DR LAMPRINOS FRANTZESKAKIS (Orcid ID : 0000-0001-8947-6934)

PROF. ANTONIO DI PIETRO (Orcid ID : 0000-0001-5930-5763)

PROF. RALPH PANSTRUGA (Orcid ID : 0000-0002-3756-8957)

Article type : Commissioned Material – Tansley Insight

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

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] University of Amsterdam, Faculty of Science, Swammerdam Institute for Life Sciences, P.O. Box 94215, 1090 GE Amsterdam, The Netherlands

[4] RWTH Aachen University, Institute of Applied Microbiology, Department of Microbial Genetics, Worringerweg 1, 52056 Aachen, Germany

[5] The Sainsbury Laboratory, University of East Anglia, Norwich Research Park, Norwich NR4 7UH, UK

[6] RWTH Aachen University, Institute for Biology I, Unit of Plant Molecular Cell Biology, Worringerweg 1, 52056 Aachen, Germany

[#] Corresponding author; e-mail panstruga@bio1.rwth-aachen.de, telephone +49 241 80 26655

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/nph.15966

Received: *8 March 2019*

Accepted: *14 May 2019*

Contents

Summary

I. Introduction

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

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

IV. Creating diversity by generating novel effectors or effector functions

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

VI. Do permissive environments foster rapid evolution?

VII. Transcriptional plasticity in stressful environments

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

IX. Ploidy and nucleotypes: finding the perfect gene dosage

X. Conclusions

Acknowledgements

References

ORCID

Lamprinos Frantzeskakis http://orcid.org/0000-0001-8947-6934

Antonio di Pietro http://orcid.org/0000-0001-5930-5763

Martijn Rep http://orcid.org/0000-0003-3608-6283

Jan Schirawski http://orcid.org/0000-0003-1615-1201

Chih-Hang Wu http://orcid.org/0000-0003-1616-1872

Ralph Panstruga http://orcid.org/0000-0002-3756-8957

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 in 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 recognized 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.

Keywords:

Phytopathogens, Genome evolution, Adaptation, Virulence factors, Dispensable chromosome

I. Introduction

Plant–microbe interactions represent a paradigm for rapid evolution (Upson *et al.*, 2018). This is particularly true for plant–pathogen interactions, where 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, which tend to be much larger and shorter, respectively, for microbes compared to 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 where 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 provided insights in the molecular mechanisms associated with evasion of plant immunity at the level of single host–pathogen gene interactions (e.g. (Rouxel & Balesdent, 2017); **Box 1**). Recent reports 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). **Figure 1** illustrates such mechanisms for eukaryotic pathogens, noting that comparable mechanisms operate in prokaryotic pathogens (e.g. box B of **Figure 1**). 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 contribute to the 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 resulted from adaptive radiation. We also mostly refer to examples of rapid evolution as it is 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 (**Figure 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), 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, which was promoted by the loss of the critical *PWT3 Avr* gene (Inoue *et al.*, 2017). However, it 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 - e.g. 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 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 *R-Avr* gene pairs thus represent genetic testimonies of rapid evolution driven by the host-pathogen arms race. Some of the respective *Avr* genes were cloned recently (Lu *et al.*, 2016; Praz *et al.*, 2017; McNally *et al.*, 2018; Saur *et al.*, 2019). Interestingly, in contrast to 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 (**Figure 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 spurious expression of non-coding sequences via a transition state termed ‘proto-gene’ (Carvunis *et al.*, 2012). This process might be kickstarted by the expression of long non-coding transcripts (lncRNAs) from TE promoters (Davis *et al.*, 2017), which 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 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 hybridization between pathogen species (see below and **Figure 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).

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 program (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). Since sRNAs are less complex and subject to fewer constraints (e.g. structural limitations) than effector proteins, they might evolve even faster. Since we are just beginning to explore pathogen and host sRNA repertoires, further studies need to determine whether they are indeed subject to rapid co-evolution in the interacting partners (Rose *et al.*, 2019).

VI. Do permissive environments foster rapid evolution?

While genomic alterations can lead to rapid shifts in the infection phenotype (**Figure 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*, where 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 non-adapted 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, hybridization of different non-adapted isolates on a common host can lead to the generation of new isolates that exhibit higher fitness or expanded host range (**Figure 2**; (Depotter *et al.*, 2016). The latter has been shown for the powdery mildew fungus *Blumeria graminis*, where the hybrid offspring of two specialized 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, hybridization has been regarded as an evolutionary dead end (Nelson, 1963), and thus the reported cases may represent rare exceptions. Sexual mating can also promote virulence via transient gene silencing and non-heritable 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 specialized form or between specialized forms 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 it is selected in, 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). Since timing of gene expression and transcript abundance are often crucial for virulence, phytopathogens may employ transcriptional plasticity to optimize infection on a given host (**Figure 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, likely accounting for the quantitative variation in virulence within this species (Palma-Guerrero *et al.*, 2017). Recent results from experimental evolution in yeast suggest 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. On the other hand, 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 (e.g. *via* HGT; (Reynolds *et al.*, 2017) and losses (Hartmann & Croll, 2017; Thynne *et al.*, 2018). Subtelomeric gene clusters are also frequently subject to epigenetic regulation (Palmer & Keller, 2010). Recently, 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, since it provides 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 are able to recognize 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 likely 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 (**Figure 2**). Thus, in spite of the recent advances, many questions still remain to be answered regarding evolution in plant–microbe interactions (**Box 2**).

