Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Plant Biology

How to win a tug-of-war: the adaptive evolution of *Phytophthora* effectors

Suomeng Dong[1] and Wenbo Ma[2,3]

The 'zigzag' model formulates some of the fundamental principles underpinning the dynamic interactions between pathogen effectors and plant immunity. As key virulence factors, effectors often exhibit a pattern of rapid evolution, presumably as a result of the host-pathogen arms race. Here, we summarize the current knowledge of mechanisms that may accelerate effector evolution in the highly successful *Phytophthora* pathogens. Recent findings on epigenetic regulation of effector genes that allows evasion of host recognition and maintenance of cost/benefit balance, and a conserved structural unit in effector proteins that may promote the evolution of virulence activities are highlighted.

Addresses
[1] Department of Plant Pathology and Key Laboratory of Plant Immunity, Nanjing Agricultural University, Nanjing, 210095, China

[2] The Sainsbury Laboratory, Norwich Research Park, Norwich, NR4 7UH, United Kingdom
[3] Department of Microbiology and Plant Pathology, University of California, Riverside, CA 92521, USA

Corresponding authors: Dong, Suomeng (smdong@njau.edu.cn), Ma, Wenbo (wenbo.ma@tsl.ac.uk)

Current Opinion in Plant Biology 2021, **62**:102027

This review comes from a themed issue on **Biotic interactions**

Edited by **Jeffery L Dangl** and **Jonathan DG Jones**

For a complete overview see the Issue and the Editorial

Available online 5th March 2021

https://doi.org/10.1016/j.pbi.2021.102027

Introduction

Diseases occur when microbial pathogens successfully defeat the plant immune system, which is composed of pattern-triggered immunity (PTI) and effector-triggered immunity (ETI) [1]. Although signaling pathways and cellular processes in PTI and ETI are highly intertwined, they tend to depend on distinct immune receptors, that is, membrane-bound pattern recognition receptors that are usually receptor-like kinase or protein (RLK or RLP) for PTI [2,3] and the intracellular nucleotide binding–leucine-rich repeat receptors (NLR) for ETI [4,5]. Activation of RLK/RLP and NLR leads to responses that restrict pathogen colonization, hence imposing a selective pressure on the pathogens for the evolution of counter-defense strategies [6].

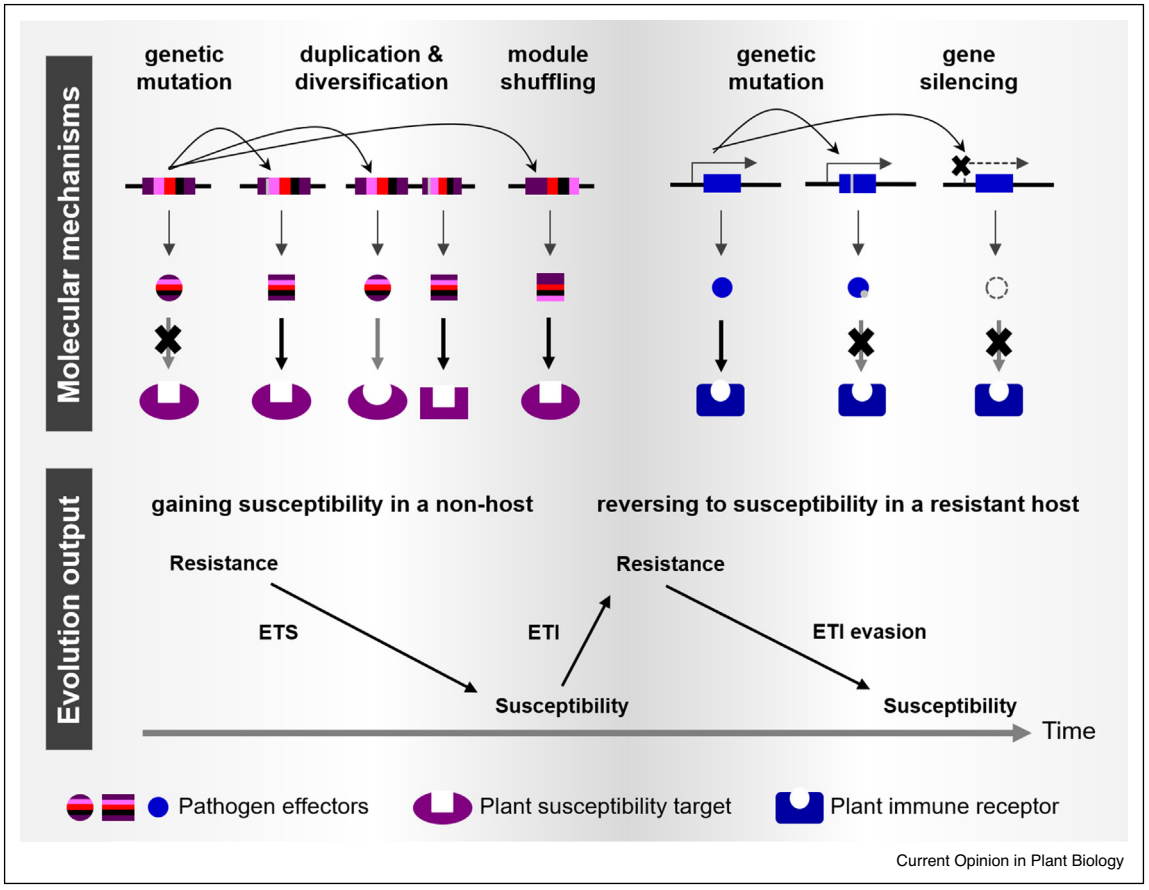
One of the central components in the zigzag model is effector-triggered susceptibility (ETS), which highlights the key role played by effectors in counter-defense [1]. Selective pressures that drive effector evolution to gain ETS are presumably from two aspects: 1) reversing resistance to susceptibility in a host with established ETI based on the recognition of specific effector(s) by NLR (s); 2) gaining susceptibility in a plant that has an effective PTI triggered by non-self molecular patterns (Figure 1). In the first scenario, the pathogen population would be under the selection to escape host recognition. In the second scenario, evolution of novel virulence activities would be selected to adapt to a new host. While these concepts are readily conceivable, the underlying mechanisms that promote the accelerated evolution of the effector repertoire in a pathogen are not well understood.

