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Review

Genome evolution in fungal plant pathogens: looking beyond the two-speed genome model

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ABSTRACT

The interaction of pathogens with their hosts creates strong reciprocal selection pressures. Pathogens often deploy an arsenal of small proteins called effectors that manipulate the plant immune system and promote disease. In the post-genomics era, a major interest has been to understand what shapes the localization of effector genes in pathogen genomes. The two-speed genome model originated with the discovery of repeat-rich and gene-sparse genome compartments with an over-representation of effector-like genes in a subset of plant pathogens. These highly polymorphic genome compartments are thought to create unique niches for effector genes and facilitate rapid adaptation. Research over the past decade has revealed a number of twists to the two-speed genome model and raised questions about the universality among plant pathogens. Here, we critically review the foundations of the two-speed model by presenting recent work on epigenetics, transposable element dynamics, and population genetics. Numerous examples have demonstrated that the location of effector genes in rapidly evolving compartments has created key adaptations. However, recent evidence suggests that the two-speed genome is unlikely to have evolved to specifically benefit the plant pathogen lifestyle. We propose that fundamental drivers of eukaryotic genome evolution have shaped both pathogen and non-pathogen genomes alike. An evolutionary genomics perspective on the two-speed genome model will open up fruitful new research avenues.

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1. Introduction

Eukaryotic microorganisms are crucial components of worldwide ecosystems. Many eukaryotic microorganisms engage in symbiotic relationships that can range from mutualistic to pathogenic. Within this continuum, tight interactions between pathogens and their hosts exert strong selection pressures (Papkou *et al.*, 2019). Hosts evolved immune systems to detect pathogen intrusions and to mount defense responses while pathogens evolved molecular tools to support host colonization (Cook *et al.*, 2015), and these everlasting co-evolutionary 'arms-races' are thus characterized by repeated cycles of adaptation and counteradaptation (Papkou *et al.*, 2019; Strotz *et al.*, 2018).

The genomic era has provided unprecedented insights into the genomic signatures of adaptation for many eukaryotic microorganisms that form symbiotic relationships with plants or animals. One of the key observations was that effectors, loci encoding secreted proteins as well as non-proteinaceous effector molecules such as secondary metabolites or small RNAs that can collectively support ecological niche colonization (Collemare *et al.*, 2019; Lo Presti *et al.*, 2015; Rovenich *et al.*, 2014; Snelders *et al.*, 2018), and other genes with roles in adaptation often co-localize within the genome (Dong *et al.*, 2015; Raffaele and Kamoun, 2012; Sánchez-Vallet et al., 2018; Seidl and Thomma, 2014, 2017). Genes localized in repeat-rich and gene-sparse regions tend to evolve faster, in contrast to slow-evolving housekeeping genes localized in repeat-poor and gene-rich regions (Dong *et al.*, 2015; Haas *et al.*, 2009; Raffaele *et al.*, 2010; Raffaele and Kamoun, 2012). The observation of this particular genome organization gave rise to the popular 'two-speed' genome model that is commonly used to describe genome organization and evolution in plant-pathogenic fungi and oomycetes (Dong *et al.*, 2015; Frantzeskakis *et al.*, 2019; Raffaele and Kamoun, 2012). Here we discuss some of the key assumptions underlying the two-speed hypothesis, highlight recent work shedding light on the evolution of this peculiar genome organization, and provide some critical re-evaluation in the post-genomics era.

2. Evolution at two speeds, a misleading concept?

A key assumption of the two-speed hypothesis underscores the presence of two discrete types of genomic regions that supposedly evolve at different speeds (Dong *et al.*, 2015). *Fast*-evolving regions in compartmentalized genomes are typically defined to be rich in repeats such as transposable elements (TEs) and to contain effector genes as has been observed in AT-isochores, sub-telomeric regions, and repeat islands embedded within chromosomes or accessory chromosomes (Fig. 1) (Akagi *et al.*, 2009; Croll and McDonald, 2012; de Jonge et al., 2012, 2013; Dutheil *et al.*, 2016; Faino *et al.*, 2016; Fokkens *et al.*, 2018; Goodwin *et al.*, 2011; Ma *et al.*, 2010; Plissonneau et al., 2016, 2018; Rouxel *et al.*, 2011; Thon *et al.*, 2006; van Dam *et al.*, 2017; Wang *et al.*, 2017). However, recent genomic analyses of diverse plant pathogens have revealed that effectors do not always cluster, that effectors and TEs do not necessarily co-localize (Frantzeskakis *et al.*, 2018; Schwessinger et al., 2018, 2020; Stam *et al.*, 2018; Wyka *et al.*, 2020), and that more than two discrete types of genomic regions can occur (Fokkens *et al.*, 2018). These observations blur the binary notion of the original two-speed hypothesis, which has been founded on observations from just a few plant pathogens (Frantzeskakis *et al.*, 2019; Raffaele *et al.*, 2010; Raffaele and Kamoun, 2012), and highlight that a clear two-speed genome compartmentalization is not the rule for all plant pathogens. It is conceivable that the plethora of different genome organizations reflects the tremendous genomic diversity in filamentous microbes, and is not *per se* tied to the *speed* assumption underling the two-speed genome hypothesis (Frantzeskakis *et al.*, 2019).

