Experimental evolution of plant-RNA virus interactions

A game of resistances and virulence

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KITP Program in *Eco-evolutionary dynamics in Nature and the lab*
UC Santa Barbara, 14/09/2017

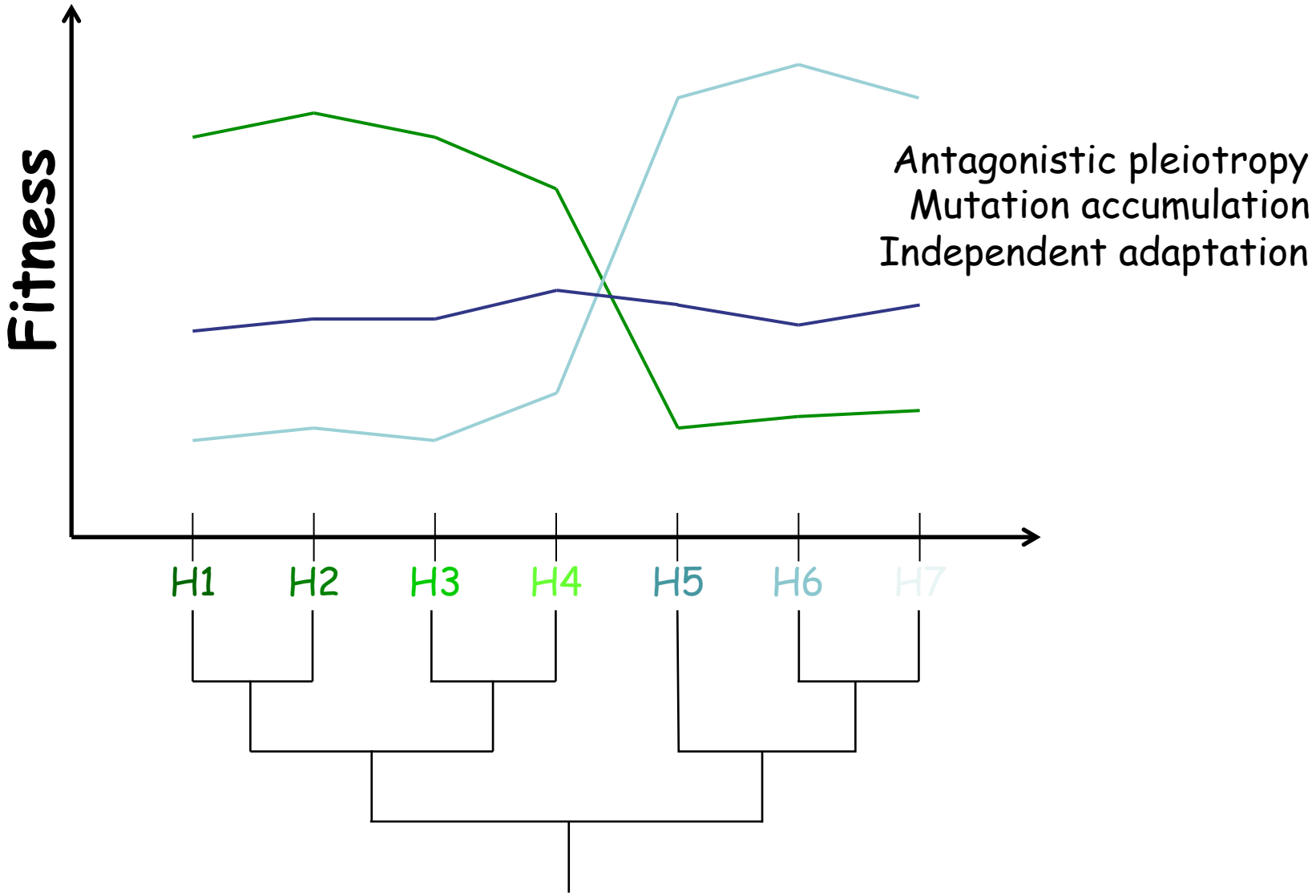
Consequences of evolving in hosts with different susceptibility to infection

- ✓ Host heterogeneity in response to infection may:
  - Select for a monomorphic generalist population.
  - A polymorphic population composed by specialists on each host genotype.
  - A monomorphic population composed by an specialist in the most abundant susceptible host.

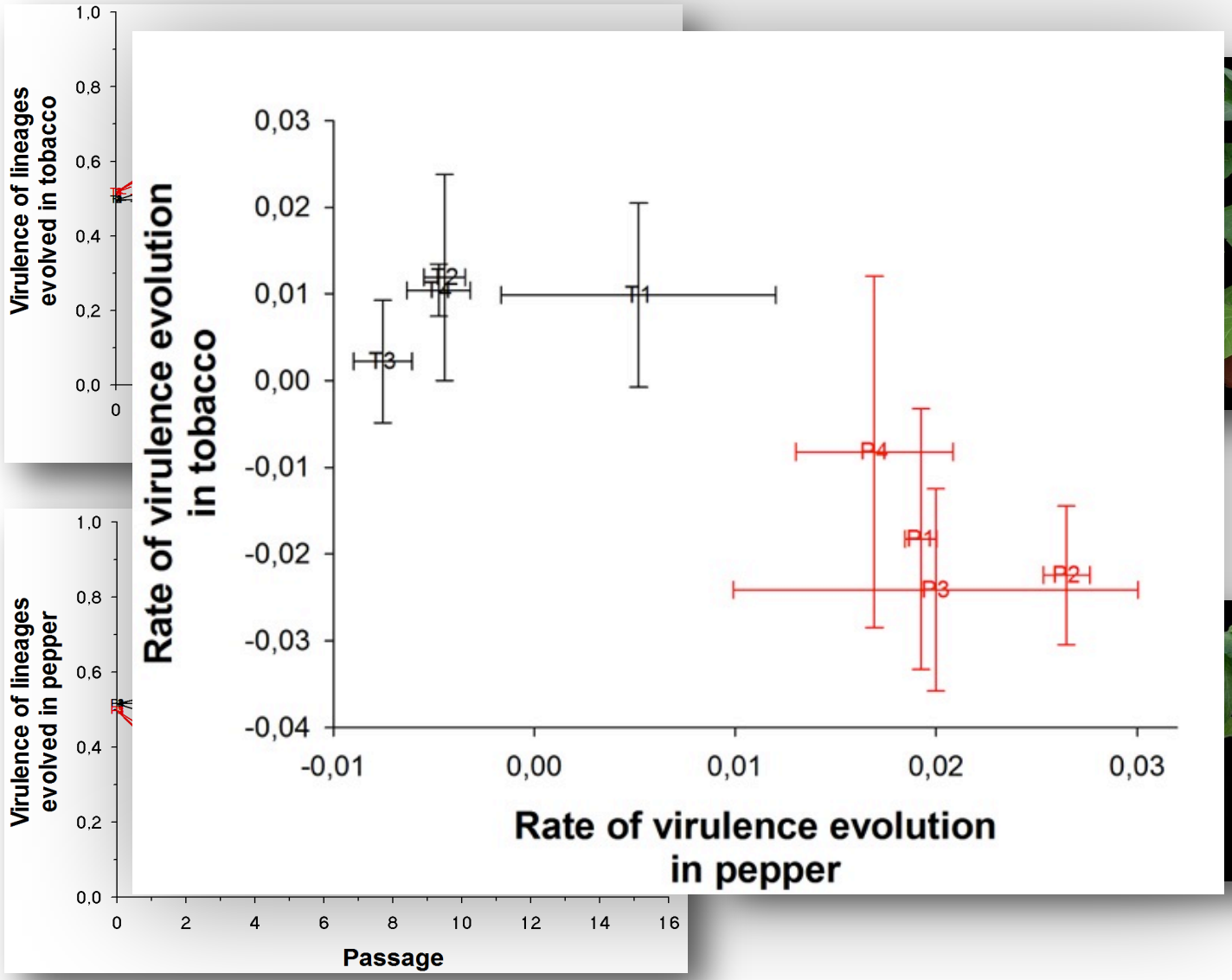
- ✓ The evolutionary outcome depends on:
  - Relative abundance and productivity of each host genotype.
  - The rate of transmission (gene flow) and whether coinfection/superinfection are frequent.
  - The strength of competition among viral phenotypes.
  - The heritability of host range (the genetic basis of virus adaptation).
  - Whether there is a fitness trade-off between host genotypes.