Filamentous pathogens in the *Phytophthora* genus offer excellent opportunities to elucidate mechanistic insight into effector evolution. *Phytophthora* are specialized into more than 100 species, and collectively, causing diseases in a wide range of plants including many economically important crops and forest trees [7]. Each *Phytophthora* species encodes hundreds to over 1000 effectors [8]. Similar to what has been observed in other microbial pathogens, effector gene complements are highly diverse between *Phytophthora* species [9,10], presumably shaped by the complicated interactions with their specific plant hosts in constant arms races. Among these effectors, the best-studied family is called the 'RXLR'. These effectors contain a conserved N-terminal motif, the RXLR motif, which is required for translocation from the pathogen to the host cytosol [11]. Here, we focus on the RXLR effectors to discuss recent findings on effector evolution in *Phytophthora*.

Invisibility cloak — how to hide from surveillance?

Effectors that activate ETI are named 'avirulence' (Avr) factors although they still maintain virulence activity in hosts that do not encode the cognate NLRs [12]. Although the use of this term can be confusing from time to time, it indicates the natural selection exerts on pathogen populations for individuals with changes in their genome that lead to regained pathogenicity. In

Figure 1



A conceptual model illustrating adaptive evolution of *Phytophthora* effectors.
Major mechanisms of effector gene evolution in *Phytophthora* corresponding to specific stages in the zigzag model are summarized. Adaptation of effectors that defeats non-host immunity is referred to as effector-triggered susceptibility (ETS), whereas mechanisms that allow the escape of host NLR-based immunity is referred to as effector-triggered immunity (ETI) evasion. In both cases, the pathogen gains pathogenicity in the plants.

Phytophthora, all currently characterized *Avr* genes encode RXLR effectors [13].

There are many examples in which *Avr* genes are lost in field isolates that have escaped the ETI conferred by specific NLR(s) [14–16]. Resequencing of sister species of *Phytophthora* revealed a range of mechanisms that contribute to a high degree of effector polymorphism including point mutation, recombination, gene duplication, deletion, and transposon activity [17–19]. Loss-of-function mutagenesis in *Avr* genes through DNA sequence changes has been well-documented in virulent strains [16,20] (Figure 1). In some cases, loss-of-function mutations of an *Avr* can be compensated by functional redundancy amongst paralogous genes, or by unrelated effectors impairing the same protein/hub/pathway, therefore the strains may not demonstrate significant reduction in virulence [14,21•]. In other cases, however, a loss-of-function mutation of *Avr* gene(s) could lead to compromised virulence of the pathogen in compatible hosts [22]. As such, it would be beneficial for the pathogen species to retain the *Avr* genes in the gene pool. Accumulating evidence has suggested gene silencing as another strategy utilized by *Phytophthora* pathogens to abrogate the negative impact of *Avr* genes in incompatible interactions.

Evading ETI by silencing the corresponding *Avr* gene(s), without changing the DNA sequences of the gene-coding region, could be advantageous because this change is easier to be reversed should a condition come up when the cognate NLR is suppressed or lost in a plant host population. The ability to retain virulence activities by recycling *Avr* genes during the highly dynamic host-pathogen co-evolution could be an important strategy for the success of a pathogen. Indeed, transcriptional silencing of *Avr* genes has been recurrently observed in field strains that successfully evaded NLR-based disease resistance [20,21•,23–25] and gene silencing could exert a pervasive effect in shaping *Phytophthora* effectoromes [21•]. In some cases, not only the open reading frame

of the effector genes but also their local flanking sequences are identical with those in the ETI-activating strains, indicating the involvement of epigenetic processes [21•,26••].

