

How Plant Genetics Taught Me To Love Microbes

Detlef Weigel

Max Planck Institute for
Developmental Biology



@PlantEvolution

Disclaimers



weigelworld.org



weigelworld

plant genetics, immunity and
evolutionary genomics.

Key Contributors



Anette Habring
Derek Lundberg

Cristina Barragan (now TSL)
Dan Koenig (now UCR)
Talia Karasov (now Univ. Utah)
Lei Li (now CAS/IGDB)
Manuela Neumann (now Bosch Global)
Julian Regalado (now Univ. Copenhagen)
Or Shalev (now Univ. Tübingen)

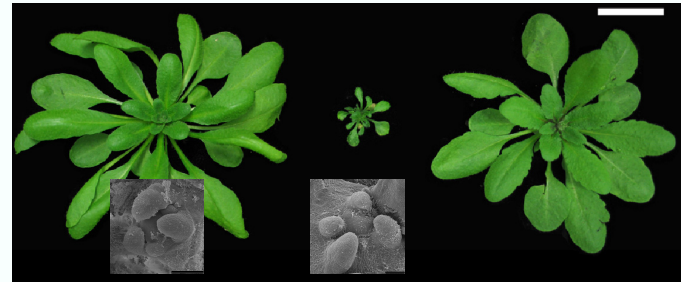
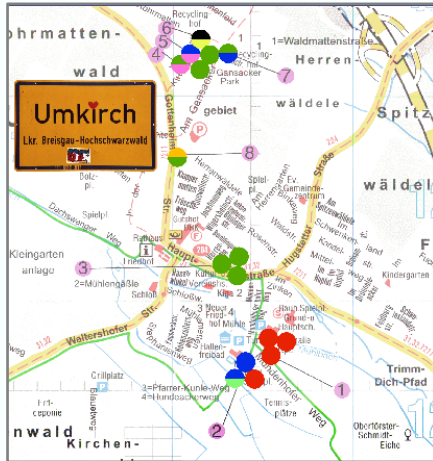
Jeff Dangl (UNC)
Eric Kemen (Univ. Tübingen)
Jonathan Jones (TSL)



Hybrid Weakness in Plants



Janne
Lempe
(JKI)



Uk-1

F₁

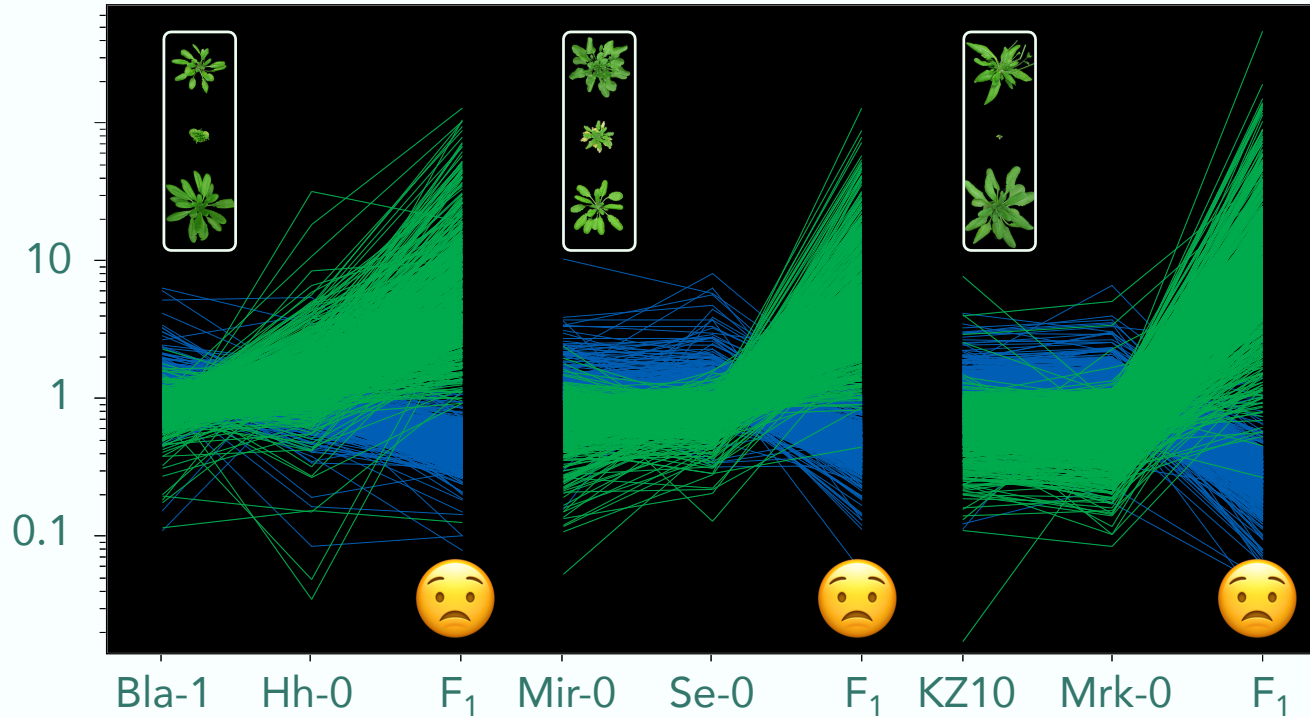
Uk-3

Several similar cases



Kirsten
Bomblies
(ETH)

Common Transcriptional Signatures



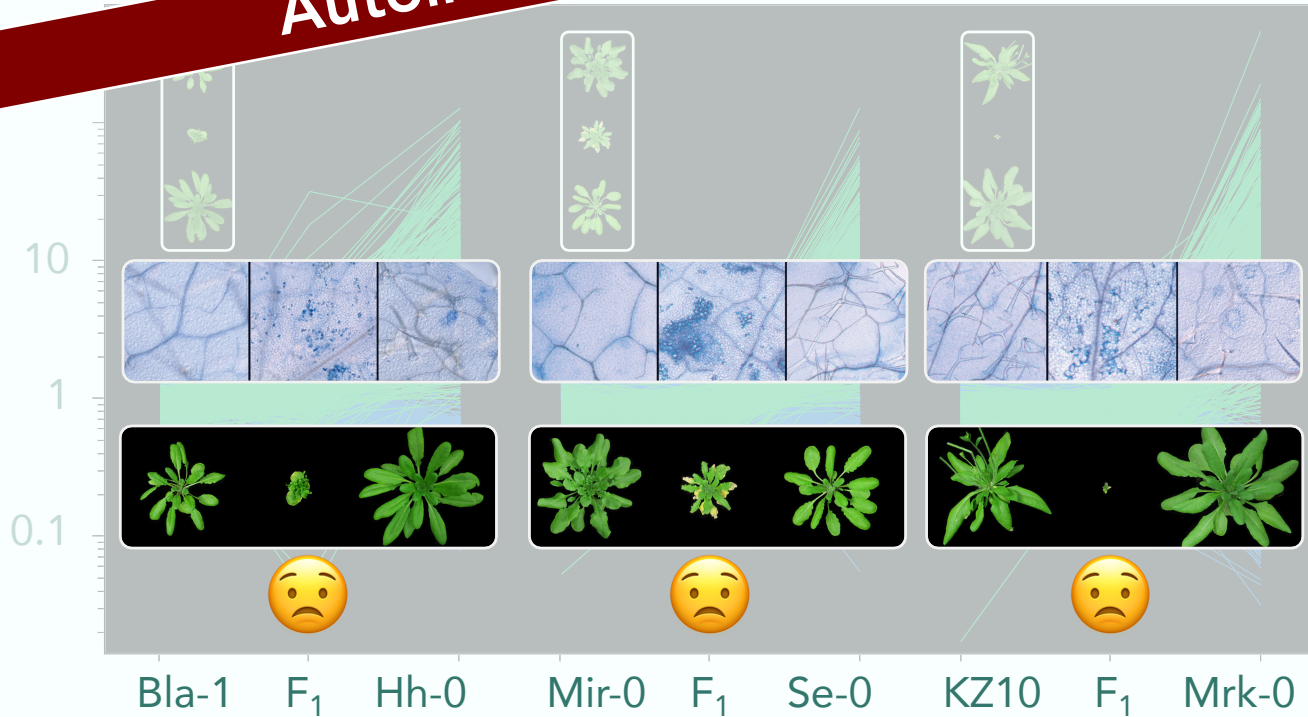
Only overrepresented GO categories relate to immune response

Bomblies, Lempe et al. (2007)

Common Transcriptional Signatures



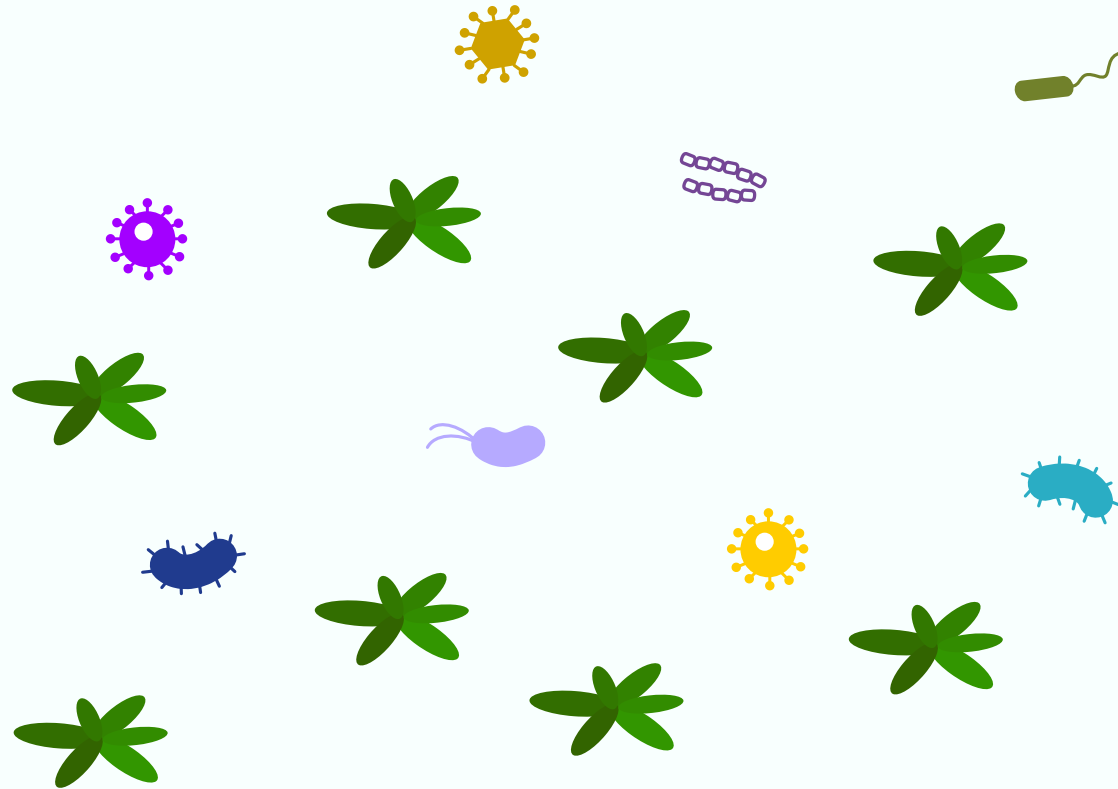
Autoimmunity



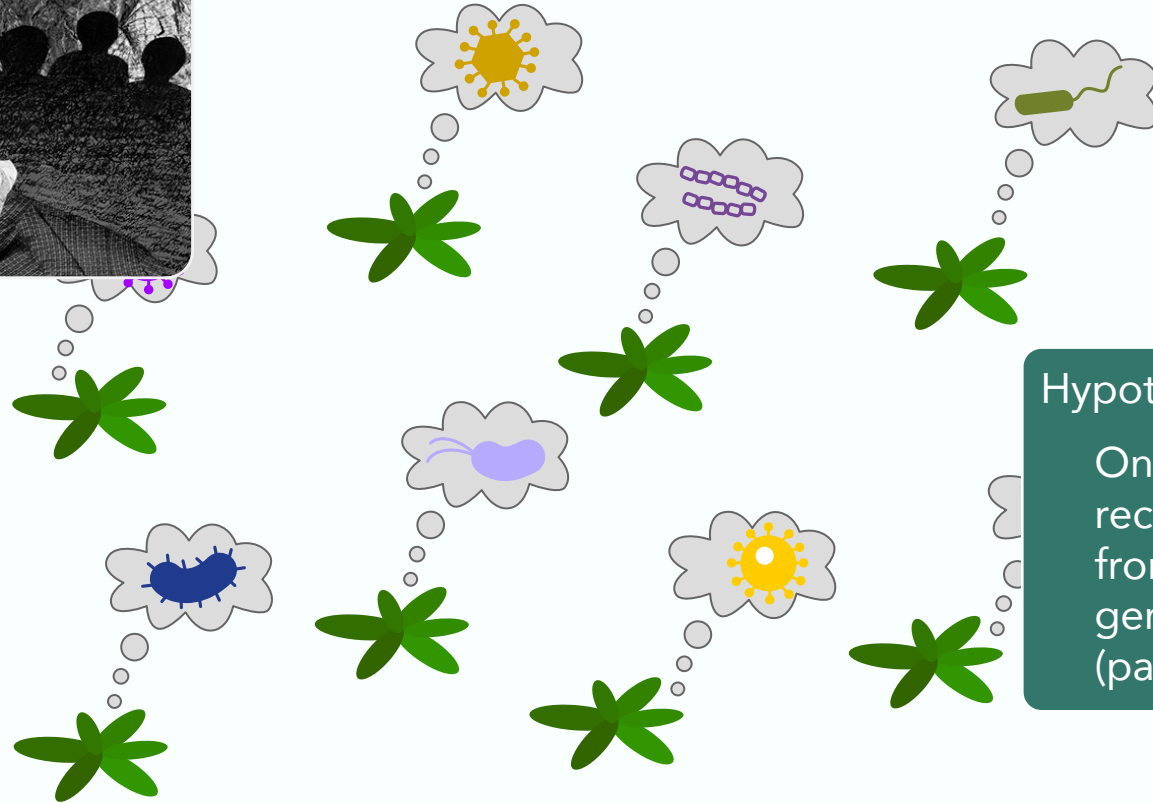
With
Dangl
lab

Bomblies, Lempe et al. (2007)

Dangerous Microbes Are Everywhere!



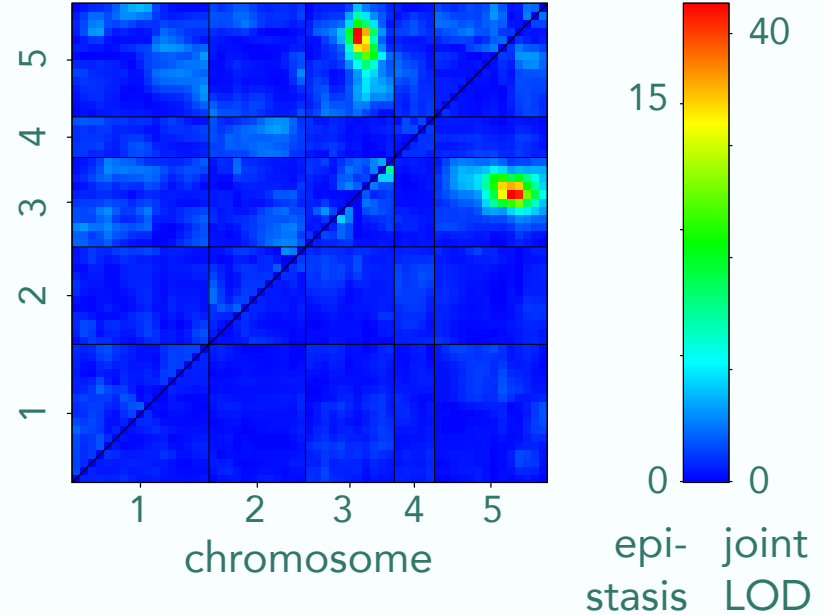
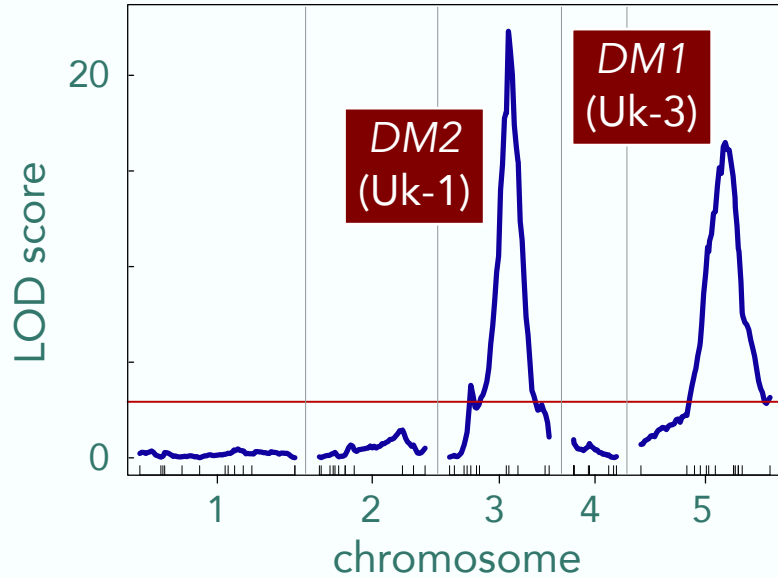
Dangerous Microbes Are Everywhere! (Are They?)