Acknowledgements

We acknowledge input from Like Fokkens, Sophien Kamoun and Eva Stukenbrock on earlier versions of this article. We thank Laura Rose for critical proofreading of the manuscript and the reviewers for constructive criticism. This review partially reflects the discussions at the workshop 'Molecular mechanisms underlying the rapid evolution of plant-microbe interactions', held at Kasteel Bloemendal, Vaals, The Netherlands (9^{th}/10^{th} February 2018). The workshop was co-funded by *New Phytologist* (as the 20^{th} New Phytologist Workshop) and the Deutsche Forschungsgemeinschaft (DFG, in the context of the priority program SPP1819 – 'Rapid evolutionary adaptation - potential and constraints' – grants PA 861/14-1 to RP and SCHI 1114/3-1 to JS).

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.

References

Azmi, NSA, Singkaravanit-Ogawa, S, Ikeda, K, Kitakura, S, Inoue, Y, Narusaka, Y, Shirasu, K, Kaido, M, Mise, K, Takano, Y. 2018. Inappropriate expression of an NLP effector in *Colletotrichum orbiculare* impairs infection on cucurbitaceae vultivars via plant recognition of the C-terminal region. *Molecular Plant-Microbe Interactions* **31**: 101–111.

Belhaj, K, Cano, LM, Prince, DC, Kemen, A, Yoshida, K, Dagdas, YF, Etherington, GJ, Schoonbeek, H-J, van Esse, HP, Jones, JDG, Kamoun, S, Schornack, S. 2016. Arabidopsis late blight: infection of a nonhost plant by *Albugo laibachii* enables full colonization by *Phytophthora infestans*. *Cellular Microbiology* **19**: e12628.

Bódi, Z, Farkas, Z, Nevozhay, D, Kalapis, D, Lázár, V, Csörgő, B, Nyerges, Á, Szamecz, B, Fekete, G, Papp, B, Araújo, H, Oliveira, JL, Moura, G, Santos, MAS, Székely, T, Balázsi, G, Pál, C. 2017. Phenotypic heterogeneity promotes adaptive evolution. *PLoS Biology* **15**: e2000644.

Borrelli, GM, Mazzucotelli, E, Marone, D, Crosatti, C, Michelotti, V, Valè, G, Mastrangelo, AM. 2018. Regulation and evolution of NLR genes: A close interconnection for plant immunity. *International Journal of Molecular Sciences* **19**: 1662.

Cai, Q, Qiao, L, Wang, M, He, B, Lin, F-M, Palmquist, J, Huang, H-D, Jin, H. 2018. Plants send small RNAs in extracellular vesicles to fungal pathogen to silence virulence genes. *Science* **360**: 1126-1129.

Cairns, T, Meyer, V. 2017. *In silico* prediction and characterization of secondary metabolite biosynthetic gene clusters in the wheat pathogen *Zymoseptoria tritici*. *BMC Genomics* **18**: 631.

Carvunis, A-R, Rolland, T, Wapinski, I, Calderwood, MA, Yildirim, MA, Simonis, N, Charloteaux, B, Hidalgo, CA, Barbette, J, Santhanam, B, Brar, GA, Weissman, JS, Regev, A, Thierry-Mieg, N, Cusick, ME, Vidal, M. 2012. Proto-genes and *de novo* gene birth. *Nature* **487**: 370–374.

Cesari, S. 2018. Multiple strategies for pathogen perception by plant immune receptors. *New Phytologist* **219**: 17–24.

Chujo, T, Lukito, Y, Eaton, CJ, Dupont, P-Y, Johnson, LJ, Winter, D, Cox, MP, Scott, B. 2019. Complex epigenetic regulation of alkaloid biosynthesis and host interaction by heterochromatin protein I in a fungal endophyte–plant symbiosis. *Fungal Genetics & Biology* **125**: 71–83.

Courville, KJ, Frantzeskakis, L, Gul, S, Haeger, N, Kellner, R, Heßler, N, Day, B, Usadel, B, Gupta, YK, van Esse, HP, Brachmann, A, Kemen, E, Feldbrügge, M, Göhre, V. 2019. Smut infection of perennial hosts: The genome and the transcriptome of the Brassicaceae smut fungus *Thecaphora thlaspeos* reveal functionally conserved and novel effectors. *New Phytologist* **222**: 1474–1492.