The silencing mechanisms deployed on *Avr* genes are best understood in the soybean root rot pathogen *Phytophthora sojae*. One example is from *PsAvr3a*, whose silencing enabled *P. sojae* strains to regain pathogenicity on soybean varieties carrying the NLR gene *Rps3a*. Virulence of these ETI-evading strains can persist over several generations, indicating that silencing of *PsAvr3a* is stable [27•]. Intriguingly, these virulent strains also show the accumulation of 25 nucleotides (nt) small RNAs (sRNAs), which are absent from *PsAvr3a* un-silenced strains. Small RNAs are ubiquitous regulators of gene expression in eukaryotes [28]. *Phytophthora* produces two classes of sRNAs that are predominantly 21-nt and 25-nt in length respectively. The 21-nt sRNAs tend to be spawned from the highly expressed gene loci, whereas the 25-nt sRNAs are mainly derived from transposable elements (TE) and repeat-rich regions [29–31]. The 21-nt and 25-nt sRNA populations also associate with distinct ARGONAUTE proteins, the key component of RNA-induced silencing complexes. Further investigations on the biological function of the 25-nt sRNAs will determine whether there is a direct link between the 25-nt sRNA production and the silencing of *PsAvr3a*.

In addition to post-transcriptional gene silencing, sRNAs can also mediate transcriptional gene silencing by guiding the establishment of heterochromatin through histone and DNA modifications in a sequence-dependent manner [32,33]. Recently, an increased level of histone H3 Lysine27 trimethylation (H3K27me3), a repressive gene expression mark, was observed at the *PsAvr1b* locus of a *P. sojae* isolate, in which the effector was silenced [26••]. Knocking down a core subunit of the H3K27me3 methylation complex resulted in the depletion of H3K27me3 at the *PsAvr1b* locus, which was concomitant with the derepression of *PsAvr1b* expression [26••]. An independent study of another *PsAvr1b*-silenced strain showed that 25-nt sRNAs were accumulated from the *PsAvr1b* locus, but not from the corresponding loci in un-silenced strains [34]. Whether there is a causal relationship between H3K27 methylation and sRNA production remains to be determined in *Phytophthora*. Nonetheless, these studies provide another example of epigenetic regulation of *Avr* genes that involves histone modification and possibly sRNAs (Figure 2a).

Mutability of genes is significantly influenced by genome environment. In some *Phytophthora* species, effector-encoding genes tend to localize to repeat-rich and hyper-methylated compartments [35,36]. A particularly interesting question is whether the physical localization of the effector genes in the genome promotes silencing mediated by sRNAs. In addition to accelerating effector evolution by promoting recombination, residence in repetitive genome regions could also allow heritable but reversible epigenetic regulation of effector gene expression, which presumably increases the adaptation potential of *Phytophthora*.

Epigenetic regulation is widely employed in eukaryotes to achieve precise and quantitative control of gene expression in a temporal-spatial manner [37]. While an expansion of effector repertoire could enhance pathogen virulence, it inevitably increases the chance of being recognized by immune receptors [38], which also undergo rapid sequence diversification and possess a broad recognition capacity [39]. Epigenetic regulation could be advantageous in preserving the virulence activity in the pathogen population while balancing this cost. An interesting observation is that genetic crosses between *PsAvr3a*-silenced and non-silenced *P. sojae* strains generate a F1 hybrid population with variable *PsAvr3a* expression levels [38,40]. Therefore, epigenetic control seems to mediate effector expression plasticity, which could be a robust strategy to enable adaptations to hosts in a short evolutionary scale.

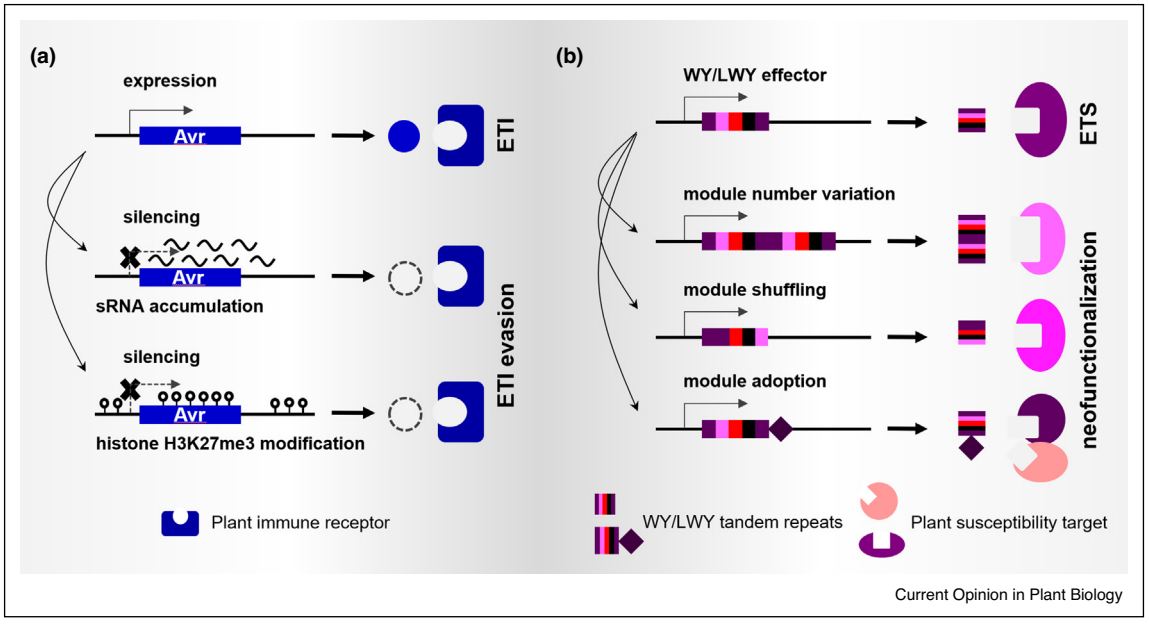
New kids in the block — where did they come from?