Hypothesis:

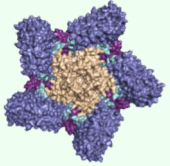
One genome recognizes something from the other genome as foreign (pathogen derived)

Simple Genetics of Hybrid Necrosis



Dangerous Mix (DM)

DM1 Encodes an NLR Immune Receptor



Nucleotide binding site
Leucine rich repeat
Receptor

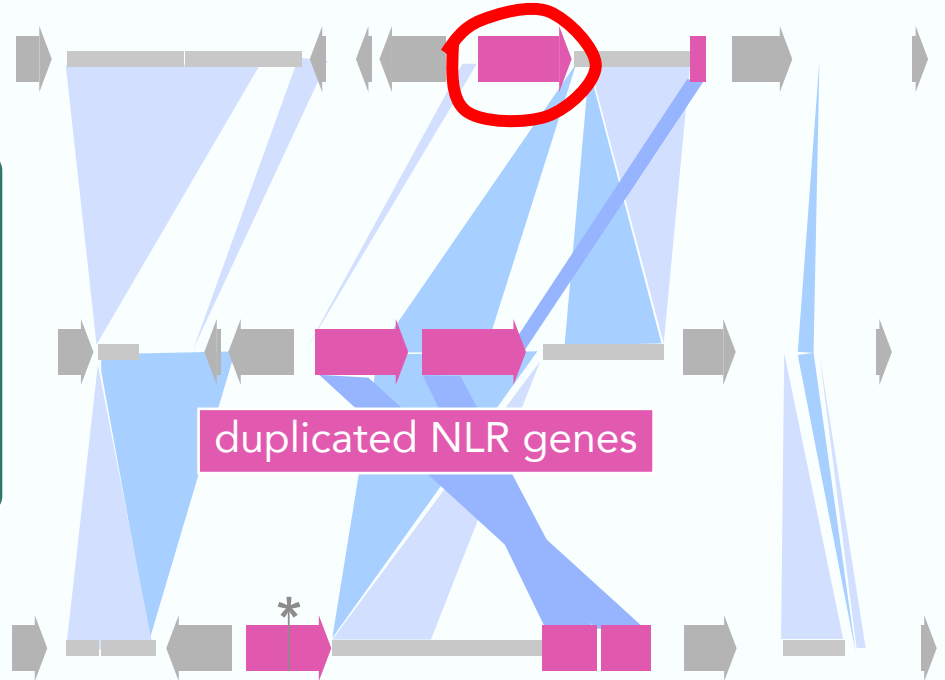
Hypothesis:

One genome recognizes something from the other genome as foreign (pathogen derived)

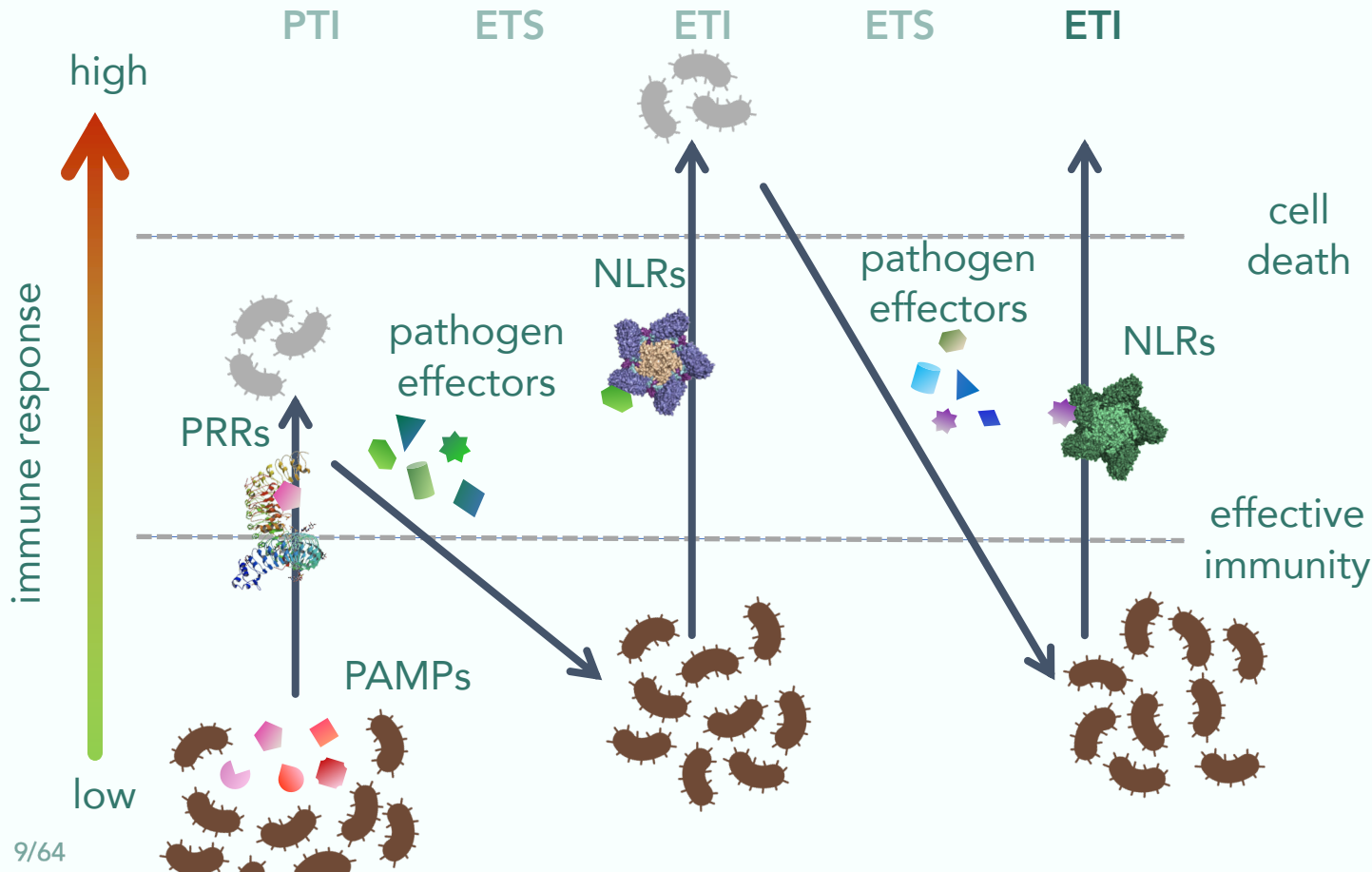
bad allele (Uk-3)

good allele (Uk-1)

20% aa differences



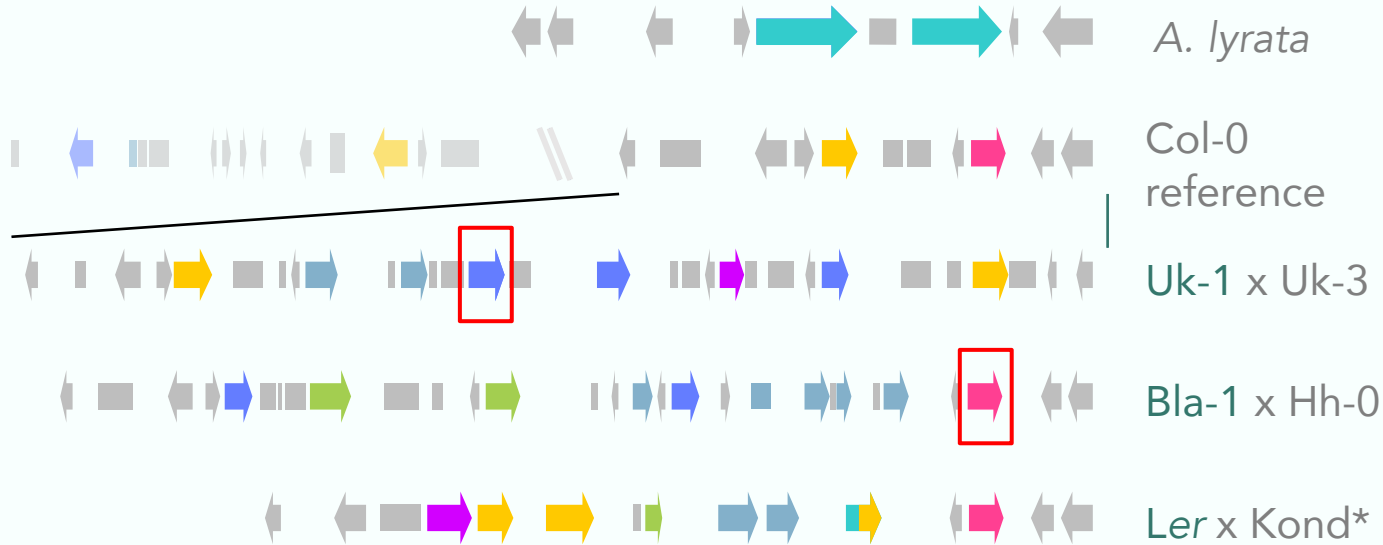
ETS-ETI Cycles in Plant Immunity



Jones & Dangl,
Nature (2006)

PTI
PAMP
Triggered
Immunity

...DM2 Encodes an NLR Immune Receptor As Well



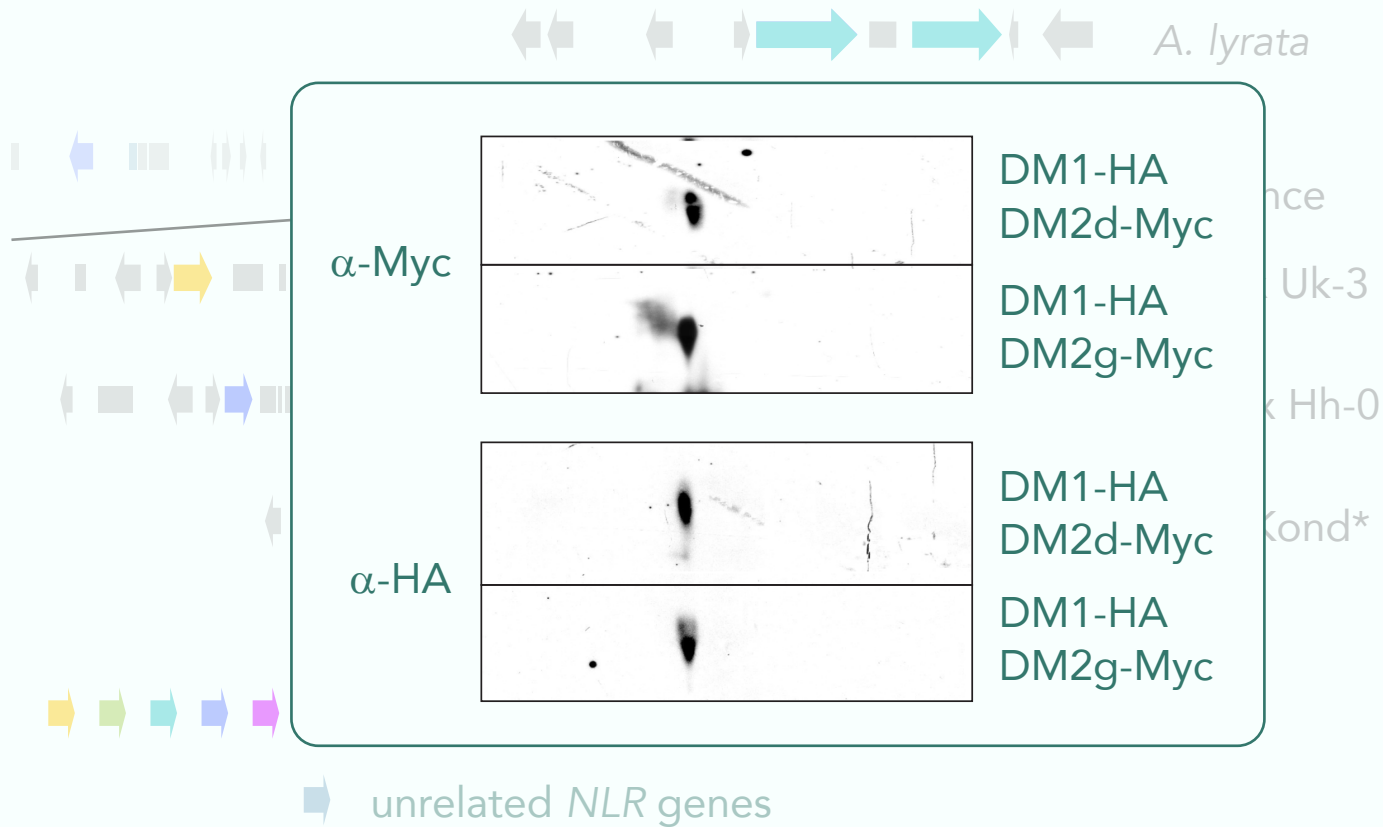
→ → → → → different *NLR* clades
→ unrelated *NLR* genes

*F₂ incompatibility
(Alcázar et al., 2009)

...DM2 Encodes an NLR Immune Receptor As Well



Diep Tran
(Inovarion)



nce
Uk-3
k Hh-0
Kond*

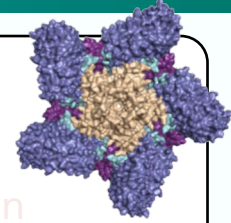
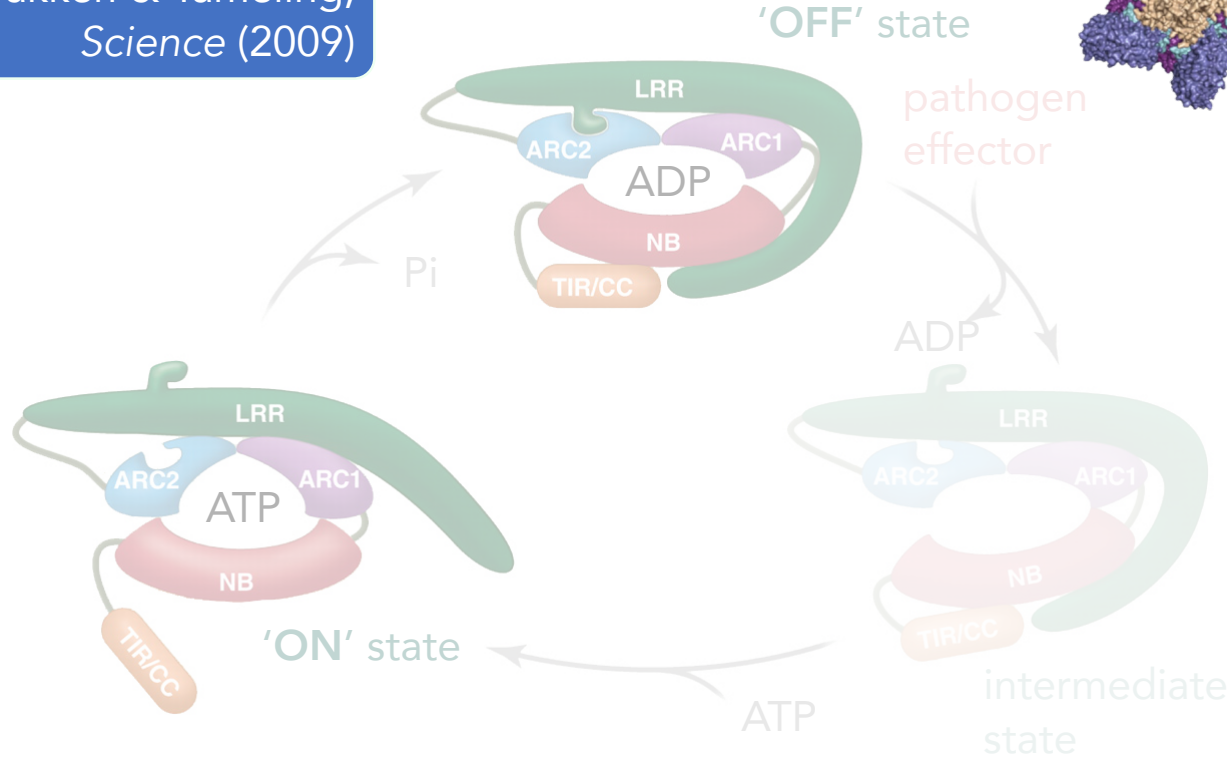
Tran et al. (2017)

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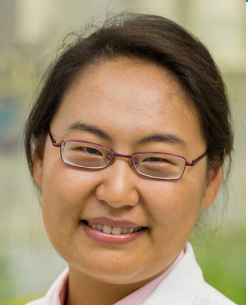
NLRs Cycle Between 'ON' and 'OFF'



Takken & Tameling,
Science (2009)



Species-wide Survey of Hybrid Necrosis



Eunyoung
Chae
(NUS)



6,409 crosses
(3,330 unique
combinations)

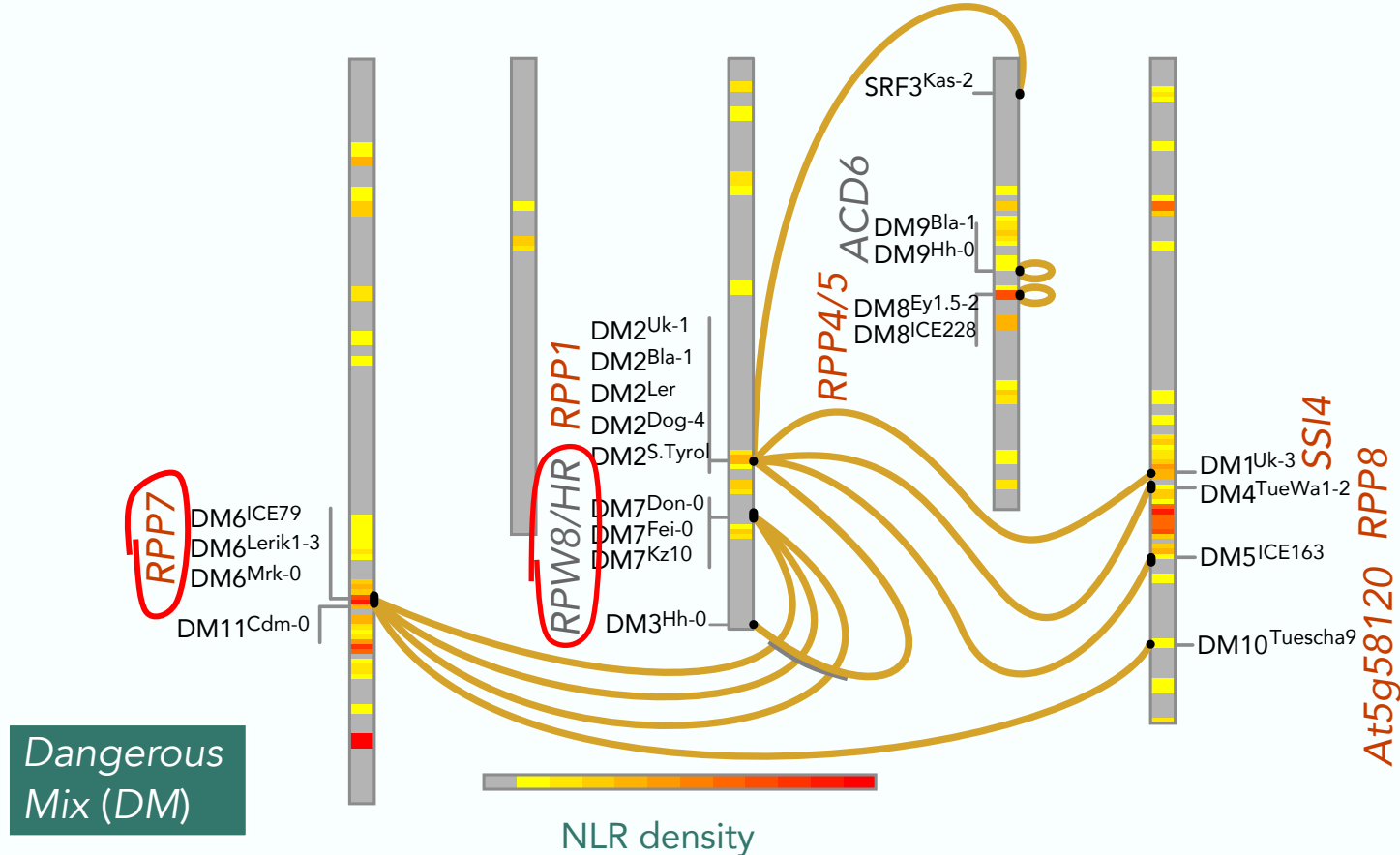
■ not tested

- hybrid
- necrosis
- classes
- geographic origins

Chae et al. (2014)