Davis, MP, Carrieri, C, Saini, HK, van Dongen, S, Leonardi, T, Bussotti, G, Monahan, JM, Auchynnikava, T, Bitetti, A, Rappsilber, J, Allshire, RC, Shkumatava, A, O'Carroll, D, Enright, AJ. 2017. Transposon-driven transcription is a conserved feature of vertebrate spermatogenesis and transcript evolution. *EMBO Reports* **18**: 1231–1247.

Depotter, JR, Seidl, MF, Wood, TA, Thomma, BP. 2016. Interspecific hybridization impacts host range and pathogenicity of filamentous microbes. *Current Opinion in Microbiology* **32**: 7–13.

Dong, S, Raffaele, S, Kamoun, S. 2015. The two-speed genomes of filamentous pathogens: waltz with plants. *Current Opinion in Plant Biology* **35**: 57–65.

Fiorin, GL, Sanchéz-Vallet, A, Thomazella, DPdT, do Prado, PFV, do Nascimento, LC, Figueira, AVdO, Thomma, BPHJ, Pereira, GAG, Teixeira, PJPL. 2018. Suppression of Plant Immunity by Fungal Chitinase-like Effectors. *Current biology : CB* **28**: 3023-3030.e5.

Fletcher, K, Gil, J, Bertier, LD, Kenefick, A, Wood, KJ, Zhang, L, Reyes-Chin-Wo, S, Cavanaugh, K, Tsuchida, C, Wong, J, Michelmore, R. 2019. Genomic signatures of somatic hybrid vigor due to heterokaryosis in the oomycete pathogen, *Bremia lactucae*. *bioRxiv*: doi: https://doi.org/10.1101/516526.

Fouché, S, Plissonneau, C, Croll, D. 2018. The birth and death of effectors in rapidly evolving filamentous pathogen genomes. *Current Opinion in Microbiology* **46**: 34–42.

Frantzeskakis, L, Dahlen, JK von, **Panstruga, R, Rose, LE. 2018.** Rapid evolution in the tug-of-war between microbes and plants. *New Phytologist* **219**: 12–14.

Frantzeskakis, L, Kusch, S, Panstruga, R. 2019. The need for speed: Compartmentalized genome evolution in filamentous phytopathogens. *Molecular Plant Pathology* **20**: 3–7.

Friesen, TL, Stukenbrock, EH, Liu, ZH, Meinhardt, S, Ling, H, Faris, JD, Rasmussen, JB, Solomon, PS, McDonald, BA, Oliver, RP. 2006. Emergence of a new disease as a result of interspecific virulence gene transfer. *Nature Genetics* **38**: 953–956.

Hartmann, FE, Croll, D. 2017. Distinct trajectories of massive recent gene gains and losses in populations of a microbial eukaryotic pathogen. *Molecular Biology and Evolution* **34**: 2808–2822.

Hartmann, FE, Sánchez-Vallet, A, McDonald, BA, Croll, D. 2017. A fungal wheat pathogen evolved host specialization by extensive chromosomal rearrangements. *ISME Journal* **11**: 1189–1204.

Hodgins-Davis, A, Townsend, JP. 2009. Evolving gene expression: From G to E to GxE. *Trends in Ecology & Evolution* **24**: 649–658.

Hua, C, Zhao, J-H, Guo, H-S. 2018. Trans-kingdom RNA silencing in plant-fungal pathogen interactions. *Molecular Plant* **11**: 235–244.

Hurni, S, Brunner, S, Buchmann, G, Herren, G, Jordan, T, Krukowski, P, Wicker, T, Yahiaoui, N, Mago, R, Keller, B. 2013. Rye *Pm8* and wheat *Pm3* are orthologous genes and show evolutionary conservation of resistance function against powdery mildew. *Plant Journal* **76**: 957–969.

Inoue, Y, Vy, TTP, Yoshida, K, Asano, H, Mitsuoka, C, Asuke, S, Anh, VL, Cumagun, CJR, Chuma, I, Terauchi, R, Kato, K, Mitchell, T, Valent, B, Farman, M, Tosa, Y. 2017. Evolution of the wheat blast fungus through functional losses in a host specificity determinant. *Science* **357**: 80–83.

Kaiser, CA, Preuss, D, Grisafi, P, Botstein, D. 1987. Many random sequences functionally replace the secretion signal sequence of yeast invertase. *Science* **235**: 312–317.

Karasov, TL, Almario, J, Friedemann, C, Ding, W, Giolai, M, Heavens, D, Kersten, S, Lundberg, DS, Neumann, M, Regalado, J, Neher, RA, Kemen, E, Weigel, D. 2018. *Arabidopsis thaliana* and *Pseudomonas p*athogens exhibit stable associations over evolutionary timescales. *Cell Host & Microbe* **24**: 168-179.e4.

Kema, GHJ, Gohari, AM, Aouini, L, Gibriel, HAY, Ware, SB, van den Bosch, F, Manning-Smith, R, Alonso-Chavez, V, Helps, J, M'Barek, SB, Mehrabi, R, Diaz-Trujillo, C, Zamani, E, Schouten, HJ, Lee, Theo A. J. van der, Waalwijk, C, Waard, MAd, Wit, PJGM de, **Verstappen, ECP, Thomma, BPHJ, Meijer, HJG, Seidl, MF. 2018.** Stress and sexual reproduction affect the dynamics of the wheat pathogen effector AvrStb6 and strobilurin resistance. *Nature Genetics* **50**: 375. https://www.nature.com/articles/s41588-018-0052-9.pdf.