A major unanswered question in pathology is how novel virulence activities arise. Studies in apoplastic effectors, which manipulate host cellular processes by functioning in the extracellular space, uncovered mechanisms related to point mutations (e.g. *Phytophthora mirabilis* PmEPIC1) and gene duplication followed by functional diversification (e.g. *P. sojae* XEG1/XLP1) [41,42]. Studies on *Phytophthora* RXLR effectors revealed another mechanism that potentially promotes neofunctionality through protein module shuffling.

Modularity is a feature shared by effectors in many pathogens. In Gram-negative bacteria that utilize the type III secretion system to inject effectors into the host cytosol, the effectors carry the N-terminal 'secretion and translocation' module and the C-terminal 'effector' module. This modularity enables 'terminal reassortment' that promotes the evolution of novel effectors with similar N-terminal modules but distinct effector domains [43]. Another well-known bacterial effector family with modularity is the Transcription Activator-like (TAL) effectors produced by *Xanthomonas* species, where combinations of repeat units with different variant residues allow specific binding of the effectors to host DNA with different lengths and nucleotide sequences [44].

It has been observed that many RXLR effectors in *Phytophthora* also contain one or more L, W and Y motifs [45•], named by the most conserved amino acid residue. The W and Y motifs are almost always co-existing in an

Figure 2



Two mechanisms that promote *Phytophthora* effector evolution highlighted in this review.
(a) Epigenetic gene silencing underpins ETI evasion. Deposition of histone H3K27me3 epigenetic marks or accumulation of endogenous small RNA at *Avr* gene loci are associated with silencing of these effectors in hosts that encode the cognate NLR immune receptor(s). *Phytophthora* strains carrying the epi-allele are able to evade ETI. **(b)** Modularity-driven effector evolution through the linear arrangement of the WY/LWY tandem repeats. Extensive polymorphism resulted from module insertion/deletion, shuffling, and recruitment of additional functional domains potentially promotes the evolution of new virulence functions by targeting novel host targets.

effector and each WY domain forms a highly similar three or four α-helix bundle [46•,47–49]. In addition to *Phytophthora*, WY domains are also enriched in effectors produced by other oomycete pathogens [46•,50]. Recently, the structure of an additional WY effector *Ps*PSR2 (*P. sojae Phytophthora* suppressor of RNA silencing 2) was solved, unearthing the contribution of a newly defined L-WY motif to the evolvability of *Phytophthora* effectors [51••].

The entire effector domain of *Ps*PSR2 consists of seven tandem repeat units. Except for the first WY unit, the following six units all have an L motif; and each of the LWY units forms a highly similar five-helix fold despite limited sequence similarity. Importantly, the L motif acts as a hinge to stabilize the concatenation of individual units, resulting in a highly organized, overall linear structure of *Ps*PSR2. The extended, non-globular architecture leads to larger surface areas, presumably promoting the interactions with (multiple) host molecules and/or the formation of novel protein complexes by simultaneous interacting with host proteins that would otherwise not interact (Figure 2b). Indeed, the first three repeat units in *Ps*PSR2 is sufficient to mediate the interaction with a host target protein Double stranded RNA-binding protein 4 (DRB4) [52]. While the function of the other four units remains unknown, they may mediate interaction(s) with additional host target(s), potentially facilitating the formation of larger protein complexes. Interestingly, effectors with the LWY units tend to have a higher number of repeats compared to effectors that do not have the WY/LWY repeats or only have the WY repeats, consistent with the potential of an increased interaction capacity [51••].

