

Evolution of *Actinidia* resistance genes

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INTRODUCTION

Pseudomonas syringae pv. *actinidiae* (Psa) is a causative agent of bacterial canker of kiwifruit (*Actinidia chinensis*). The first two discovered lineages Psa-1 and Psa-2, have caused localized epidemics in orchards in Japan and South Korea, respectively. However, the more recently emergent pandemic lineage Psa-3 has caused considerable damage in kiwifruit orchards worldwide. In all these cases the evidence points towards the endemic populations of wild *Actinidia* serving as a natural reservoir of Psa¹. While there is growing understanding of Psa-3 virulence and effector repertoire, little is known about the other described Psa lineages. And so far, no *Actinidia* nucleotide-binding and leucine-rich repeat (NLR) gene that interacts with Psa effectors has been identified.

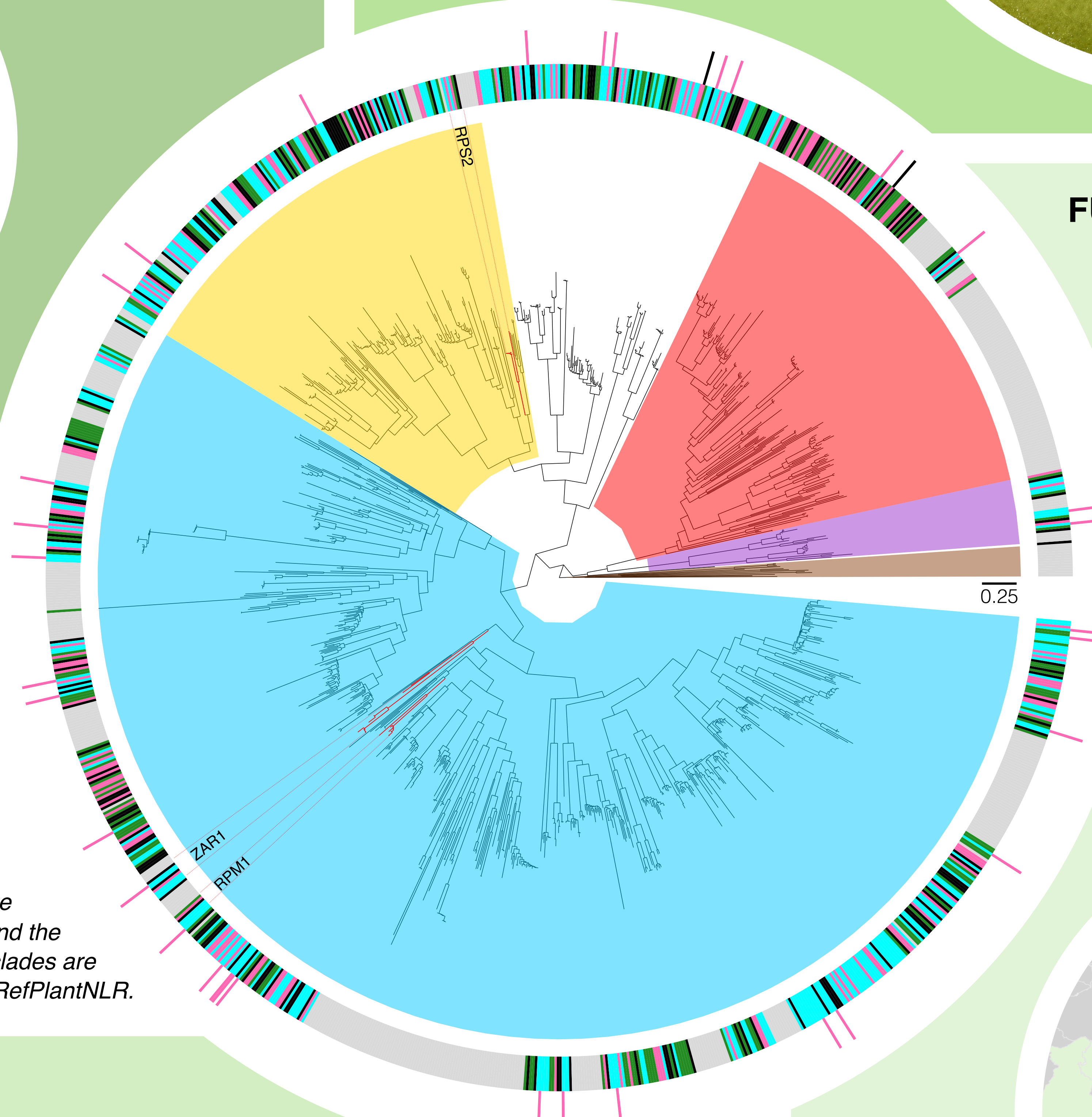
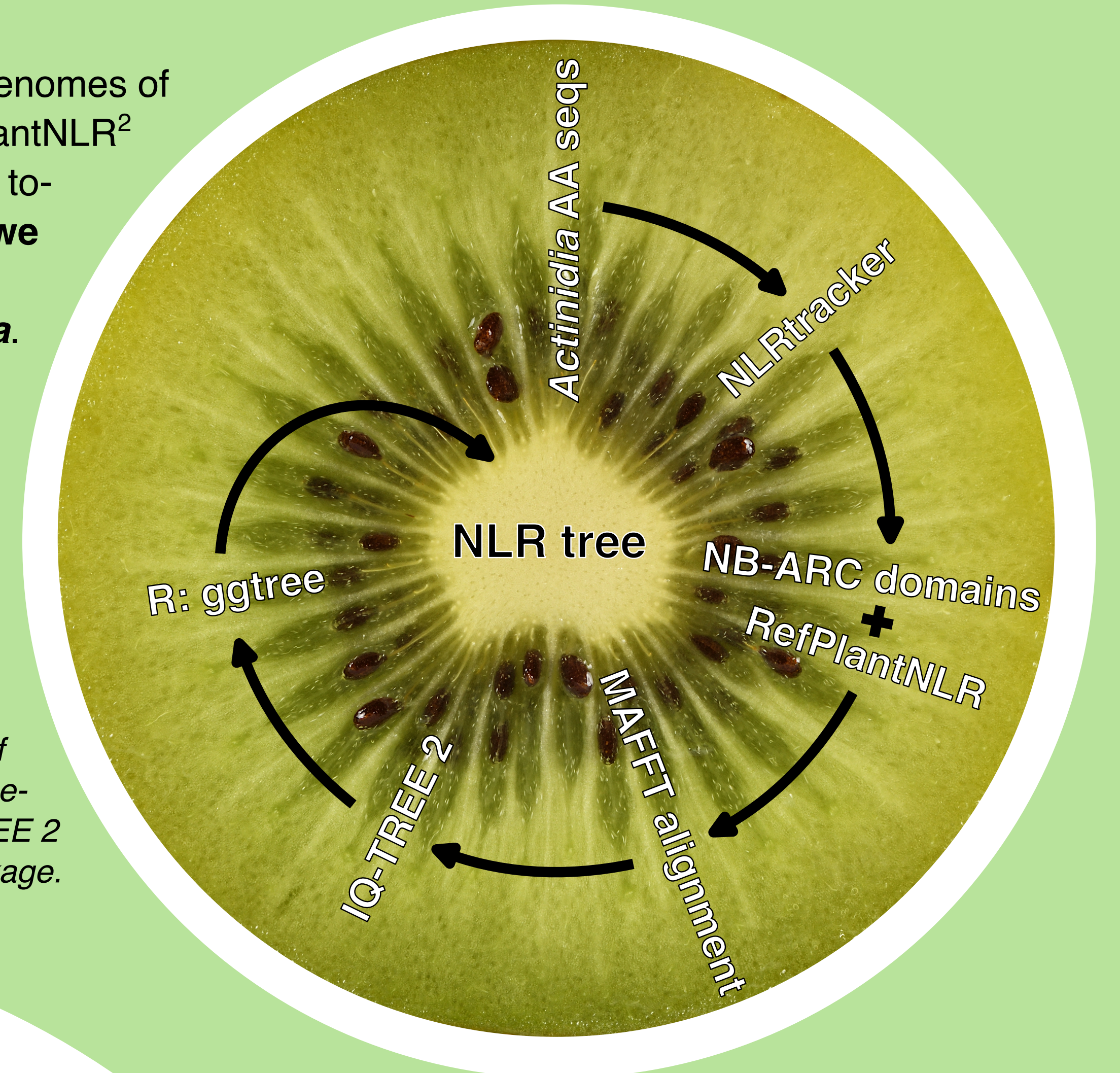


