Rapid evolution in plant-microbe interactions - a molecular genomics perspective

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Author for correspondence:
Ralph Panstruga
Tel: +49 241 80 26655
Email: panstruga@bio1.rwth-aachen.de

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Lamprinos Frantzeskakis[1], Antonio Di Pietro[2], Martijn Rep[3], Jan Schirawski[4], Chih-Hang Wu[5] and Ralph Panstruga[6]

[1]DOE Joint Genome Institute, Walnut Creek, CA 94598, USA; [2]Departamento de Genética and Campus de Excelencia Agroalimentario (ceiA3), Universidad de Córdoba, 14071, Córdoba, Spain; [3]Faculty of Science, Swammerdam Institute for Life Sciences, University of Amsterdam, PO Box 94215, 1090 GE, Amsterdam, the Netherlands; [4]Microbial Genetics, Institute of Applied Microbiology, RWTH Aachen University, Aachen, Germany; [5]The Sainsbury Laboratory, University of East Anglia, Norwich Research Park, Norwich, NR4 7UH, UK; [6]Unit of Plant Molecular Cell Biology, Institute for Biology I, RWTH Aachen University, Worringerweg 1, Aachen 52056, Germany

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Summary

Rapid (co-)evolution at multiple timescales is a hallmark of plant–microbe interactions. The mechanistic basis for the rapid evolution largely rests on the features of the genomes of the interacting partners involved. Here, we review recent insights into genomic characteristics and mechanisms that enable rapid evolution of both plants and phytopathogens. These comprise fresh insights in allelic series of matching pairs of resistance and avirulence genes, the generation of novel pathogen effectors, the recently recognised small RNA warfare, and genomic aspects of secondary metabolite biosynthesis. In addition, we discuss the putative contributions of permissive host environments, transcriptional plasticity and the role of ploidy on the interactions. We conclude that the means underlying the rapid evolution of plant–microbe interactions are multifaceted and depend on the particular nature of each interaction.

I. Introduction

Plant–microbe interactions represent a paradigm for rapid evolution (Upson *et al.*, 2018). This is particularly true for plant–pathogen interactions, in which the molecular warfare between plants and microbial intruders drives the fixation of beneficial allelic variants in either genomic pool (Frantzeskakis *et al.*, 2018). While pathogens profit from alterations that allow a

better escape from or suppression of plant defence, plants in turn benefit from innovations that improve their immune capacities (Borrelli *et al.*, 2018). Critical factors are population sizes and generation times that tend to be much larger and shorter, respectively, for microbes compared with plants. Microbial populations are therefore more likely to experience new mutations, resulting in a higher evolutionary pace. This imbalance is exacerbated in the context of modern agriculture in which monocultures further limit genetic diversity in plants. Despite advances in plant breeding and agricultural practices, pathogens are still able to re-emerge after a few crop seasons, or even expand their host range and/or geographic distribution (McDonald & Stukenbrock, 2016). The phenotypic consequences of rapid pathogen evolution are well known, and earlier studies have provided insights into the molecular mechanisms associated with evasion of plant immunity at the level of single host–pathogen gene interactions (Rouxel & Balesdent, 2017; Box 1). Recent reports have additionally brought forward models of how genome compartments of plant pathogens might enhance the rate at which such changes occur (Frantzeskakis *et al.*, 2019; Box 1). These events are essentially mirrored in plant genomes, where, in particular, resistance (*R*) gene clusters can be subject to rapid evolution, in part by very similar means (Borrelli *et al.*, 2018). Fig. 1 illustrates such mechanisms for eukaryotic pathogens, noting that comparable mechanisms operate in prokaryotic pathogens (for example box II of Fig. 1a). In prokaryotes, plasmids represent additional vehicles for the rapid transfer of virulence-related genes even across species borders (Schierstaedt *et al.*, 2019). In this review, we highlight recent examples of genomic features that have contributed to rapid evolution in the context of plant–microbe interactions. We primarily focus on evolutionary events that occur in host and pathogen populations within a few tens of generations but, in some instances, also cover examples that have resulted from adaptive radiation. We also mostly refer to examples of rapid evolution as observed in agricultural environments, warranting that such events might be more rare in natural ecosystems due to the more stable and/or more complex host–microbe warfare in natural settings (Karasov *et al.*, 2018).