The LWY tandem repeats are found in numerous RXLR effectors, which are widely present in *Phytophthora* species. Sequence conservation of the LWY motifs is restricted to a small number of buried residues that are essential to maintain the fold of individual units, and stabilize unit concatenation and the overall linear structure of the effectors. This high level of sequence plasticity on the surface residues may promote divergent evolution of the effectors and facilitate functional differentiation. Many LWY effectors are chimeras of LWY units with diversified surface residues, adding another layer of potentiality to evolve novel activities [51••]. Indeed, *Phytophthora* effectors with different WY/LWY domain(s) or domain combinations demonstrate distinct molecular functions in host plants [52–55]. Moreover, unrelated functional domain(s) can be recruited to WY/LWY effectors outside the repeat region [56], further expanding the diversity of effector function (Figure 2b). These findings suggest a role of the WY/LWY motif in the generation of a diverse effector repertoire in *Phytophthora*.

Future perspectives — a race in the new era

Specific interactions of pathogen effectors and their host targets or immune receptors determine whether disease will occur. Although steady progress has been made in understanding their virulence mechanisms, how effectors rapidly evolve in the context of host-pathogen arms race remain largely enigmatic. Recent findings in *Phytophthora* RXLR effectors provide new opportunities to elucidate mechanisms underlying effector evolution.

A budding area in effector biology is epigenetic regulation. Although evasion of host recognition by *Avr* gene silencing has been repeatedly observed, direct links between histone modification, sRNA production, and gene silencing have not been formed and factors that modulate the epigenetic regulations yet to be identified. Looking forward, large-scale, multi-dimensional analyses of epigenome and transcriptome using cutting-edge technologies such as long-reads RNA sequencing, ATAC (Assay for Transposase-Accessible Chromatin)-seq, and probe-enriched effector sequencing (Penseq) [57] in field isolates of *Phytophthora*, will provide key information that facilitates an overall understanding of the expression dynamics of effector genes during natural infection of hosts encoding different NLRs. Results from these analyses will then pave the foundation for in-depth genetic studies to uncover the underlying mechanisms of effector gene regulation. The establishment of CRISPR/Cas9-based genome editing technology in some *Phytophthora* species enhances the functional characterization of genes with known or newly identified functions in epigenetic regulation on modulating effector expression.

Tandem repeats are hot spots for recombination-based gene evolution. Although modularity is widely present in effectors, how recombination may accelerate the evolution of novel virulence activities is largely unknown. The WY/LWY effectors of *Phytophthora* and other oomycete pathogens offer a unique opportunity to elucidate repeat-driven evolution in pathogenicity. In particular, the overall linear structure of the LWY effectors makes it possible to assign specific activities and host targets to distinct repeat units or unit combinations using a systemic approach. The rich resources of *Phytophthora* genome sequences and an accumulating knowledge of the virulence activities of the WY/LWY effectors will propel a fundamental understanding of effector evolution through modularity-mediated neofunctionalization and multifunctionalization.

Author contributions

W. M. and S. D. drafted and edited the manuscript.

Conflict of interest statement

Nothing declared.

Acknowledgements

Authors would like to thank Kangping Li (Nanjing Agricultural University) for assistance in organizing the literature. Research on *Phytophthora* effectors in the W.M. group has been supported by National Science Foundation (NSF) grant IOS-1758889, USDA-NIFA award # 2018-67014-28488, and the Gatsby Charitable Foundation. S.D. has been supported by grants from the Chinese National Science Fund (31972252, 31721004).

Declaration of Competing Interest

The authors report no declarations of interest.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Jones JD, Dangl JL: **The plant immune system**. *Nature* 2006, **444**:323-329.
2. Zhou JM, Zhang Y: **Plant immunity: danger perception and signaling**. *Cell* 2020, **181**:978-989.
3. Couto D, Zipfel C: **Regulation of pattern recognition receptor signalling in plants**. *Nat Rev Immunol* 2016, **16**:537-552.
4. Adachi H, Derevnina L, Kamoun S: **NLR singletons, pairs, and networks: evolution, assembly, and regulation of the intracellular immunoreceptor circuitry of plants**. *Curr Opin Plant Biol* 2019, **50**:121-131.
5. Jones JD, Vance RE, Dangl JL: **Intracellular innate immune surveillance devices in plants and animals**. *Science* 2016, **354**.
6. Upson JL, Zess EK, Białas A, Wu CH, Kamoun S: **The coming of age of EvoMPMI: evolutionary molecular plant-microbe interactions across multiple timescales**. *Curr Opin Plant Biol* 2018, **44**:108-116.
7. Kamoun S, Furzer O, Jones JD, Judelson HS, Ali GS, Dalio RJ, Roy SG, Schena L, Zambounis A, Panabières F *et al.*: **The Top 10 oomycete pathogens in molecular plant pathology**. *Mol Plant Pathol* 2015, **16**:413-434.
8. McGowan J, Fitzpatrick DA: **Genomic, network, and phylogenetic analysis of the oomycete effector arsenal**. *mSphere* 2017, **2**.
9. Franceschetti M, Maqbool A, Jiménez-Dalmaroni MJ, Pennington HG, Kamoun S, Banfield MJ: **Effectors of filamentous plant pathogens: commonalities amid diversity**. *Microbiol Mol Biol Rev* 2017, **81**.
10. Pritchard L, Birch PR: **The zigzag model of plant-microbe interactions: is it time to move on?** *Mol Plant Pathol* 2014, **15**:865-870.
11. Whisson SC, Boevink PC, Moleleki L, Avrova AO, Morales JG, Gilroy EM, Armstrong MR, Grouffaud S, van West P, Chapman S *et al.*: **A translocation signal for delivery of oomycete effector proteins into host plant cells**. *Nature* 2007, **450**:115-118.
12. Białas A, Zess EK, De la Concepcion JC, Franceschetti M, Pennington HG, Yoshida K, Upson JL, Chanclud E, Wu CH, Langner T *et al.*: **Lessons in effector and NLR biology of plant-microbe systems**. *Mol Plant Microbe Interact* 2018, **31**:34-45.
13. Anderson RG, Deb D, Fedkenheuer K, McDowell JM: **Recent progress in RXLR effector research**. *Mol Plant Microbe Interact* 2015, **28**:1063-1072.
14. Yin W, Dong S, Zhai L, Lin Y, Zheng X, Wang Y: **The *Phytophthora sojae* Avr1d gene encodes an RxLR-dEER effector with presence and absence polymorphisms among pathogen strains**. *Mol Plant Microbe Interact* 2013, **26**:958-968.
15. Zhang F, Chen H, Zhang X, Gao C, Huang J, Li L, Shen D, Wang L, Huang C, Ye W *et al.*: **Genome analysis of two newly emerged potato late blight isolates shed lights on pathogen adaptation and provides tools for disease management**. *Phytopathology* 2020, **111**:96-107.