- ✓ In terms of virulence:
  - Strong host immunity may exacerbate selection for virulence (S. Gandon, A. Read…).
  - Virulence escalates up in an homogeneous population (D. Ebert, R. Regoes…).
  - An heterogeneous population cannot maintain virulence at intermediate levels in all host genotypes (M. Nowak, T. Day…).
  - Whether virulence escalates up or slides down to intermediate levels ultimately depends on the trade-off in replication between host genotypes.

Fitness tradeoffs across hosts

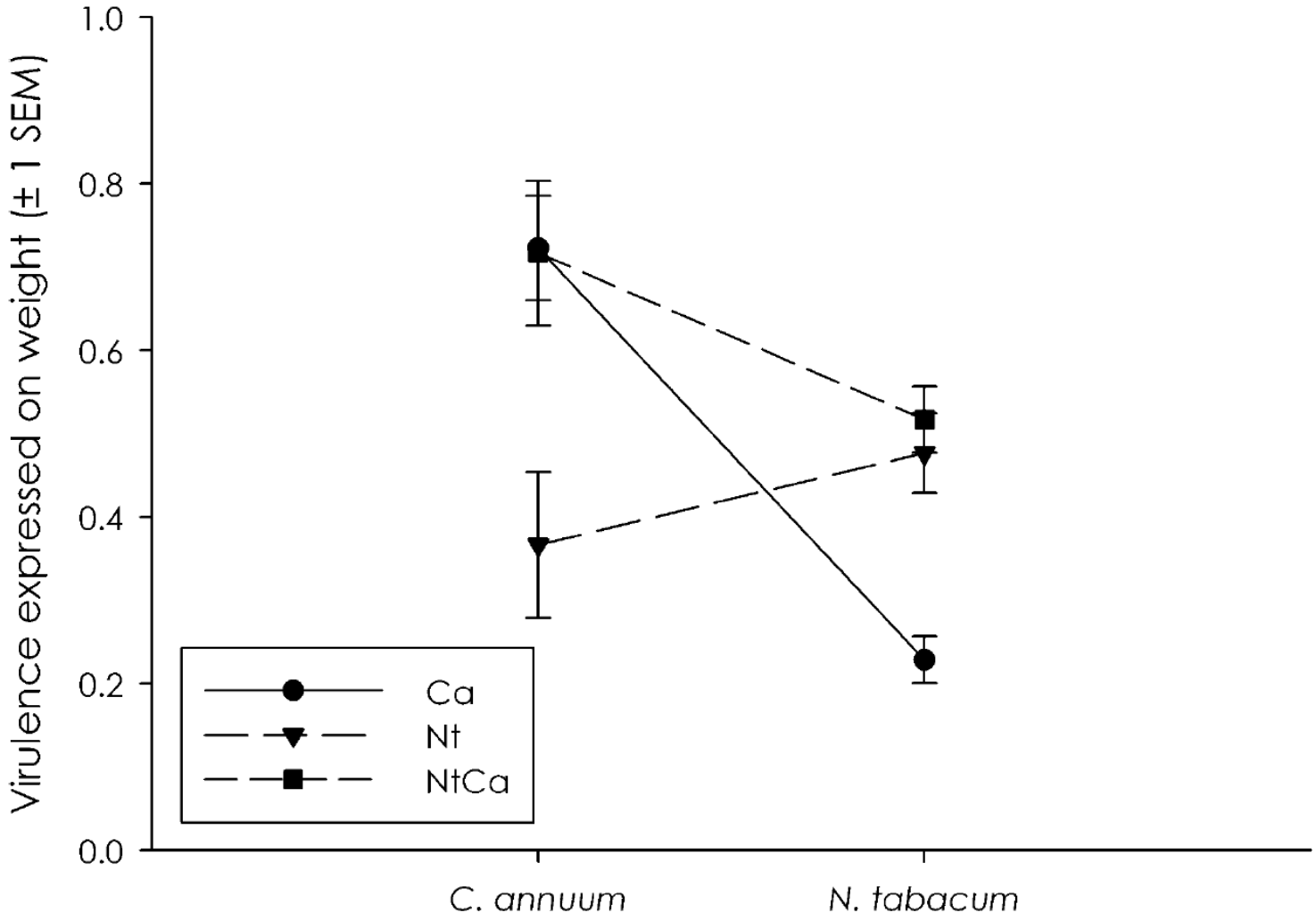


Evolution in a new single host promotes specialization and pays the cost of host-range expansion.



Agudelo-Romero *et al.* 2008 *Infect. Genet. Evol.*
Bedhomme *et al.* 2012 *Mol. Biol. Evol.*

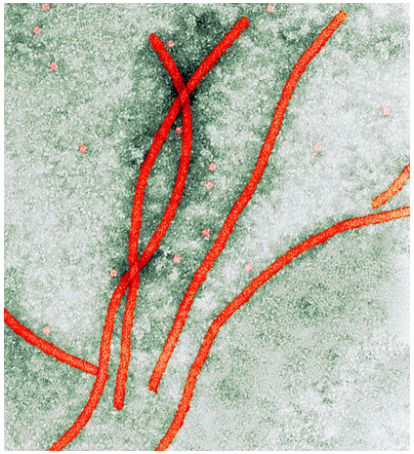
Generalists evolve under host-switching regimes without a fitness trade-off across hosts.

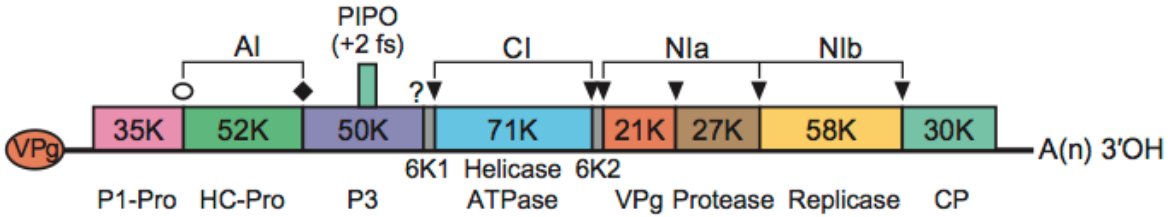


Bedhomme *et al.* 2012 *Mol. Biol. Evol.*

1. TEV/*Arabidopsis thaliana*, an experimental model for studying virus emergence and adaptation

The *A. thaliana* Ler-0 – TEV pathosystem





✓ TEV has a moderately wide host range infecting around 149 species from 19 families, although most belong to the *Solanaceae*.

✓ *Arabidopsis* is a *Brassicaceae*, which belongs to a different order than the *Solanaceae* within the class *Magnoliopsida*. Therefore, adaptation of TEV to *A. thaliana* represents a jump in host species at the taxonomic level of orders.

✓ *A. thaliana* ecotypes vary in their susceptibility to TEV due to the presence of at least five resistance dominant loci.