↑ Fig. 1: The first typical symptoms of bacterial canker of kiwifruit caused by Psa are dark, angular leaf spots surrounded by yellow halo.

METHODS

We leveraged publicly available genomes of *Actinidia*. By employing the RefPlantNLR² database of reference plant NLRs together with the NLRtracker tool², we assessed the NLR repertoire within three species of *Actinidia*. For the analysis of effector repertoires across known Psa lineages, we conducted a thorough review of over 50 articles.

→ Fig. 2: NLRtracker annotates NLRs from protein predictions and extracts their NB-ARC domains. We then align these domains together with domains of RefPlantNLR using MAFFT. The phylogenetic NLR tree is constructed by IQ-TREE 2 and visualized in R utilizing ggtree package.



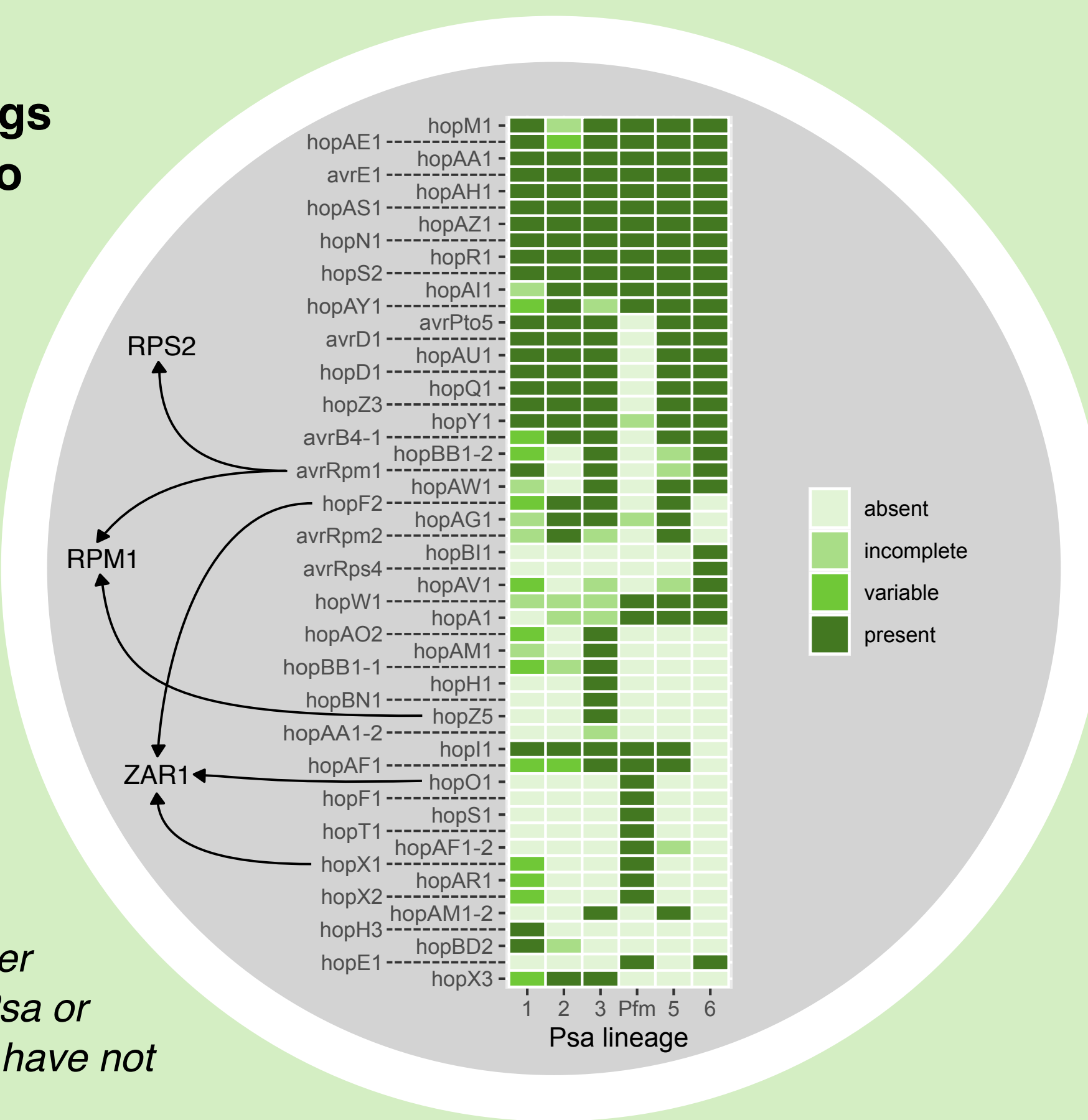
NLR clades	NLR source
Non-plant NLRs	A. eriantha
CC _R -NLRs	A. rufa
TIR-NLRs	Acc HY
CC _{G10} -NLRs	Acc R5
CC-NLRs	RefPlantNLR