16. Vleeshouwers VG, Raffaele S, Vossen JH, Champouret N, Oliva R, Segretin ME, Rietman H, Cano LM, Lokossou A, Kessel G *et al.*: **Understanding and exploiting late blight resistance in the age of effectors**. *Annu Rev Phytopathol* 2011, **49**:507-531.

17. Raffaele S, Farrer RA, Cano LM, Studholme DJ, MacLean D, Thines M, Jiang RH, Zody MC, Kunjeti SG, Donofrio NM *et al.*: **Genome evolution following host jumps in the Irish potato famine pathogen lineage**. *Science* 2010, **330**:1540-1543.

18. Knaus BJ, Tabima JF, Shakya SK, Judelson HS, Grünwald NJ: **Genome-wide increased copy number is associated with emergence of dominant clones of the Irish potato famine pathogen *Phytophthora infestans***. *mBio* 2020, **11**.

19. Goss EM, Press CM, Grünwald NJ: **Evolution of RXLR-class effectors in the oomycete plant pathogen *Phytophthora ramorum***. *PLoS One* 2013, **8**:e79347.

20. Zhang X, Liu B, Zou F, Shen D, Yin Z, Wang R, He F, Wang Y, Tyler BM, Fan W *et al.*: **Whole genome re-sequencing reveals natural variation and adaptive evolution of *Phytophthora sojae***. *Front Microbiol* 2019, **10**:2792.

21. • Pais M, Yoshida K, Giannakopoulou A, Pel MA, Cano LM, Oliva RF, Witek K, Lindqvist-Kreuze H, Vleeshouwers V, Kamoun S: **Gene expression polymorphism underpins evasion of host immunity in an asexual lineage of the Irish potato famine pathogen**. *BMC Evol Biol* 2018, **18**:93

This study revealed the importance of effector epi-alleles in breaking NLR-based resistance in a pandemic clonal lineage of *Phytophthora infestans,* generalizing the pioneering work from *Phytophthora sojae*. Importantly, this work illustrates how gene silencing can exert a pervasive effect on the effectorome.

22. Huang J, Gu L, Zhang Y, Yan T, Kong G, Kong L, Guo B, Qiu M, Wang Y, Jing M *et al.*: **An oomycete plant pathogen reprograms host pre-mRNA splicing to subvert immunity**. *Nat Commun* 2017, **8**:2051.

23. Qutob D, Tedman-Jones J, Dong S, Kuflu K, Pham H, Wang Y, Dou D, Kale SD, Arredondo FD, Tyler BM *et al.*: **Copy number variation and transcriptional polymorphisms of *Phytophthora sojae* RXLR effector genes Avr1a and Avr3a**. *PLoS One* 2009, **4**: e5066.

24. Na R, Yu D, Chapman BP, Zhang Y, Kuflu K, Austin R, Qutob D, Zhao J, Wang Y, Gijzen M: **Genome re-sequencing and functional analysis places the *Phytophthora sojae* avirulence genes Avr1c and Avr1a in a tandem repeat at a single locus**. *PLoS One* 2014, **9**:e89738.

25. Shan W, Cao M, Leung D, Tyler BM: **The Avr1b locus of *Phytophthora sojae* encodes an elicitor and a regulator required for avirulence on soybean plants carrying resistance gene Rps1b**. *Mol Plant Microbe Interact* 2004, **17**:394-403.