Kessler, D, Sang, H, Bousquet, A, Hulvey, JP, Garcia, D, Rhee, S, Hoshino, Y, Yamada, T, Jung, G. 2018. Nucleic adaptability of heterokaryons to fungicides in a multinucleate fungus, *Sclerotinia homoeocarpa*. *Fungal Genetics & Biology* **115**: 64–77.

Kettle, AJ, Batley, J, Benfield, AH, Manners, JM, Kazan, K, Gardiner, DM. 2015. Degradation of the benzoxazolinone class of phytoalexins is important for virulence of *Fusarium pseudograminearum* towards wheat. *Molecular Plant Pathology* **16**: 946–962.

Krishnan, P, Ma, X, McDonald, BA, Brunner, PC. 2018. Widespread signatures of selection for secreted peptidases in a fungal plant pathogen. *BMC Evolutionary Biology* **18**: 7.

Kronstad, JW, Leong, SA. 1989. Isolation of two alleles of the *b* locus of *Ustilago maydis*. *Proceedings of the National Academy of Sciences of the United States of America* **86**: 978–982.

Lei, Y, Wang, M, Wan, A, Xia, C, See, DR, Zhang, M, Chen, X. 2017. Virulence and molecular characterization of experimental isolates of the stripe rust pathogen (*Puccinia striiformis*) indicate somatic recombination. *Phytopathology* **107**: 329–344.

Li, Y, Shen, H, Zhou, Q, Qian, K, van der Lee, Theo, Huang, S. 2017. Changing ploidy as a strategy: The Irish potato famine pathogen shifts ploidy in relation to its sexuality. *Molecular Plant-Microbe Interactions* **30**: 45–52.

Lilley, CJ, Maqbool, A, Wu, D, Yusup, HB, Jones, LM, Birch, PRJ, Banfield, MJ, Urwin, PE, Eves-van den Akker, S. 2018. Effector gene birth in plant parasitic nematodes: Neofunctionalization of a housekeeping glutathione synthetase gene. *PLoS Genetics* **14**: e1007310.

Lu, X, Kracher, B, Saur, IML, Bauer, S, Ellwood, SR, Wise, R, Yaeno, T, Maekawa, T, Schulze-Lefert, P. 2016. Allelic barley MLA immune receptors recognize sequence-unrelated avirulence effectors of the powdery mildew pathogen. *Proceedings of the National Academy of Sciences of the United States of America* **113**: E6486-E6495.

Ma, LJ, van der Does, HC, Borkovich, KA, Coleman, JJ, Daboussi, MJ, Di Pietro, A, Dufresne, M, Freitag, M, Grabherr, M, Henrissat, B, Houterman, PM, Kang, S, Shim, WB, Woloshuk, C, Xie, XH, Xu, JR, Antoniw, J, Baker, SE, Bluhm, BH, Breakspear, A, Brown, DW, Butchko, RAE, Chapman, S, Coulson, R, Coutinho, PM, Danchin, EGJ, Diener, A, Gale, LR, Gardiner, DM, Goff, S, Hammond-Kosack, KE, Hilburn, K, Hua-Van, A, Jonkers, W, Kazan, K, Kodira, CD, Koehrsen, M, Kumar, L, Lee, YH, Li, LD, Manners, JM, Miranda-Saavedra, D, Mukherjee, M, Park, G, Park, J, Park, SY, Proctor, RH, Regev, A, Ruiz-Roldan, MC, Sain, D, Sakthikumar, S, Sykes, S, Schwartz, DC, Turgeon, BG, Wapinski, I, Yoder, O, Young, S, Zeng, QD, Zhou, SG, Galagan, J, Cuomo, CA, Kistler, HC, Rep, M. 2010. Comparative genomics reveals mobile pathogenicity chromosomes in Fusarium. *Nature* **464**: 367–373.

McDonald, BA, Stukenbrock, EH. 2016. Rapid emergence of pathogens in agro-ecosystems: Global threats to agricultural sustainability and food security. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **371**: 20160026.

McNally, KE, Menardo, F, Lüthi, L, Praz, CR, Müller, MC, Kunz, L, Ben-David, R, Chandrasekhar, K, Dinoor, A, Cowger, C, Meyers, E, Xue, M, Zeng, F, Gong, S, Yu, D, Bourras, S, Keller, B. 2018. Distinct domains of the AVRPM3$^{A2/F2}$ avirulence protein

from wheat powdery mildew are involved in immune receptor recognition and putative effector function. *New Phytologist* **218**: 681–695.

