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The Genome Biology of Effector Gene Evolution in Filamentous Plant Pathogens

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Abstract

Filamentous pathogens, including fungi and oomycetes, pose major threats to global food security. Crop pathogens cause damage by secreting effectors that manipulate the host to the pathogen's advantage. Genes encoding such effectors are among the most rapidly evolving genes in pathogen genomes. Here, we review how the major characteristics of the emergence, function, and regulation of effector genes are tightly linked to the genomic compartments where these genes are located in pathogen genomes. The presence of repetitive elements in these compartments is associated with elevated rates of point mutations and sequence rearrangements with a major impact on effector diversification. The expression of many effectors converges on an epigenetic control mediated by the presence of repetitive elements. Population genomics analyses showed that rapidly evolving pathogens show high rates of turnover at effector loci and display a mosaic in effector presence-absence polymorphism among strains. We conclude that effective pathogen

containment strategies require a thorough understanding of the effector genome biology and the pathogen's potential for rapid adaptation.

INTRODUCTION

The emergence of filamentous pathogens, including fungi and oomycetes, is a major threat to sustainable crop production. Damage by pathogenic fungi and oomycetes is ubiquitous and affects all major food crops. Efforts to control pathogen outbreaks are defeated with worrisome frequency. Chemicals sprayed to control filamentous pathogens lose their efficacy within years of the first application (44, 63). In parallel, the deployment of resistant crop varieties often fails because of rapid evolutionary changes in pathogen populations that gave rise to genotypes that defeat the host resistance mechanisms. Among serious pathogen outbreaks, breakdown in host resistance was at the origin of the global spread of wheat stem rust and the emergence of wheat blast in South America and beyond (51). Yet many more pathogens pose a sustained threat to crop yields even if these pathogens do not cause epidemics (3).

Understanding the mechanisms underlying the threat of pathogens to crops requires both a mechanistic understanding of the infection process and an appreciation of the evolutionary trajectory of host-pathogen interactions. Despite extensive differences in the biology of plant infections, filamentous pathogens share common themes in their infection processes. A major determinant of a pathogen's ability to infect a host plant is determined by a set of proteins called effectors (59). The advent of large-scale genomics, transcriptomics, and epigenetics studies revolutionized our understanding of commonalities in effector functions and evolution. In this review, we outline both the latest advances in our understanding of effector functions and the evolutionary trajectories that enabled pathogens to adapt rapidly. We show that a mechanistic understanding of pathogen genomes is a crucial link between function and evolutionary trajectory. We first introduce the major biological roles of effectors and their transcriptional regulation during infection. We then show how effector genes emerge in pathogen genomes and how genome compartmentalization is crucially linked to the mechanisms generating variability in effector loci. Finally, we suggest directions for future research to build upon our emerging understanding of effector genome biology in plant pathogens.

THE BIOLOGICAL FUNCTIONS OF EFFECTORS DURING HOST COLONIZATION

Plants interact with pathogenic microorganisms through a wealth of molecules. The main components of this molecular dialogue are microbial effectors and host immune receptors (**Figure 1**). During host colonization, pathogen molecules are exposed to the host, and host endogenous molecules are released and trigger host immune responses. Thus, successful colonization of a plant is dependent on a pathogen's ability to surmount or suppress host immune responses. Pathogens secrete effectors, which are molecules that manipulate the host cell biology, repress the host immune response, or shield the pathogen to support growth and colonization (**Figure 1**) (48). Because of their highly specific roles during infection, effector expression is tightly regulated throughout the infection cycle (38, 43, 55, 78, 89, 98) and effectors need to be transported to either the host apoplast or cytoplasm (58). To defend against a pathogen manipulating the host's physiology, plants produce resistance proteins that are activated by the presence of effectors and induce strong resistance responses (16). Thus, effectors play an integral role in host-pathogen interactions and