A second key assumption of the 'two-speed' hypothesis is that genes embedded in repeat-rich regions are assumed to evolve *faster* compared with genes residing in the core genome. However, what *speed* implies is often glossed over. Does speed imply a higher mutation rate per generation? Or does speed imply stronger positive selection? Repeat-rich genomic regions in plant pathogens are often enriched for structural variations, copy-number variations, or sequence polymorphisms (Faino *et al.*, 2016; Frantzeskakis *et al.*, 2018; Plissonneau *et al.*, 2018; Raffaele *et al.*, 2010; Schirawski *et al.*, 2010; Thon *et al.*, 2006). Furthermore, *fast*-evolving genes such as effectors are often identified by genome-wide scans for positive selection (Aguileta *et al.*, 2009; Sánchez-Vallet et al., 2018). Nevertheless, not all effectors evolve under positive selection or even show signs of increased sequence polymorphisms. For example, *in planta* expressed effector genes in the vascular wilt pathogen *Verticillium dahliae* display few sequence polymorphisms and are not evolving under positive selection but spur frequent gene presence/absence polymorphisms between different *V. dahliae* strains (de Jonge et al., 2012, 2013; Depotter *et al.*, 2019; Faino *et al.*, 2016; Kombrink *et al.*, 2017). The common observation of genes under positive selection and high degree of polymorphisms in repeat-rich genomic regions are the result of selection and thus are not *per se* an indication for speed. To our knowledge, genomic mutation rates have not yet been rigorously assessed in plant pathogens. Such experiments would require carefully controlled conditions and well-designed sequencing strategies to avoid false positives. Furthermore, mutation rates cannot easily be estimated from population sequencing data. Consequently, we know little about whether mutation rates truly differ between genome compartments, and how they differ compared to genomes lacking a clear compartmentalization. Most observed differences in polymorphisms in repeat-rich regions can be explained by mutation accumulation due to relaxed selection. In line with Frantzeskakis and colleagues (2019), we therefore propose to refer to these regions as 'dynamic compartments' rather than '*fast*-evolving' regions.

Apart from plant pathogens, compartmentalized genomes have been found in many bacteria and eukaryotes where co-expressed genes, genes that operate in the same biological process, or genes involved in adaptation cluster together (Batada and Hurst, 2007; Gokcumen *et al.*, 2011; Hurst *et al.*, 2004; Yeaman, 2013) (Fig. 1). In plants and animals, for example, genes with roles in immunity often cluster and are