Meile, L, Croll, D, Brunner, PC, Plissonneau, C, Hartmann, FE, McDonald, BA, Sánchez-Vallet, A. 2018. A fungal avirulence factor encoded in a highly plastic genomic region triggers partial resistance to septoria tritici blotch. *New Phytologist* **219**: 1048–1061.

Menardo, F, Praz, CR, Wyder, S, Ben-David, R, Bourras, S, Matsumae, H, McNally, KE, Parlange, F, Riba, A, Roffler, S, Schaefer, LK, Shimizu, KK, Valenti, L, Zbinden, H, Wicker, T, Keller, B. 2016. Hybridization of powdery mildew strains gives rise to pathogens on novel agricultural crop species. *Nature Genetics* **48**: 201–205.

Nelson, RR. 1963. Interspecific hybridization in the fungi. *Mycologia* **55**: 104–123.

Nottensteiner, M, Zechmann, B, McCollum, C, Hückelhoven, R. 2018. A barley powdery mildew fungus non-autonomous retrotransposon encodes a peptide that supports penetration success on barley. *Journal of Experimental Botany* **69**: 3745–3758.

Palma-Guerrero, J, Ma, X, Torriani, SFF, Zala, M, Francisco, CS, Hartmann, FE, Croll, D, McDonald, BA. 2017. Comparative transcriptome analyses in *Zymoseptoria tritici r*eveal significant differences in gene expression among strains during plant infection. *Molecular Plant-Microbe Interactions* **30**: 231–244.

Palmer, JM, Keller, NP. 2010. Secondary metabolism in fungi: Does chromosomal location matter? *Current Opinion in Microbiology* **13**: 431–436.

Pennington, HG, Jones, R, Kwon, S, Bonciani, G, Thieron, H, Chandler, T, Luong, P, Morgan, SN, Przydacz, M, Bozkurt, T, Bowden, S, Craze, M, Wallington, EJ, Garnett, J, Kwaaitaal, M, Panstruga, R, Cota, E, Spanu, PD. 2019. The fungal ribonuclease-like effector protein CSEP0064/BEC1054 represses plant immunity and interferes with degradation of host ribosomal RNA. *PLoS Pathogens* **15**: e1007620.

Piasecka, A, Jedrzejczak-Rey, N, Bednarek, P. 2015. Secondary metabolites in plant innate immunity. Conserved function of divergent chemicals. *New Phytologist* **206**: 948–964.

Plissonneau, C, Benevenuto, J, Mohd-Assaad, N, Fouché, S, Hartmann, FE, Croll, D. 2017a. Using population and comparative genomics to understand the genetic basis of effector-driven fungal pathogen evolution. *Frontiers in Plant Science* **8**: 119.

Plissonneau, C, Blaise, F, Ollivier, B, Leflon, M, Carpezat, J, Rouxel, T, Balesdent, M-H. 2017b. Unusual evolutionary mechanisms to escape effector-triggered immunity in the fungal phytopathogen *Leptosphaeria maculans*. *Molecular Ecology* **26**: 2183–2198.

Prats, E, Carver, Timothy L W, Lyngkjær, MF, Roberts, PC, Zeyen, RJ. 2006. Induced inaccessibility and accessibility in the oat powdery mildew system: insights gained from use of metabolic inhibitors and silicon nutrition. *Molecular Plant Pathology* **7**: 47–59.

Praz, CR, Bourras, S, Zeng, F, Sánchez-Martín, J, Menardo, F, Xue, M, Yang, L, Roffler, S, Böni, R, Herren, G, McNally, KE, Ben-David, R, Parlange, F, Oberhaensli, S, Flückiger, S, Schäfer, LK, Wicker, T, Yu, D, Keller, B. 2017. *AvrPm2* encodes an

RNase-like avirulence effector which is conserved in the two different specialized forms of wheat and rye powdery mildew fungus. *New Phytologist* **213**: 1301–1314.

Praz, CR, Menardo, F, Robinson, MD, Müller, MC, Wicker, T, Bourras, S, Keller, B. 2018. Non-parent of origin expression of numerous effector genes indicates a role of gene regulation in host adaption of the hybrid triticale powdery mildew pathogen. *Frontiers in Plant Science* **9**: 49.

Qutob, D, Chapman, BP, Gijzen, M. 2013. Transgenerational gene silencing causes gain of virulence in a plant pathogen. *Nature Communications* **4**: 1349.

Reynolds, HT, Slot, JC, Divon, HH, Lysøe, E, Proctor, RH, Brown, DW. 2017. Differential retention of gene functions in a secondary metabolite cluster. *Molecular Biology and Evolution* **34**: 2002–2015.

Roca, MG, Read, ND, Wheals, AE. 2005. Conidial anastomosis tubes in filamentous fungi. *FEMS Microbiology Letters* **249**: 191–198.

Rose, LE, Overdijk, EJR, van Damme, M. 2019. Small RNA molecules and their role in plant disease. *European Journal of Plant Pathology* **153**: 1–14.

Rouxel, T, Balesdent, M-H. 2017. Life, death and rebirth of avirulence effectors in a fungal pathogen of Brassica crops, *Leptosphaeria maculans*. *New Phytologist* **214**: 526–532.