II. Loss of avirulence: a frequent type of rapid evolutionary adaptation

A common type of plant resistance follows the 'gene-for-gene' concept and mechanistically often relies on the direct or indirect perception of pathogen strain-specific secreted effector proteins, termed avirulence (Avr) factors, by host genotype-specific immune sensors, termed resistance (R) proteins (Cesari, 2018). Perception typically depends on bimolecular interactions, and therefore loss of recognition can occur upon mutation of the *Avr* gene, leading from an avirulent to a virulent allele (Fig. 1). Recently reported examples include SNPs (Lu *et al.*, 2016; Plissonneau *et al.*, 2017b; Zhong *et al.*, 2017; Meile *et al.*, 2018), deletions (Hartmann *et al.*, 2017), transposable element (TE) insertions (Wu *et al.*, 2015; Zhang *et al.*, 2015), and epigenetic gene silencing (Qutob *et al.*, 2013) of avirulence genes, all of which can result in a gain of virulence. An instance of great agronomical relevance is the emergence of the rice blast fungus as a novel pathogen of wheat that was promoted by the loss of the critical *PWT3 Avr* gene (Inoue *et al.*, 2017). However, it

Box 1. Mutational events and genomic features enabling rapid evolution

AT-rich isochores: A large genomic region with an overrepresentation of adenine- thymine base pairs, usually coinciding with deactivated repetitive elements by the RIP mechanism.
Chromosomal polysomy or length polymorphism: Core or dispensable chromosomes can become duplicated. Also homologous chromosomes between isolates of the same species can have significant length variation.
Chromosomal rearrangements: Large-scale differences in gene order and organisation in a genome.
Conditionally dispensable chromosomes: Accessory chromosomes that, unlike core chromosomes, are not essential for the organism. In the case of phytopathogens, these often harbour virulence genes.
Copy number variation (CNV): Differences regarding the copy number of a given gene in a genome, for example in comparisons between individuals of a population.
***De novo* genes:** Species-specific (orphan) genes originating from sequences that did not have any coding potential before.
Epigenetic modification of gene expression: Epigenetic mechanisms can repress or release gene expression in a nonheritable manner. They can have a limited effective range (for example a single gene; RNA interference (RNAi)-based silencing) or extend to entire chromosomal regions (for example epigenetic silencing of subtelomeric regions due to histone modifications).
Horizontal gene/chromosome transfer (HGT/HCT): Transfer of genetic material (either single genes or entire chromosomes) from a donor organism to an acceptor organism that are not in parent-offspring relation.
Hybridisation: Mating of organisms of different varieties or species to create a hybrid.
Insertions/deletions (indels): Typically small stretches of DNA that are present/absent in comparison to a reference sequence. Indels can result in frame shifts when present in a coding region.
Polyploidization: Acquisition of one or more additional sets of chromosomes in a cell or organism.
Repeat-induced point mutation (RIP): Fungal genome defence mechanism to limit transposon activity by mutating cytosines in repetitive sequences.
RIP leakage: Spreading of RIP from duplicated sequences into neighbouring nonrepetitive regions.
Single nucleotide polymorphisms (SNPs): Genomic base pair exchanges, which in the case of coding regions may result in amino acid replacements, premature stop codons or mis-splicing. SNPs can be the result of rare DNA polymerase replication errors during mitosis/meiosis or DNA damage.
Transposable elements (TEs): Mobile genetic elements that can 'jump' around in genomes. Transposition events can lead to gene inactivation, but also to gene activation or duplication or even emergence of a new gene.