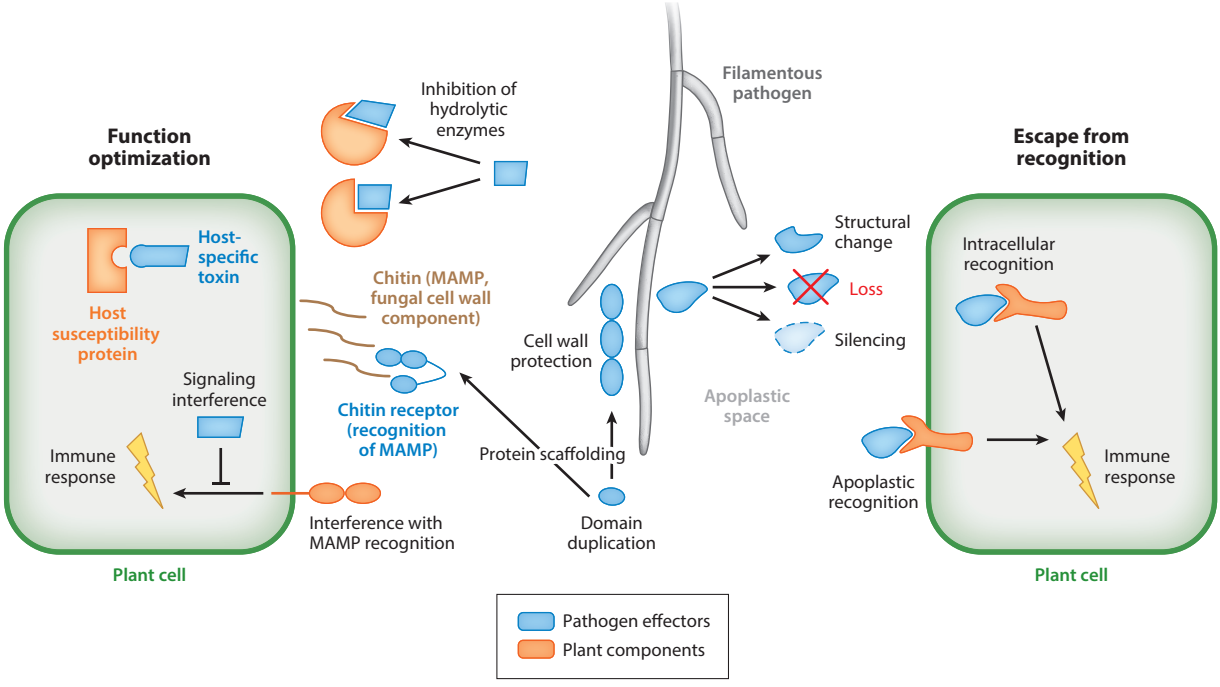


Figure 1

Effectors evolve toward optimizing their function (*left*) or escaping from recognition (*right*). Changes in the structural conformation can help in acquiring new targets. For example, effectors of *Phytophthora infestans* and the closely related species *Phytophthora mirabilis* evolved to inhibit hydrolytic enzymes of new hosts (28). Effectors, such as the LysM effectors, also evolve through domain duplication and changes in the scaffolding (93). These conserved fungal effectors interfere with the recognition of chitin, which is an essential component of the fungal cell wall. Some effectors target host susceptibility proteins and act as necrosis-inducing toxins. Effectors can manipulate signaling pathways and prevent the host from triggering a strong immune response. The second major route of effector evolution is the escape from host recognition. Some changes in the conformation of effectors can maintain essential functions while enabling avoidance of detection by a receptor. Abbreviation: MAMP, microbe-associated molecular pattern.

can impact the outcome of an infection both positively and negatively depending on the host genotype. The recognition of a pathogen effector prevents the infection on a cognate host. The discovery that host and pathogen genotypes play a crucial role in disease was originally described as the gene-for-gene interaction (33). As a result of the tight association between effectors and the success of pathogens, genes encoding effectors are often swiftly evolving and can be the target of rapidly fluctuating selection pressures (8, 49, 113).

The function of most filamentous pathogen effectors is unknown. A major challenge in predicting the function of effectors is that most effectors lack conserved domains or homologs in other species. Yet unrelated effectors in different pathogen species often have common targets, suggesting convergent evolution in the host infection process (73). Furthermore, some unrelated effectors share structural similarities (23, 34) and other characteristic features, such as small size and a signal peptide for secretion. The functions of well-characterized effectors are highly diverse and include the protection of fungal cell walls from hydrolytic enzymes (**Figure 1**) (25, 109). Additionally, cell wall–degrading enzymes (84), protease inhibitors (28), interactors with the ubiquitin-proteasome system (79), and disruptors of the hormone signaling pathway (26) can act as effectors. Hence, effectors can have profound impacts on both the host's metabolism and its ability to mount defenses.

The effector repertoire of a pathogen is a major determinant of host specialization (47, 61, 83, 86). A successful pathogen must continuously maintain the ability to escape host recognition and maintain virulence (i.e., the ability to reproduce on the host). Evolution toward evasion of recognition and functional optimization is achieved by sequence modification, gene deletion, alteration of expression of existing effector genes, and the gain of new effectors (**Figure 2**) (59). Through the same process, pathogens evolved existing effectors or gained new effectors to specialize on a new host (83). Within species, many effectors can be functionally redundant. Redundancy may arise from recent effector gene duplications and is hypothesized to serve a crucial role in the bet-hedging of plant pathogens. The emergence of a new host genotype that recognizes a specific effector can be highly detrimental to the pathogen's ability to cause disease. However, if several effectors target the same host pathway, the pathogen populations can adapt by losing the gene encoding the recognized effector. The loss of an effector helps the pathogen to evade recognition but does not compromise functionality in targeting the host pathway (59, 112). Indeed, effectors of the same pathogen have evolved to disable the same host pathway. For example, in *Zymoseptoria*