Rufián, JS, Macho, AP, Corry, DS, Mansfield, JW, Ruiz-Albert, J, Arnold, DL, Beuzón, CR. 2018. Confocal microscopy reveals in planta dynamic interactions between pathogenic, avirulent and non-pathogenic *Pseudomonas syringae* strains. *Molecular Plant Pathology* **19**: 537–551. https://onlinelibrary.wiley.com/doi/full/10.1111/mpp.12539.

Saur, IM, Bauer, S, Kracher, B, Lu, X, Franzeskakis, L, Müller, MC, Sabelleck, B, Kümmel, F, Panstruga, R, Maekawa, T, Schulze-Lefert, P. 2019. Multiple pairs of allelic MLA immune receptor-powdery mildew AVR_A effectors argue for a direct recognition mechanism. *eLife* **8**: e44471.

Schierstaedt, J, Bziuk, N, Kuzmanović, N, Blau, K, Smalla, K, Jechalke, S. 2019. Role of plasmids in plant–bacteria interactions. *Current Issues in Molecular Biology* **30**: 17–38.

Schulze-Lefert, P, Panstruga, R. 2011. A molecular evolutionary concept connecting nonhost resistance, pathogen host range, and pathogen speciation. *Trends in Plant Science* **16**: 117–125.

Seeholzer, S, Tsuchimatsu, T, Jordan, T, Bieri, S, Pajonk, S, Yang, WX, Jahoor, A, Shimizu, KK, Keller, B, Schulze-Lefert, P. 2010. Diversity at the *Mla* powdery mildew resistance locus from cultivated barley reveals sites of positive selection. *Molecular Plant-Microbe Interactions* **23**: 497–509.

Sperschneider, J, Dodds, PN, Gardiner, DM, Singh, KB, Taylor, JM. 2018. Improved prediction of fungal effector proteins from secretomes with EffectorP 2.0. *Molecular Plant Pathology* **19**: 2094–2110.

Srichumpa, P, Brunner, S, Keller, B, Yahiaoui, N. 2005. Allelic series of four powdery mildew resistance genes at the *Pm3* locus in hexaploid bread wheat. *Plant Physiology* **139**: 885–895.

Thynne, E, Mead, O, Chooi, Y-H, McDonald, M, Solomon, P. 2018. Acquisition and loss of secondary metabolite clusters shaped the evolutionary path of three recently emerged phytopathogens of wheat. *bioRxiv*: doi: https://doi.org/10.1101/283416.

Upson, JL, Zess, EK, Białas, A, Wu, C-H, Kamoun, S. 2018. The coming of age of EvoMPMI: Evolutionary molecular plant-microbe interactions across multiple timescales. *Current Opinion in Plant Biology* **44**: 108–116.

van Dam, P, Fokkens, L, Ayukawa, Y, van der Gragt, M, Ter Horst, A, Brankovics, B, Houterman, PM, Arie, T, Rep, M. 2017. A mobile pathogenicity chromosome in *Fusarium oxysporum* for infection of multiple cucurbit species. *Scientific Reports* **7**: 9042.

Wei, FS, Wong, RA, Wise, RP. 2002. Genome dynamics and evolution of the Mla (powdery mildew) resistance locus in barley. *Plant Cell* **14**: 1903–1917. Wei et al. (Plant Cell 2002).pdf.

Weiberg, A, Wang, M, Lin, F-M, Zhao, H, Zhang, Z, Kaloshian, I, Huang, H-D, Jin, H. 2013. Fungal small RNAs suppress plant immunity by hijacking host RNA interference pathways. *Science* **342**: 118–123.

Wu, J, Kou, Y, Bao, J, Li, Y, Tang, M, Zhu, X, Ponaya, A, Xiao, G, Li, J, Li, C, Song, M-Y, Cumagun, CJR, Deng, Q, Lu, G, Jeon, J-S, Naqvi, NI, Zhou, B. 2015. Comparative genomics identifies the *Magnaporthe oryzae* avirulence effector *AvrPi9* that triggers *Pi9*-mediated blast resistance in rice. *New Phytologist* **206**: 1463–1475.

Yomtovian, I, Teerakulkittipong, N, Lee, B, Moult, J, Unger, R. 2010. Composition bias and the origin of ORFan genes. *Bioinformatics* **26**: 996–999.

Yoshida, K, Schuenemann, VJ, Cano, LM, Pais, M, Mishra, B, Sharma, R, Lanz, C, Martin, FN, Kamoun, S, Krause, J, Thines, M, Weigel, D, Burbano, HA. 2013. The rise and fall of the *Phytophthora infestans* lineage that triggered the Irish potato famine. *eLife* **2**: e00731.

Zhang, S, Wang, L, Wu, W, He, L, Yang, X, Pan, Q. 2015. Function and evolution of *Magnaporthe oryzae* avirulence gene *AvrPib* responding to the rice blast resistance gene *Pib*. *Scientific Reports* **5**: 11642.