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NLR Immune Receptors Almost Always Causal



Multiple Pairs of *RPP7* – *RPW8/HR* Interactions



Cristina
Barragan
(TSL)



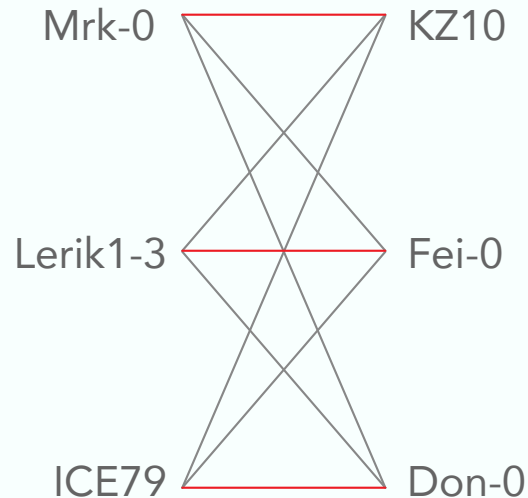
Parent 1

F₁

Parent 2

RPW8/HR

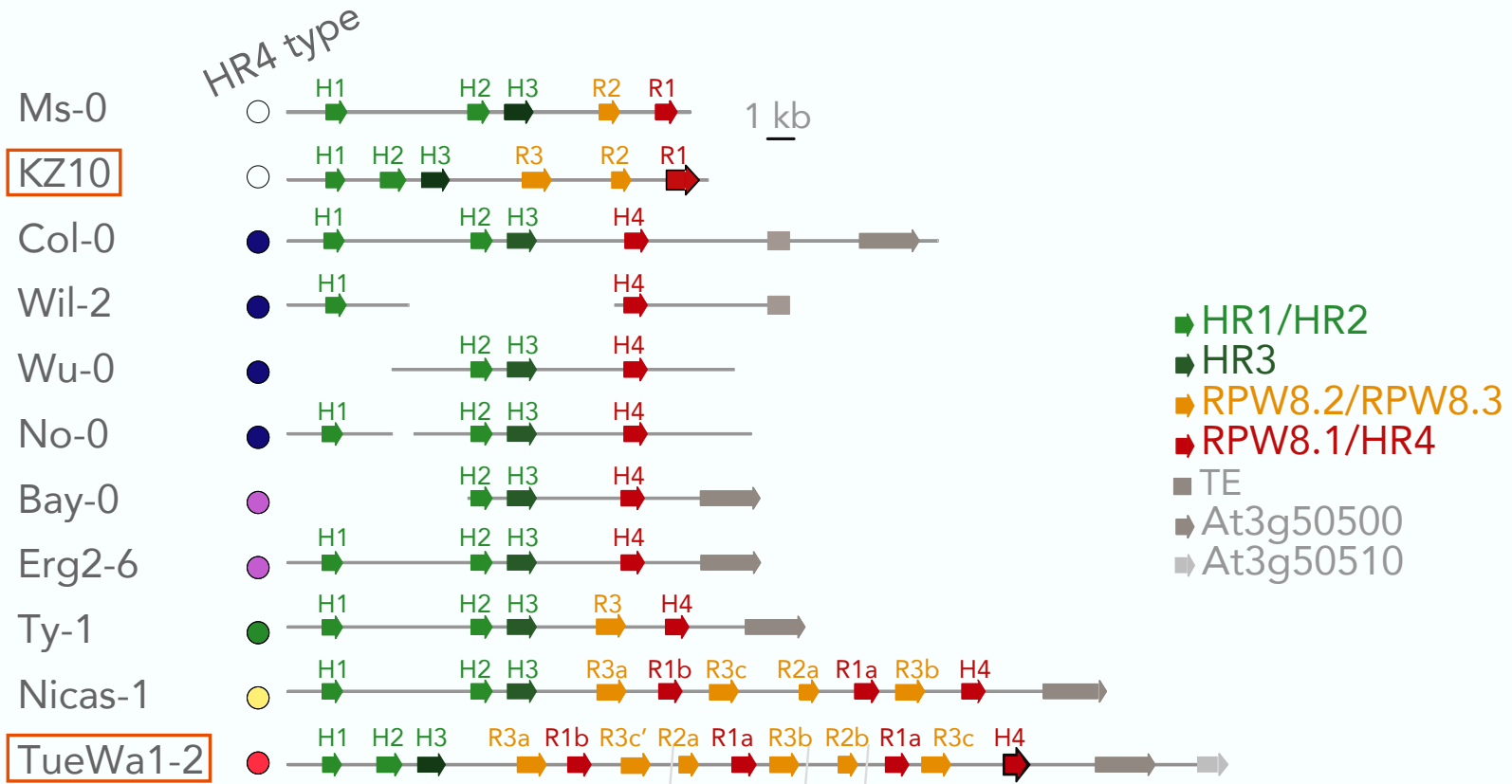
RPP7



Barragan et al. (2019)

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Extreme Sequence Diversity at *RPW8/HR*

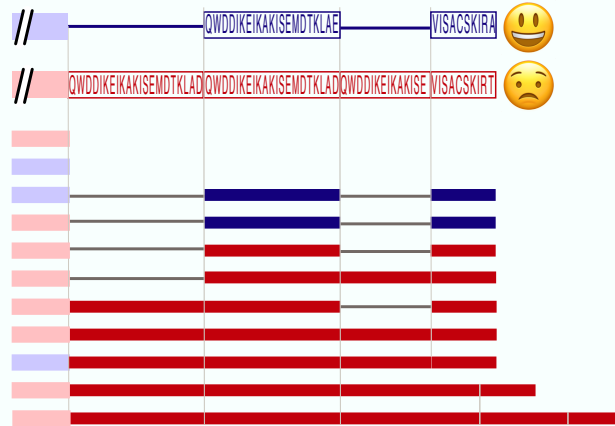


(similar to Fei-0)

RPW8/HR Coding Sequences Causal



causal RPW8/HR repeats



repeat #



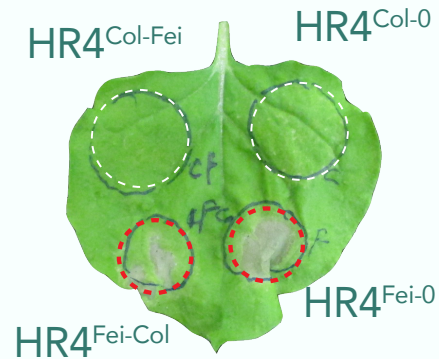
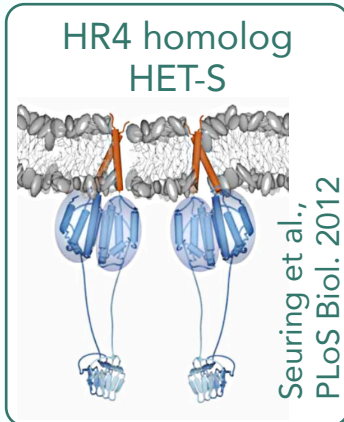
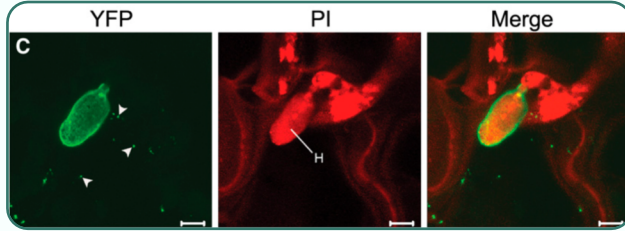
RPW8/HR4 Proteins: Potentially Direct Role in Defense



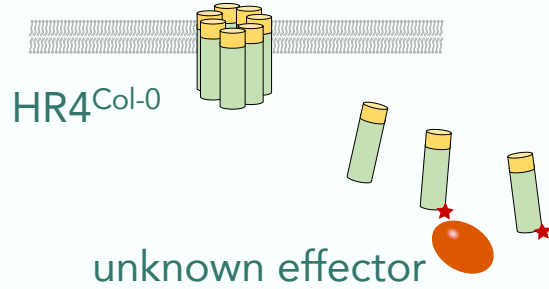
The Plant Cell, Vol. 21: 2898–2913, September 2009, www.plantcell.org © 2009 American Society of Plant Biologists

Specific Targeting of the *Arabidopsis* Resistance Protein RPW8.2 to the Interfacial Membrane Encasing the Fungal Haustorium Renders Broad-Spectrum Resistance to Powdery Mildew^{W, GA}

Wenming Wang, Yingqiang Wen, Robert Berkey, and Shunyuan Xiao¹



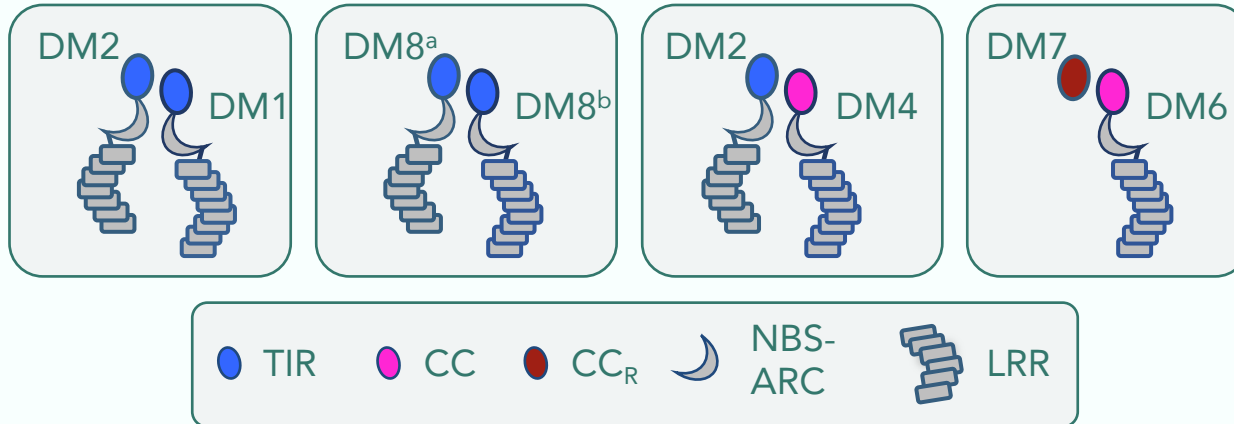
Hypothesis: HR4^{Fei-0} Mimics Effector-modified HR4^{Col-0}



A Wide Range of (Direct?) NLR Interactions



Potentially promiscuous NLR interactions limit possible immune receptor combinations in a single genotype



Genome-wide functional analyses of plant coiled-coil NLR-type pathogen receptors reveal essential roles of their N-terminal domain in oligomerization, networking, and immunity

NLR network mediates immunity to diverse plant pathogens

Chih-Hang Wu^a, Ahmed Abd-El-Halim^b, Tolga O. Bozkurt^{c,d}, Khaoula Belhaj^b, Ryohei Terauchi^{d,e}, Jack H. Vossen^b, and Sophien Kamoun^{a,1}

Tadeusz Wróblewski^{1*}, Laurentiu Spiridon², Eliza Cristina Martin², Andrei-Jose Petrescu², Keri Cavanaugh¹, Maria José Truco¹, Huaqin Xu¹, Dariusz Gozdowski³, Krzysztof Pawłowski³, Richard W. Michelmore^{1,4,5}, Frank L.W. Takken^{6*}

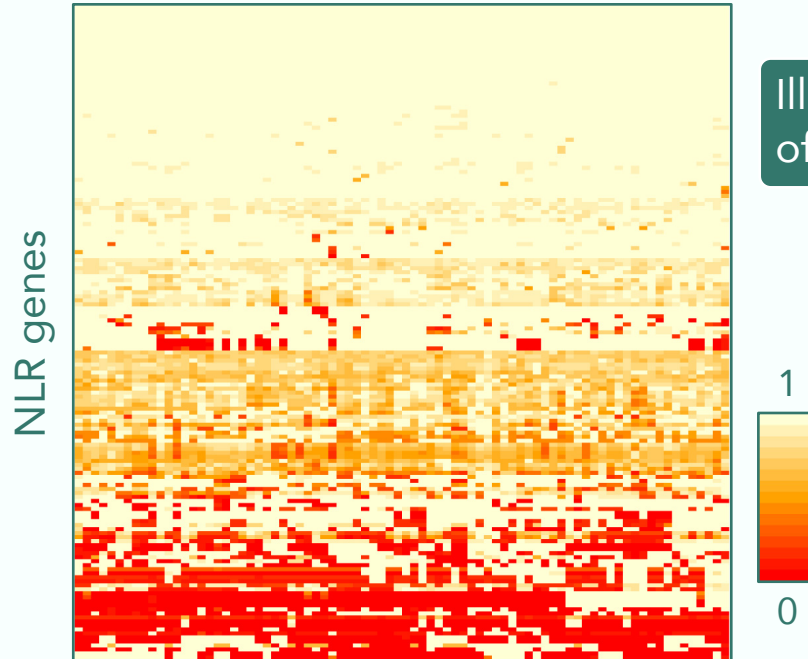
How polymorphic are NLR genes?

NLR Genes Are Very Polymorphic



Darya
Karelina

1001 Genomes accessions (phase 1)

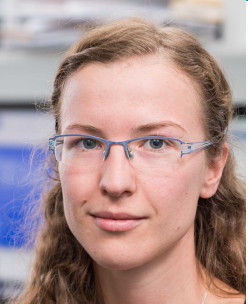


Illumina read coverage
of reference NLR genes



Karelina PhD thesis (2019)

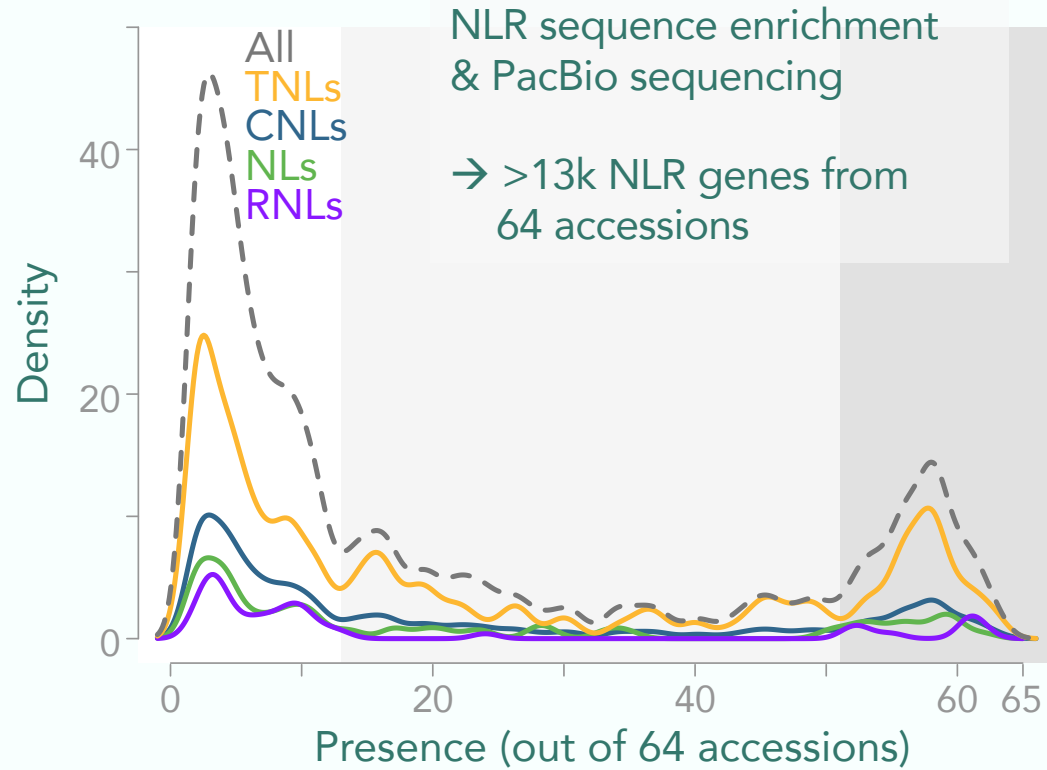
NLR Genes Are Very Polymorphic



Anna-Lena
Van de Weyer
(LaboKlin)



Felix
Bemm
(KWS)

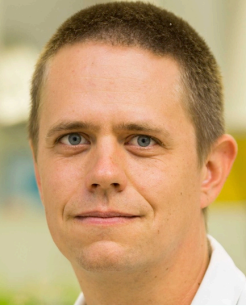


With
Dangl &
Jones
labs

Van de Weyer et al. (2019)

Why are NLR genes so diverse?