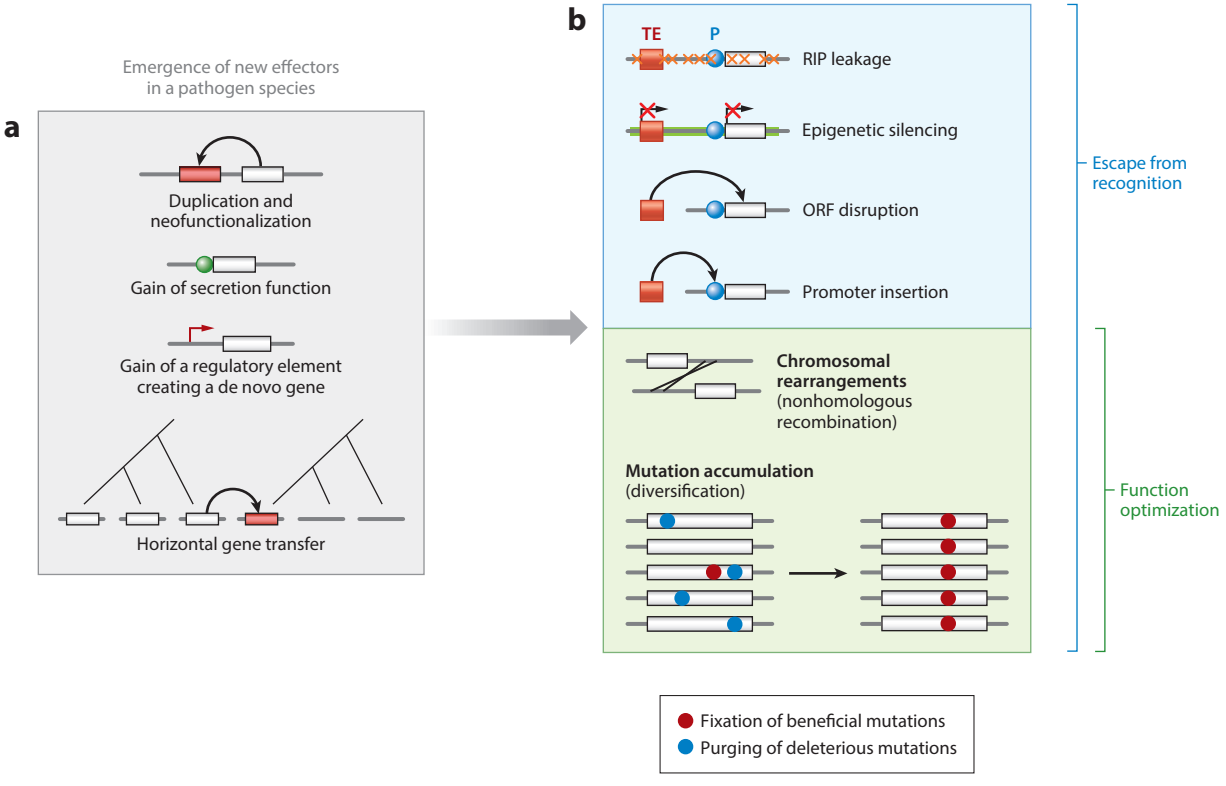


Figure 2

The evolutionary birth and death of effectors. New effectors can emerge through gene duplication or the gain of a secretion function. Effector genes may also evolve de novo from noncoding sequences through the gain of a regulatory element or be acquired horizontally from a different pathogen species. Effector genes can undergo rapid sequence evolution upon recognition of the encoded effector by the host. The major mechanism leading to the loss of an effector gene is the presence and activity of nearby transposable elements (TEs). The effects of the transposable elements can include repeat-induced point (RIP) mutations, epigenetic silencing or the disruption of the gene sequence. Escape from recognition can also be mediated by chromosomal rearrangements or the fixation of beneficial mutations. Rearrangements and selection for beneficial mutations are also major routes for effectors to optimize their function. Abbreviations: ORF, open reading frame; P, promoter regions.

tritici, two LysM effectors are essential for preventing chitin degradation by host hydrolytic enzymes (64), and two apoplastic effectors of the potato late blight pathogen *Phytophthora infestans* share the papain-like cysteine protease C14 as their target (52). Similarly, redundancy in effector functions has been observed in other pathogens such as *Ustilago maydis* (74), *Melampsora lini* (57), and *Magnaporthe oryzae* (72).

SYNCHRONIZING TRANSCRIPTIONAL CONTROL OF EFFECTORS

Upon infection, filamentous pathogens undergo a tightly controlled transcriptional reprogramming to express the required machinery to colonize the host. As different sets of effectors are required for different stages of pathogen development and host colonization, transcriptional control of individual effector genes is highly stage, host, and tissue specific. For example, in *Colletotrichum higginsianum*, effectors favoring cell viability are expressed in prepenetration appressoria during the early biotrophic phase, and genes involved in cell death are activated during the transition to necrotrophy (55, 75). Similarly, transcriptional control of effectors in other pathogens such as *Leptosphaeria maculans*, *U. maydis*, *Puccinia striiformis*, *Melampsora larici-populina*, *Z. tritici*, and *M. oryzae* is highly specific to individual stages of the infection or to the colonized host tissue (9, 29, 42, 69, 91, 98).

The regulation of effector genes also depends on the host genotype and the nature of the interaction. Host-specific expression of effector genes was found in *Z. tritici* and *Blumeria graminis* f. sp. *hordei* (43, 53). Gene expression differed significantly between compatible and incompatible hosts. In compatible interactions, the pathogen was expressing a large set of effectors at high levels, whereas in incompatible interactions most of the infection gene repertoire was downregulated. Furthermore, in *Z. tritici*, the infection on wheat showed a highly specific upregulation of rapidly evolving genes, including effector candidates (53). Intriguingly, different pathogen genotypes of the same species can show different patterns of effector gene expression over the course of an infection. An invasive lineage of *P. infestans* called *13_A2*, which rapidly displaced other European lineages, showed expression polymorphisms compared to other strains, particularly for effectors (17). Genetically different strains of *Z. tritici* share a core set of jointly upregulated genes during the infection of wheat (77, 78). However, a considerable number of effectors and other virulence factors have a strain-specific transcription profile. Such variation in gene regulation could therefore be a major determinant of the outcome of infections. In *Z. tritici*, the differential regulation of effector genes among strains is mirrored in differentially regulated host genes when confronted by genetically different strains (62). Genes encoding receptor-like kinases and pathogenesis-related (PR) proteins are jointly upregulated during the early asymptomatic phase. However, additional defense-related genes show differential expression depending on which *Z. tritici* genotype is infecting the host.

Effector gene expression can be regulated by a variety of mechanisms, including specific transcription factors. However, only a few transcription factors affecting effector gene expression have been identified in fungi so far (reviewed in 104). The best-understood transcription factors include the Sge1/Ros1 orthologs. Sge1 is a positive regulator of virulence genes in many fungal species, including *Fusarium oxysporum* f. sp. *lycopersici*, *Verticillium dahliae*, *Z. tritici*, *Botrytis cinerea*, *Cladosporium fulvum*, and *M. oryzae* (12, 66–68, 76, 94). The ortholog of Sge1 in *U. maydis* activates the switch to the later phases of infection, including sporogenesis. *Sge1* negatively regulates expression of effector genes associated with biotrophic development and positively regulates effector genes linked to spore formation (108). In *Alternaria brassicicola*, the zinc cluster transcription factor AbPf2 regulates the expression of 33 genes encoding secreted proteins, including eight putative effectors (14). Remarkably, orthologs of AbPf2 in *Parastagonospora nodorum* and *Pyrenophora*

tritici-repentis positively regulate the horizontally transferred necrotrophic effectors *SnToxA* and *ToxA*, respectively, indicating that orthologs of AbPf2 constitute a conserved signaling component that regulates effectors in necrotrophic fungi (92).

In fungi, the most extreme form of physical clustering and tight transcriptional regulation is found for genes encoding components of the biosynthetic pathway of secondary metabolites (6), which can act as toxins during infection. Genes in clusters are often jointly regulated by a single transcription factor. Physical clustering of effector genes was found, e.g., in *F. oxysporum*, where effector genes reside on accessory chromosomes, which are highly polymorphic chromosomes not shared by all members of the species. The effectors require FTF1, a transcription factor encoded by a gene colocated on an accessory chromosome. Interestingly, in *F. oxysporum*, Sge1 is also essential for expression of accessory chromosome effectors but is encoded on a core chromosome (110). Hence, *F. oxysporum* accessory chromosomes are only partially transcriptionally autonomous, with consequences for their mobility among lineages (see below).