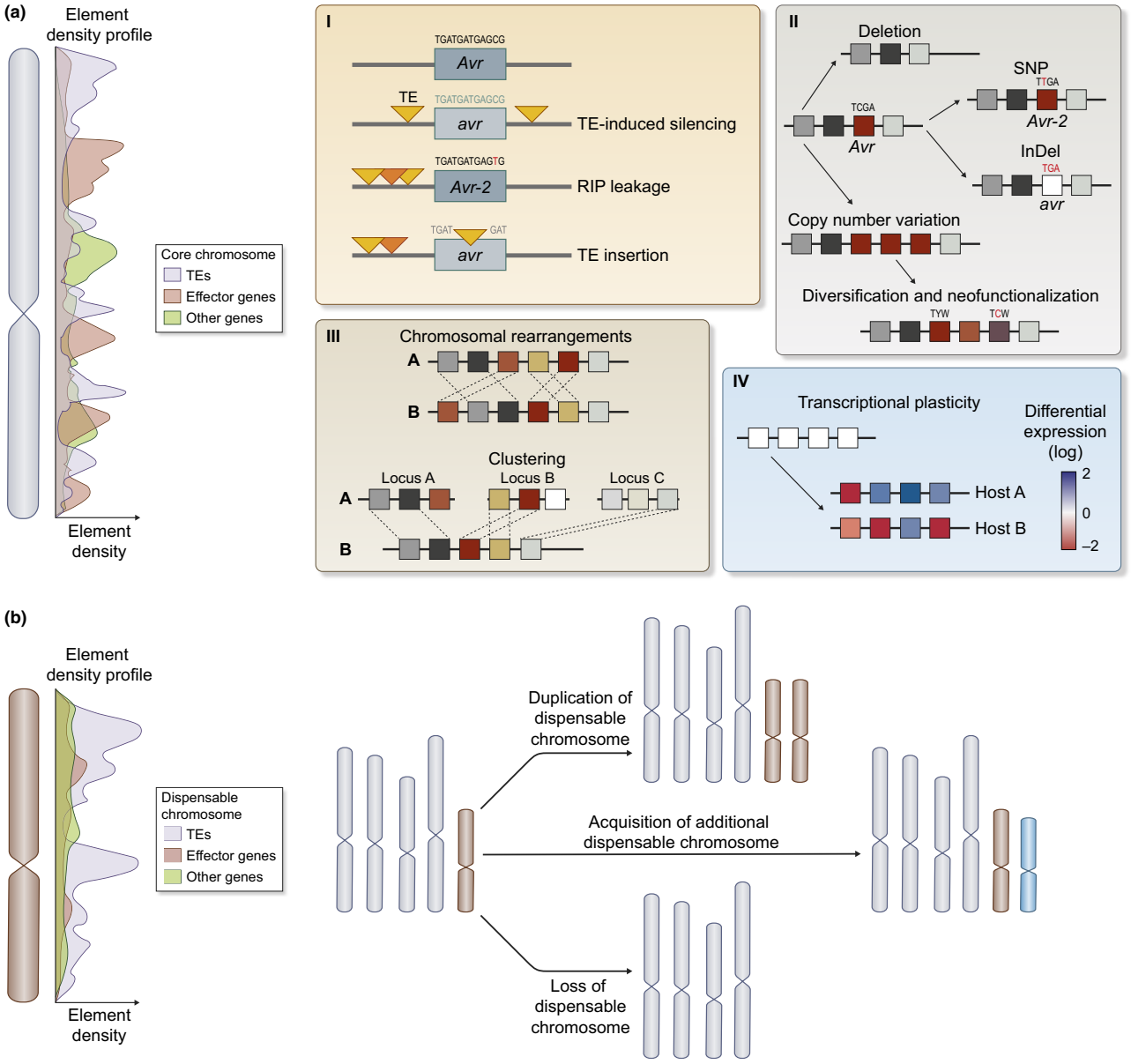

Fig. 1 A consensus view of the rapidly evolving phytopathogen genome. In this figure, several genomic features/processes enabling rapid evolution are summarised, exemplarily for a hypothetical core chromosome (greyish colour, a) and a dispensable chromosome, a type of chromosomes found in some filamentous pathogens (brownish colour, b). The transposable element (TE), effector and nonvirulence-associated ('Other genes') gene profiles for these two types of chromosomes are depicted as density graphs next to the chromosome schemes, illustrating gene-rich and TE-poor genomic compartments (or the opposite), as well as compartmentalisation of effector genes. (a) The fate of individual effector genes is illustrated for an exemplary avirulence gene (*Avr*). Coloured boxes I to III show events that can happen at specific chromosomal loci in dependence of the proximity to different elements (for example to TEs; box I), or depending on the specific location (for example subtelomeric region; box III). In box I, the *Avr* gene is affected by TEs (yellow and orange triangles) in several ways as it might happen at loci populated by repetitive elements. These events can be: DNA methylation-based silencing (indicated by grey nucleotide sequence) induced by insertion of elements flanking the gene ('TE-induced silencing'); alteration of the nucleotide sequence (indicated by red letter in nucleotide sequence) resulting from repeat-induced point mutation (RIP), a fungal genome defence mechanism targeting flanking repetitive elements ('RIP leakage'), possibly resulting in a different allele (*Avr*-2); or disruption of the sequence by insertion of a TE ('TE insertion'), which may likewise cause silencing (grey nucleotide sequence). In box II, several alterations are presented for a given *Avr* gene (red square) that are not necessarily related to the activity of repetitive elements. Some of the events shown here can lead to different effector alleles (for example 'single nucleotide polymorphism (SNP)' → *Avr*-2), complete deactivation of the sequence by frameshift mutations ('InDel', here resulting in a premature TGA stop codon), or complete removal of the sequence ('Deletion'). Alternatively, gene duplication ('Copy number variation') can lead to multiple *Avr* gene copies. Duplication events can be either recent, giving rise to identical copies (shown by the same colour), or older, enabling more sequence divergence (shown by different shades of the same colour). In the latter case, novel functions