Restricted TEV Movement genes (*RTM*)

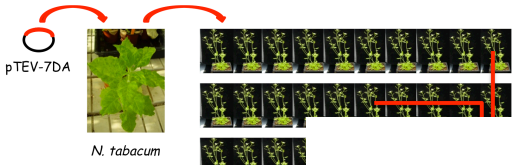
(J.C. Carrington, F. Revers *et al.*)

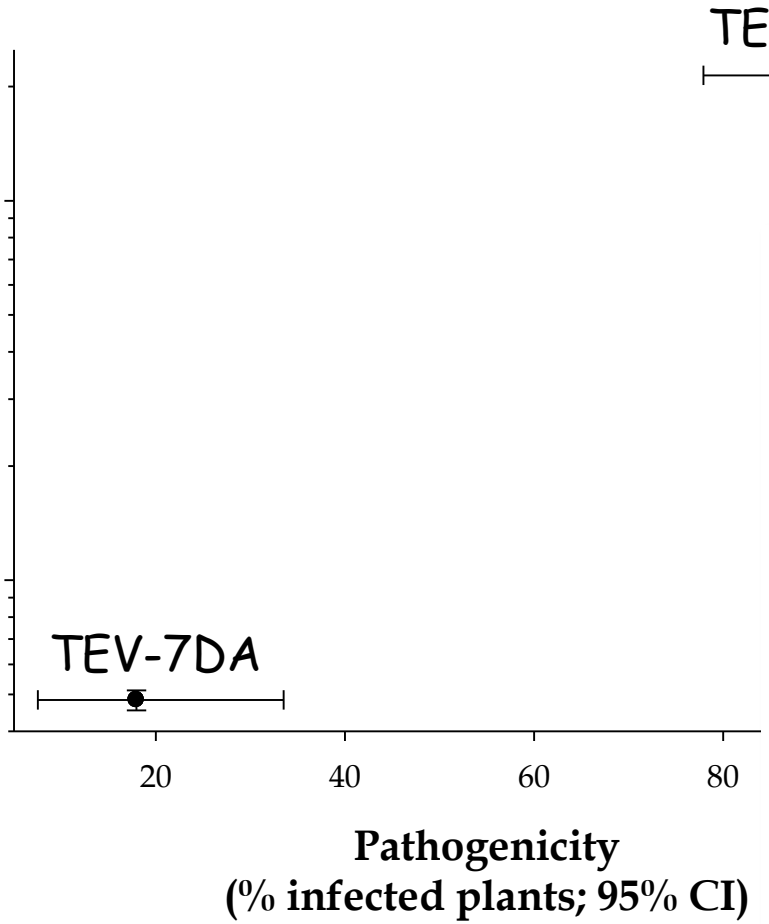
Characteristics of the *RTM* loci		
RTM1	Phloematic jacalin-related lectin-like protein -- carbohydrates binding, parasite recognition and stress response	Chromosome 1
RTM2	Phloematic HSP-like protein with a transmembrane domain -- stress response	Chromosome 3
RTM3	Meprin and TRAF homology (MATH) domain-containing protein -- regulation of protein processing and ubiquitination	Chromosome 3
RTM4	?	Chromosome 1
RTM5	?	Chromosome 2

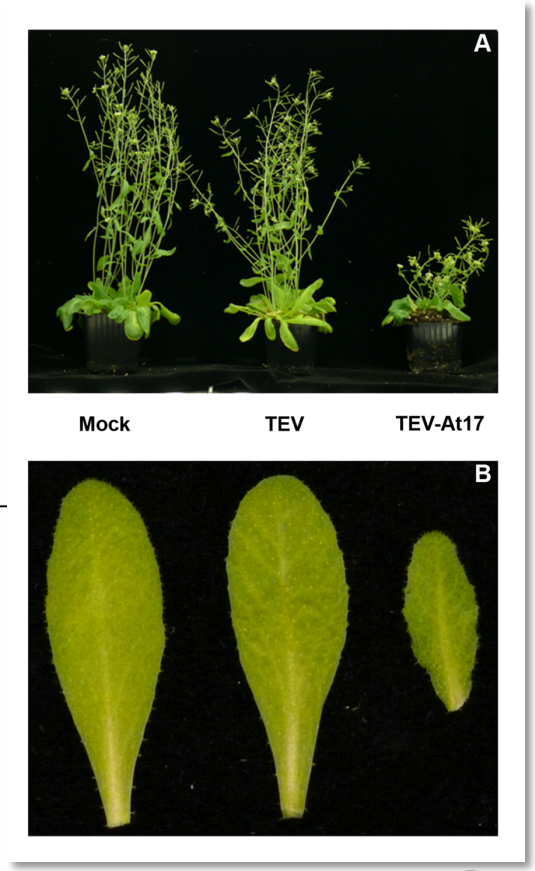
✓ Wildtype alleles at all loci abolish systemic movement, whereas homozygous mutations in anyone locus would allow it in more or less extent.

✓ Ler-0 (*rtm1/rtm1*).