Diversity in *Capsella*



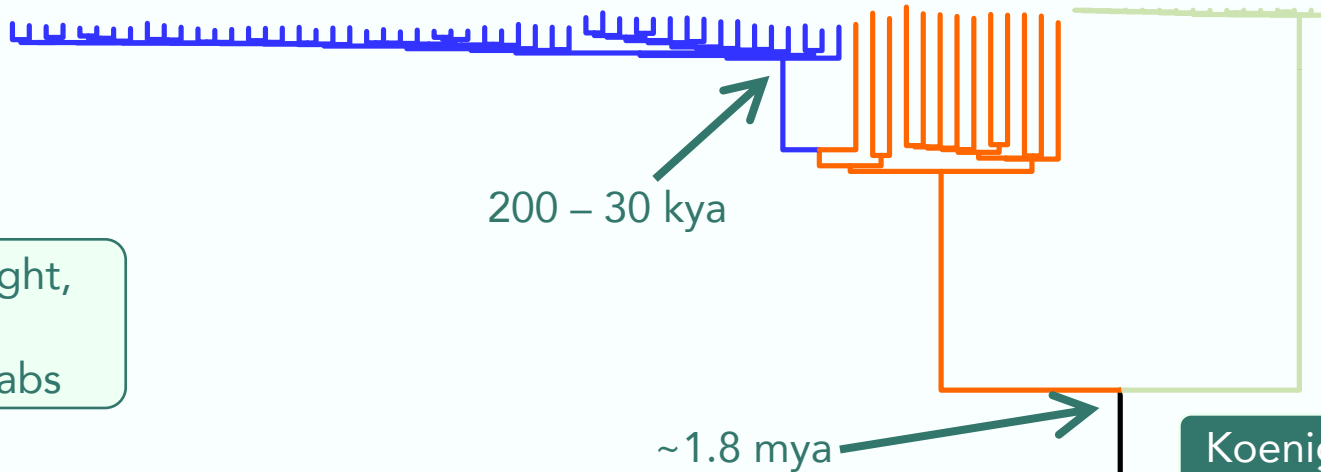
Daniel Koenig
(UC Riverside)

Many trans-specific alleles } genomes

C. rubella

C. grandiflora

C. orientalis



With Wright,
Slotte &
Neuffer labs

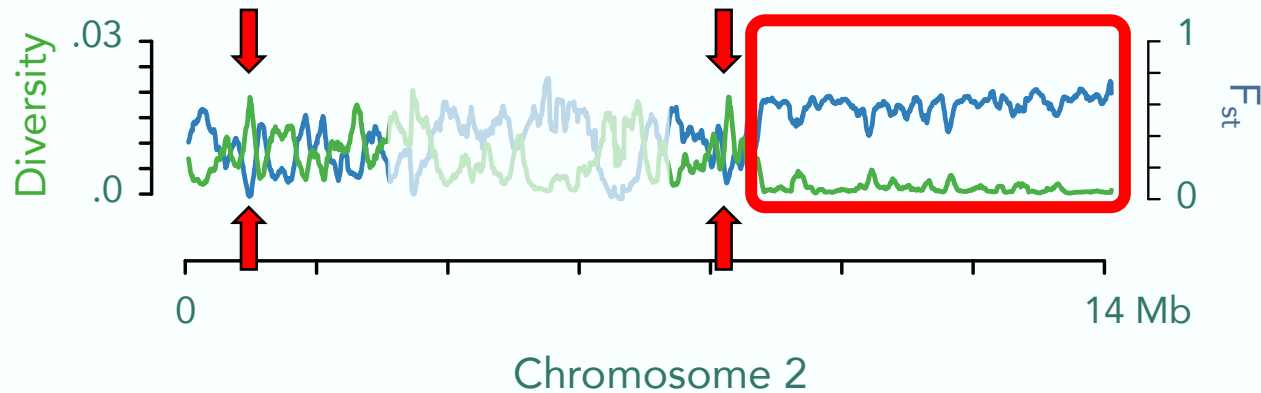
Koenig et al. (2019)

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Nonrandom Distribution of Trans-specific Alleles



- Diversity within *C. rubella*
- Differentiation between *C. rubella* & *C. grandiflora*



Nonrandom Distribution of Trans-specific Alleles



- Diversity within *C. rubella*

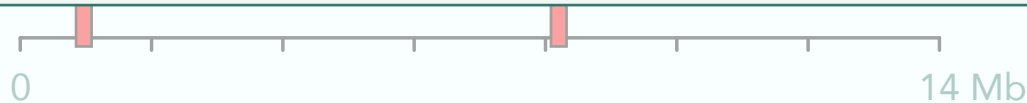
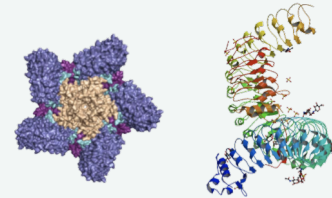
~2% of total genome

21 regions

9 overlap with NLR immune receptor genes

5 overlap with other immune genes incl. Pattern Recognition Receptors

Several of these with known function in *A. thaliana*



Chromosome 2

Replication?

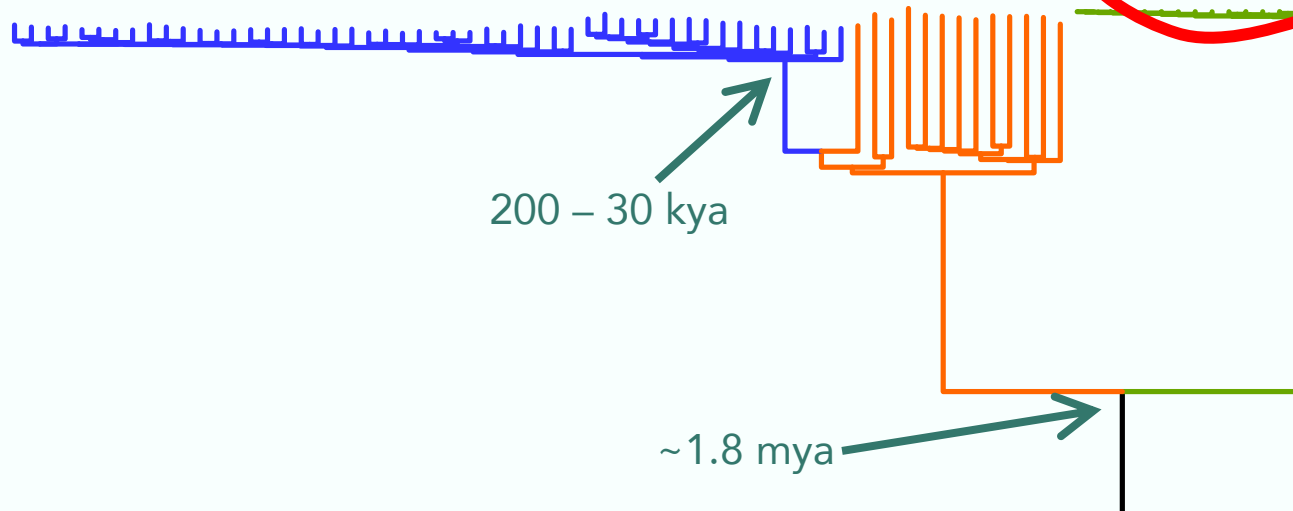


Distance tree from whole-genomes

C. rubella

C. grandiflora

C. orientalis






Many Trans-Specific Alleles in *C. orientalis* as well



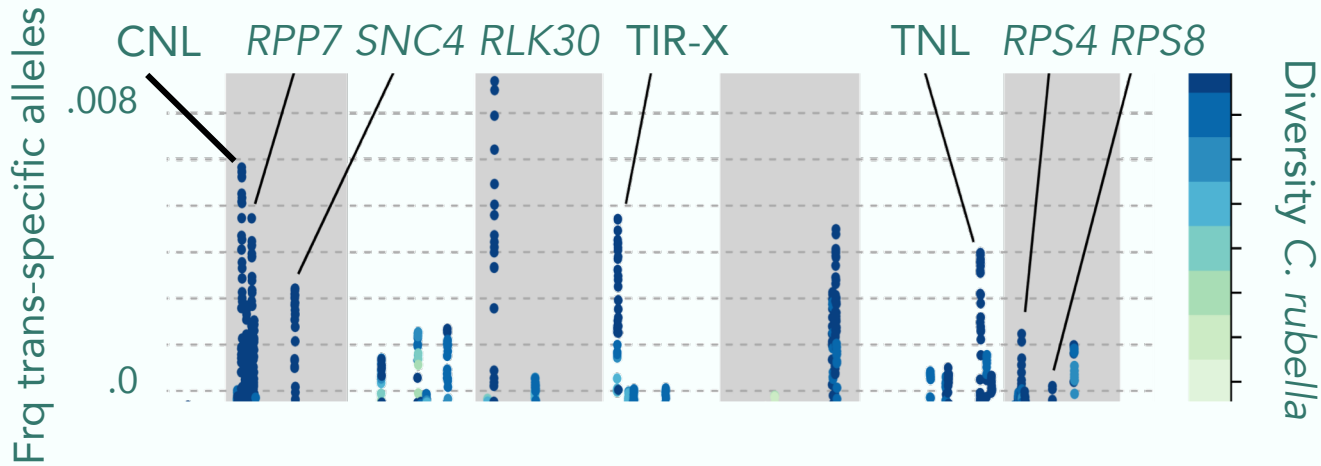
61,543 species-specific SNPs



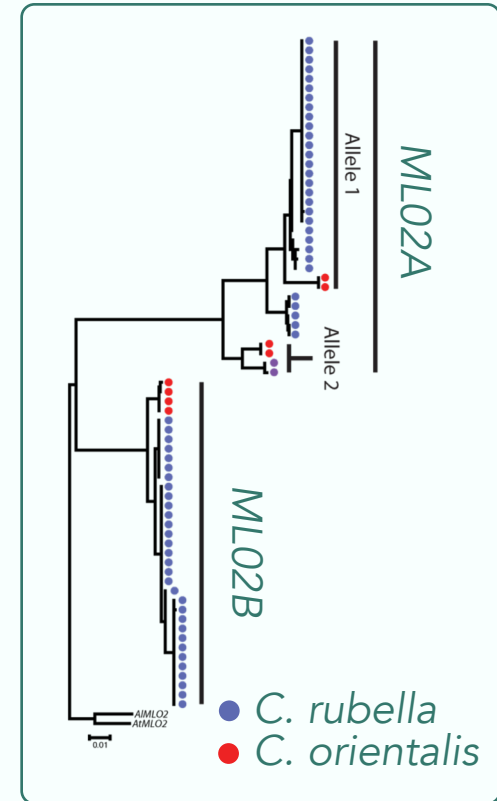
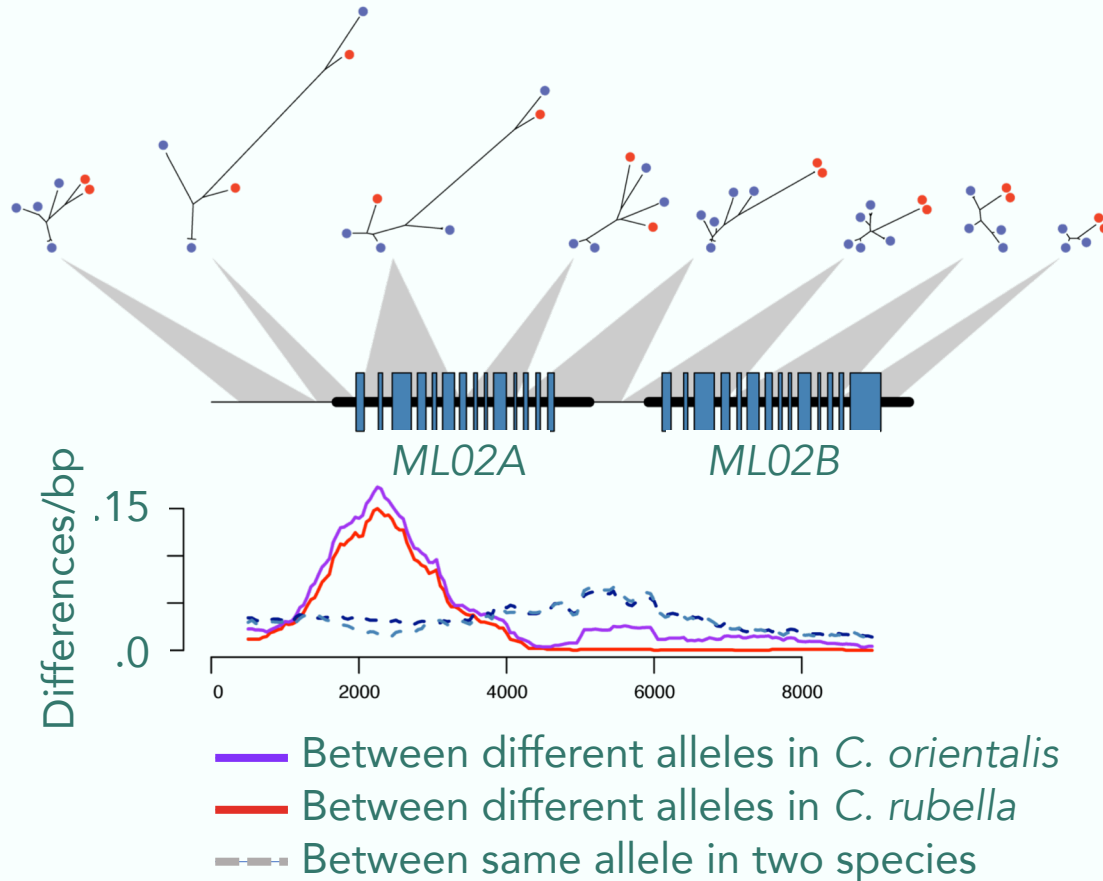
9,911 trans-specific SNPs

-  Shared with *C. grandiflora*
-  Shared with *C. rubella*
-  Shared with both species

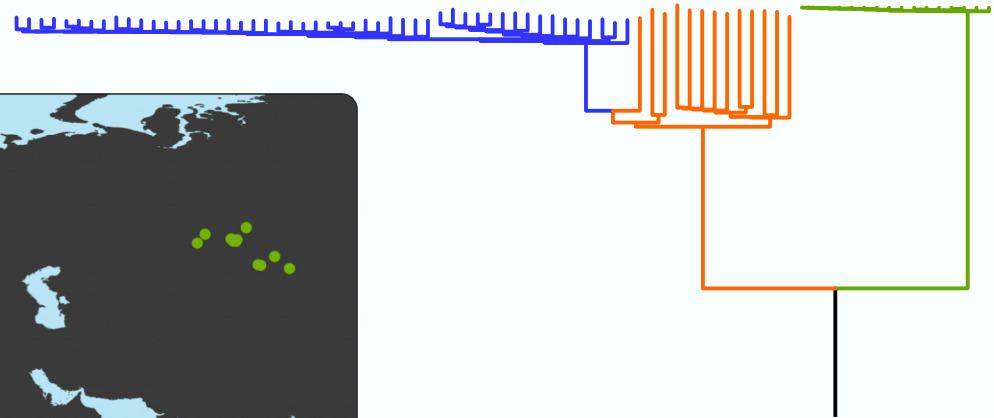
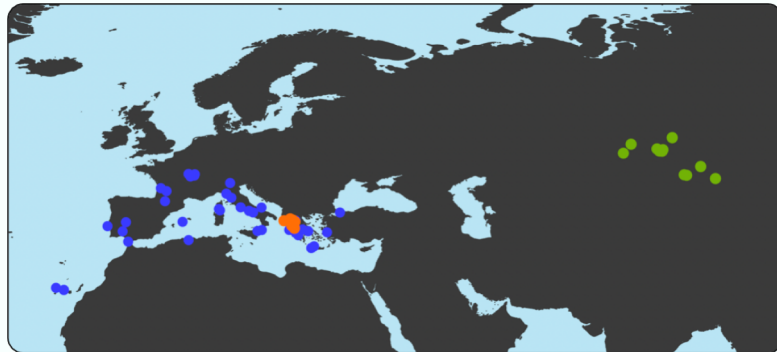
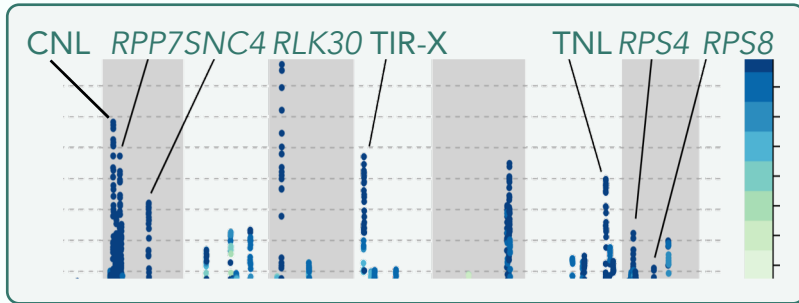
Diversity at Same Immune Receptor Genes



Ancient Balanced Polymorphism at *MLO2*

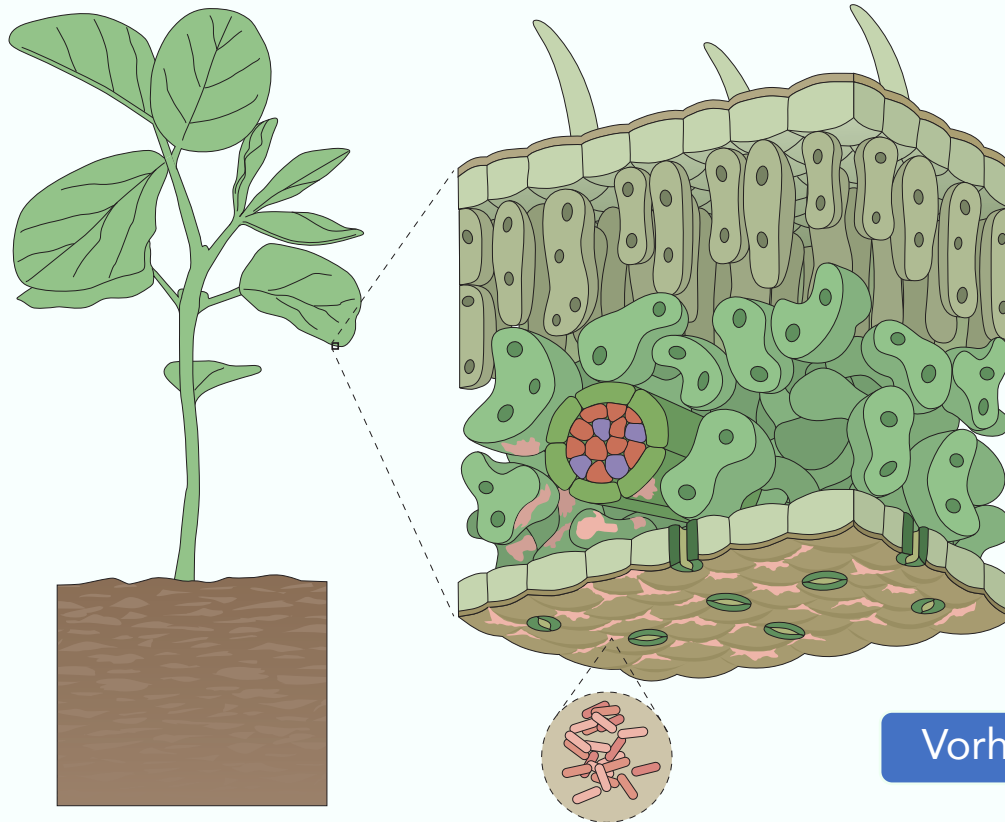


But Why The Same Loci (and Often Even Same Alleles)?