ORIGIN OF EFFECTOR GENES IN PATHOGEN GENOMES

Our understanding of effector evolution in pathogens was largely inferred by analyzing functional variation for a small number of effector genes. Adaptation to a new host and evasion from recognition can require changes in the effector repertoire, but the mechanisms through which a pathogen species can gain new effectors remains largely unexplored (**Figure 2**). The best-characterized example of a gain in the effector gene repertoire is the transfer of *ToxA* between three unrelated wheat pathogens: *P. nodorum*, *P. tritici-repentis*, and *Bipolaris sorokiniana* (35, 65). Isolates carrying *ToxA* were significantly more virulent than the isolates lacking the effector. The *V. dahliae* effector *Ave1* was postulated to be of plant origin and is localized in lineage-specific regions of the genome that lack synteny with other strains and are rich in transposable elements (TEs) (25, 32). In a different *V. dahliae* strain that is highly virulent on cotton, a lineage-specific region that might have originated from *F. oxysporum* f. sp. *vasinfectum* conferred increased virulence on cotton but not on other hosts (11). Among different *F. oxysporum* strains, entire chromosomes can be transferred and confer virulence to nonpathogenic recipient strains (61). Despite these fascinating cases of effector gene gains in different pathogens, we lack a general understanding of the overall importance and main mechanisms leading to horizontal gene transfer. The available evidence strongly suggests that necrotrophic pathogens are far more susceptible to the acquisition of effector genes, particularly if the effector gene encodes a host-specific toxin.

In addition to horizontal gene transfer, gene duplications followed by mutations were shown to generate effector genes in the smut fungus *U. maydis* (30). Highly repetitive compartments containing TEs favored gene duplications and the formation of virulence gene clusters through nonhomologous recombination (**Figure 2**). Effectors can also emerge through rearrangements that lead to the gain of a sequence encoding a signal peptide, as found for the effector encoded by *Zt80707* in *Z. tritici* (83). Despite evidence that gene duplications and other types of sequence rearrangements create novel effectors, the origins of most effector genes are difficult to trace back (39, 80). The genome of *Z. tritici* harbors hundreds of effector-like genes without evidence for a homologous sequence in closely related species (40). Hence, many of these effector-like genes emerged after speciation of the closely related sister species *Zymoseptoria pseudotritici* and *Zymoseptoria ardabiliae* (102). In *Z. tritici*, effector-like genes were among the most polymorphic and frequently deleted genes among isolates of the same species (45). The predominantly short sequences of effector genes and lack of conserved domain structures raise intriguing questions regarding how effector genes first emerged in genomes and how evolutionary processes optimized their function.

GENOME COMPARTMENTALIZATION AND THE CRADLES FOR EFFECTOR GENE DIVERSIFICATION

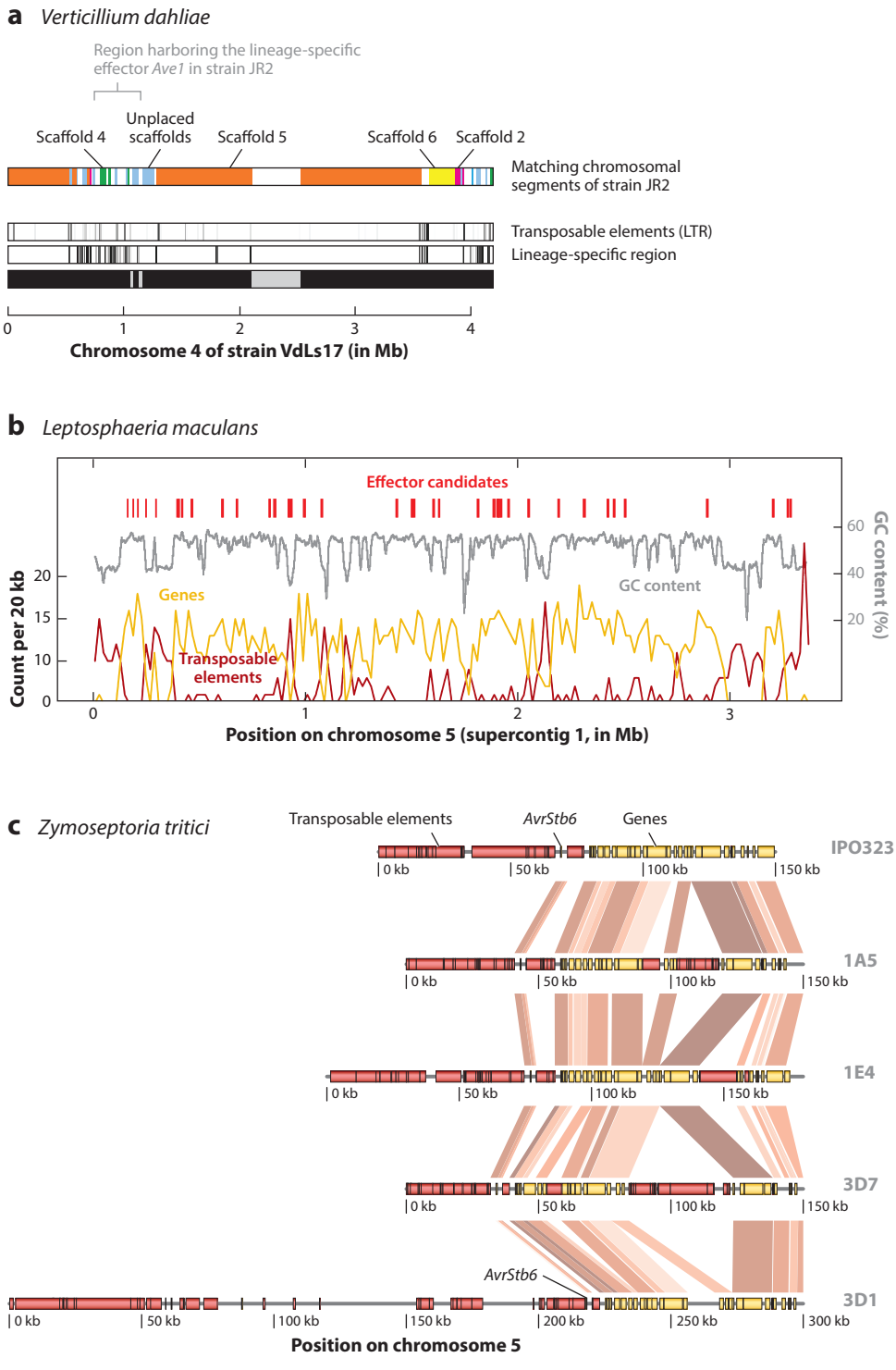
A major advance in our understanding of effector evolution came from complete genome assemblies. Third-generation sequencing strategies led to a fascinating set of (nearly) perfectly assembled chromosomal sequences for *Fusarium graminearum*, *V. dahliae*, *C. higginsianum*, *B. cinerea*, and *Z. tritici* (21, 32, 54, 81, 82, 111). The analyses of these complete genomes shed light on the extent and structure of TE blocks, the structure of accessory chromosomes and subtelomeric sequences. Finished genomes showed that TE content had often been underestimated in prior genome assemblies (e.g., the finished *C. higginsianum* genome contains 7% TEs but was estimated to have only 1.2% in the first assembly) (21, 31). TEs caused chromosomal length variation within species and contributed to genome expansions among closely related taxa (87).