Experimental evolution of TEV in Ler-0



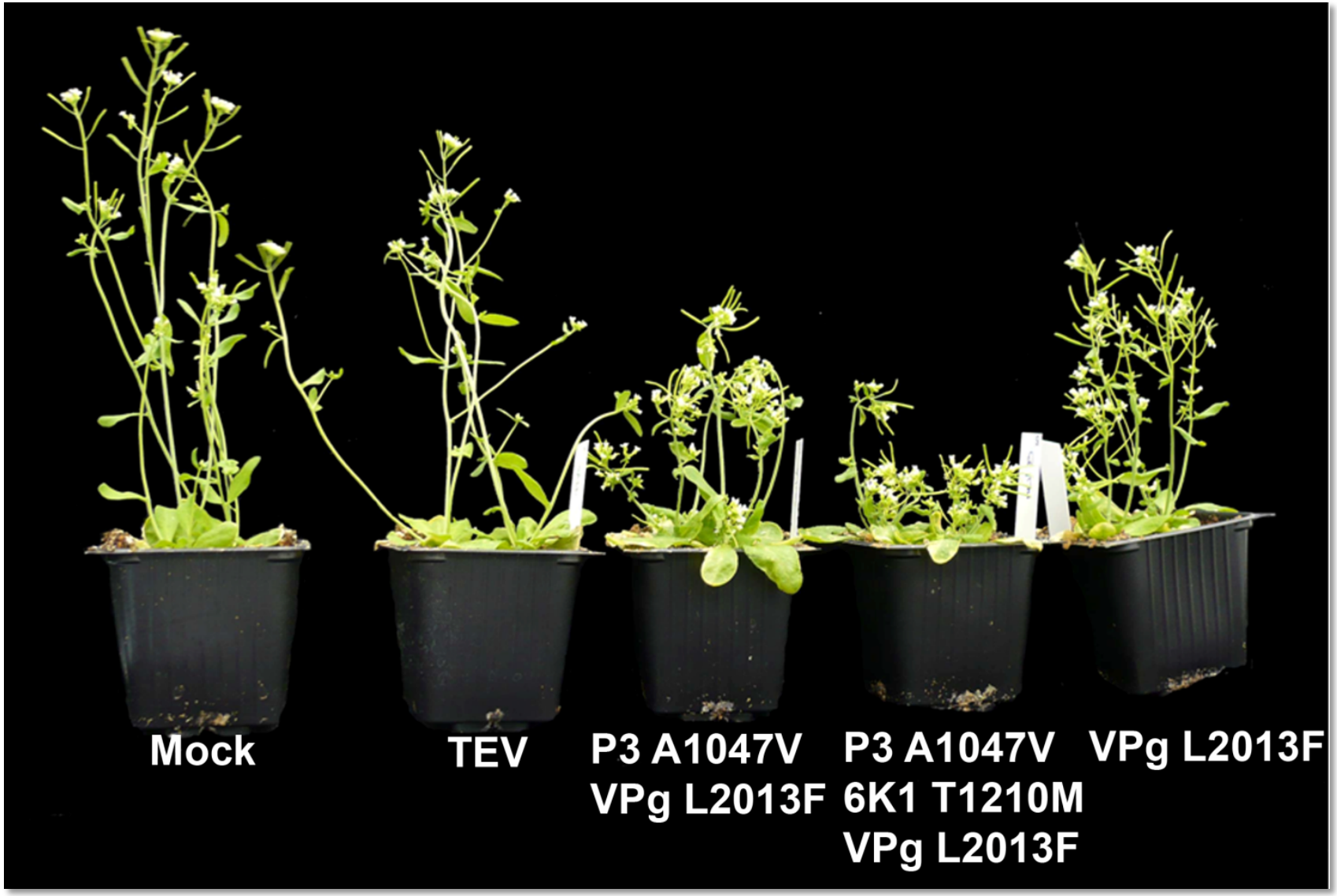




Agudelo-Romero *et al.* 2008 *PLoS ONE*

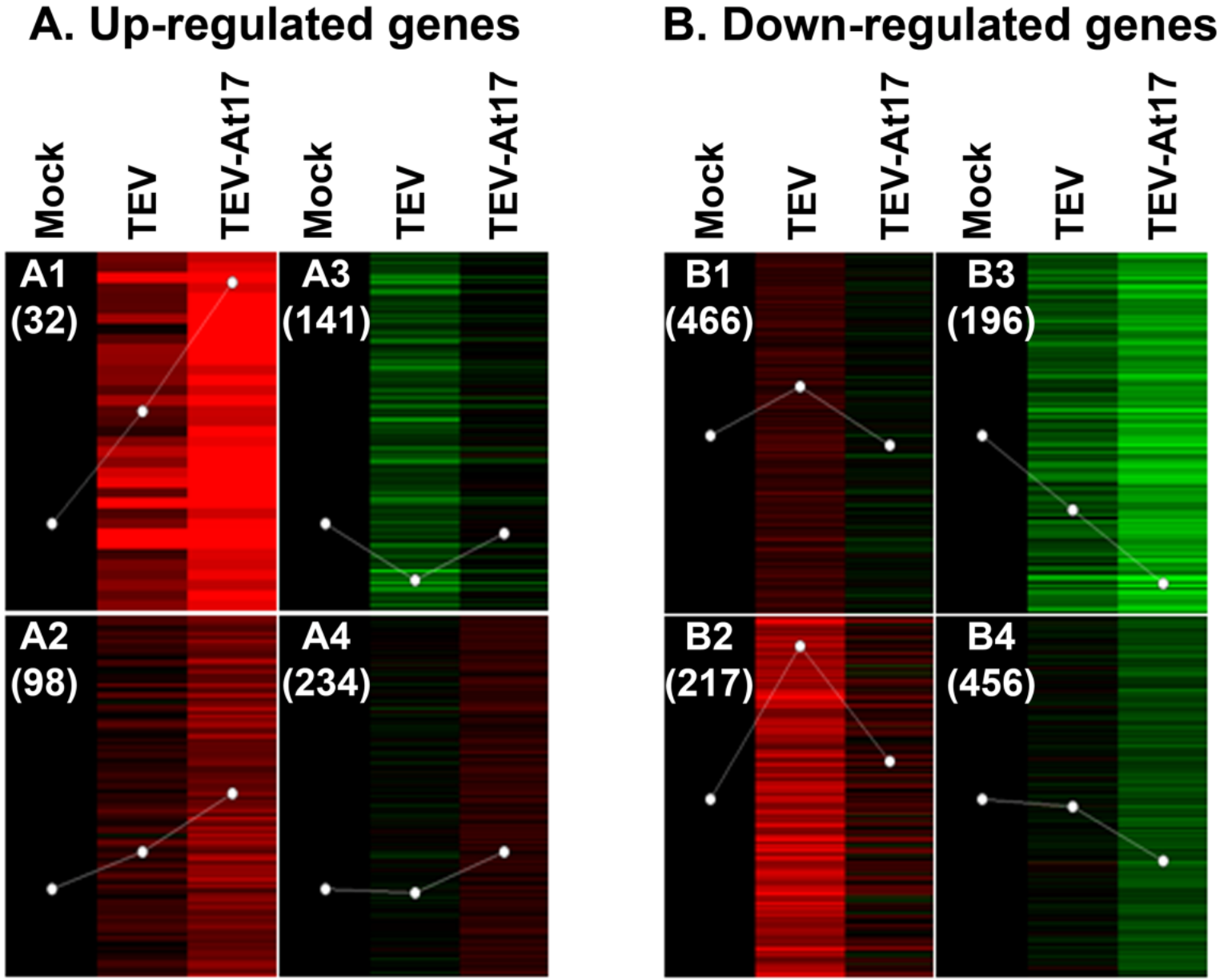
Molecular changes in TEV-*At*17 genome

Nucleotide change	Cistron and aa change	Symptoms severity
U357C	P1 synonymous	-
C3140U	P3 A1047V	-
C3629U	6K1 T1210M	-
C6037U	VPg L2013F	+
C6666U	NIa-Pro synonymous	-
C6906U	NIa-Pro synonymous	-
	A1047V/T1210M	-
	A1047V/L2013F	++
	T1210M/L2013F	++
	A1047V/T1210M/L2013F	+++



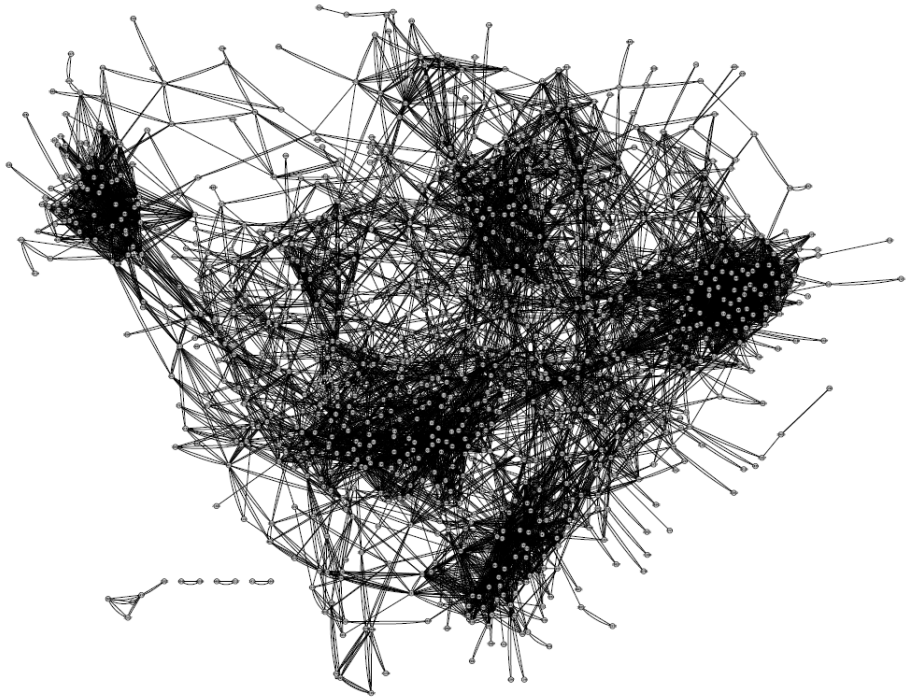
Agudelo-Romero *et al.* 2008 *PLoS ONE*

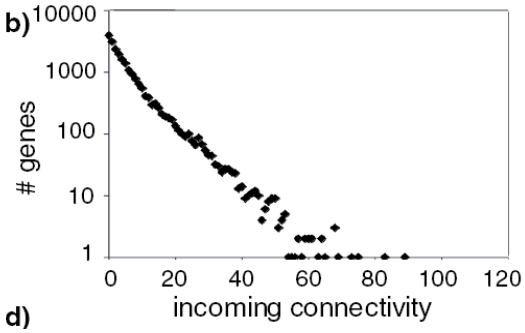
Changes in the plant response to infection