might be assumed for some of these copies ('Diversification & neofunctionalisation'). In box III, chromosomal rearrangements between two closely related isolates (or species) A and B are shown, leading either to the disruption of synteny (top) or gene clustering (bottom). This type of variation does not exclusively affect *Avr* genes. Box IV illustrates differential expression of four *Avr* genes ('Transcriptional plasticity'), which can occur independently of chromosomal location. (b) Possible events associated with dispensable chromosomes. Often smaller in size and with a different repetitive element profile than core chromosomes, dispensable chromosomes are more prone to loss or duplication and can also be horizontally transferred (here shown in brownish and blue colour), potentially altering the virulence or host range of a pathogen.

remains unclear whether these genomic events indeed affect virulence genes more frequently than housekeeping genes (Box 2). While SNPs, deletions or TE insertions occur throughout the lifespan of an organism and throughout the entire genome, the genomic context of a gene – for example its proximity to recombination hotspots or TE insertions – might introduce a site bias.

III. Allelic series of *R-Avr* gene pairs: testimonies of an ongoing arms race

In some cases of *Avr–R* gene pairs, extended allelic series encoding polymorphic protein variants have been reported. Prominent examples include the powdery mildew *R* gene loci *Mla* and *Pm3* of barley and wheat, respectively. Both are complex genetic loci that have evolved over a period of > 7 million years through a variety of duplication, inversion and transposon-insertion events (Wei *et al.*, 2002; Hurni *et al.*, 2013), each providing numerous recognition specificities (Srichumpa *et al.*, 2005; Seeholzer *et al.*, 2010). This, in turn, has driven the evolution of new *Avr* gene variants in the pathogen. These allelic series of *Avr–R* gene pairs therefore represent genetic testimonies of rapid evolution driven by the host–pathogen arms race. Some of the respective *Avr* genes have been cloned recently (Lu *et al.*, 2016; Praz *et al.*, 2017; McNally *et al.*, 2018; Saur *et al.*, 2019). Interestingly, by contrast with the sequence-related allelic *Mla* gene variants residing at a single locus, the cognate *Avr* genes in the barley powdery mildew pathogen are spread throughout the genome and encode sequence-unrelated effectors probably engaging in direct interactions with their respective R proteins (Saur *et al.*, 2019).