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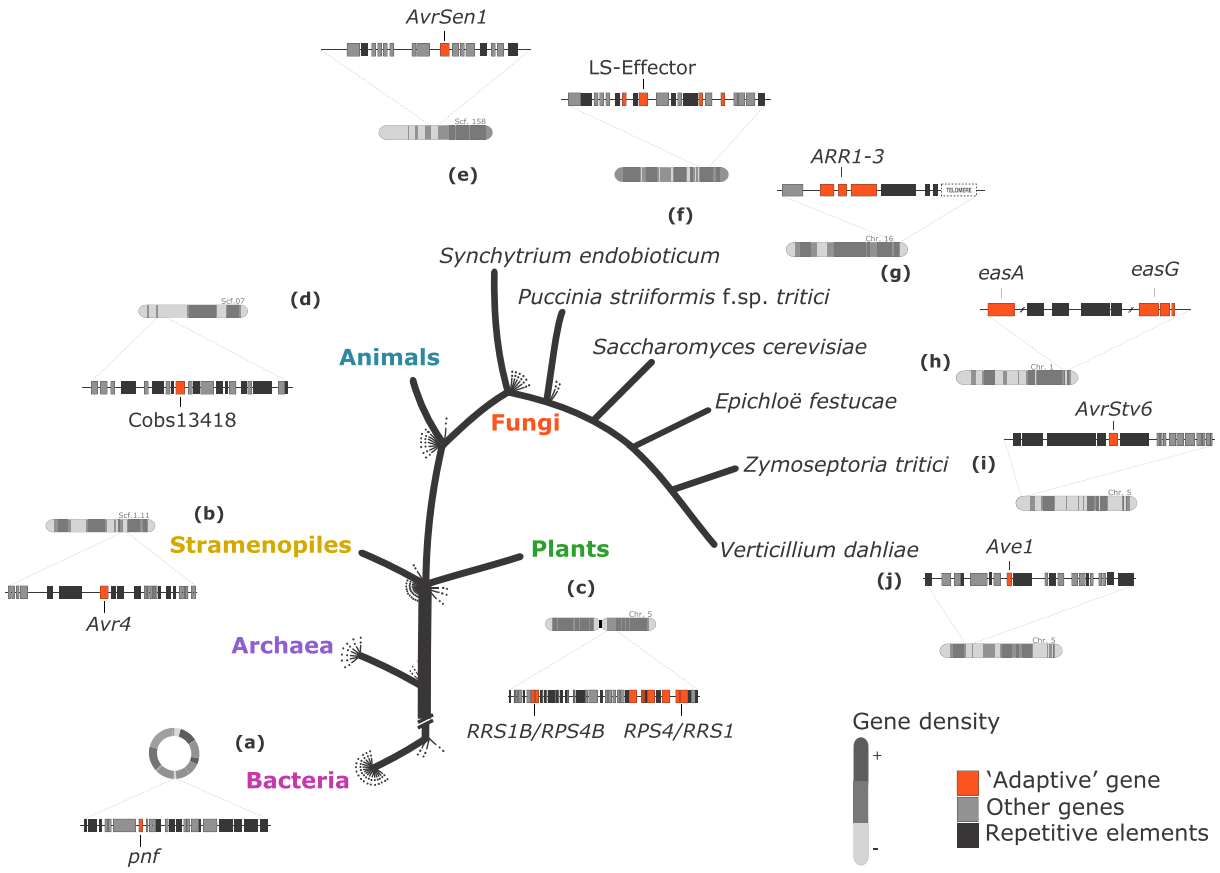

Fig. 1 – Dynamic genomic compartments occur in many branches of the tree of life. Illustration of the tree of life depicting the relationship between bacteria, archaea, and eukaryotes highlights few (a–d) major non-fungal taxa (bacteria, Stramenopiles, animals, plants) and members of the fungal taxa (e) Chytridiomycetes, (f) Basidiomycetes, and (g–j) Ascomycetes. These diverse taxonomic groups contain species that have adapted to different ecological niches and lifestyles. The species differ in their precise genome organizations, but dynamic compartments are enriched to be encode genes will roles in virulence (a–b) (Duchaud *et al.*, 20 03; Haas *et al.*, 2009; Heermann and Fuchs, 2008; Raffaele *et al.*, 2010; Waterfield *et al.*, 2002), immunity (c) (Kawakatsu *et al.*, 2016; van Wersch and Li, 2019), or environmental responses (d) (Schrader et al., 2014). A spectrum of diverse dynamic genomic compartments have been reported in diverse fungi (e–j) ranging from genomes without clear genomic compartments (e–f) (Schwessinger *et al.*, 2018; van de Vossenberg et al., 2019a, 2019b; Xia *et al.*, 2018) to clearly defined genome compartments (g–j) (Brown *et al.*, 2010; de Jonge et al., 2012, 2013; Faino *et al.*, 2016; Fleetwood *et al.*, 2011; Kema *et al.*, 2018; Peter *et al.*, 2018; Plissonneau *et al.*, 2018; Winter *et al.*, 2018; Yue *et al.*, 2017; Zhong *et al.*, 2017). Importantly, irrespective of the details of the genome compartmentalization, effector genes and other genes with roles in adaptation in fungal plant pathogens and also other species typically co-localize with transposable elements (b–j).

located in dynamic regions (Gokcumen *et al.*, 2011; Kawakatsu *et al.*, 2016; Laun *et al.*, 2006; Leister, 2004; Mascher *et al.*, 2017; Roach *et al.*, 2005; Seidl and Thomma, 2017). Notably, irrespective in which species genome compartmentalization has been observed, this phenomenon has nearly always been linked to the occurrence of repetitive genomic elements.