Zhong, Z, Marcel, TC, Hartmann, FE, Ma, X, Plissonneau, C, Zala, M, Ducasse, A, Confais, J, Compain, J, Lapalu, N, Amselem, J, McDonald, BA, Croll, D, Palma-Guerrero, J. 2017. A small secreted protein in *Zymoseptoria tritici* is responsible for avirulence on wheat cultivars carrying the Stb6 resistance gene. *New Phytologist* **214**: 619–631.

Figure 1: A consensus view of the rapidly evolving phytopathogen genome.

In this figure, several genomic features/processes enabling rapid evolution are summarized, exemplarily for a hypothetical core chromosome (blue, **a**) and a dispensable chromosome, a type of chromosomes found in some filamentous pathogens (red, **b**). The transposable element (TE), effector and non-virulence-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 compartmentalization 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 (e.g. to TEs; box I), or depending on the specific location (e.g. 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: (a) DNA methylation-based silencing (indicated by grey nucleotide sequence) induced by insertion of elements flanking the gene ('TE-induced silencing'); (b) alteration of the nucleotide sequence (indicated by red letter in nucleotide sequence) resulting from repeat-induced point mutation (RIP), a fungal genome defense mechanism targeting flanking repetitive elements ('RIP leakage'), possibly resulting in a different allele (*Avr*-2); or (c) 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 (e.g. '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 color), or older, enabling more sequence divergence (shown by different shades of the same color). In the latter case, novel functions might be assumed for some of these copies ('Diversification & neofunctionalization'). 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 chromosome. 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 red or dark blue), potentially altering the virulence or host range of a pathogen.

Figure 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 harboring resistance gene R_1 (yellowish wilted plant), but avirulent on a 'Host 1' genotype harboring 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). Horizontal gene transfer (HGT) of single effectors or an effector cluster (light blue dots), or hybridization with a different pathogen species followed by reshuffling of the parental effector pool, can extend the host range of a non-adapted pathogen to previously unaccessible plant genotypes.

Box 1. Mutational events and genomic features enabling rapid evolution.

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.

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.

Copy number variation (CNV): Differences regarding the copy number of a given gene in a genome, e.g. in comparisons between individuals of a population.

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.

Epigenetic modification of gene expression: Epigenetic mechanisms can repress or release gene expression in a non-heritable manner. They can have a limited effective range (e.g. a single gene; RNA interference (RNAi)-based silencing) or extend to entire chromosomal regions (e.g. epigenetic silencing of subtelomeric regions due to histone modifications).

Repeat-Induced Point mutation (RIP): Fungal genome defense mechanism to limit transposon activity by mutating cytosines in repetitive sequences

RIP leakage: Spreading of RIP from duplicated sequences into neighboring nonrepetitive regions

Chromosomal rearrangements: Large-scale differences in gene order and organization in a genome.

AT-rich isochores: A large genomic region with an overrepresentation of adenine- thymine base pairs, usually coinciding with deactivated repetitive elements by the repeat-induced point mutation (RIP) mechanism.

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.

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.

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.

Polyploidization: Acquisition of one or more additional sets of chromosomes in a cell or organism.

Hybridization: Mating of organisms of different varieties or species to create a hybrid.

***De novo* genes:** Species-specific (orphan) genes originating from sequences that did not have any coding potential before.

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 versus 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?