Many pathogen genomes share a bipartite structure of conserved and rapidly evolving compartments. Elevated rates of sequence evolution are often caused by increases in local point mutation rates, segregating presence/absence polymorphisms, the activity of TEs, and sequence rearrangements (71). In some pathogens, such as *B. graminis* (100), these compartments are extensive and contain a large number of TEs. Smut fungi, including *U. maydis*, have small genomes with compact TE compartments. The TE-rich and gene-poor compartments can include accessory chromosomes, such as in *F. oxysporum* f. sp. *lycopersici* (61), clusters of duplicated genes (e.g., smut fungi) (30), TE-rich genomic islands (e.g., *V. dahliae*) (32), highly repetitive subtelomeric regions (e.g., *Fusarium fujikuroi*) (13), and AT-rich isochores (e.g., *L. maculans*) (89). Genome compartmentalization enabled variable evolutionary rates across the genome and is a widely shared feature among eukaryotes (60). In many fungal pathogens, effector genes are located in or adjacent to these rapidly evolving regions (**Figure 3**). This preferential location of effector genes was formulated into the two-speed genome model of pathogen evolution and received strong support with the analysis of an increasing number of filamentous pathogen genomes (27, 86). TE-rich compartments provide a favorable environment for the diversification of the effector gene repertoires, leading to the rapid rise of gain-of-virulence genotypes.

The two-speed genome organization shields essential genes in the core genome from high rates of deleterious mutations, whereas effector genes undergo rapid evolutionary change in the accessory genome (20). Hence, the major evolutionary advantage to compartmentalize effectors is that locally elevated mutation rates can be more easily tolerated. Pathogen adaptation through effector evolution is largely limited by the rate of functional mutations (see below). Hence, local increases in functional mutation rates should enable pathogens to adapt more rapidly to overcome, e.g., host resistance. Mutation rate variations stem largely from repeat-induced point (RIP) mutations, which are a premeiotic mechanism that rapidly mutates copies of TEs and other nearly identical sequences (37, 89). Furthermore, nonhomologous recombination among repeat sequences can generate structural polymorphism (24, 56). As both of these sources of mutations can be highly deleterious, a crucial step for pathogens to adapt through mutation accumulation is to disentangle beneficial from deleterious mutations. At high mutation rates, each pathogen genotype likely harbors multiple mutations with highly variable effects on the pathogen's fitness. Hence, for the two-speed genome to provide an evolutionary advantage to the pathogen, the reshuffling of variants through recombination may be a key attribute. A largely unexplored aspect of the two-speed genome is variation in the recombination rate itself. Studies on *Z. tritici* and *F. graminearum* revealed strong hot spots of recombination focused on narrow tracts of the chromosomes (19, 56). In both species, hot spots colocalized with genes encoding secreted proteins and other proteins putatively involved in host interactions. A comparison of two independent crosses of *Z. tritici* isolates showed that hot spots were only partially conserved (19). Hence, genomic factors determining the rates of recombination are at least somewhat variable.

Figure 3

The association of effector genes, genomic plasticity, and genome compartmentalization in pathogen genomes. (*a*) In *Verticillium dahliae*, the effector gene *Ave1* is located in a lineage-specific region rich in transposable elements (TEs) (24). (*b*) Genes encoding effector candidates (small secreted proteins) are associated with AT-rich isochores and blocks of TEs in *Leptosphaeria maculans* (89). (*c*) The effector gene *AvrStb6* of *Zymoseptoria tritici* is located in the subtelomere of chromosome 5 (81, 116). A comparison of five completely assembled genomes shows how *AvrStb6* is surrounded by TEs and that the entire subtelomeric region is highly dynamic among the different strains. Figure elements were adapted with permission from References 24, 81, and 89. Abbreviations: GC, guanine-cytosine; LTR, long terminal repeat.



Many successful pathogens have only minor repetitive compartments, or their effector genes are only weakly associated with the repetitive compartments. Such pathogens include *Leptosphaeria biglobosa* (41) and *Sclerotinia sclerotiorum* (1). Even when effector genes are not located within TE-rich regions, TE transpositions can influence the outcome of host-pathogen interactions. *M. oryzae* gained virulence by a TE insertion in the coding sequence of a gene encoding the recognized effector Avr-Pi9 and by altering the transcription of the *Avr-Pib* gene through a TE insertion in the 5′ region of the gene (114, 115). Expanding the framework of how effector evolution and genome compartmentalization are linked to pathogen evolution represents a major area for future research.

STANDING GENETIC VARIATION AND THE RAPID EVOLUTION OF EFFECTORS WITHIN SPECIES

Plants impose strong selection pressure on pathogen populations to evade detection (59, 103). Selection can be particularly strong against recognized effectors that trip the wire and are deleterious to infection of cognate hosts (5). As the process of selecting for favorable new pathogen genotypes is continuous in agricultural systems, genomic analyses of extant pathogen populations can reveal the process of pathogen adaptation. Examples of rapid breakdown of host resistance are numerous. Some of the best-studied crops include the loss of resistance in oilseed rape and barley to *L. maculans* and *B. graminis* f. sp. *hordei*, respectively (7, 88). *L. maculans* regained virulence in less than three years after the deployment of new resistance genes (90). In field populations, loss-of-function variants of the *AvrLm6* or *AvrLm4-7* effector genes rose to high frequencies to defeat the newly deployed *Rlm6* and *Rlm7* resistance genes (22, 36). Loss-of-function variants were primarily generated by high rates of RIP.