IV. Creating diversity by generating novel effectors or effector functions

It is widely believed that effector repertoires are key determinants of pathogen host spectra (Fig. 2; Schulze-Lefert & Panstruga, 2011). Given the high number of effectors present in phytopathogen species and their typically low sequence conservation, even between closely related species, *de novo* gene birth might be an important driving force in creating effector diversity (Plissonneau *et al.*, 2017a). Such novel genes can arise from the spurious expression of non-coding sequences via a transition state termed a 'proto-gene' (Carvunis *et al.*, 2012). This process might be kick-started by the expression of long noncoding transcripts (lncRNAs) from TE promoters (Davis *et al.*, 2017), and this situation may explain the frequently observed physical association between TEs and effector genes (Dong *et al.*, 2015). Proto-genes may then acquire secretion signals from random sequences (Kaiser *et al.*, 1987). In fact, many effector genes share common characteristics with reported proto-genes, such as a small size or amino acid composition bias (Yomtovian *et al.*, 2010; Sperschneider *et al.*, 2018). An intriguing example for a *de novo* gene birth is a virulence effector gene of the barley powdery mildew pathogen, which apparently had originated from a non-autonomous retrotransposon (Nottensteiner *et al.*, 2018). Phytopathogens can also acquire new effector genes by different means (Fouché *et al.*, 2018), including horizontal gene transfer (HGT; as in the case of *ToxA*; Friesen *et al.*, 2006), horizontal chromosome transfer (HCT, as in the case of *Fusarium oxysporum*; Ma *et al.*, 2010; van Dam *et al.*, 2017), or hybridisation between pathogen species (see Section VI below and Fig. 2). Similarly, the neofunctionalization of endogenous genes with housekeeping functions for the purpose of virulence was recently suggested for a subset of secreted peptidases in the *Zymoseptoria* species complex (Krishnan *et al.*, 2018). An extension of this concept is the evolution of catalytically inactive variants of secreted proteins. Examples include the functional conversion of a glutathione synthetase in a plant–parasitic nematode (Lilley *et al.*, 2018), enzymatically inactive fungal chitinases that sequester immunogenic chitin fragments (Fiorin *et al.*, 2018), or the large family of catalytically inactive RNase-like effector proteins in cereal powdery mildews (Pennington *et al.*, 2019).

Box 2. Ten interesting questions for the exploration of rapid evolution in plant–microbe interactions in the molecular genomics era

1 How 'rapid' (in quantitative terms and relative to other systems) is rapid evolution in plant–microbe interactions?
2 Are these mechanisms of rapid evolution (Box 1) predominantly used in microbes vs plants?
3 How does rapid evolution differ between natural ecosystems and agricultural environments?
4 How fast evolving and diverse is secondary metabolism between isolates or ecotypes of the same species?
5 What is the mechanistic basis of transcriptional plasticity in phytopathogens?
6 Do sRNAs rapidly co-evolve in interacting organisms?
7 How widespread is flexibility in ploidy among phytopathogens?
8 Can polyploidy provide a selective advantage?
9 Do plant-associated microbial communities affect the rapid adaptation of phytopathogens?
10 To what extent do insights obtained from rapid evolution of phytopathogens reflect the situation in symbionts?

V. Small RNA warfare: a novel attribute of plant–microbe interactions

The cross-kingdom exchange of small RNAs (sRNAs) recently emerged as a novel tier of mutual molecular manipulation in plant–microbe interactions. The seminal discoveries that fungal sRNAs can be transferred into plant cells to promote virulence (Weiberg *et al.*, 2013), and *vice versa* that plants deliver sRNAs to fungal pathogens as part of their defence programme (Cai *et al.*, 2018), have added a new level of complexity to our understanding of plant disease. In either case the transmitted sRNAs can provoke gene silencing in the respective opponent (Hua *et al.*, 2018). As sRNAs are less complex and subject to fewer constraints (for example structural limitations) than effector proteins, they might evolve even faster. As we are just beginning to explore pathogen and host sRNA repertoires, further studies are needed to determine whether they are indeed subject to rapid co-evolution in the interacting partners (Rose *et al.*, 2019).