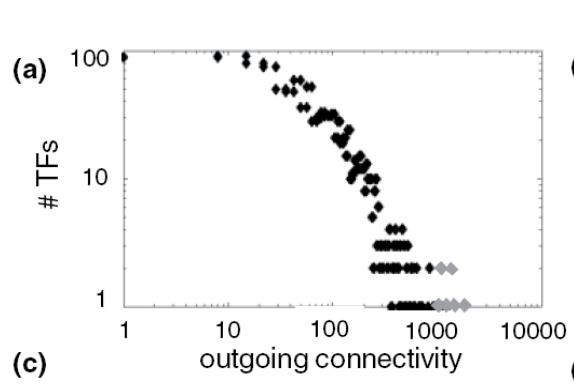


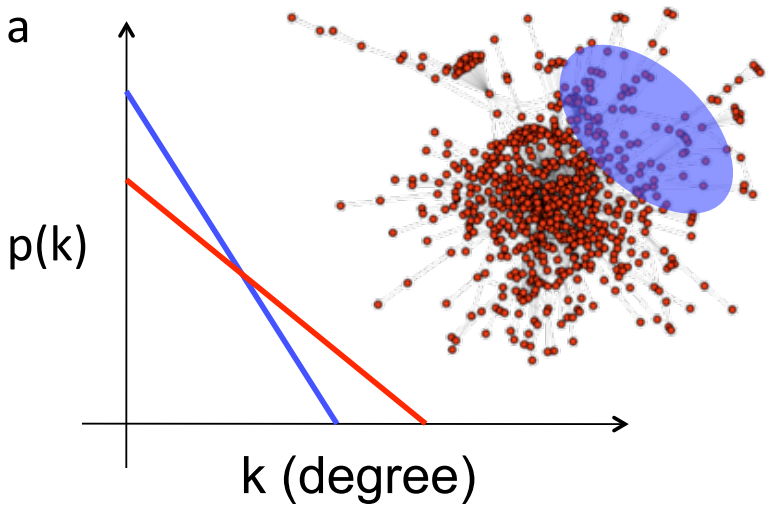
Agudelo-Romero *et al.* 2008 *PLoS ONE*

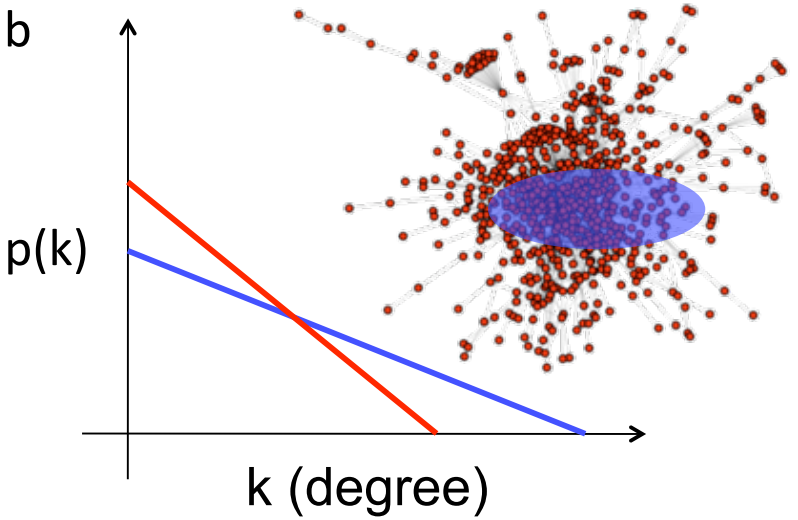
Over-represented GO terms in SOMs B1+B2	GO level	Differentially expressed (%)	Total genes in the class (%)	P
Response to wounding	4	4.26	0.76	< 0.001
Response to hormone stimulus	4	9.09	4.85	0.048
Cell-to-cell signaling	4	1.42	0.19	0.050
Response to cold	5	4.82	1.43	0.008
Response to bacterium	5	3.54	0.82	0.009
Thigmotropism	5	0.64	0.00	0.048
Hyperosmotic salinity response	6	2.47	0.27	0.010
Protein modification process	6	24.69	15.02	0.010
Response to light intensity	6	2.06	0.27	0.047
Protein amino acid phosphorylation	7	26.56	14.38	0.002
MAPKKK cascade	7	1.56	0.07	0.047
Systemic acquired resistance	8	5.38	0.47	0.013
Activation of innate immune resistance	9	10.53	0.61	0.015





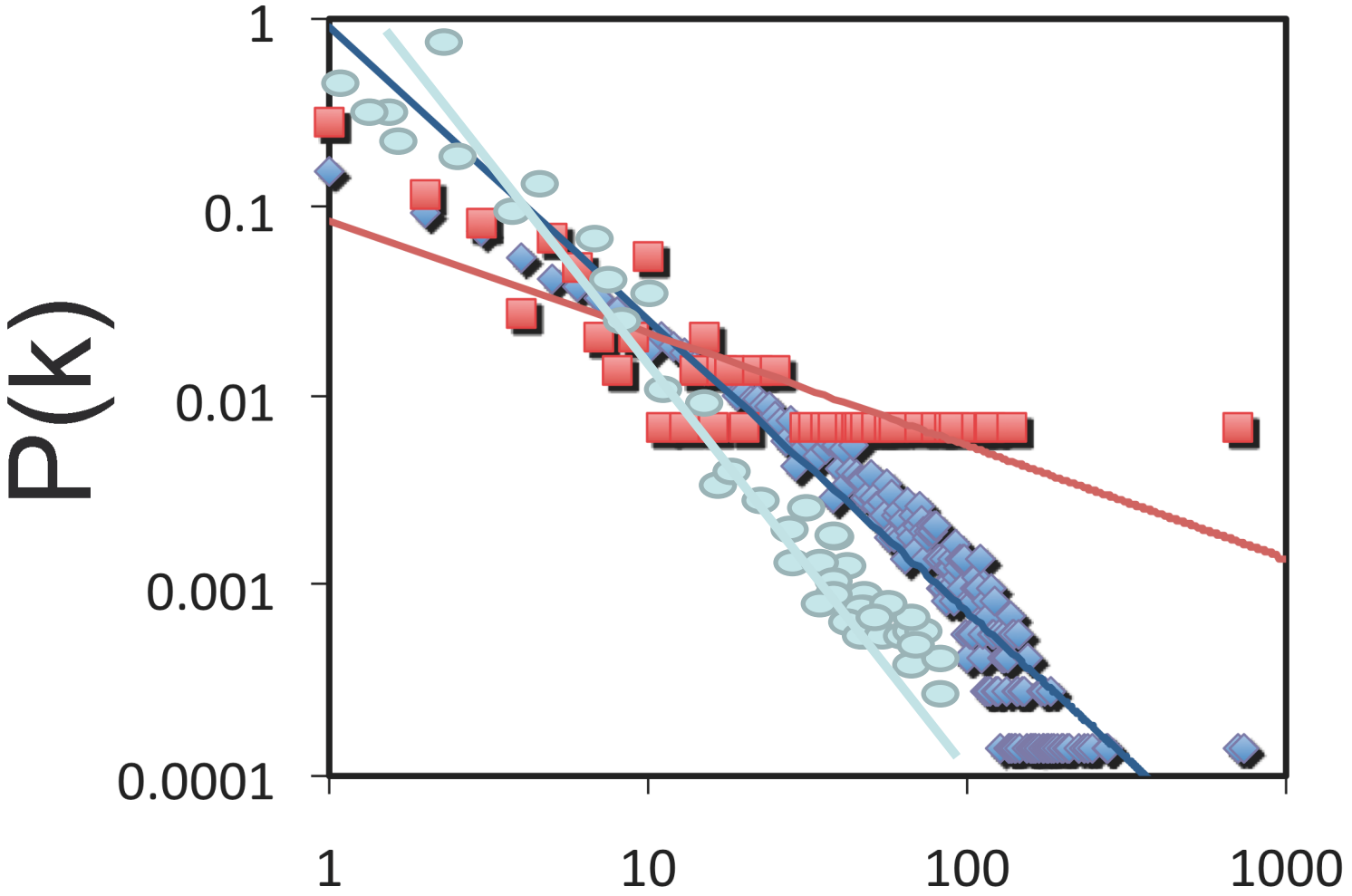






Carrera *et al.* 2009 *Genome Biol.*
Elena *et al.* 2011 *Curr. Opin. Plant Biol*