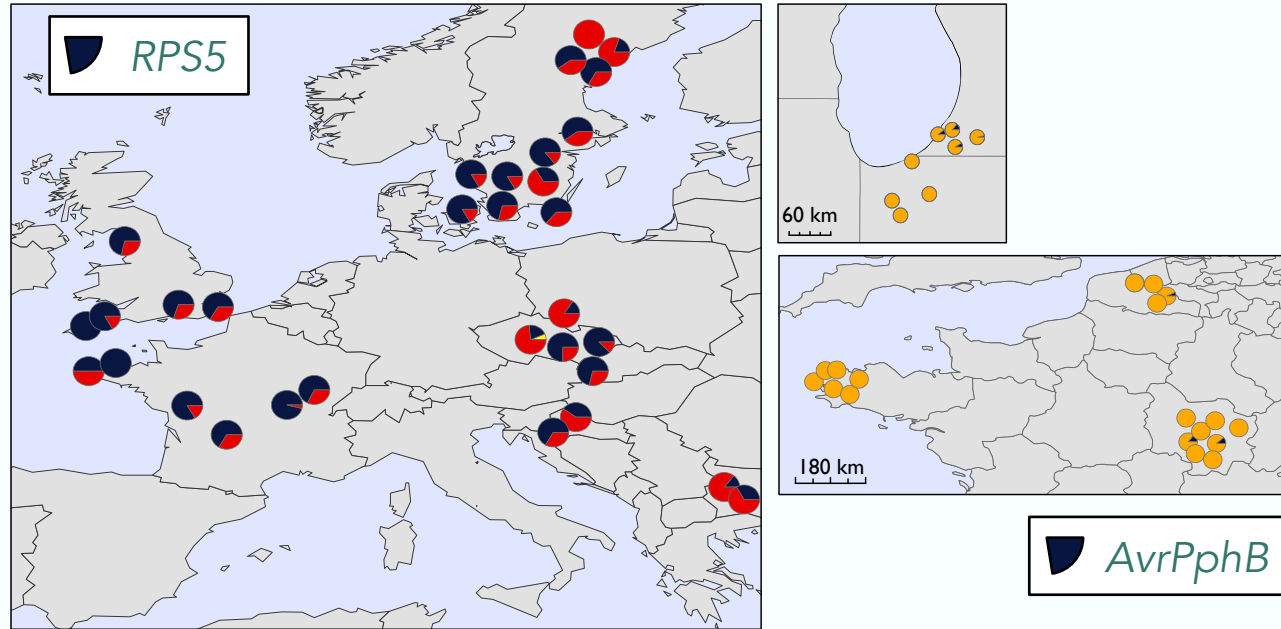
What drives immune system diversification in the wild?

The Plant Phyllosphere



Vorholt, *Nat. Rev. Microbiol.* (2014)

The Difficulty of Matching Effectors and NLRs in Nature



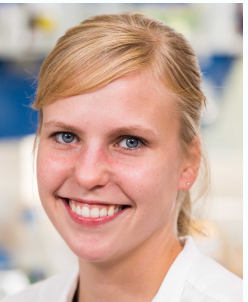
Karasov, ..., Bergelson, *Nature* (2014)

Who Is There?



Talia Karasov
(Univ. of Utah)

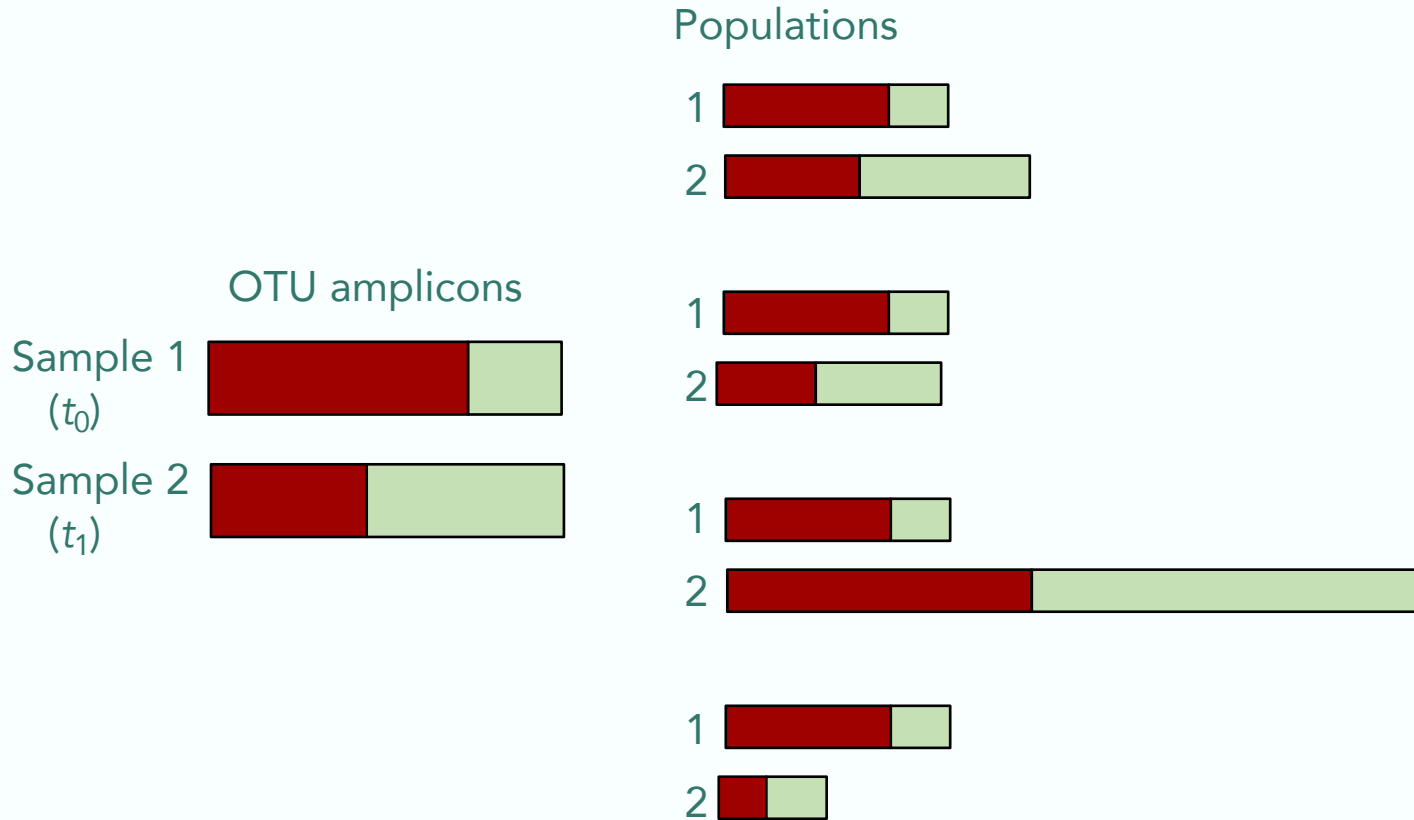
Manuela Neumann
(Bosch Global)



Sampling the phyllosphere of *A. thaliana*



Excursion: OTU Amplicon Data Are Compositional



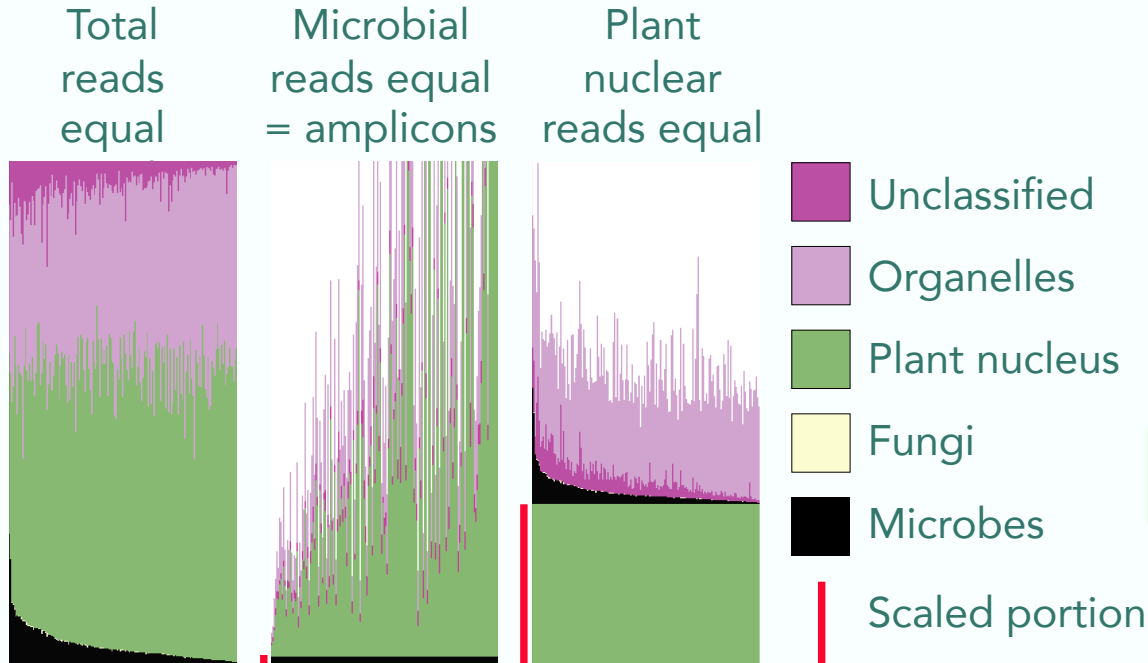
Metagenome Shotgun Sampling



Julian
Regalado
(Univ. Copenhagen)



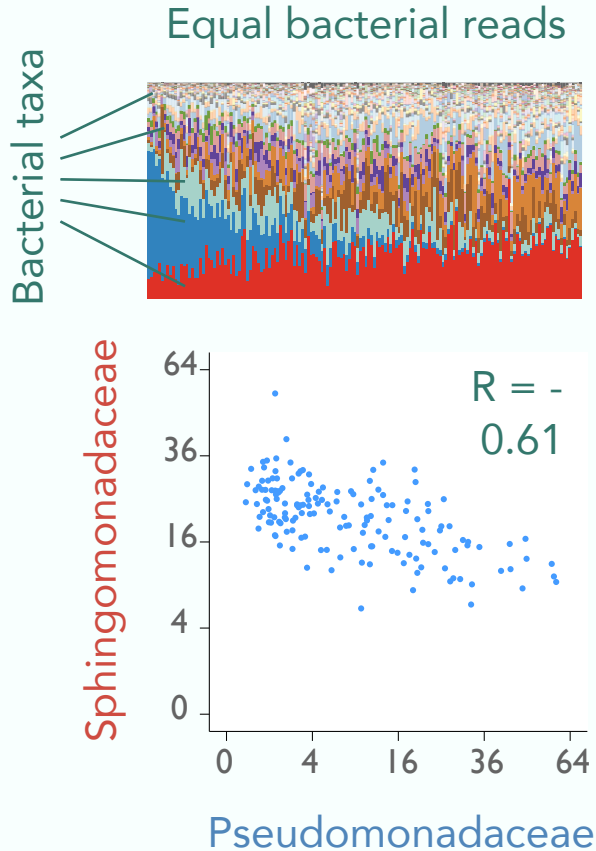
Derek
Lundberg



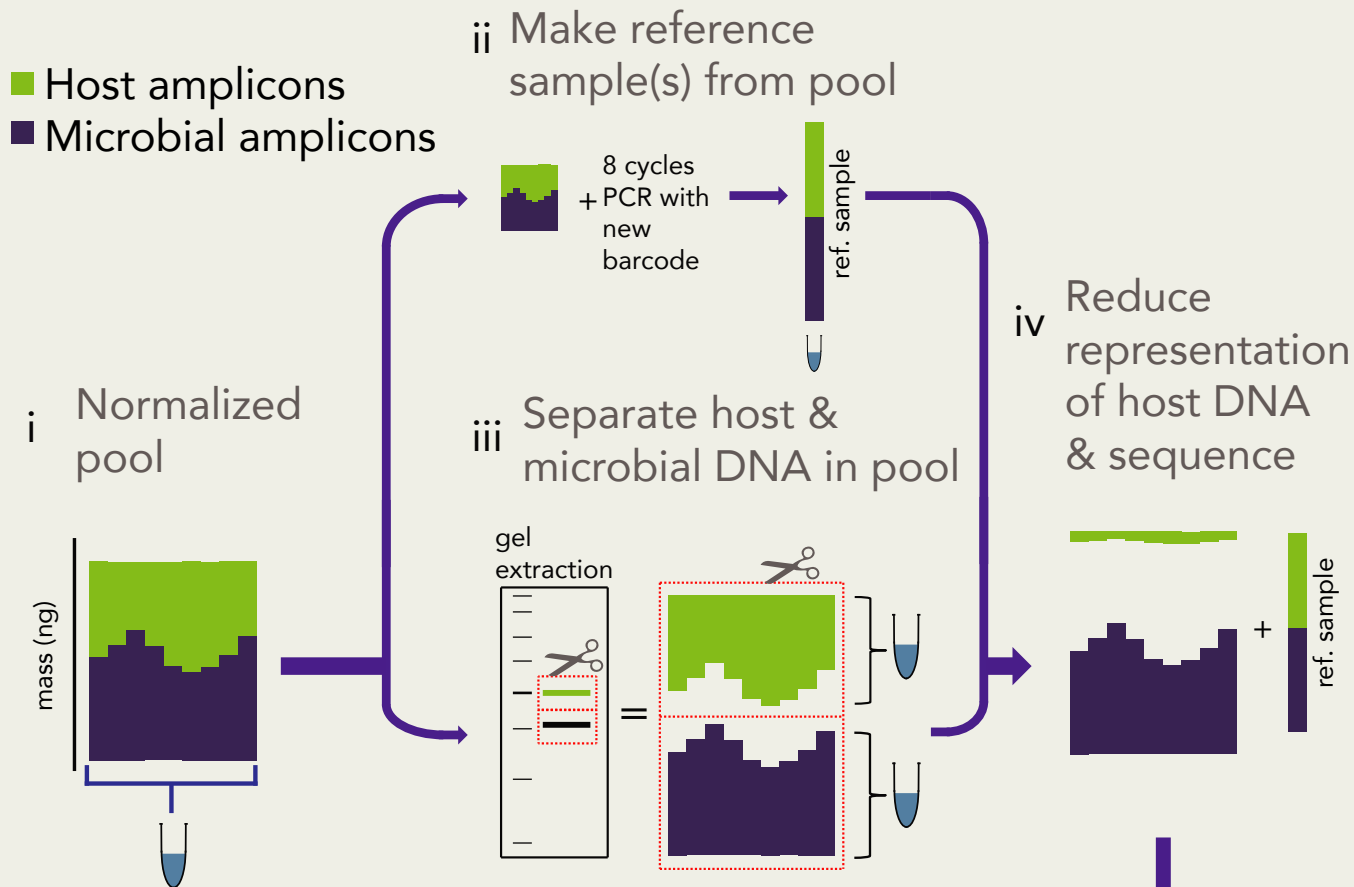
Regalado, Lundberg
et al., (2020)

Karasov et al.,
bioRxiv (2019)

Absolute Data Can Lead to Very Different Conclusions



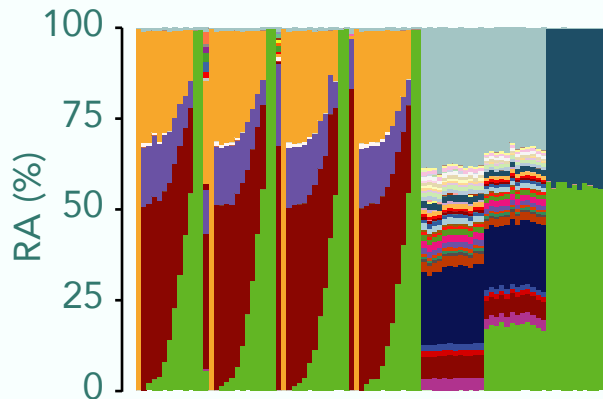
hamPCR: Host Associated Microbe PCR



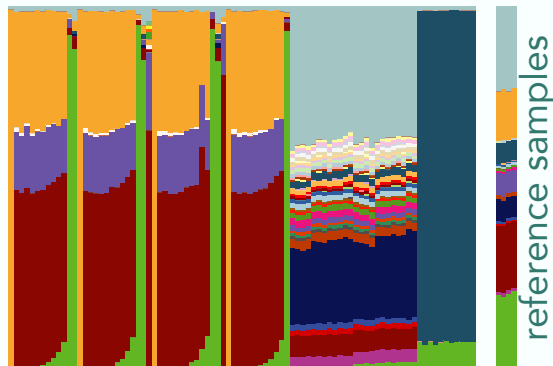
hamPCR: Host Associated Microbe PCR



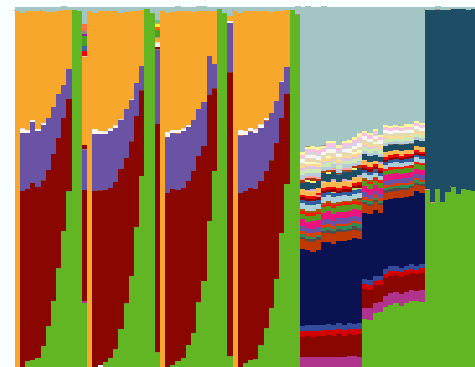
Original library



Reduced host amplicon library
with reference samples



Reconstruction



■ *A. thaliana* GI

■ Low abundance

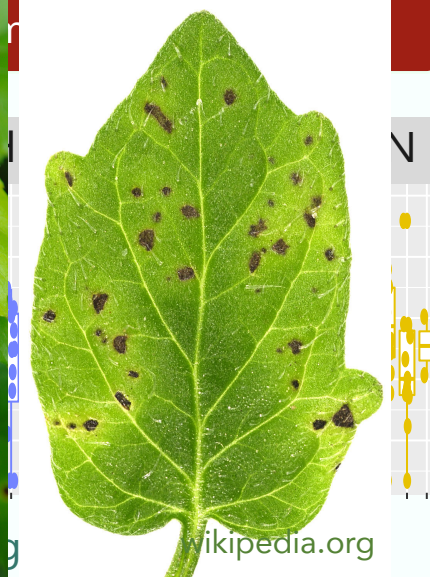
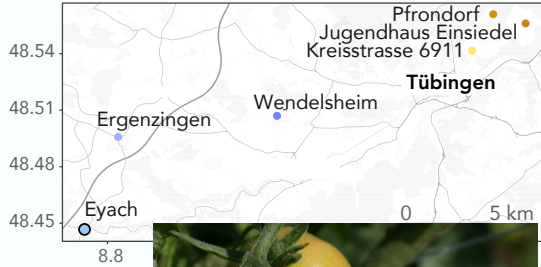
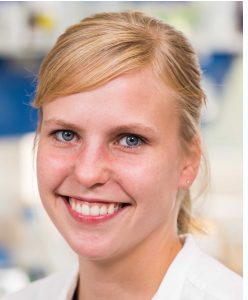
(All other colors bacterial ASVs)

So, Who Is There?