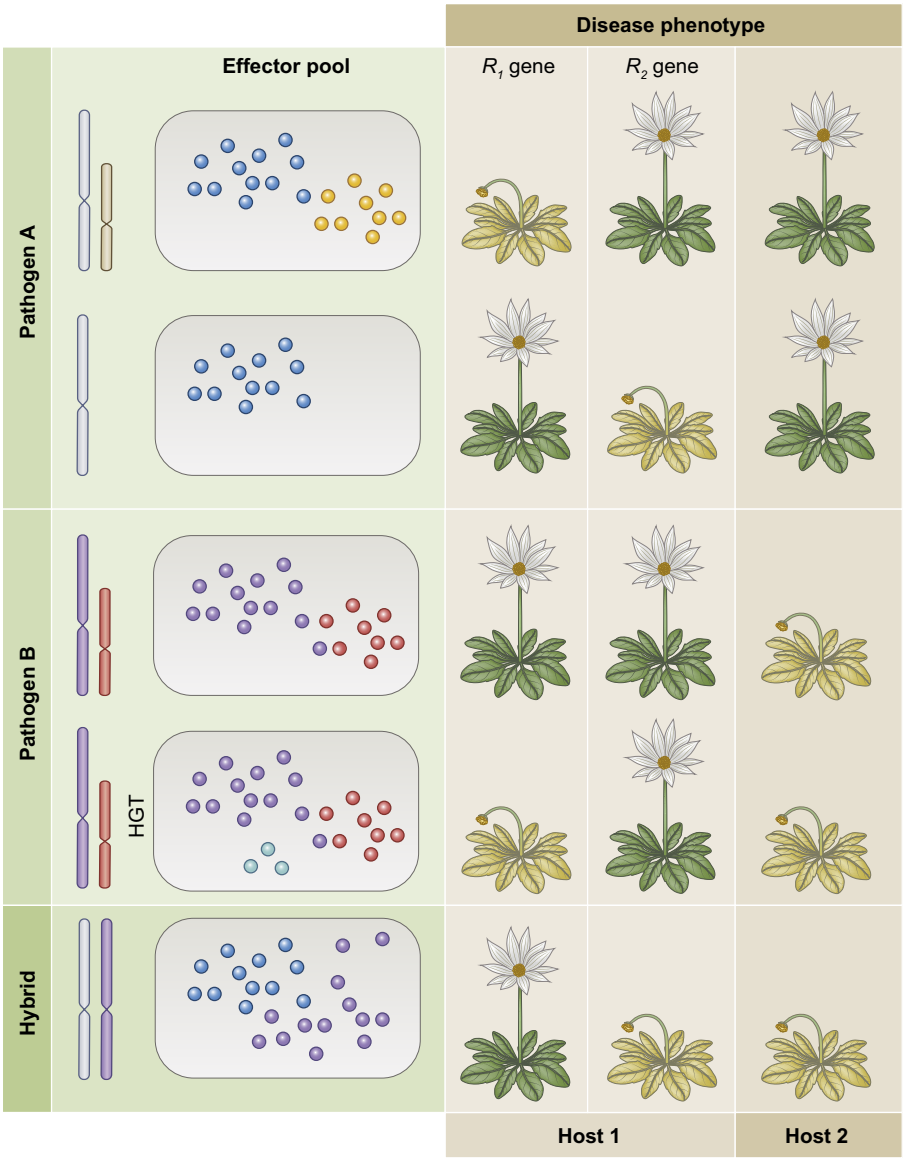

Fig. 2 Rapid adaptation and the effector pool. Different adaptation mechanisms can have large- and/or small-scale effects on the effector pool. In this example 'Pathogen A', which carries a number of effectors encoded on core (blue dots) and accessory chromosomes (yellow dots), is virulent on a 'Host 1' genotype harbouring resistance gene R_1 (yellowish wilted plant), but avirulent on a 'Host 1' genotype harbouring resistance gene R_2, which matches one of the 'Pathogen A' effectors, as well as on a different host species ('Host 2'; green vigorous plant). Loss of an accessory chromosome eliminates one or several effectors, resulting in a change of virulence on 'Host 1'. Meanwhile, 'Pathogen B' is adapted to 'Host 2' but not to 'Host 1', and has a different effector suite encoded by core and dispensable chromosomes (purple and red dots, respectively). HGT of single effectors or an effector cluster (light blue dots), or hybridisation with a different pathogen species followed by reshuffling of the parental effector pool, can extend the host range of a nonadapted pathogen to previously unaccessible plant genotypes.