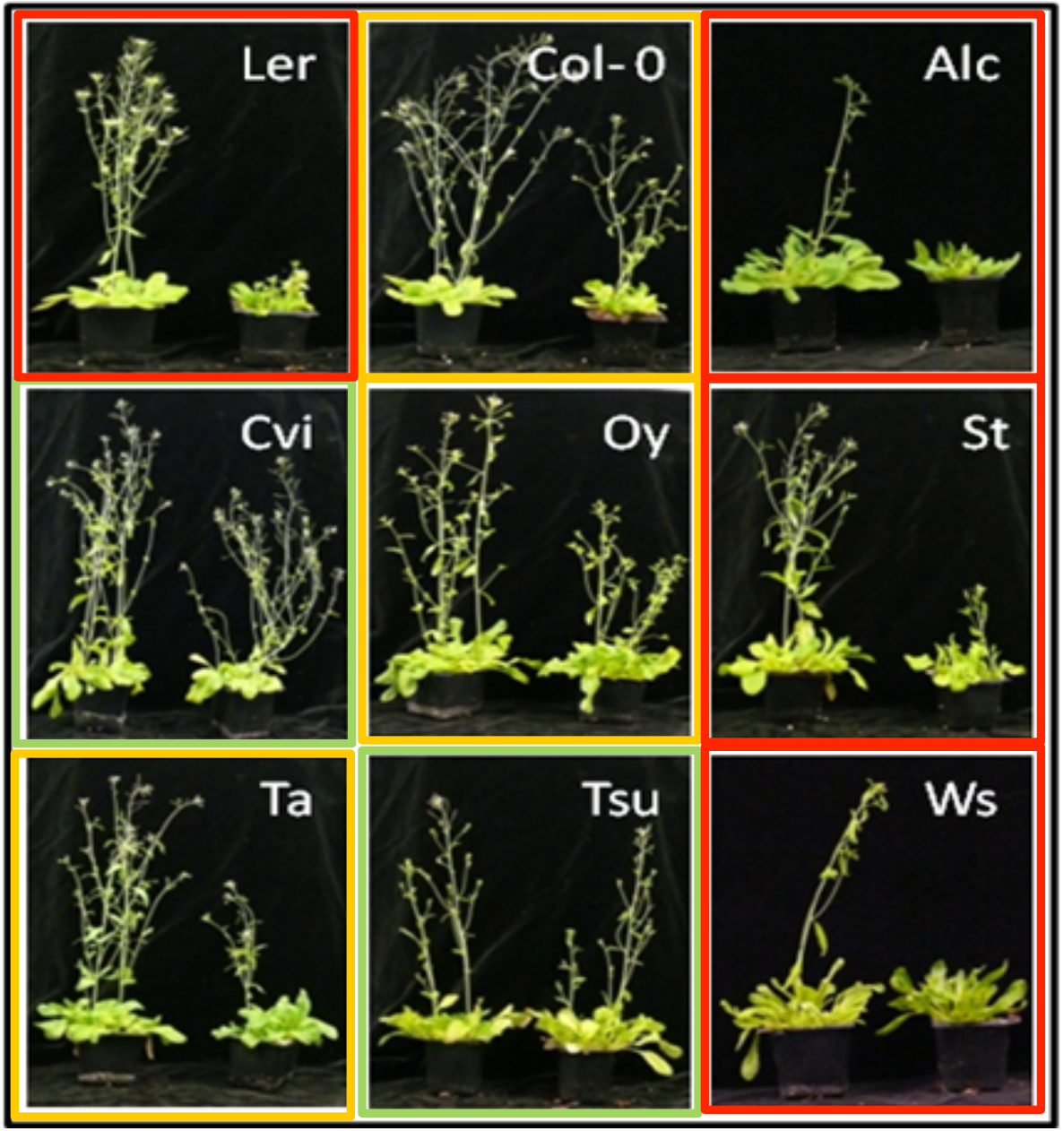
Targeting hubs in the host regulatory network



Rodrigo *et al.* 2012 *PLoS ONE*

2. Erasing differences in susceptibility to infection

Accession	TEV[1]	TEV-*At*17	*P*[2]	Category[3]
Alc-0	0			
Col-0	0			
Cvi-0	0			
Di-2	0			
Ei-2	0 ??			
Ga-0	0			
Ler-0	0.180 ± 0.104			
Oy-0	0			
Sorbo-0	0			
St-0	0.429 ± 0.121			
Ta-0	0			
Tsu-0	0			
Ws-0	0			
Wt-1	0			



