen cell walls from hydrolysis by the chitinases of its host plant tomato[103]. *Avr4* alleles that evade recognition by the tomato surface receptor-like protein Cf4 contain SNPs, typically cysteine to tyrosine replacements[104]. These stealthy Avr4 proteins are less stable in the tomato apoplast because of the loss of one disulphide bridge, but they retain their ability to bind chitin and presumably to contribute to virulence[103,105].

The AvrL567 effector of the flax rust fungus *Melampsora lini* binds and activates the flax immune receptors L5, L6 and L7, but some variants with multiple amino acid substitutions escape recognition while maintaining a stable structure[106]. Wang *et al.*[107] discovered that AvrL567 polymorphic residues associated with recognition differences map to the effector surface and probably form multiple contact points with the receptors. However, in the absence of a known virulence activity for AvrL567, the extent to which these amino acid replacements affect virulence is unknown.

Pseudogenization
A process through which genes lose their ability to code for proteins, either by mutation or by loss of expression.

Copy number variation
(CNV). A form of genetic polymorphism in which the number of copies of a gene is modified within a genome.

The hundreds of annotated oomycete RXLR effectors show extreme amino acid sequence diversity, although ~44% of *Phytophthora* spp. RXLR proteins contain a conserved α-helical fold, the 'WY domain', in their C-terminal effector domain[62,108] (FIG. 4b). The Avr3a family of WY domain RXLR effectors occurs in several *Phytophthora* spp., including *P. infestans* and *P. sojae*[62]. Avr3a family members that are differentially recognized by potato and soybean resistance proteins exhibit a number of polymorphisms on their surface that contribute to the differences in activity[62,63] (FIG. 4b).

***Chimeric proteins.*** Modularity is a common feature of filamentous pathogen effectors, particularly in the oomycete RXLR and CRN families[11,73] (FIG. 4). These modules can be assorted independently into chimeric or repeat-containing proteins. The oomycete CRN proteins are prime examples of chimeric effectors, with the host-translocation N-terminal domain fused to 36 different C-terminal domains, which are sometimes combined within a single protein[11] (FIG. 4c). CRN domain shuffling probably generates new effector activities. For example, CRN proteins with a DXW–DXX–DXS domain combination trigger cell death in plants, but those with a DXX–DXS or DHB–DXX–DHA combination do not[11]. In the RXLR effectors, the C-terminal WY domain is associated with the N-terminal translocation domain and carries out the effector activity[62–64] (FIG. 4b). The WY domain can occur as a single unit, such as in the Avr3a protein family[62], be duplicated as in the *H. arabidopsidis* ATR1 effector[64], or even occur as tandem repeats of up to 11 units in some annotated effectors[62]. The WY domain can also associate with other domains at either terminus. *H. arabidopsidis* ATR1 contains an additional helical domain before the two WY domains and mutations that alter recognition by the immune receptor RPP1 occur in both regions[64]. RXLR effector modularity also occurs at the level of protein oligomerization. The *P. infestans* PexRD2 effector occurs as a dimer of WY domains[62]. The extent to which RXLR effector oligomerization impacts activity, and whether these proteins form hetero-oligomers when they are delivered inside plant cells, remains an open question. A systematic study of protein domain combinations in 67 eukaryotes revealed a high number of oomycete-specific combinations and expansions, supporting the existence of active remodelling of signalling and interaction networks by recombination in oomycetes[109].