Talia Karasov
(Univ. of Utah)

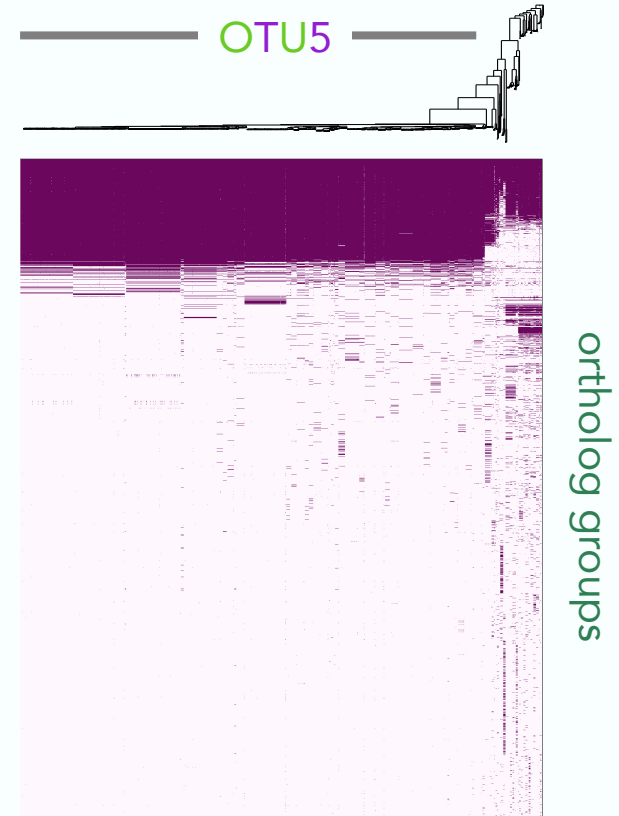
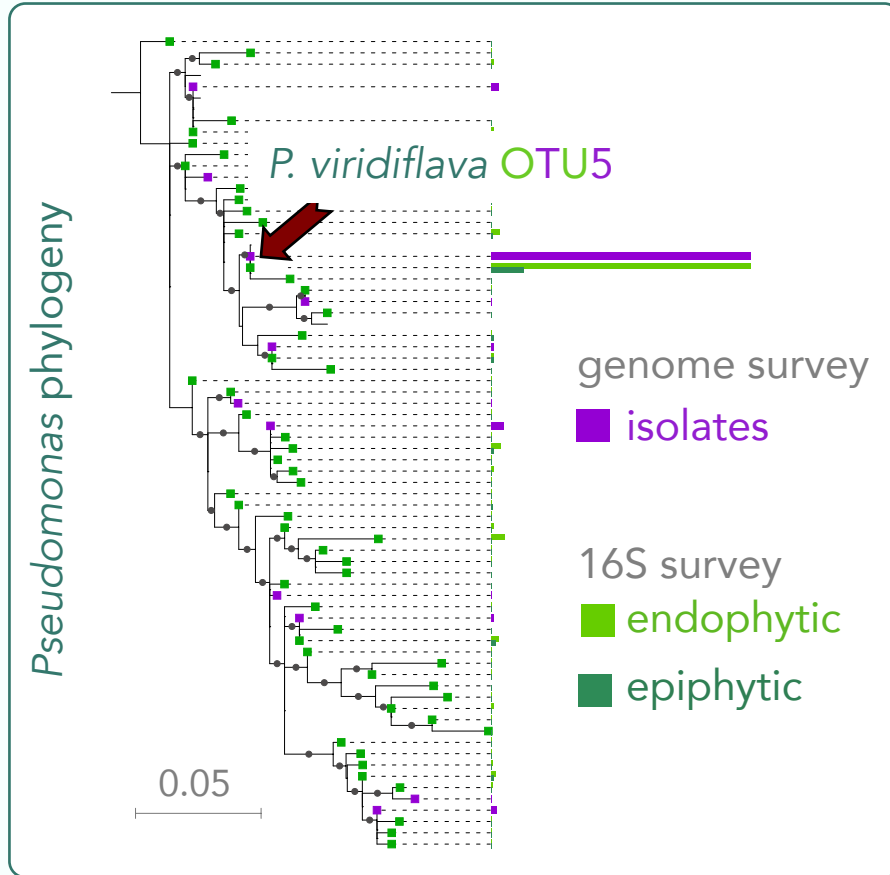
Manuela Neumann
(Bosch Global)



With
Kemen
lab:
Juliana
Almario

Karasov et al. (2018)

A Single Dominant *Pseudomonas* OTU

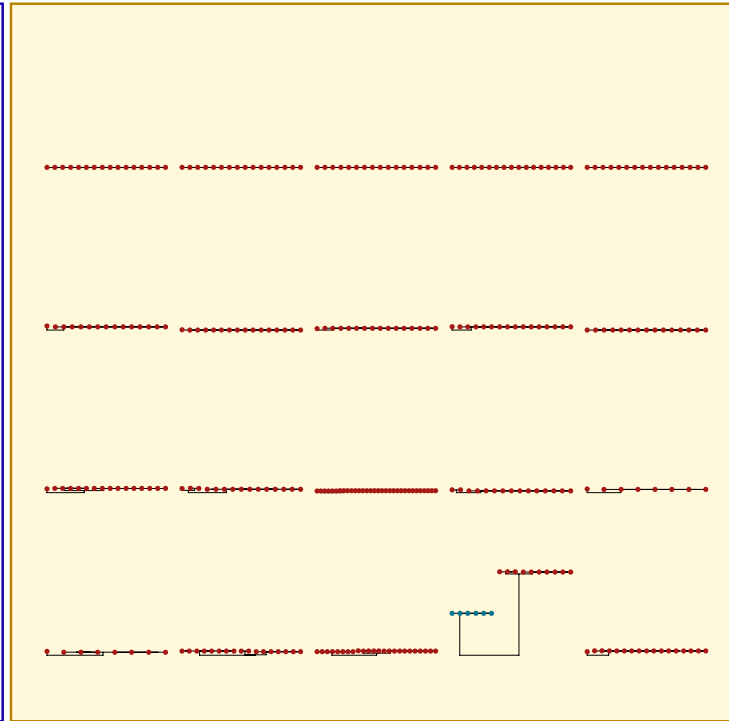
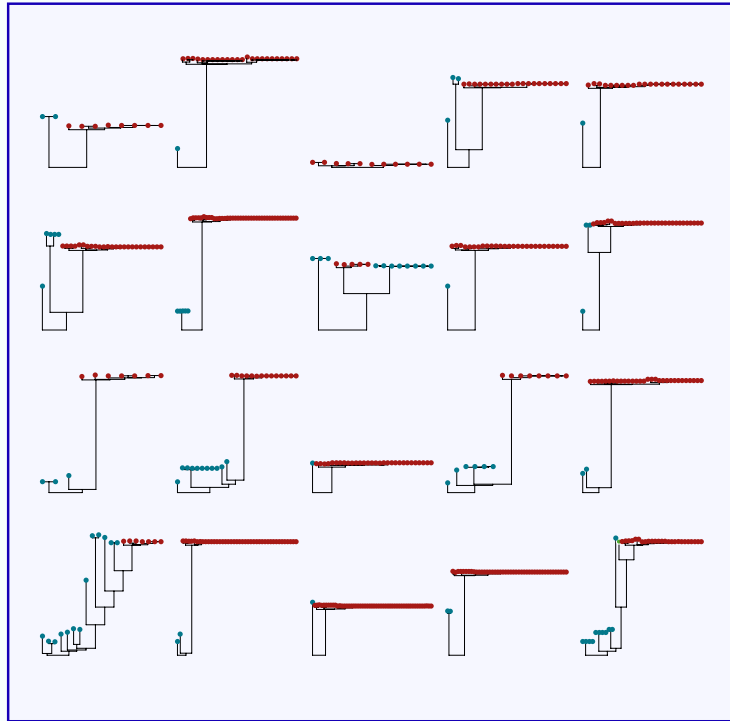


Clonal Expansions Within Individual Plants



December 2015

March 2016



I 0.2

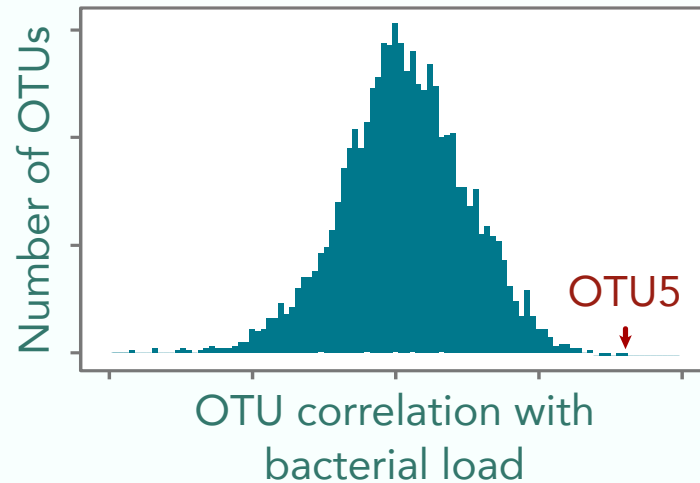
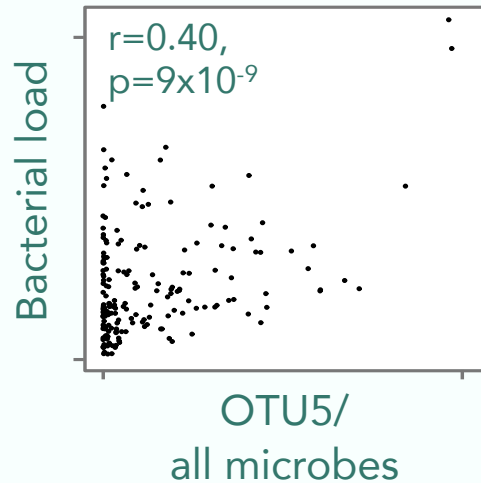
■ non-OTU5
■ OTU5

based on whole-genome sequences

OTU5 Drives Bacterial Load in *A. thaliana*



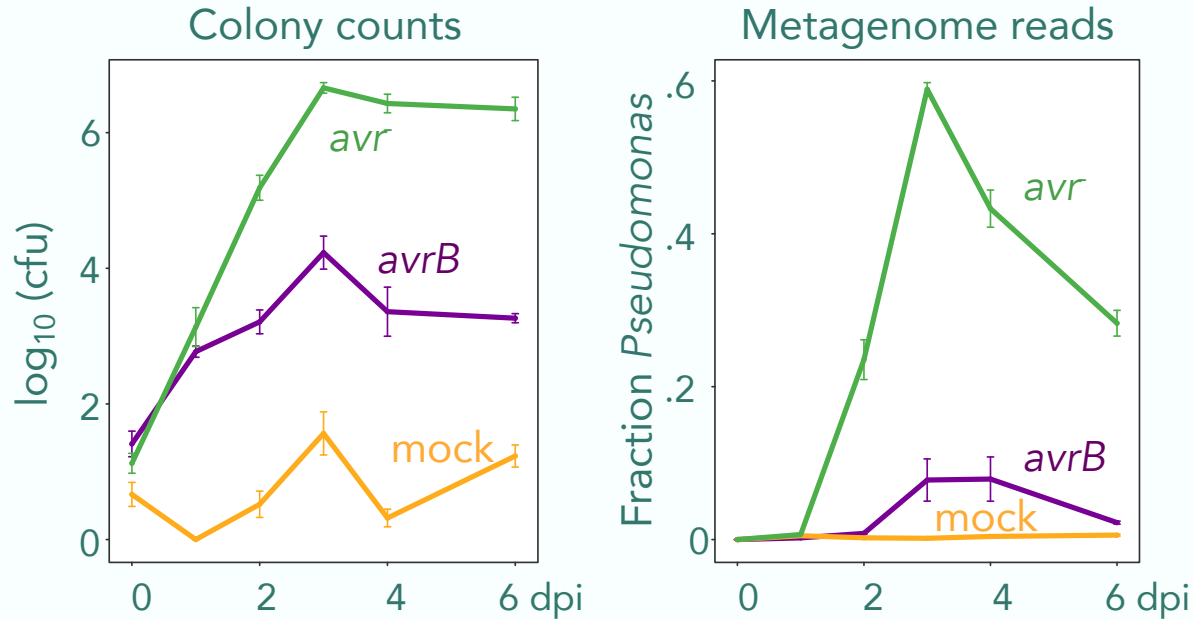
$$\text{bacterial load} = \frac{\text{microbial reads}}{\text{plant reads}}$$



Pseudomonas Loads In the Lab



Laboratory infection with *Pst* DC3000



10% *Pseudomonas* reads = 500 bacteria/mm² leaf

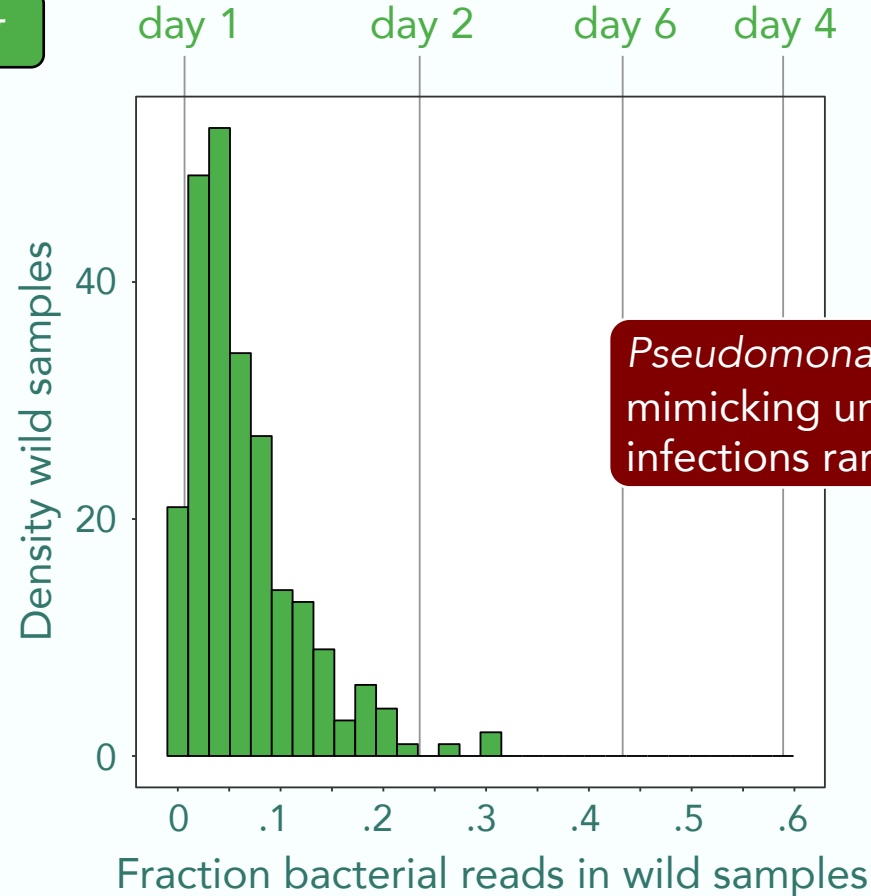
Karasov et al.,
bioRxiv (2019)

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How Does This Compare to the Wild?



Lab infection *avr*

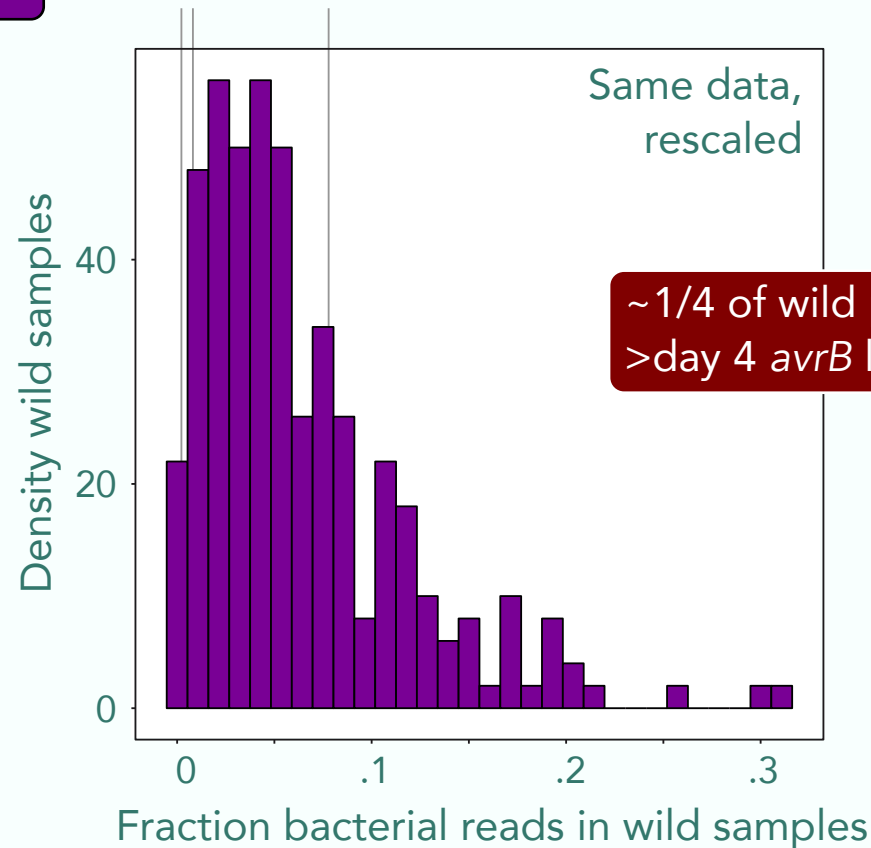


How Does This Compare to the Wild?



Lab infection *avrB*

day 1 day 2 day 4

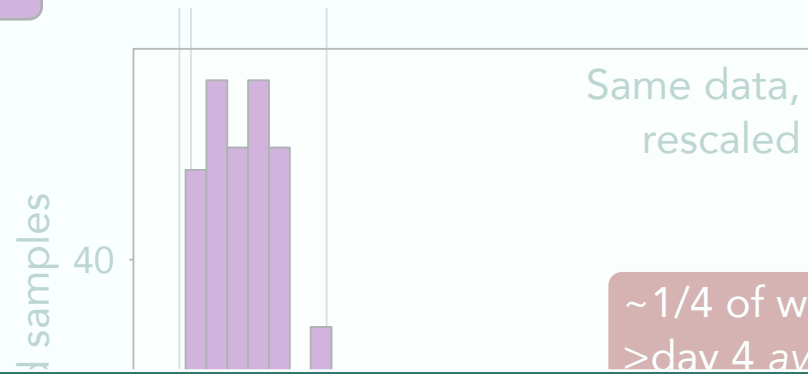


How Does This Compare to the Wild?