VI. Do permissive environments foster rapid evolution?

While genomic alterations can lead to rapid shifts in the infection phenotype (Fig. 1), the trajectory of a given plant–microbe interaction does not solely depend on intrinsic genome characteristics. Certain host environments that allow the coexistence of virulent and avirulent strains could promote the exchange of genetic information through sexual or asexual mechanisms such as HGT. Host plant defences are often attenuated in the presence of a virulent pathogen that is able to suppress the immune response – a phenomenon known as 'induced accessibility' (Prats *et al.*, 2006). An illustrative example is provided by the bacterial pathogen *Pseudomonas syringae*, in which the presence of a virulent strain suppresses *in trans* the host defences triggered by a co-inoculated avirulent strain (Rufián *et al.*, 2018). Similarly, the oomycete *Albugo laibachii* renders Arabidopsis susceptible to the nonadapted pathogen *Phytophthora infestans* (Belhaj *et al.*, 2016). It is likely that these circumstances may further promote the exchange of genetic material between different pathogen strains or species, thereby leading to the rapid acquisition of novel virulence determinants. In *Zymoseptoria tritici*, mating of virulent and avirulent strains can occur even in a resistant host, resulting in the maintenance of avirulence alleles as balanced polymorphisms in subsequent generations (Kema *et al.*, 2018). Similarly, hybridisation of different nonadapted isolates on a common host can lead to the generation of new isolates that exhibit higher fitness or an expanded host range (Fig. 2; Depotter *et al.*, 2016). The latter has been shown for the powdery mildew fungus *Blumeria graminis*, in which the hybrid offspring of two specialised pathogenic forms of wheat and rye led to the emergence of a new pathogenic form (f.sp. *triticale*) able to infect the new host triticale (Menardo *et al.*, 2016). Historically, hybridisation has been regarded as an evolutionary dead end (Nelson, 1963), and therefore the reported cases may

represent rare exceptions. Sexual mating can also promote virulence via transient gene silencing and nonheritable changes in the effector repertoire. For example, transient silencing of the effector *Avr3a* in the oomycete *Phytophthora sojae* was reported in offspring of crosses between avirulent and virulent strains, thereby allowing the pathogen to evade host immune detection in soybean (Qutob *et al.*, 2013). Finally, asexual exchange of genes between individuals of the same or different fungal species can occur through conidial or hyphal fusions termed anastomoses (Roca *et al.*, 2005). This process has been proposed as a possible mechanism for the transfer of the *ToxA* virulence gene between the wheat pathogens *Pyrenophora tritici-repentis* and *Stagonospora nodorum* (Friesen *et al.*, 2006). Similarly in the stripe rust pathogen *Puccinia striiformis*, somatic recombination between different isolates of the same specialised form or between specialised forms have generated novel virulence specificities (Lei *et al.*, 2017).

VII. Transcriptional plasticity in stressful environments

Transcriptional plasticity is another aspect of rapid evolution that is not strictly dependent on heritable genomic changes. Because a single genotype can have multiple transcriptional phenotypes depending on the environment in which it is selected, it was suggested that genes that are under strong selection are more likely to display variable expression levels between populations, species or isolates (Hodgins-Davis & Townsend, 2009). As the timing of gene expression and transcript abundance are often crucial for virulence, phytopathogens may employ transcriptional plasticity to optimise infection on a given host (Fig. 1; Azmi *et al.*, 2018). This form of adaptation will lead to isolates with diverse transcriptional profiles, which could also have different fitness optima on the same host. Indeed, individual isolates of the same *forma specialis* of *B. graminis* show considerable differences in expression levels of effector genes during infection (Praz *et al.*, 2018). Similarly, in *Z. tritici*, 20–30% of the genes are differentially regulated between individual isolates during infection of the same host, and are likely to account for the quantitative variation in virulence within this species (Palma-Guerrero *et al.*, 2017). Recent results from experimental evolution in yeast have suggested that variation in expression levels of genes associated with a trait under selection might be highly advantageous for survival and rapid adaptation (Bódi *et al.*, 2017). Although epigenetic changes are thought to play a major role in this phenomenon, the molecular mechanisms underlying transcriptional plasticity in phytopathogens still need to be uncovered.