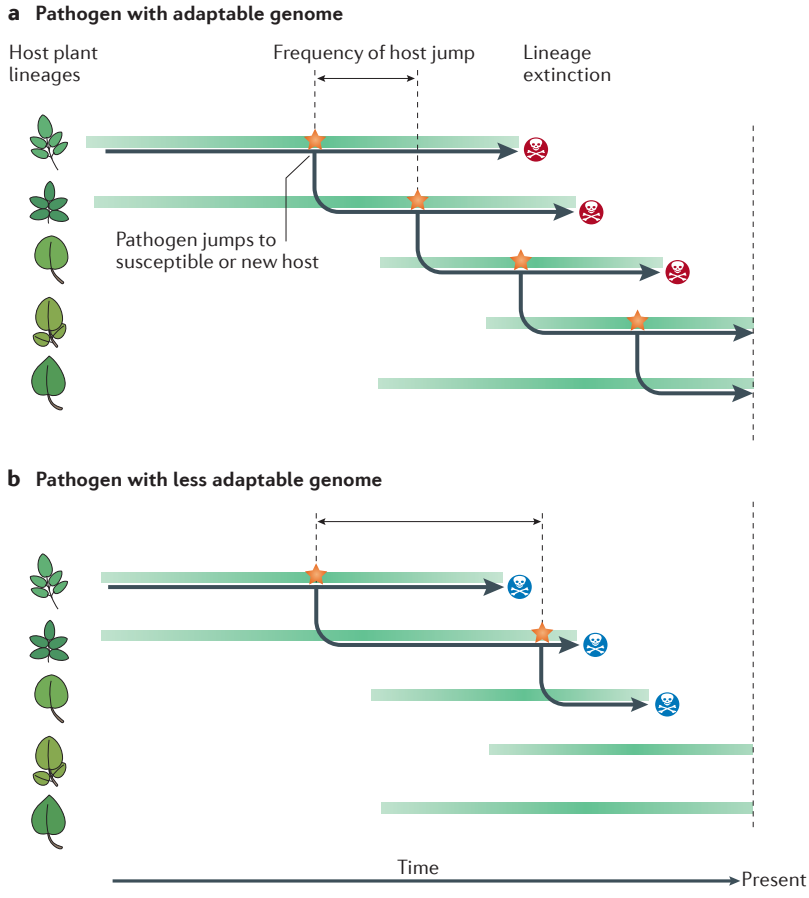



Figure 5 | **Jump or die: a model to account for the macroevolutionary persistence of host-specialized filamentous pathogens.** The model illustrates the evolutionary fate of pathogen lineages with (**a**) highly adaptable genomes and (**b**) less adaptable genomes as they evolve on various lineages of host plants. Given that extinction of the host population or species is fatal to a host-specific pathogen lineage, the frequency of jumps to susceptible or new hosts must be crucial for macroevolutionary persistence. Therefore, for a host-specific plant pathogen lineage to survive over a long evolutionary timescale into the modern biota, the frequency of host jumps must be greater than the frequency of host-population extinction. The same model applies if we consider that host populations could evolve to become fully resistant to the pathogen (host-lineage extinction is then replaced by the host lineage becoming fully resistant to the pathogen).

***New genes and functions.*** HGT enables the acquisition of new genes and functions not only from close relatives but also from phylogenetically unrelated organisms. There is evidence that filamentous plant pathogens may have acquired genes from distant species. In one example[110] it was proposed that an *in planta*-induced cutinase gene was acquired by an ancestral *Phytophthora* species from an actinobacterial source. Richards *et al.*[111] suggested that HGT played an important part in the evolution of plant parasitism in the oomycetes. A genome-wide HGT screen revealed 34 cases of relatively ancient HGT between fungi and oomycetes[111]. Candidate genes acquired by HGT encode secreted proteins, plant cell wall hydrolases and proteins involved in heterotrophic growth[111]. More striking is the recent interspecific transfer of the *ToxA* toxin gene from one wheat fungal pathogen, *Stagonospora nodorum*, to another, *Pyrenophora tritici-repentis*[112]. This single HGT event is thought to have occurred not long before 1941 and was responsible for the emergence of the wheat tan spot disease in the 1940s[112]. The proximity of the *ToxA* gene to a hAT family TE may have contributed to the transfer and integration of this gene into the *P. tritici-repentis* genome[112].

Chromosomes can also be transferred horizontally in fungi. As already discussed, in *A. alternata*, the ability to produce the host-specific toxin AAL and to infect tomato was acquired by horizontal transfer of a complete pathogenicity chromosome[56]. Mobile CDCs are thought to be the key determinant of host-specificity in *F. oxysporum*[40]. Following the pattern of

Box 3 | **Outstanding questions in comparative genomics of filamentous plant pathogens**

- Which cues, internal or external, drive the expansion of pathogen genomes? How are bipartite, 'two-speed' genomes generated? What are the local constraints that govern the emergence of repeat- and effector-rich regions?
- How often do host jumps occur and which factors and mutations favour them? How do pathogens adapt to their new hosts following host jumps? What makes obligate biotrophs dependent on their hosts?
- How do the genomes and effector reservoirs of wide host-range biotrophic filamentous pathogens differ from those of host-specific species?
- What is the exact role of transposable elements in the evolution and regulation of effector genes?
- Are there specific molecular mechanisms that increase mutation rates and favour plasticity?
- What is the extent of expression polymorphism between filamentous pathogen strains that differ in virulence and host-specificity? To what extent does epigenetic imprinting contribute to the evolution and regulation of effector genes?
- To what extent does modularity contribute to the emergence of new complex virulence functions?

chromosome inheritance would prove useful to clarify the mechanisms underlying their transfer and their contribution to virulence in natural populations.