Evolutionary change at effector loci is favored by a rapid decay in linkage disequilibrium. The primary factors leading to low linkage disequilibrium in the genome are regular sexual cycles and large population sizes. Positive selection of effector gene variants in a sexual pathogen can act primarily on beneficial mutations at the effector locus itself and is not hampered by deleterious mutations in the same genetic background (71). In the clonal wheat yellow rust pathogen *P. striiformis* f. sp. *tritici*, European races were replaced by new races originating from sexually recombining populations coming from the center of diversity in the near-Himalayan region instead of through fixing new variants within Europe (50). In contrast, in many sexual pathogens, such as *L. maculans* and *Z. tritici*, better-adapted genotypes typically emerge locally through recombination instead of through gene flow of immigrants (22).

Standing genetic variation is the second major factor fueling pathogen adaptation. Many of the fastest responses to selection in filamentous pathogens were observed in highly polymorphic species. The gain of virulence on *Stb6* wheat cultivars was observed within three years in the case of the cultivar gene (18). The gain of virulence was driven by mutations at the highly polymorphic effector *AvrStb6* (**Figure 3*c***) (116). This effector gene showed almost exclusively nonsynonymous substitutions (16 out of 18 SNPs over a coding sequence length of 249 bp). As gain of virulence was observed repeatedly across the world, it is likely that pathogen populations harbored standing genetic variation at this locus prior to the introduction of *Stb6* cultivars. A major contributor to the high levels of polymorphism was likely the subtelomeric location of *AvrStb6* (116). The gene is within a few dozen kilobases of the telomere and is embedded in large clusters of TEs. An analysis of multiple completely assembled genomes identified large chromosomal rearrangements in close proximity to *AvrStb6*, even among isolates collected from the same field (**Figure 3*c***). This example follows well the prediction from the two-speed genome model that effectors are preferentially localized in a rapidly evolving chromosomal segment. A separate study of another major *Z. tritici*

effector revealed a variation on the theme of effector localization near repetitive elements. The effector encoded by the gene *Zt_8_609* is likely recognized by a resistance protein in the wheat cultivar Toronit, with *Z. tritici* evading recognition by the adaptive loss of the effector gene (47). In one of the first applications of genome-wide association studies in filamentous pathogens, the effector gene could be localized to the boundary region of a large cluster of TEs. Nonhomologous recombination among the nearby repetitive elements generated a mosaic of deletion polymorphisms within the species, including the complete excision of the repetitive element cluster (47). As a consequence of these rearrangements, *Zt_8_609* was lost from populations as an adaptive response to infect the cognate cultivar. Although the gene *Zt_8_609* has a clear association with TEs, the gene is embedded in a twilight zone between a highly repetitive and a highly conserved chromosomal region.

Adaptive loss of effector genes is another major route of pathogen evolution (**Figure 2**). Such gene losses are fueled by extensive deletion polymorphisms segregating in pathogen populations. Whole-genome analyses of *M. oryzae*, *Z. tritici*, and *V. dahliae* populations revealed substantial variation in gene content (i.e., copy-number variation) (10, 24, 45). In *Z. tritici*, gene deletion polymorphism affected more than 10% of all genes in the genome and affected both effectors and genes with conserved functions such as secondary metabolite production pathways (45). Variation in gene content among pathogens raises intriguing questions about the true extent of functional variation within pathogen species. Complete genome analyses of both asexual pathogens such as *V. dahliae* and sexual pathogens such as *Z. tritici* revealed that a pathogen species harbors a substantial number of accessory genes that are not shared among all strains (31) (**Figure 3**). Such accessory or orphan genes tend to be in clusters and show, on average, lower levels of expression and sequence conservation (82). Intriguingly, accessory genes were enriched for effector candidate genes (31, 82). Core genes, which are shared among all strains of a species, and accessory genes, which are found in only a subset of strains, can be combined into a pangenome for the species. Analyses of five complete and annotated *Z. tritici* genomes revealed that the species had a core set of genes that was substantially smaller than reported for the reference genome alone (81). However, the total number of unique genes in the pangenome was significantly larger when compared to the gene content known from the reference genome. The construction of the pangenome revealed that a highly polymorphic pathogen such as *Z. tritici* segregates an extensive accessory genome among members of the same species. Such highly plastic genomes create a cradle for adaptive evolution of rapidly evolving effector genes.

GENOME DEFENSES: INTERPLAY OF EPIGENETICS AND EFFECTOR GENE EVOLUTION

In addition to sequence polymorphism, gain of virulence can be triggered by the silencing of a gene encoding a recognized effector. In *Phytophthora sojae*, small RNAs were involved in transgenerational epigenetic silencing of *Avr3a* (85). Another route to silencing is through the impact of TEs on gene expression. Genomes have evolved strategies, such as packaging of TEs into heterochromatin, to protect the genomes from the deleterious activity of TE transposition. As a side effect, genes located in the vicinity of TEs usually have low expression. As an example, genes located in TE-rich AT isochores of *L. maculans* or accessory chromosomes of *Z. tritici* show low expression in vitro (53, 89). Interestingly, the genes associated with TE-rich regions can be upregulated upon infection, as found in *L. maculans* during infection of oilseed rape leaves (89) and *Z. tritici* (**Figure 4**). Waves of expression of different TE-associated genes were identified in *C. higginsianum* (21). In all three species, the genes influenced epigenetically by the presence of TEs included effector genes and secondary metabolite gene clusters.