3. Do transposable element insertion dynamics create compartmentalized genomes?

Repetitive sequences such as TEs are ubiquitous in eukaryotes (Wicker *et al.*, 2007), although TE content and distribution can remarkably differ between species. For example, the TE content in fungi can differ between very streamlined genomes as found in the yeast *Saccharomyces cerevisiae* (~3.3%) (Carr *et al.*, 2012) and genomes with a TE content above 80% such as the ectomycorrhiza *Cenococcum geophilum* (Peter *et al.*, 2016) or the wheat powdery mildew *Blumeria graminis f. sp. tritici* (Müller et al., 2019). Genomes are colonized by actively transposing TEs, but these evolutionary young TEs are not evenly distributed in fungal genomes (Cook *et al.*, 2020; Faino *et al.*, 2016; Muszewska *et al.*, 2019). Disruptive impact of new TE insertions triggers purifying selection, while insertions into non-coding regions should have a less dramatic impact on host fitness (Lynch, 2007). Thus, TE insertions are likely under relaxed selection in gene-poor and repeat-rich regions. The number of TE insertions in gene-poor regions

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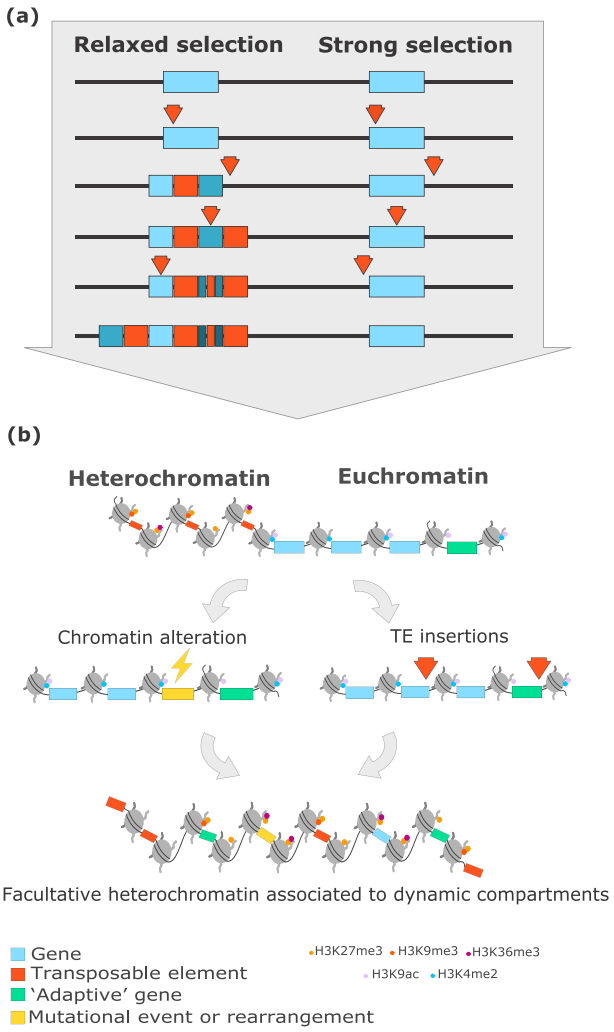

Fig. 2 – Origin and evolution of dynamic compartments in filamentous plant pathogens. (a) Transposable element (TE) insertions events (red arrow) occur in theory randomly throughout the genome. In gene-poor regions and under relaxed selection these insertions would be tolerated. Over evolutionary time, the number of TE insertions will increase as additional TE insertions would be tolerated, and consequently genes (blue boxes) located in these regions would be 'trapped' in proximity to TEs. The TE accumulation could over longer time contribute to the formation of dynamic compartments. In gene-rich regions, strong selection is expected to purge TE insertions and TE content would maintain constant over time. (b) It is conceivable that the chromatin landscape contributes to the formation of dynamic compartments. Genomic rearrangements, e.g. mediated by TEs, could modify the chromatin landscape and induce the spread of heterochromatin, thereby altering the boundaries of different chromatin conformations (Wang et al., 2014, 2015). Furthermore, TE insertions could induce chromatin-based silencing mechanisms aimed to further suppress their spreading (Rebollo et al., 2011; Sentmanat and Elgin, 2012), and thereby induce heterochromatin formation and spread.

may slowly increase, while deleterious TE insertions into genes or regulatory regions will be purged very rapidly from the population (Fig. 2). Furthermore, random processes can act on the population frequency of inserted TEs. If a pathogen population undergoes a bottleneck or colonizes a new habitat, chance events can drastically increase or decrease the frequency of a TE at a specific locus within the population.