Chuma *et al.*[53] proposed an attractive model to explain how predominantly asexual plant pathogens, such as the rice blast fungus *M. oryzae*, regain deleted avirulence effector genes. They proposed that parasexual exchange of genetic material enabled the recovery of 'lost' effector genes in asexual lineages. This model is strongly supported by a comprehensive population study that revealed that the *Avr-Pita* effector gene has experienced a number of translocations in *Magnaporthe grisea* and related *Pyricularia* species, most likely as a consequence of its recovery by lateral transfer[53].

***CNV.*** CNV has been noted in effector genes of *P. infestans* and *P. sojae*. In the *P. infestans Pi3.4* locus, CNV was proposed to contribute to the assembly of novel genes leading to the emergence of virulent races[113]. As discussed above, multiple near-identical copies of *Avr1a* and *Avr3a* are frequent in *P. sojae* strains[99]. CNV is also prevalent among RXLR effector genes in *P. sojae*[99]. However, the extent to which these CNV polymorphisms alter pathogen phenotype is unclear. Unlike oomycetes, CNV in phytopathogenic fungi has not been reported.

## Why bigger can be better

The genomes of filamentous plant pathogens have diverse architectures that have favoured the evolution of features that increase plasticity. Most pathogens, including some filamentous plant pathogens like the smut fungi, have evolved compact genomes[2–4,14]. However, one remarkable pattern in other filamentous plant pathogens is the convergent evolution towards large genomes infested with repetitive elements in deep lineages of host-specific plant pathogens, such as *Phytophthora* spp., downy mildews, rust fungi and powdery mildews (FIG. 1). Which evolutionary trade-offs drive this evolutionary trend and counterbalance the cost of maintaining these large genomes? The plasticity conferred by the TEs is thought to be adaptive, enhancing the evolutionary potential of pathogen lineages independently of genetic drift[114]. But this creates a conundrum because natural selection cannot maintain genes for future use[115–117]. This conundrum is solved by the evolutionary concept known as clade selection (also called species selection) that was put forward by George C. Williams[115,118]. Williams proposed that lineages that produce new species at a high frequency and, therefore, are better at avoiding extinction, will dominate the biota compared with lineages that are prone to extinction[115,118]. By extension, it is reasonable to assume that pathogen lineages with large, flexible genomes are likely to adapt faster during co-evolution, continuously producing virulent pathotypes that keep up with the nimble and highly effective plant immune system, and occasionally yielding genotypes that can jump to new host populations or even new host species.

In FIG. 5, we describe a model that accounts for the macroevolutionary persistence of host-specialized filamentous pathogens inspired by the clade selection concept[115]. We propose that clade selection opposes the advantages conferred by smaller, compact genomes and underlies the evolutionary trend towards larger, plastic genomes. Lineages with less adaptable genomes have an increased probability of extinction and, therefore, suffer a macroevolutionary disadvantage. Deep phylogenetic lineages of host-specific filamentous plant pathogens have been purged of the less adaptable genomes, explaining why today's biota of biotrophic plant pathogens appears depleted in lineages with compact and stable genomes. Our model illustrates how pathogens with an elevated ability to produce genotypes that infect resistant plants, whether hosts or non-hosts, benefit from a macroevolutionary advantage. Such lineages are less likely to disappear due to the emergence of host resistance or extinction of the host population. Indeed, given that a high incidence of resistance or of host population or species extinction is fatal to host-specific pathogen lineages, the frequency of host jumps must be a critical measure of macroevolutionary persistence. In the future, it will be interesting to challenge this model, for instance by determining the degree to which genome size is linked to lineage age in filamentous plant pathogens.

## Outlook

The first batch of genome sequences of plant pathogenic fungi and oomycetes marked the emergence of a new research field centred on the genome biology of these

Clade selection
A macroevolutionary concept that proposes that a clade carrying a certain set of genes can survive over a longer time relative to another clade. Also known as species selection.

Macroevolutionary
The evolution of a group of organisms (clade) over long periods of time.

important pathogens. The genome sequences have revealed a lot of new information about the evolution of these fascinating microorganisms and the genomic features that underlie their success. Most strikingly, several lineages of filamentous plant pathogens, particularly the biotrophs, are remarkable among pathogenic organisms in displaying an evolutionary trend towards bigger, TE-rich genomes. Nonetheless, many interesting and important questions remain to be addressed (see BOX 3 for an overview). In the future, targeted genome sequencing projects of specific clades and populations will help to answer these questions. More insights will also undoubtedly arise from the imminent deluge of filamentous pathogen genome sequences and assemblies driven by second- and third-generation sequencing.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**Competing interests statement**
The authors declare no competing financial interests.

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