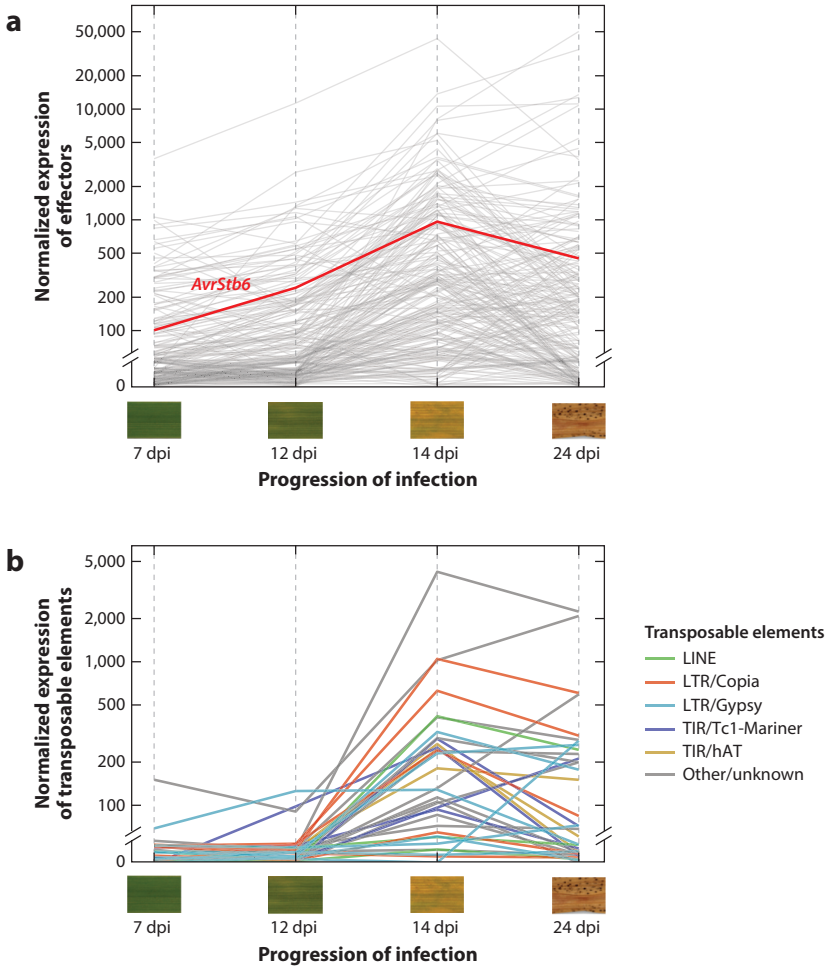


Figure 4

The correlated expression of (*a*) effector gene candidates and (*b*) transposable elements (TEs) and in *Zymoseptoria tritici* over the course of an infection cycle on wheat. At 7 and 12 days post-infection (dpi) most TEs and effectors are only weakly expressed. Effector genes, including *AvrStb6*, show a characteristic peak of expression at the onset of necrosis (14 dpi). TEs of distinct orders and superfamilies are expressed in synchrony with the peak of infection. Abbreviations: hAT, hobo/Activator/Tam3; LINE, long interspersed element; LTR, long terminal repeat; TIR, terminal inverted repeat; Tc1, *Caenorhabditis elegans* transposon 1.

Upregulation of genes associated with TEs under specific conditions suggests that either not all TE sequences are heterochromatic or chromatin structure in these regions is under a complex and dynamic regulation that responds to different biotic or abiotic factors (32, 96, 97). Genome-wide studies of the chromatin structure in fungi and oomycetes are still sparse. A pioneering analysis of the chromatin structure was performed in *Z. tritici* under axenic conditions. In this species, TE-rich compartments in core and accessory chromosomes are associated with two histone modifications typical for heterochromatin, i.e., trimethylation of lysine 9 and 27 of histone H3 (H3K9me3 and H3K27me3, respectively) (95). Generating genome-wide histone maps in conjunction with transcriptomic studies in vitro and in planta will dramatically improve our understanding of effector gene regulation.

With accumulating evidence for epigenetic regulation of effector genes, understanding heterochromatin dynamics in pathogens becomes a crucial area of investigation (**Figure 4**). Studies conducted in two species showing different lifestyles were particularly instructive. In *Epichloë festucae*, an endophyte of *Lolium perenne*, genes underlying the production of toxic alkaloids in planta exhibit reduced levels of H3K9me3 and H3K27me3 methylation upon infection compared to axenic cultures (15). Similarly, in the oilseed rape pathogen *L. maculans*, H3K9me3 represses genes (most notably, effector genes) located in TE-rich compartments during axenic growth. This suggests that the concerted expression of effector genes during leaf infection is governed by chromatin remodeling (99). *L. maculans* candidate effector genes located in distinct genomic regions were expressed at distinct stages of the interaction with its host (38, 89). Although effector genes expressed early during infection of oilseed rape are located in TE-rich compartments and are influenced by H3K9me3, genes upregulated during stem infection do not seem to be influenced by the same histone methylation (38, 99). The control mechanisms associated with different waves of effector expression raise questions on the overall role of chromatin-based regulation. The silencing of some effector genes prior to the infection of the host raises questions regarding the possible costs of expressing effector genes in the absence of a host. Future studies will need to disentangle why some effector genes evolved transcriptional regulation by chromatin state changes instead of being solely controlled by transcription factors.

PLACING THE EVOLUTIONARY DYNAMICS OF EFFECTOR GENES IN A CONCEPTUAL FRAMEWORK

A large body of literature-devised models describe the evolutionary trajectory of effector genes as an interplay with host resistance loci. At the extremes, either of two dynamics are usually expected: the so-called arms race model consisting of recurrent fixation of effector alleles by selective sweeps and transient effector gene polymorphism (4, 49, 113), and the trench warfare model with the cycling of effector allele frequencies and long-term maintenance of polymorphism (49, 101, 113). An important step toward making these models more realistic was to include the effect of pathogen and host population sizes (i.e., genetic drift). These refined models suggest that the host-pathogen dynamics are unlikely to be realistically described by either of the two extreme scenarios but rather form a continuum between the two extremes (107). Whether a pathosystem is more likely to follow one of the two scenarios (arms race versus trench warfare) depends on the relative strength of two particular types of selection. Negative frequency–dependent selection favors the rarer phenotype over the more common phenotype (a typical scenario if pathogens specialize on the most common host). If the fitness conferred by a specific effector allele is determined by the frequency of the matching host resistance gene frequency, then the negative frequency–dependent selection is termed indirect. Negative indirect frequency–dependent selection favors cycling and often results in arms races (105). Negative direct frequency–dependent selection (ndFDS) occurs if the frequency of an allele influences its own contribution to fitness and often leads to a trench warfare scenario. Furthermore, the high fitness cost of carrying effector alleles is a crucial factor in combination with ndFDS to achieve trench warfare dynamics and long-term maintenance of polymorphism. The trench warfare scenario is more likely to occur in wild host-pathogen systems (8). Because agricultural systems are often characterized by homogeneous synchronized landscapes of fixed crop population sizes and a low cost of virulence, the predominant outcome is arms race dynamics (8).