Lab infection *avrB*

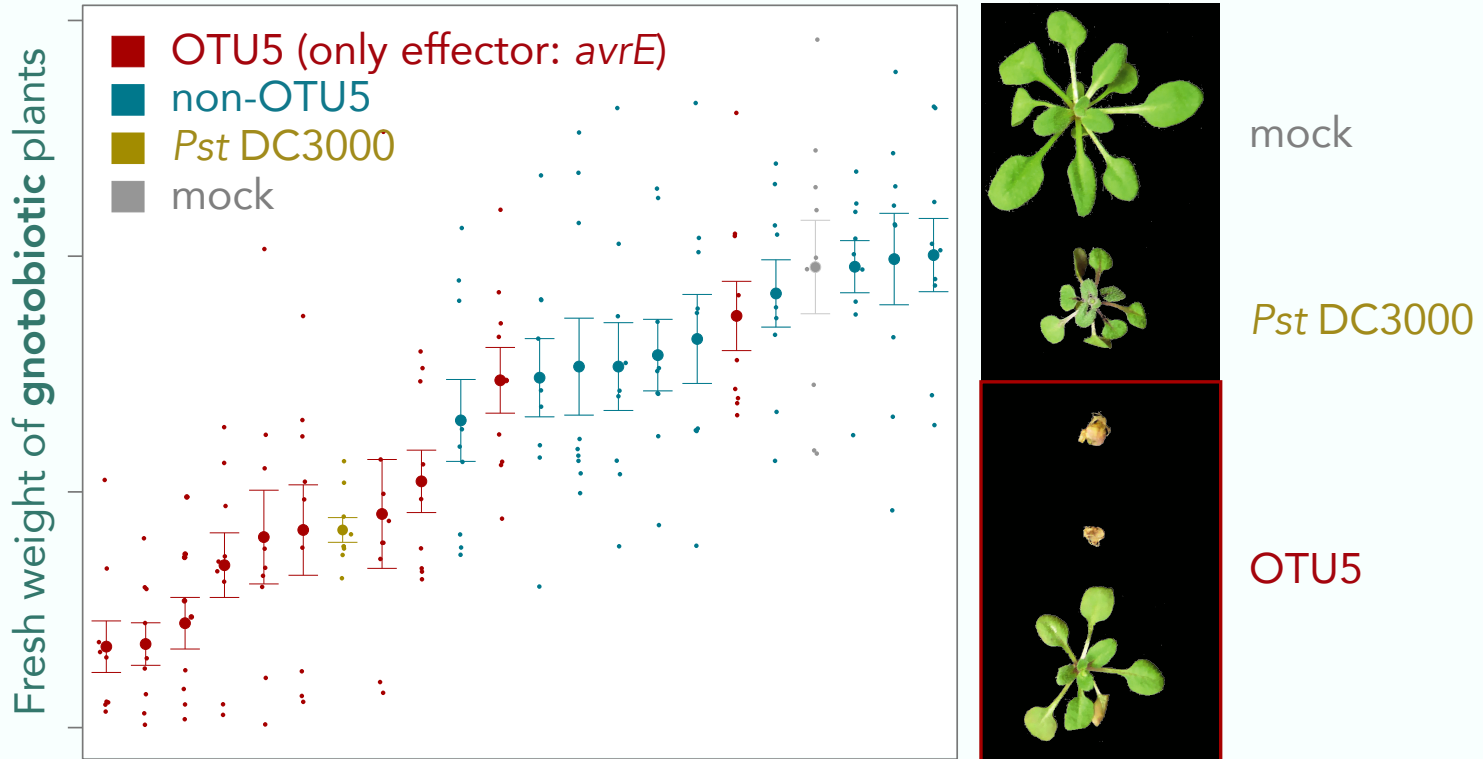
day 1 day 2 day 4



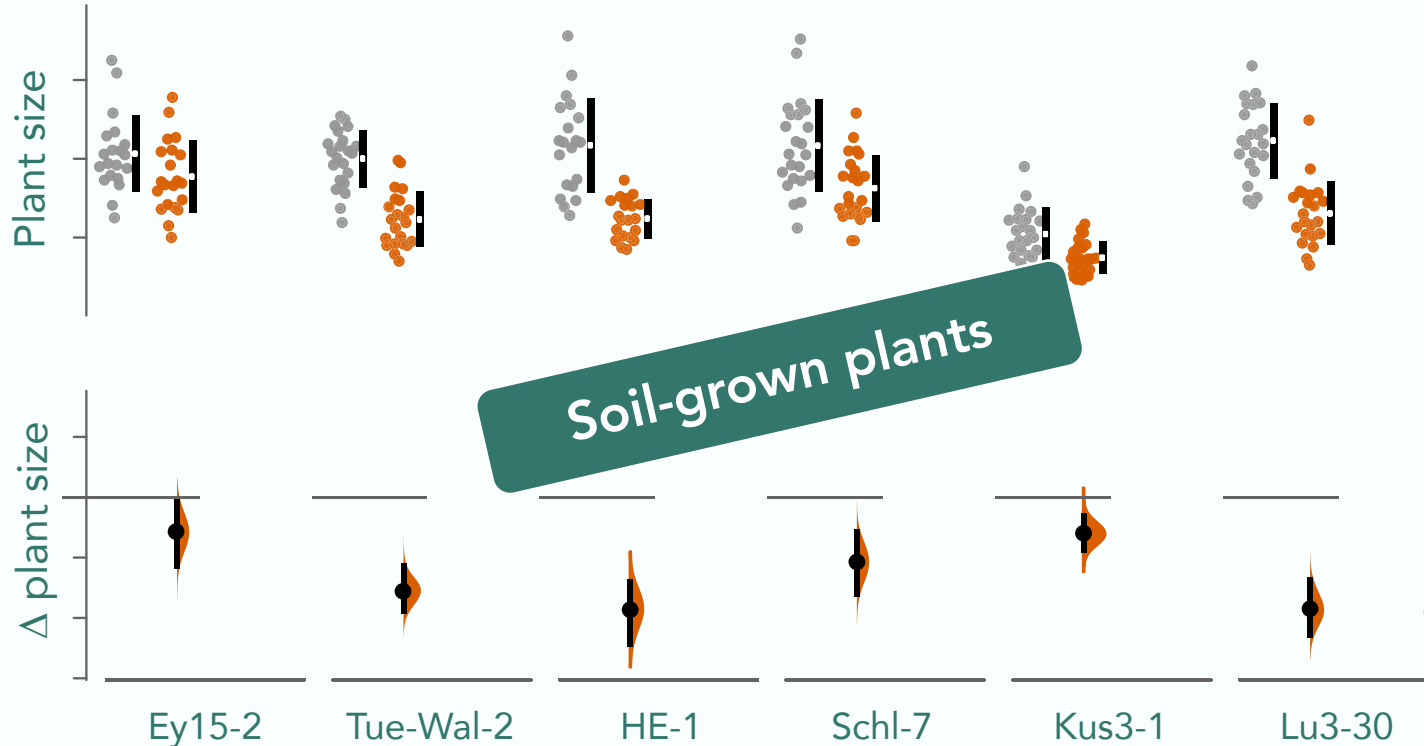
Microbial loads in wild plants are typically intermediate between compatible & incompatible interactions



Phenotypic Differentiation Among OTU5 Members



Is *Pseudomonas* OTU5 Truly a Pathogen?

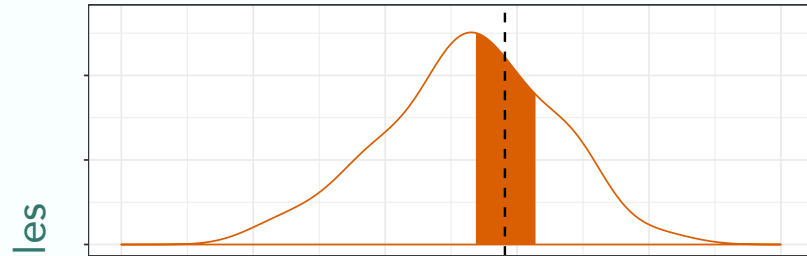


Or Shalev
(Uni Tü)

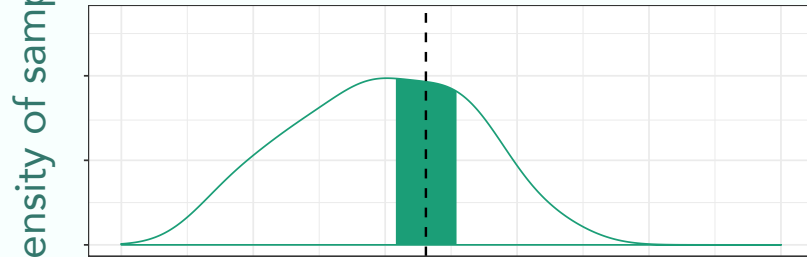


Shalev et al., *bioRxiv* (2021)

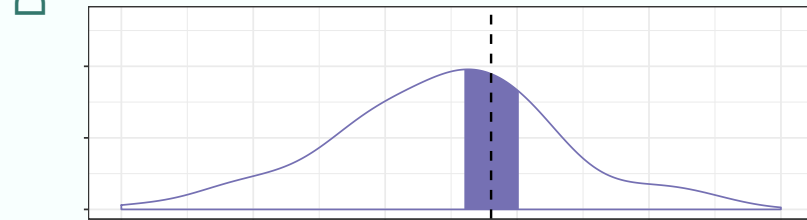
Commensals Do Not Reduce Total Load



PathoCom (7 OTU5)



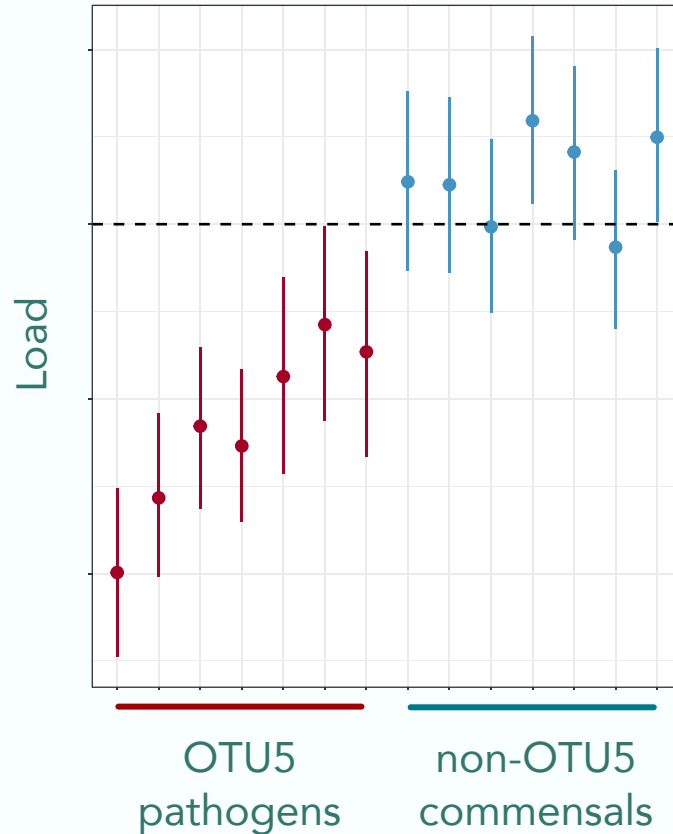
CommenCom (7 non-OTU5)



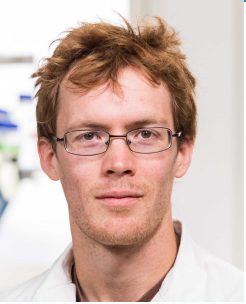
MixedCom (7+7)

Total load

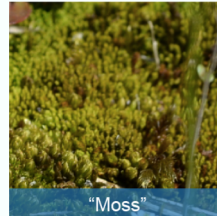
...But They Reduce Load of OTU5 Pathogens



So, Who Drives *Pseudomonas* OTU5 Evolution?



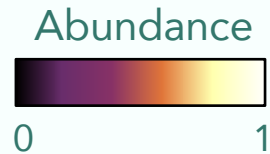
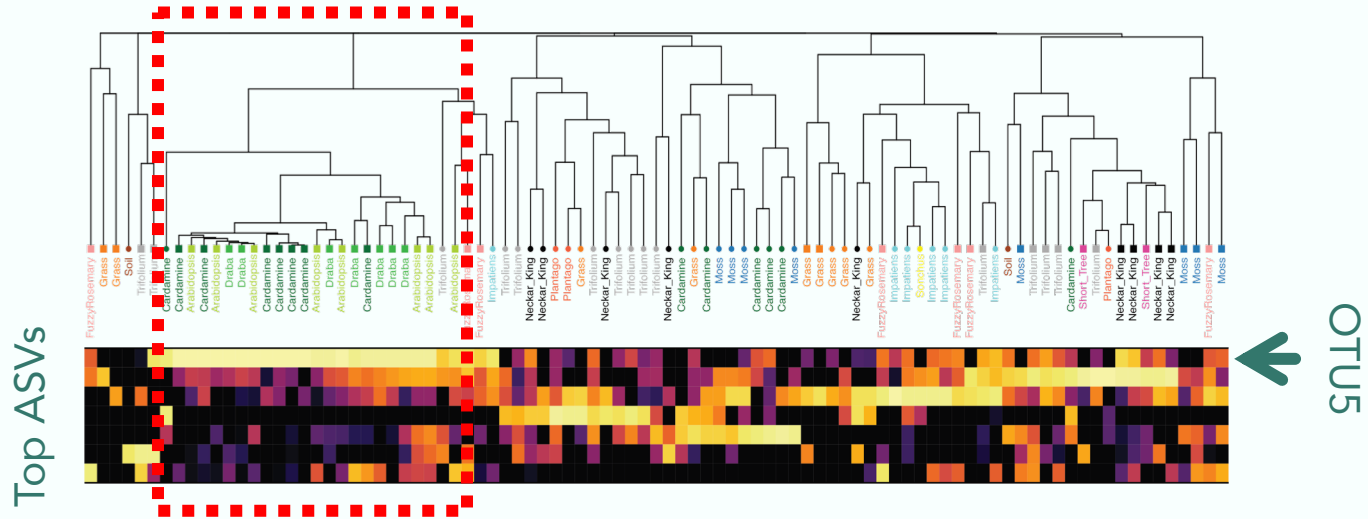
Derek
Lundberg



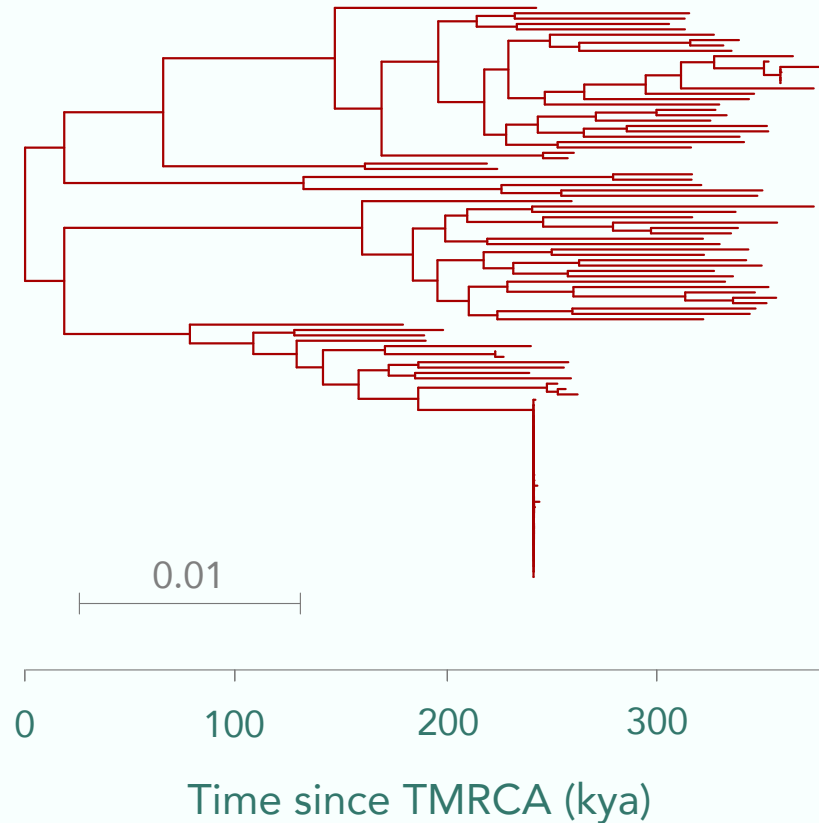
Pseudomonas Found on Many Brassicaceae



Samples clustered by Bray Curtis dissimilarity (2 seasons)
16S rDNA *Pseudomonas* abundance matrix



Pseudomonas OTU5 Much Older Than Local *A. thaliana*



What next?