VIII. Secondary metabolites: another rapidly evolving weapon in the plant–microbe warfare

Biosynthesis and delivery of secondary metabolites from both partners crucially determines the outcome of a plant–microbe interaction. Pathogens often deploy phytotoxins to interfere with plant metabolism and immunity or to kill host cells. Conversely, plants produce an array of antimicrobial secondary metabolites to fight off putative invaders. Frequently, plant pathogens are able to detoxify host antimicrobial compounds through specific enzymes encoded by genes or gene clusters in the phytopathogens' genomes, as for example degradation of benzoxazolinones by *Fusarium pseudograminearum* (Kettle *et al.*, 2015). In plants, the occurrence of secondary metabolites is often restricted to individual phylogenetic lineages such as single families or genera, suggesting that the respective biosynthetic pathways undergo rapid evolution (Piasecka *et al.*, 2015). In phytopathogen genomes, genes associated with the biosynthesis of secondary metabolites are frequently enriched in subtelomeric regions, a location that may facilitate diversification of the metabolic products by gene rearrangements or mutations (Cairns & Meyer, 2017). Subtelomeres of filamentous fungi are typically rich in repetitive regions and TEs, and consequently often undergo chromosomal rearrangements. Accordingly, such clusters are hotspots for gene gains (for example through HGT; Reynolds *et al.*, 2017) and losses (Hartmann & Croll, 2017; Thynne *et al.*, 2019). Subtelomeric gene clusters are also frequently subject to epigenetic regulation (Palmer & Keller, 2010). Recently, the deletion of heterochromatin protein-1 (HepA) in *Epichloë festucae* was shown to result in deregulation of ergot alkaloids and indole diterpene biosynthesis clusters, significantly distorting the balance in the interaction of this species with its host (Chujo *et al.*, 2019).

IX. Ploidy and nucleotypes: finding the perfect gene dosage

Carrying more than one genome copy can be advantageous for eukaryotic pathogens, as this might provide evolutionary flexibility through enhanced allelic variation. A heterokaryotic state in the fungus *Sclerotinia homoeocarpa* was found to provide improved fungicide resistance (Kessler *et al.*, 2018). Similar benefits could be envisaged for pathogenesis. For example, isolates of the smut fungus *Thecaphora thlaspeos* that are able to infect the same host may carry different effector repertoires (Courville *et al.*, 2019). These isolates are able to mate and form infectious dikaryons, thereby expanding their pathogenicity range to additional host ecotypes. Alternatively, selection by host *R* genes could act negatively on the nucleotype content. Recently it was reported that heterokaryotic isolates of the oomycete *Bremia lactucae* have higher fitness than homokaryons on susceptible hosts, whereas homokaryons performed better on hosts carrying *R* genes that were able to recognise effectors encoded by one of the two genomes (Fletcher *et al.*, 2019). Ploidy can also change within a relatively small number of generations. In *Phytophthora infestans*, triploidy was found to dominate the modern asexual lineages identified in fields of solanaceous crops, although they could rapidly revert to diploidy upon stress (Yoshida *et al.*, 2013; Li *et al.*, 2017). Interestingly, diploid strains of *Ustilago maydis* are less virulent than their dikaryotic counterparts (Kronstad & Leong, 1989), suggesting that the effects of gene dosage may differ in each case.

X. Conclusions

Although interacting species co-evolve rapidly, the molecular/genetic mechanisms underlying each case or each adaptive 'step' can

be different. In phytopathogens the speed and trajectory of adaptive events is likely to depend on the host environment, the peculiarities of their life cycle, the characteristics of each genome and on how these features are reflected in the respective effector pool (Fig. 2). Therefore, in spite of the recent advances, many questions still remain to be answered regarding evolution in plant–microbe interactions (Box 2).

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Author contributions

RP and LF conceived the review. LF and RP drafted the manuscript. ADP, MR, JS and C-HW edited the manuscript. All authors have read and approved the final version.

ORCID

Antonio Di Pietro https://orcid.org/0000-0001-5930-5763
Lamprinos Frantzeskakis https://orcid.org/0000-0001-8947-6934
Ralph Panstruga https://orcid.org/0000-0002-3756-8957
Martijn Rep https://orcid.org/0000-0003-3608-6283
Jan Schirawski https://orcid.org/0000-0003-1615-1201
Chih-Hang Wu https://orcid.org/0000-0003-1616-1872

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