Effector genes accumulate two types of mutations that can be used to infer different processes of evolutionary dynamics (**Figure 2**) (107). The first type of mutation changes the properties of a given effector (e.g., escaping recognition). These mutations are often nonsynonymous substitutions or insertions and deletions. The precise changes in protein structure can be highly informative

about the recognition by a ligand. The second type of mutation is neutral or nearly neutral (i.e., synonymous or weakly deleterious nonsynonymous substitutions). These latter mutations can be used to infer the past selective history at the effector locus. The allele frequencies in populations depend on the coevolutionary dynamics and on the linkage disequilibrium with functional mutations in the same effector gene. Trench warfare dynamics are expected to generate signatures of balancing selection (i.e., an excess of polymorphism and intermediate allele frequencies), whereas arms race dynamics generate selective sweeps (i.e., an excess of low-frequency variants and depletion of nucleotide variation). These predictions form the basis for genome scans for selection in pathogen populations (2, 46, 70).

The speed of the arms race dynamics, and thus the turnover of alleles, depends on the strength of selection. As favorable new alleles emerge and become fixed, a population continuously returns to a monomorphic stage at an effector locus. Hence, to restart new coevolutionary cycles requires new mutations to enter the population. The time until a new mutation arrives is a function of the population mutation rate (i.e., the product of the genomic mutation rate and the population size). Hence, an increase in population mutation rates accelerates the speed of arms race dynamics (107). However, there is an upper limit. If the mutation rate at effector genes becomes high due to, e.g., high rates of TE insertions or RIP mutations, the effector genes are likely to suffer mutational meltdowns (**Figure 2**). A meltdown occurs because new variants are introduced at rates exceeding the ability of selection to favor beneficial variants and eliminate deleterious variants. Conversely, the speed of trench warfare dynamics depends only very weakly on the population mutation rates (107).

As indicated earlier, the size of pathogen populations is a crucial determinant of the evolutionary dynamics at effector loci. The population size relevant for evolutionary dynamics is not the census size (e.g., the number of spores produced during a season) but rather the effective population size. The effective population size describes a smaller, idealized population that undergoes random mating at each generation and includes the effect of bottlenecks. In crop pathogens, the effective population size and census size can differ by several orders of magnitude. Pathogens that reproduce by releasing billions of spores at the end of a growing season often undergo so-called sweepstakes reproduction, in which chance events largely determine which individuals truly contribute to the next generation (106). Many emerging rusts with essentially clonal reproduction are also likely to have reduced effective population sizes compared to highly sexual pathogens such as *L. maculans* and *Z. tritici*. Pathogens with large effective population sizes can rapidly disentangle beneficial from deleterious mutations at effector loci through recombination and selection. In contrast, pathogens with small effective population sizes are likely to suffer from the accumulation of deleterious mutations and nonfunctionalization of essential effectors.

CONCLUSIONS

Devising durable pathogen control strategies is a primary challenge to improve agricultural yields and sustainability. An improved understanding of effector functions will enable a wide range of approaches that can be used to improve breeding for disease resistance. For example, purified effectors can be used to efficiently screen cultivars for susceptibility to pathogen toxins or to identify broad-spectrum resistance that pathogens cannot surmount without high fitness penalties. Genomic analyses of effectors provided fundamental insights into the processes underlying rapid evolution, including the role of genome architecture in the emergence of evolutionary novelty. Effectors were found predominantly in the most dynamic compartments of the genome (**Figure 3**). The elevated rates of sequence evolution in these regions contributed to the astonishing levels of effector diversity found within pathogen species. In addition to genetic diversity, effector evolution was often associated with the evolutionary trajectory of TEs in the genome.

These selfish elements serendipitously translocated, deleted, amplified, or silenced individual effectors, leading to structural variation and the potential for epigenetic control of effector expression (**Figures 2** and **4**). The genetic uniformity in agricultural ecosystems coupled with the deployment of major effect resistance loci that recognize effectors or their functions created enormous selection pressure to continuously adapt effector functions, including the ability to avoid detection. In some pathogens, the interplay of TEs, epigenetic regulation, and structural variation led to the emergence of rapidly evolving regions covering a large fraction of the pathogen genome. The preferential location of effector genes in these regions was termed the two-speed genome and proved to be a very useful concept to describe patterns of genomic organization. This tight association between rapidly evolving regions and major determinants of virulence also opened up a ripe area for future investigations. Achieving a better understanding of the emergence of effectors and their associated genomic compartments will require new research that combines population genomics, epigenomics, transcriptomics, and functional analyses. The implementation of this combined research approach in coming years will not only revolutionize our understanding of proximate effector functions but also reveal the ultimate mechanisms through which filamentous pathogens undergo major evolutionary innovations within contemporary time frames.

SUMMARY POINTS

1. Pathogen virulence is governed by effectors that target well-defined pathways across different hosts.
2. Resistant crops repeatedly failed because of rapid sequence evolution at effector loci in pathogen populations.
3. The costs to pathogens expressing specific effectors and hosts expressing specific resistance proteins have profound impacts on the trajectory of host-pathogen coevolution.
4. The expression of effectors is highly synchronized to maximize the exploitation of the host and is often governed by chromatin remodeling that mediates reactivation of effector genes upon host colonization.
5. Strong selection to rapidly generate and fix beneficial mutations in effector genes led to the preferential location of these genes in repeat-rich regions of the genome.
6. Population-level genome sequencing revealed extreme levels of sequence and structural variation in effector-rich chromosomal regions that can fuel virulence evolution.

FUTURE DIRECTIONS

1. More efficient tools for genetic transformation and phenotypic screening will enable large-scale characterizations of individual effector loci. Such investigations lay the groundwork for a comprehensive understanding of effector functions within pathogen species.
2. Analyzing how changes in chromatin structure impact the regulation of effector genes will establish a model for how effector genes can be recruited into a common regulatory framework.

3. Multiple complete and annotated conspecific genomes will fill large gaps in our understanding of functional diversity within pathogen species (e.g., through the construction of pathogen pangenomes). Intraspecific comparative genome analyses will provide insights into how highly polymorphic regions were generated and continue to evolve through sequence rearrangements.
4. Linking the trajectory of individual effectors from their first emergence and functional changes to the emergence of pathogen two-speed genomes will provide fascinating insights into the biology of rapidly evolving genomes.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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