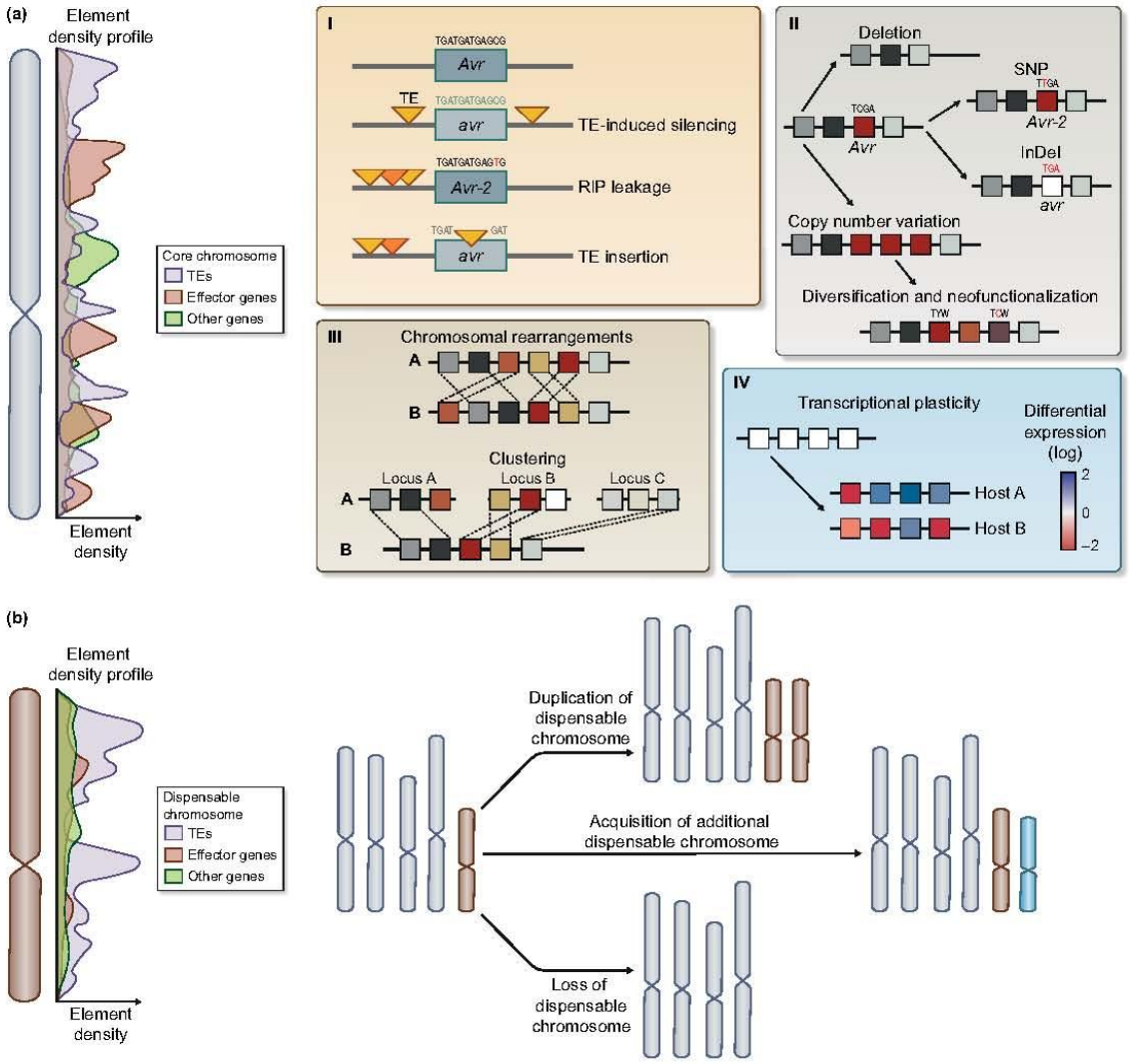

Figure 1

Tansley Insight 29503

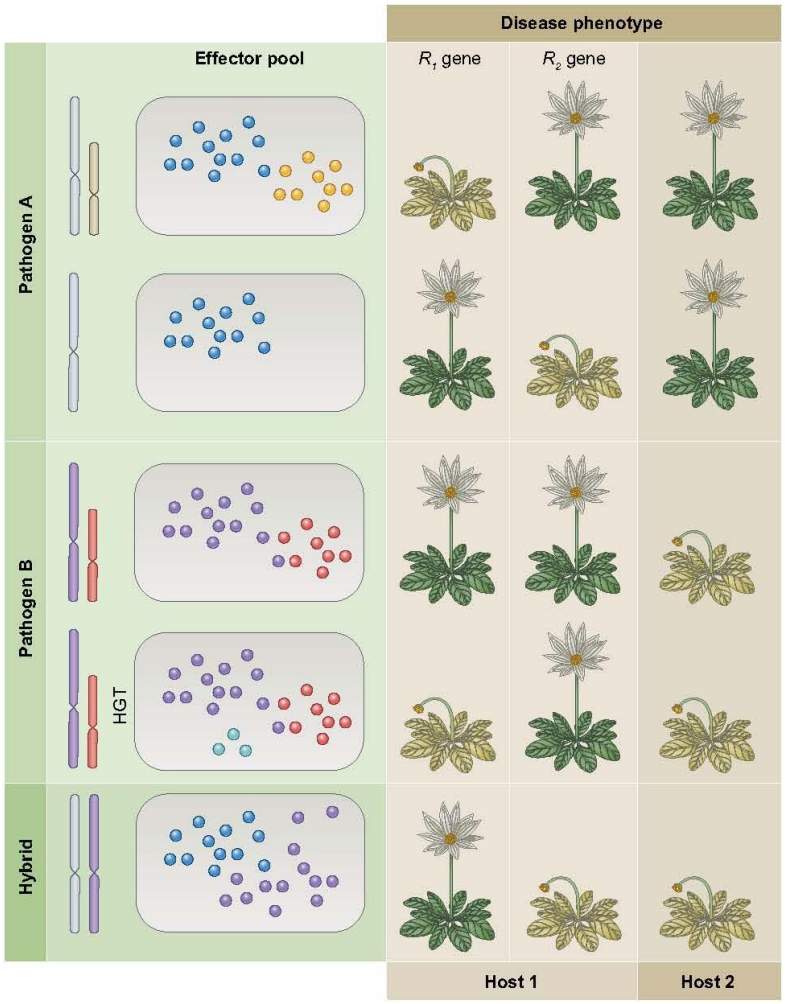

Figure 2

Tansley Insight 29503