Understanding and Predicting PATHOgen COMMunities



Quantitative genetics & ecological genomics

QTL mapping in nature
Pathobiota & host genetics
Community ecology



Fabrice Roux, CNRS

Molecular biology & genomics-driven genetics

1001 Genomes
Plant immunity
Pathogen within-species diversity



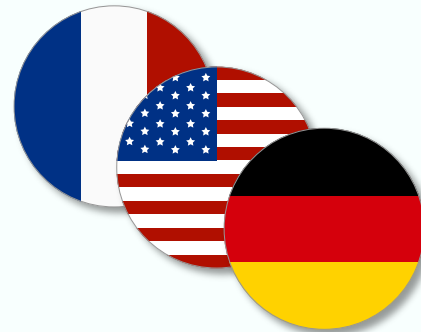
Detlef Weigel, Max Planck

Evolutionary ecology & co-evolutionary genomics

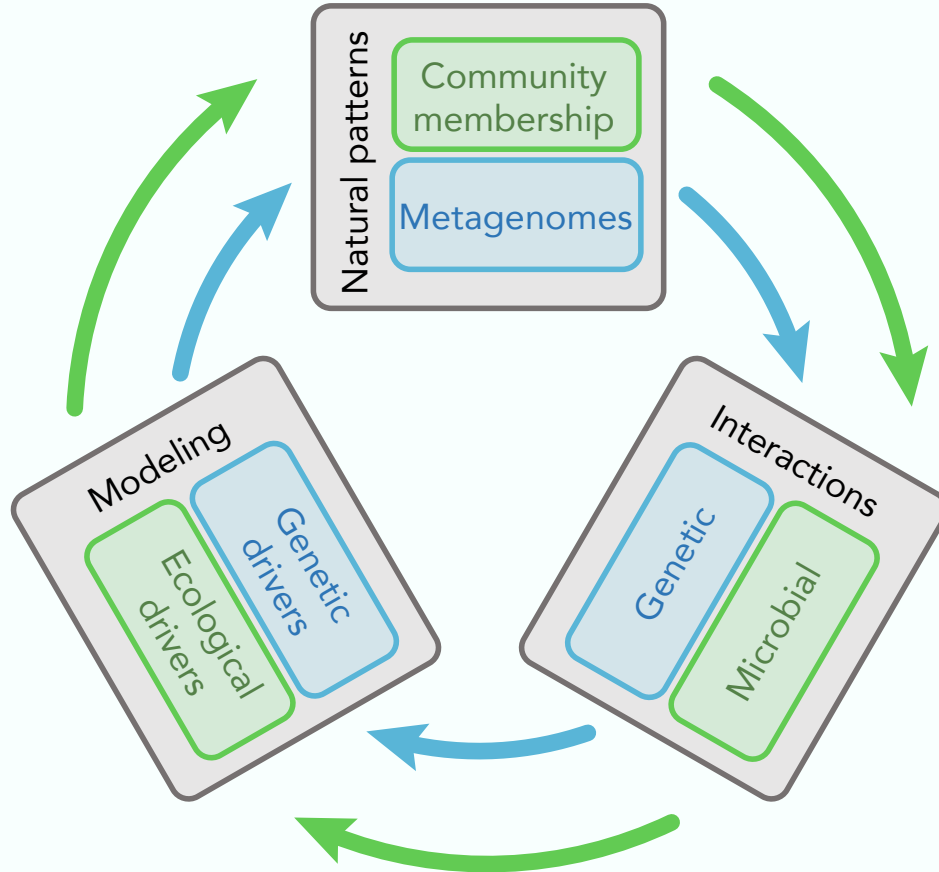
Field station for transgenics
Plant-pathogen co-evolution
Modeling collaborators



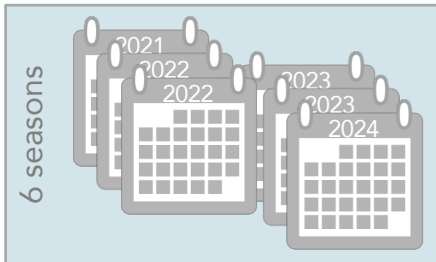
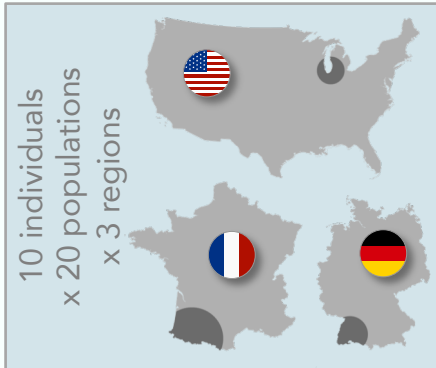
Joy Bergelson, NYU



The PATHOCOM Approach



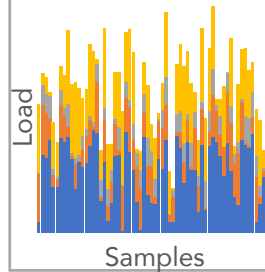
Aim 1: Collecting Real-World Data Across Time & Space



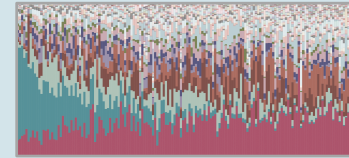
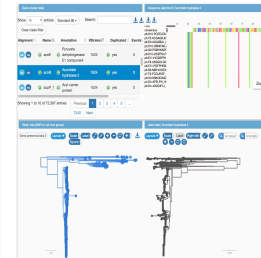
3,600 leaf samples



Bacterial load
(WGS)



Intraspecific
diversity (PEN-seq)



16S & ITS &
metagenomes



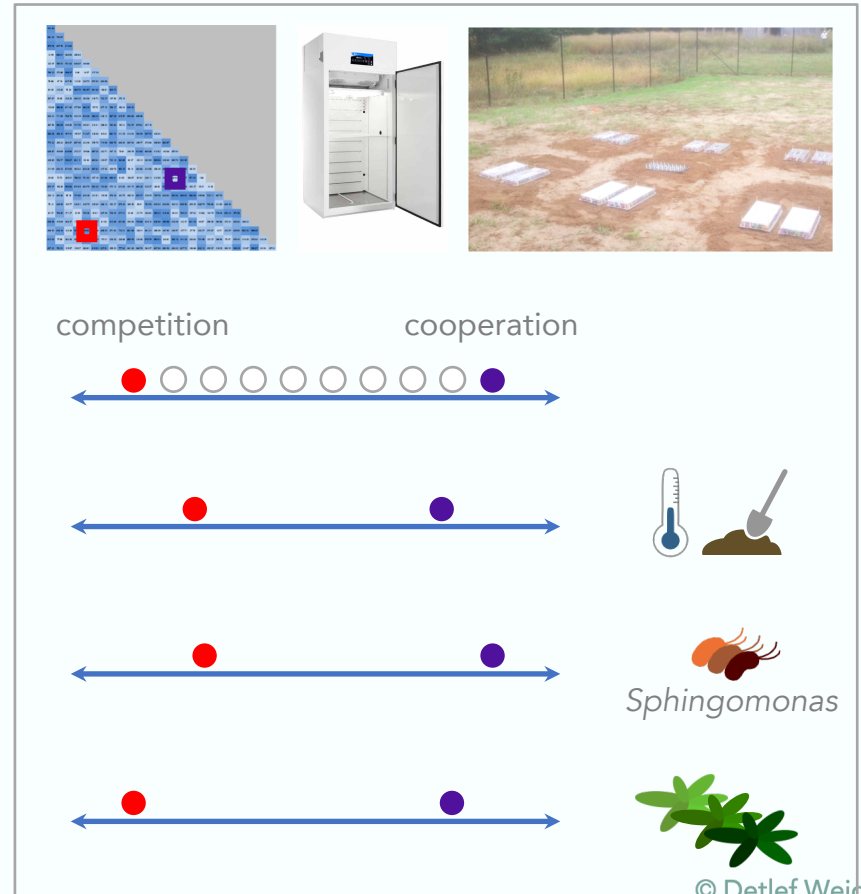
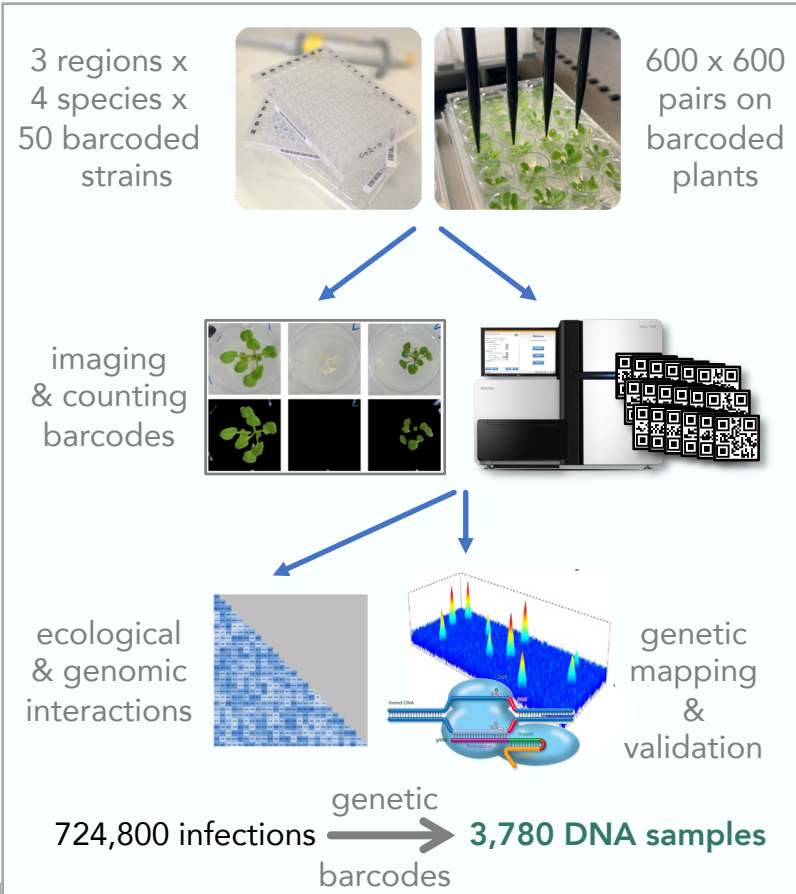
weather

soil

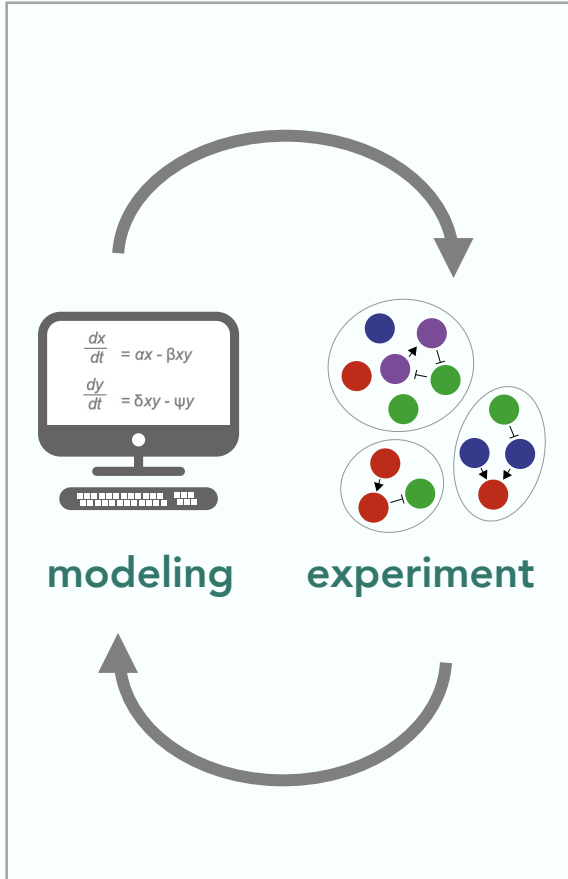


companion species

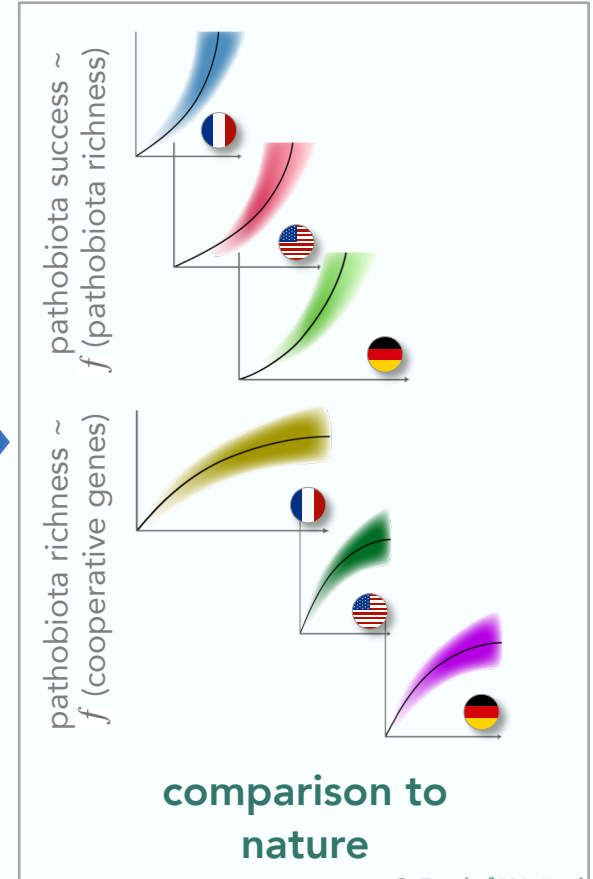
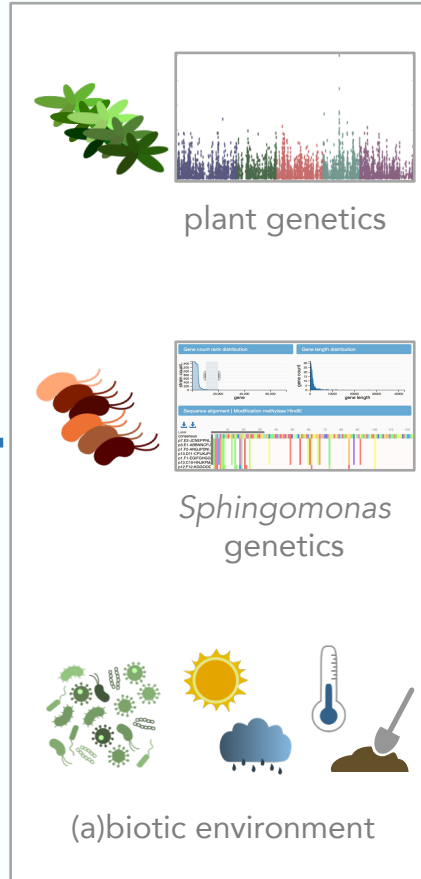
Aim 2&4: Bacteria-Bacteria-Environment Interactions



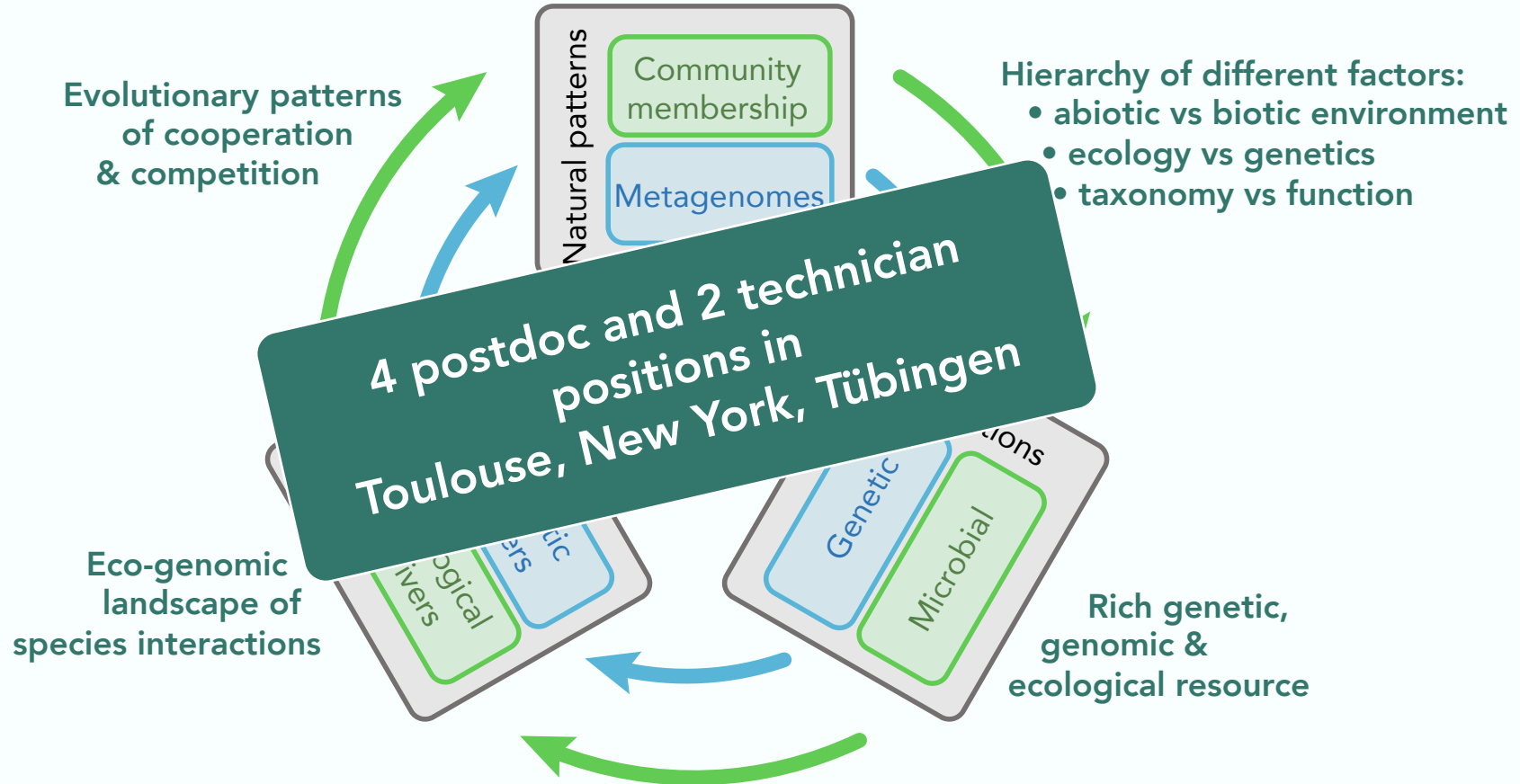
Aims 3&5: From Models to Predicting the Real World



+



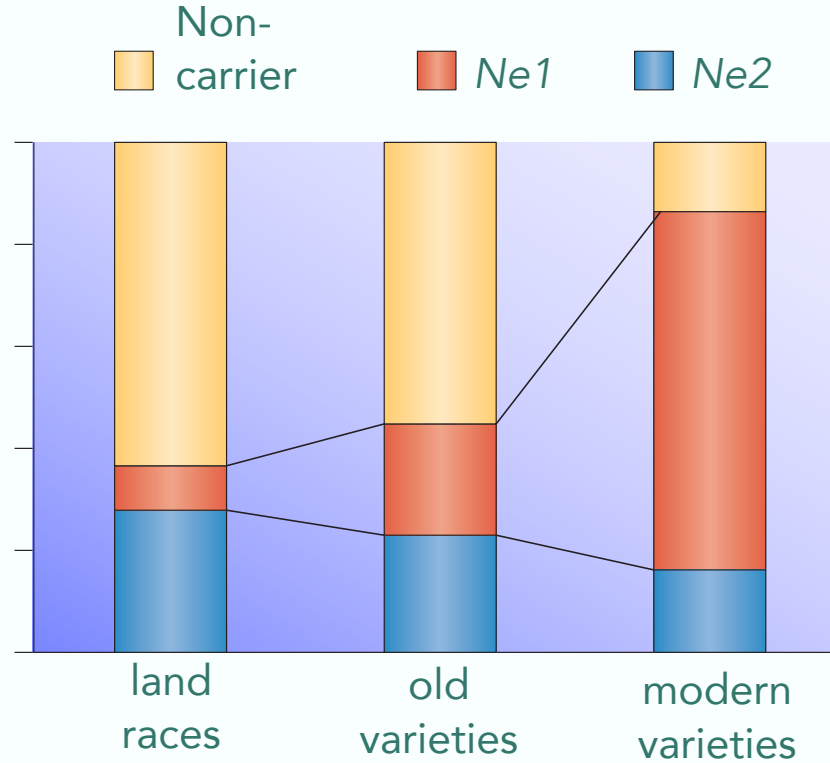
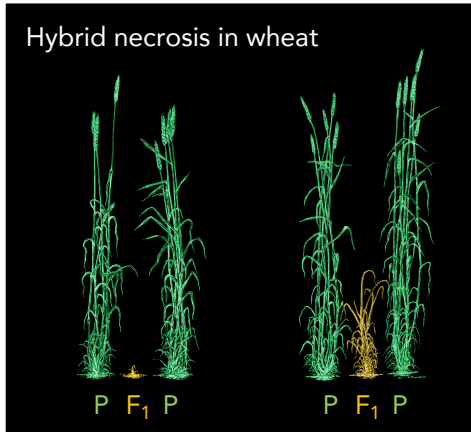
Understanding Key Drivers of Host-Pathobiota Dynamics







Historical Selection on Wheat *Ne* Genes



Could this eventually lead to speciation?