26. •• Wang L, Chen H, Li J, Shu H, Zhang X, Wang Y, Tyler BM, Dong S: **Effector gene silencing mediated by histone methylation underpins host adaptation in an oomycete plant pathogen**. *Nucleic Acids Res* 2020, **48**:1790-1799

This study illustrates a model in which the transcriptional polymorphisms of *Phytophthora* avirulence effectors are correlated with the status of a repressive histone methylation mark, highlighting chromatin epigenetic variation as a mechanism that regulates effector gene expression.

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

This is the first report that discovered a link between effector gene silencing and the accumulation of small RNAs, highlighting a role of epigenetic modulation in determining the virulence type of *Phytophthora* pathogens.

28. Castel SE, Martienssen RA: **RNA interference in the nucleus: roles for small RNAs in transcription, epigenetics and beyond**. *Nat Rev Genet* 2013, **14**:100-112.

29. Åsman AK, Fogelqvist J, Vetukuri RR, Dixelius C: ***Phytophthora infestans* Argonaute 1 binds microRNA and small RNAs from effector genes and transposable elements**. *New Phytol* 2016, **211**:993-1007.

30. Jia J, Lu W, Zhong C, Zhou R, Xu J, Liu W, Gou X, Wang Q, Yin J, Xu C *et al.*: **The 25-26 nt small RNAs in *Phytophthora parasitica* are associated with efficient silencing of homologous endogenous genes**. *Front Microbiol* 2017, **8**:773.

31. Fahlgren N, Bollmann SR, Kasschau KD, Cuperus JT, Press CM, Sullivan CM, Chapman EJ, Hoyer JS, Gilbert KB, Grünwald NJ *et al.*: ***Phytophthora* have distinct endogenous small RNA populations that include short interfering and microRNAs**. *PLoS One* 2013, **8**:e77181.

32. Lewsey MG, Hardcastle TJ, Melnyk CW, Molnar A, Valli A, Urich MA, Nery JR, Baulcombe DC, Ecker JR: **Mobile small RNAs regulate genome-wide DNA methylation**. *Proc Natl Acad Sci U S A* 2016, **113**:E801-810.

33. Mao H, Zhu C, Zong D, Weng C, Yang X, Huang H, Liu D, Feng X, Guang S: **The Nrde pathway mediates small-RNA-directed histone H3 lysine 27 trimethylation in *Caenorhabditis elegans***. *Curr Biol* 2015, **25**:2398-2403.

34. Wang Q, Li T, Zhong C, Luo S, Xu K, Gu B, Meng Y, Tyler BM, Shan W: **Small RNAs generated by bidirectional transcription mediate silencing of RXLR effector genes in the oomycete *Phytophthora sojae***. *Phytopathol Res* 2019, **1**.

35. Haas BJ, Kamoun S, Zody MC, Jiang RH, Handsaker RE, Cano LM, Grabherr M, Kodira CD, Raffaele S, Torto-Alalibo T *et al.*: **Genome sequence and analysis of the Irish potato famine pathogen *Phytophthora infestans***. *Nature* 2009, **461**:393-398.

36. Chen H, Shu H, Wang L, Zhang F, Li X, Ochola SO, Mao F, Ma H, Ye W, Gu T *et al.*: ***Phytophthora* methylomes are modulated by 6mA methyltransferases and associated with adaptive genome regions**. *Genome Biol* 2018, **19**:181.

37. Gómez-Díaz E, Jordà M, Peinado MA, Rivero A: **Epigenetics of host-pathogen interactions: the road ahead and the road behind**. *PLoS Pathog* 2012, **8**:e1003007.

38. Na R, Gijzen M: **Escaping host immunity: new tricks for plant pathogens**. *PLoS Pathog* 2016, **12**:e1005631.

39. Barragan AC, Weigel D: **Plant NLR diversity: the known unknowns of pan-NLRomes**. *Plant Cell* 2020 http://dx.doi.org/10.1093/plcell/koaa002.

40. Shrestha SD, Chapman P, Zhang Y, Gijzen M: **Strain specific factors control effector gene silencing in *Phytophthora sojae***. *PLoS One* 2016, **11**:e0150530.

41. Dong S, Stam R, Cano LM, Song J, Sklenar J, Yoshida K, Bozkurt TO, Oliva R, Liu Z, Tian M *et al.*: **Effector specialization in a lineage of the Irish potato famine pathogen**. *Science* 2014, **343**:552-555.

42. Ma Z, Zhu L, Song T, Wang Y, Zhang Q, Xia Y, Qiu M, Lin Y, Li H, Kong L *et al.*: **A paralogous decoy protects *Phytophthora sojae* apoplastic effector PsXEG1 from a host inhibitor**. *Science* 2017, **355**:710-714.

43. Stavrinides J, Ma W, Guttman DS: **Terminal reassortment drives the quantum evolution of type III effectors in bacterial pathogens**. *PLoS Pathog* 2006, **2**:e104.