Mild

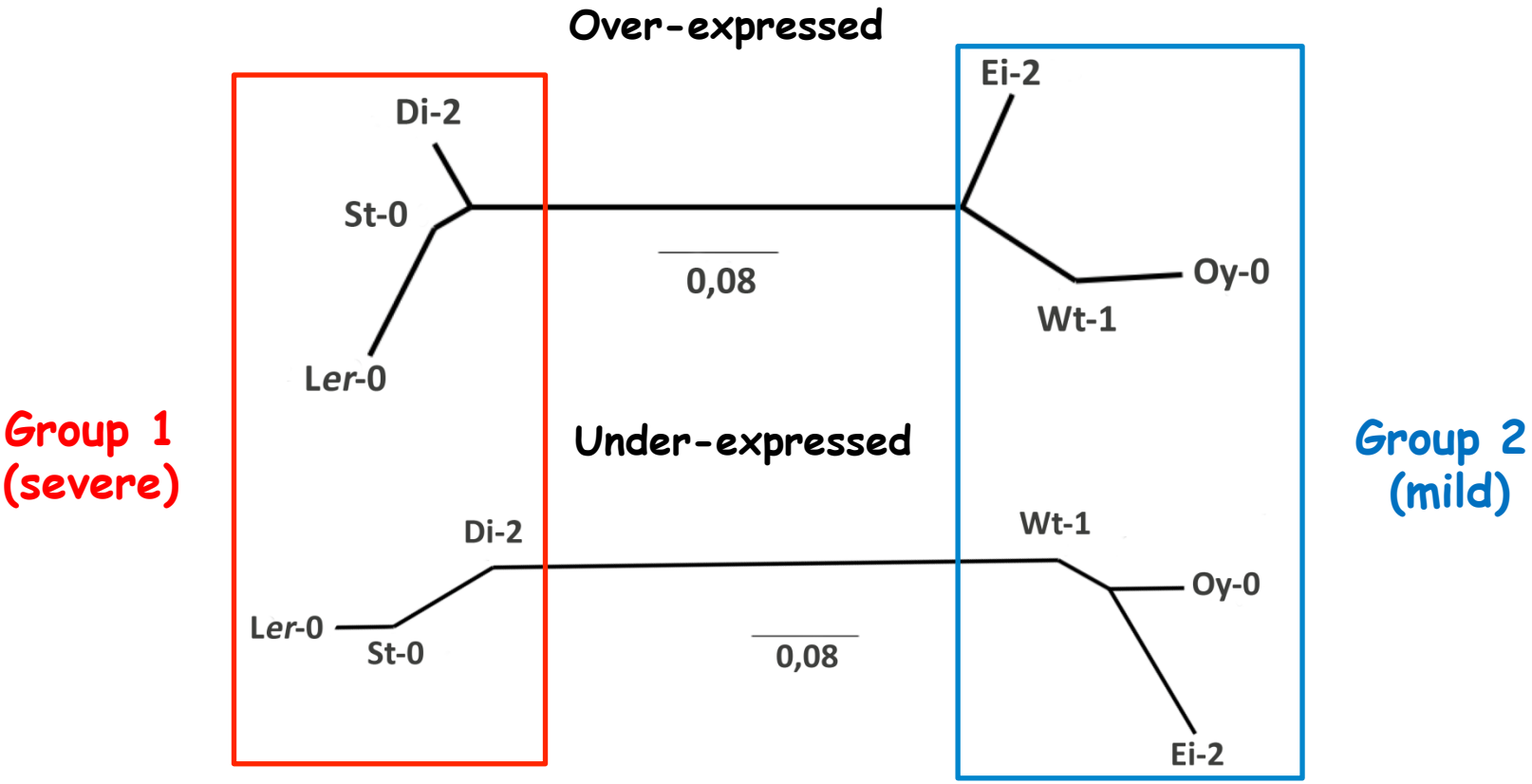
Moderate

Severe

Lalić *et al.* 2010 *Phil. Trans. R. Soc. B*

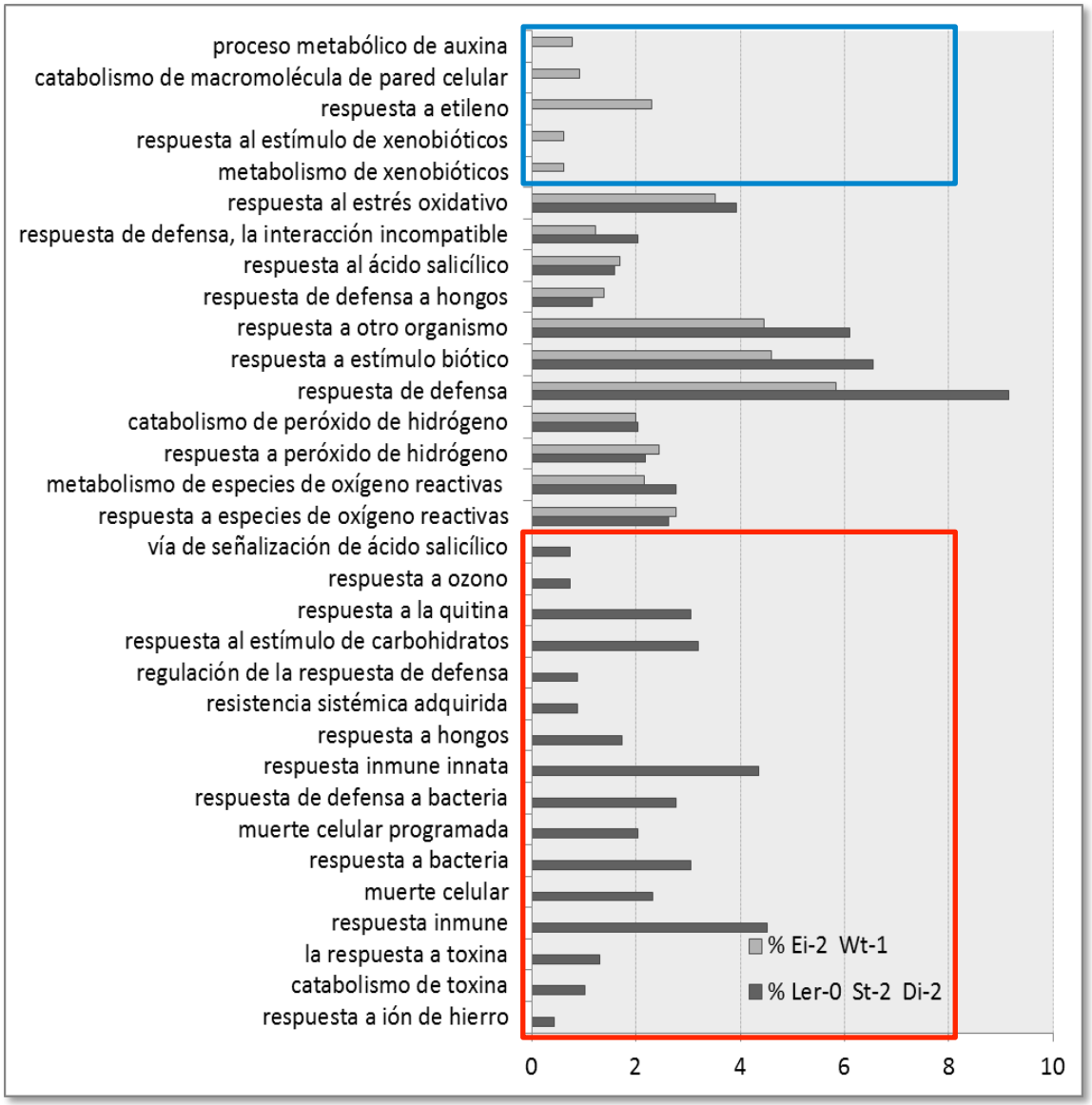
- ✓ Transcriptomic analysis (Agilent 4 × 44) of different ecotypes infected with TEV-*At*17.

- ✓ NJ dendograms from the pairwise similarity matrix computed between lists of (A) over- and (B) under-expressed genes.



Hillung *et al.* 2012 *Front. Microbiol.*

Functional categories among over-expressed



- ✓ **Similarities:**
  - 5 related to oxidative stress
  - 6 related to defense responses
- ✓ **Differences:**
  - 16 processes exclusive of **Group 1**: more defense
  - 5 processes exclusive of **Group 2**: metabolism → tolerance