TE insertions tend to accumulate in accessory chromosomes or accessory compartments in core chromosomes (Croll and McDonald, 2012; Sánchez-Vallet et al., 2018). In the fungal pathogens *Fusarium oxysporum*, *Leptosphaeria maculans*, and *Zymoseptoria tritici*, accessory chromosomes or accessory compartments are generally characterized by low gene density, low gene expression, facultative heterochromatin, and enrichment of species-specific genes (Feurtey et al., 2019; Fokkens et al., 2018; Plissonneau et al., 2018; Soyer et al., 2014). Relaxed purifying selection on accessory genes could have allowed TEs to accumulate. Frantzeskakis and colleagues (2019) argued purifying selection should remain strong on housekeeping genes regardless of their localization and accessory genes should universally experience more relaxed selection. However, how did effectors end up or emerge in close proximity to TEs? Is it a consequence of relaxed selection against TE insertions or selection favoring the localization of effectors in dynamic regions? The challenge will be to disentangle these two fundamentally linked phenomena in the future.

4. Dynamic chromatin for dynamic compartments

Active TEs have a strong mutagenic potential, and consequently TE-rich regions are often highly condensed into heterochromatin to suppress TE activity (Grewal and Jia, 2007). The TE-rich dynamic compartments in filamentous microbes are typically associated with condensed heterochromatin (Connolly et al., 2013; Cook et al., 2020; Janevska et al., 2018; Möller et al., 2019; Schotanus et al., 2015; Wang et al., 2017). However, facultative heterochromatin can rapidly switch to an open euchromatic state depending on developmental stage or environmental cues, and therefore act as an important modulator of effector gene expression (Soyer et al., 2014, 2019), secondary metabolite gene clusters (Chujo et al., 2019; Chujo and Scott, 2014; Collemare and Seidl, 2019; Pfannenstiel and Keller, 2019), or genes encoding proteins with various roles in carbohydrate metabolism (Fokkens et al., 2018).

Next to playing an important role in modulating gene expression, chromatin has been hypothesized to influence genome evolution (Seidl et al., 2016). Typically, heterochromatin is thought to suppress recombination (Grewal and Jia, 2007). However, this observation is inconsistent with data from pathogen genomes where heterochromatic regions are often unstable or enriched in copy number variations (Hastings et al., 2009; Möller et al., 2019; Plissonneau et al., 2018; Schotanus et al., 2015; Seidl et al., 2016). Heterochromatic regions are considered to be more prone to DNA breakage (Sasaki et al., 2014). Heterochromatic regions also often co-localize within the nucleus (Galazka et al., 2016).

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Hence, it is conceivable that chromatin structure can directly influence genetic variation. Furthermore, heterochromatic regions are often associated with higher densities of single nucleotide polymorphisms compared with euchromatic regions (Fokkens *et al.*, 2018; Makova and Hardison, 2015; Schuster-Böckler and Lehner, 2012; Wang *et al.*, 2017), which is likely due to altered accessibility for DNA repair machineries (Sun *et al.*, 2016). Thus, it has been hypothesized that heterochromatin serves as cradle for genomic variability (Liu *et al.*, 2020; Seidl *et al.*, 2016; Yasuhara *et al.*, 2005). However, the roles of chromatin in the formation and evolution of dynamic compartments is still elusive. Is the association between dynamic compartments and heterochromatin a consequence of heterochromatin formation to suppress and silence recent TE insertions (Rebollo *et al.*, 2011; Sentmanat and Elgin, 2012; Stuart *et al.*, 2016)? Alternatively, heterochromatin and higher degrees of polymorphism could directly drive the formation of the dynamic regions (Fig. 2), for instance by the spread of heterochromatic regions (Dixon *et al.*, 2012; Wang et al., 2014, 2015) or by co-opting heterochromatic genomic regions such as sub-telomeres (Hocher and Taddei, 2020; Juárez-Reyes and Castaño, 2019). These hypotheses are not mutually exclusive, yet it remains challenging to experimentally elucidate how chromatin states affect genome compartmentalization dynamics and the embedded genes.