44. Hummel AW, Wilkins KE, Wang L, Cernadas RA, Bogdanove AJ: **A transcription activator-like effector from *Xanthomonas oryzae* pv. *oryzicola* elicits dose-dependent resistance in rice**. *Mol Plant Pathol* 2017, **18**:55-66.

45. • Jiang RH, Tripathy S, Govers F, Tyler BM: **RXLR effector reservoir in two *Phytophthora* species is dominated by a single rapidly evolving superfamily with more than 700 members**. *Proc Natl Acad Sci U S A* 2008, **105**:4874-4879

This bioinformatic analysis discovered the widely spread effector motifs L, W and Y from two *Phytophthora* species, which were later found to be prevalent not only in *Phytophthora* but also other oomycete pathogens. The authors proposed that these motifs may facilitate pathogen adaptation to plant hosts.

46. • Win J, Krasileva KV, Kamoun S, Shirasu K, Staskawicz BJ, Banfield MJ: **Sequence divergent RXLR effectors share a structural fold conserved across plant pathogenic oomycete species**. *PLoS Pathog* 2012, **8**:e1002400

This paper systemically analyzed the structures of oomycete effectors harboring the WY domain(s) and proposed that the conserved WY fold is critical for effector functions by providing both structural stability and plasticity.

47. Boutemy LS, King SR, Win J, Hughes RK, Clarke TA, Blumenschein TM, Kamoun S, Banfield MJ: **Structures of *Phytophthora* RXLR effector proteins: a conserved but adaptable fold underpins functional diversity**. *J Biol Chem* 2011, **286**:35834-35842.

48. Guo B, Wang H, Yang B, Jiang W, Jing M, Li H, Xia Y, Xu Y, Hu Q, Wang F *et al.*: ***Phytophthora sojae* effector PsAvh240 inhibits host aspartic protease secretion to promote infection**. *Mol Plant* 2019, **12**:552-564.

49. Maqbool A, Hughes RK, Dagdas YF, Tregidgo N, Zess E, Belhaj K, Round A, Bozkurt TO, Kamoun S, Banfield MJ: **Structural basis of host autophagy-related protein 8 (ATG8) binding by the Irish potato famine pathogen effector protein PexRD54**. *J Biol Chem* 2016, **291**:20270-20282.

50. Wood KJ, Nur M, Gil J, Fletcher K, Lakeman K, Gann D, Gothberg A, Khuu T, Kopetzky J, Naqvi S *et al.*: **Effector prediction and characterization in the oomycete pathogen *Bremia lactucae* reveal host-recognized WY domain proteins that lack the canonical RXLR motif**. *PLoS Pathog* 2020, **16**: e1009012.

51. •• He J, Ye W, Choi DS, Wu B, Zhai Y, Guo B, Duan S, Wang Y, Gan J, Ma W *et al.*: **Structural analysis of *Phytophthora* suppressor of RNA silencing 2 (PSR2) reveals a conserved modular fold contributing to virulence**. *Proc Natl Acad Sci U S A* 2019, **116**:8054-8059

This work reports the crystal structure of a *Phytophthora* RXLR effector, leading to the discovery of a conserved concatenation mechanism conferred by the L motif of the LWY modules that are arranged as highly organized tandem repeats. This finding provides novel insights into mechanisms underlying effector diversification and adaptation.

52. Hou Y, Zhai Y, Feng L, Karimi HZ, Rutter BD, Zeng L, Choi DS, Zhang B, Gu W, Chen X *et al.*: **A *Phytophthora* effector suppresses trans-kingdom RNAi to promote disease susceptibility**. *Cell Host Microbe* 2019, **25**:153-165.e5.

53. He Q, McLellan H, Hughes RK, Boevink PC, Armstrong M, Lu Y, Banfield MJ, Tian Z, Birch PRJ: ***Phytophthora infestans* effector SFI3 targets potato UBK to suppress early immune transcriptional responses**. *New Phytol* 2019, **222**:438-454.

54. Zhang P, Jia Y, Shi J, Chen C, Ye W, Wang Y, Ma W, Qiao Y: **The WY domain in the *Phytophthora* effector PSR1 is required for infection and RNA silencing suppression activity**. *New Phytol* 2019, **223**:839-852.

55. Ye W, Ma W: **Filamentous pathogen effectors interfering with small RNA silencing in plant hosts**. *Curr Opin Microbiol* 2016, **32**:1-6.

56. Dagdas YF, Belhaj K, Maqbool A, Chaparro-Garcia A, Pandey P, Petre B, Tabassum N, Cruz-Mireles N, Hughes RK, Sklenar J *et al.*: **An effector of the Irish potato famine pathogen antagonizes a host autophagy cargo receptor**. *eLife* 2016, **5**.

57. Lin X, Song T, Fairhead S, Witek K, Jouet A, Jupe F, Witek AI, Karki HS, Vleeshouwers VGAA, Hein I *et al.*: **Identification of Avramr1 from *Phytophthora infestans* using long read and cDNA pathogen-enrichment sequencing (PenSeq)**. *Mol Plant Pathol* 2020, **21**:1502-1512.