→ Fig. 3: Phylogenetic tree of NLRs from three *Actinidia* species as indicated by the ring around the tree (Acc = *A. chinensis* var. *chinensis*). NLR clades are highlighted according to NLR classification in RefPlantNLR.

RESULTS

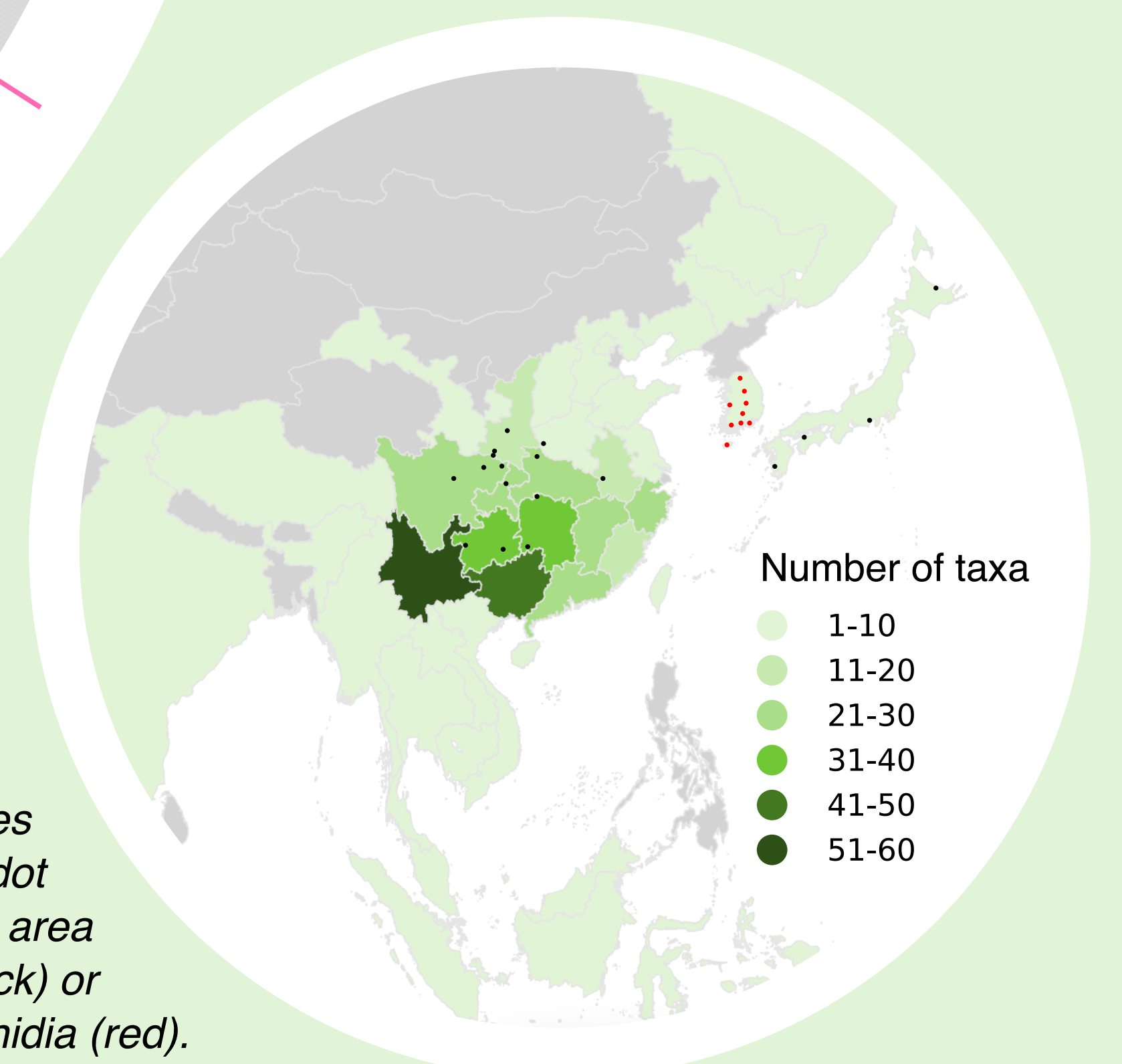
- considerable NLR expansion of putative CC_{G10}-NLRs in the genus *Actinidia*
- we discovered three kiwifruit NLR homologs that can recognize effector homologs also found in Psa
- the pandemic Psa-3 lineage has effectors that are recognized by all three NLRs for which we found kiwifruit homologs

→ Fig. 4: Presence/absence scheme of effectors across all Psa lineages (Psa-1 to Psa-6). Pfm is a former Psa-4 lineage, reclassified as *P. syringae* pv. *actinidifoliorum*. Arrows pointing to NLRs (RPS2, RPM1, and ZAR1) indicate that a particular effector was shown to be recognized by certain NLR in another system. Given effectors could have originated from Psa or other *P. syringae* pathovars. However, kiwifruit NLRs have not been used so far for experimental validation.



FUTURE DIRECTIONS

To address the knowledge gap of Psa-*Actinidia* co-evolution in the wild, we plan to do whole genome sequencing of both the pathogen and the host from natural populations. This enables us to uncover the Psa effectors and *Actinidia* immunity genes circulating in the wild. For this purpose we collected samples across South Korea last year, obtaining both host tissue and live Psa isolates. Additionally, we can take advantage of our collection of Psa isolates from China and Japan.



→ Fig. 5: Map of *Actinidia* native range. Taxa richness corresponds to shades of green. Each dot marks sampling area of only Psa (black) or both Psa & *Actinidia* (red).

FUNDING



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REFERENCES

¹McCann et al. (2017). Origin and Evolution of the Kiwifruit Canker Pandemic. *Genome Biol Evol.* doi: 10.1093/gbe/evx055

²Kourelis et al. (2021). RefPlantNLR is a comprehensive collection of experimentally validated plant disease resistance proteins from the NLR family. *PLoS Bio.* doi: 10.1371/journal.pbio.3001124

ACKNOWLEDGEMENT

Kevin D Murray, Luisa Teasdale