5. The chicken and egg problem: dynamic compartments and genes underlying rapid adaptation

Effector genes and other genes with roles in rapid adaptation are often located in dynamic compartments (Dong *et al.*, 2015; Raffaele and Kamoun, 2012) (Fig. 1). In line with the two-speed genome hypothesis, the presence of adaptive genes in TE-rich dynamic compartments is considered beneficial (Seidl and Thomma, 2017). However, the evolutionary transitions towards a compartmentalized genome architecture each have to be advantageous and favored by selection over less compartmentalized genome architectures. At what stage did a pathogen species harbor different variants of a genome architecture and selection could favor one over the other? Did a compartmentalized genome architecture emerge through random events (*e.g.* massive TE proliferation) in some ancestral pathogen species and selection favored the pathogen species carrying two-speed genomes? Is this compartmentalization reversible? Resolving such questions will clarify our understanding of why a compartmentalized genome is thought to be beneficial. Understanding mechanisms driving genome evolution can be fruitful to answer these major questions. TEs could influence effectors through leaky TE defense mechanisms such as repeat-induced point mutation (Fudal *et al.*, 2009; Galagan and Selker, 2004), by altering the expression of the effector (Chuong *et al.*, 2016; Omrane *et al.*, 2017), through disturbance through by ectopic recombination (Devos *et al.*, 2002), by leaking of silencing (Hollister and Gaut, 2009), or even by creating effector genes *de novo* (Nottensteiner *et al.*, 2018; Sabelleck and Panstruga, 2018). Deletion of a TE-rich region carrying recognized effectors enables plant pathogens such as *Z. tritici* or *V. dahliae* to increase virulence on a specific host genotype (de Jonge *et al.*, 2012; Hartmann *et al.*, 2017). Conceivably, these processes could potentially also reduce fitness, e.g. by deleting, mutating, or silencing effector genes. Thus, what are the selective advantages of the association between effector genes or other genes with adaptive roles and TE-rich dynamic compartments, and how did dynamic compartments emerge in the first place?

TE-rich compartmentalized genomes are commonly observed in nature (Lynch, 2007) and their maintenance (e.g. replication and transcription) could be evolutionary costly (Bennetzen and Wang, 2014; Oliver and Greene, 2009; Schrader and Schmitz, 2019), and thus it is conceivable that TE-rich compartments directly or indirectly contribute to higher pathogen fitness. It has been proposed that over evolutionary time, compartmentalized genomes can evolve from a random genome through TE-mediated rearrangements, which increases the probability of rapid adaptation to novel environments (Crombach and Hogeweg, 2007). Thus, over evolutionary timescales these TE-rich dynamic compartments can emerge and are maintained as they positively contribute to evolvability (Cuypers and Hogeweg, 2012; Fablet and Vieira, 2011; Fouché et al., 2020; Kidwell and Lisch, 2001; Slotkin and Martienssen, 2007). Due their increased evolvability, these regions can serve as cradles for adaptive genome evolution.

6. Conclusions

Plant pathogenic fungi provide some of the most fascinating examples of how genome evolution is linked to phenotypic trait evolution. Effector genes encoded in fast evolving genome compartments can undergo rapid rearrangements, silencing, and activations due to the unique genomic environment. However, recent genome analyses across plant pathogenic fungi suggest that no unifying rules exist how effector genes are localized in the genome. We suggest that the evolutionary history, mode of reproduction, and population structure have strongly influenced characteristics of the pathogen genome. Such features include chromatin landscape along chromosomes, the dynamics of TE insertions, how selection can act on beneficial mutations, and how random processes such as founder effects structure polymorphism within a species. A major unanswered question is how associations of effector genes and fast evolving genome compartments have evolved in some lineages but not others. A crucial missing link is convincing evidence for the stepwise re-localization of effector genes to fast-evolving genome compartments or for the emergence (*i.e.* birth) of effector genes in fast evolving genome compartments. Suitable pathogen systems with multiple closely related species and high-quality genome and population resources will be critical to address these questions in the future.

Declaration of Competing Interest

The authors declare no conflict of interest.

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