Hillung *et al.* 2012 *Front. Microbiol.*

3. The extent and cost of local adaptation

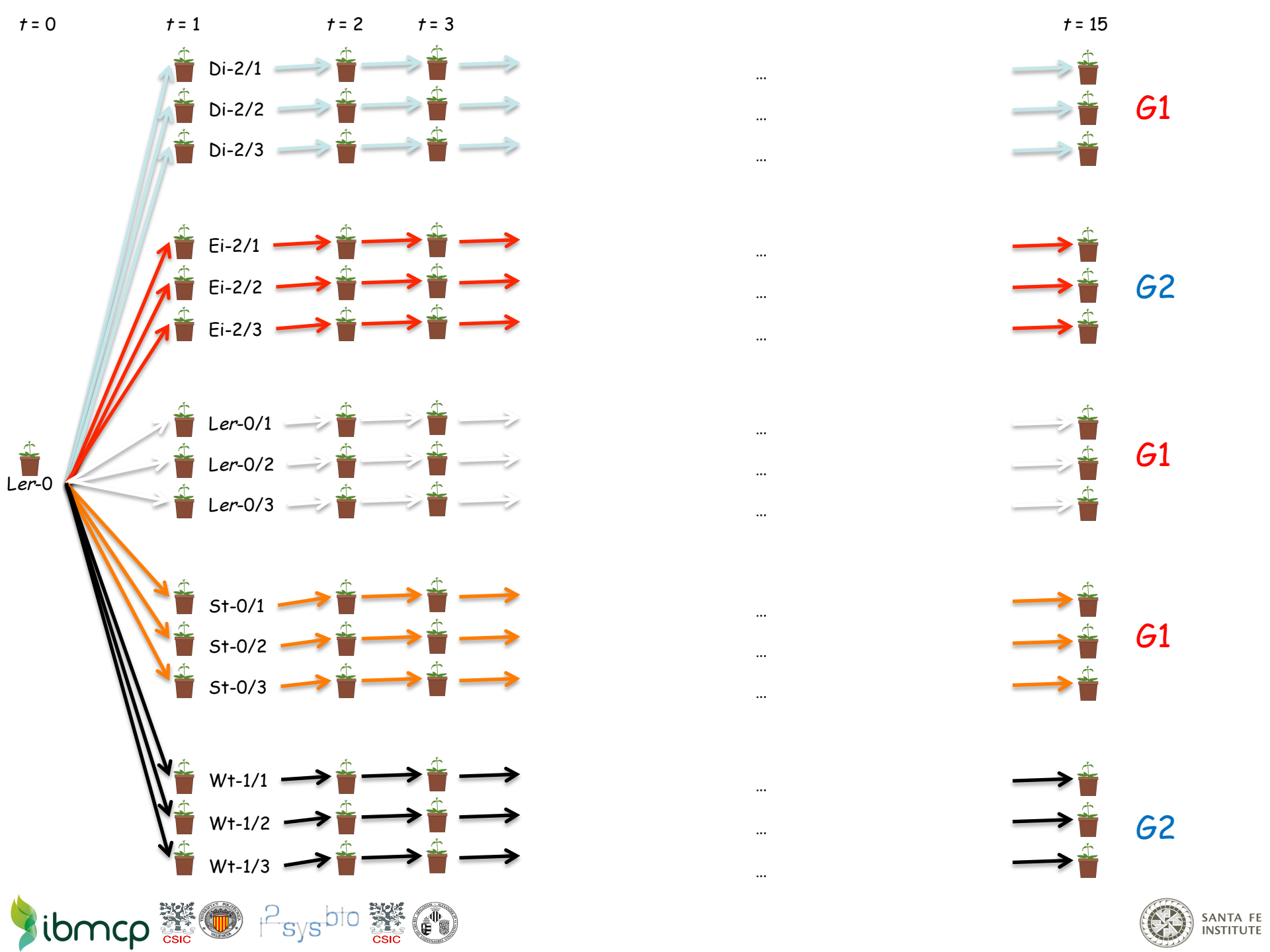
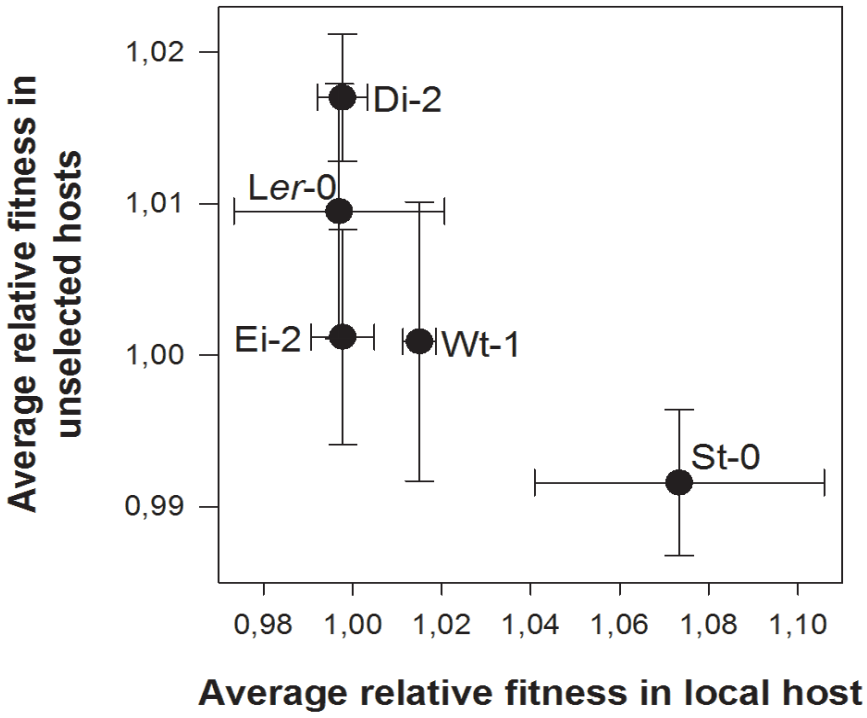
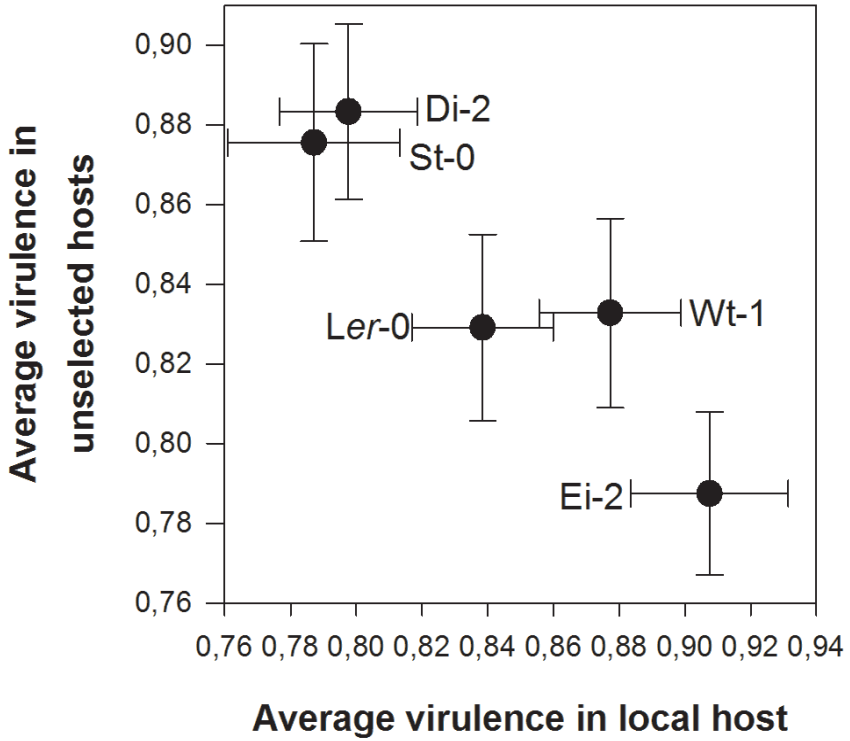

t = 0
t = 1
t = 2
t = 3
t = 15
Ler-0
Di-2/1
Di-2/2
Di-2/3
Ei-2/1
Ei-2/2
Ei-2/3
Ler-0/1
Ler-0/2
Ler-0/3
St-0/1
St-0/2
St-0/3
Wt-1/1
Wt-1/2
Wt-1/3
G1
G2
G1
G1
G2

Table S1. Relative fitness values for each evolved lineage measured on each alternative host genotype. The gray shadow indicates tests of adaptation to the local host. Values are the average of a number of infection assays (between five and nine) and errors correspond to ±1 SEM. Asterisks indicate cases in which the value is significantly different from the value estimated for the ancestral TEV-*At*17b isolate (one-sample *t*-tests, $P < 0.05$; significance levels corrected by the FDR method).

		Test host				
Local host	**Lineage**	**Di-2**	**Ei-2**	**L*er*-0**	**St-0**	**Wt-1**
Di-2	1	0.990±0.000*	0.984±0.002*	0.998±0.021	1.044±0.015*	1.061±0.036
	2	0.995±0.012	0.988±0.004*	1.002±0.000*	1.024±0.028	1.021±0.004*
	3	1.009±0.004*	0.987±0.015	1.000±0.021	1.041±0.010*	1.054±0.027
Ei-2	1	1.018±0.002*	1.006±0.004	0.954±0.024	1.024±0.015	0.953±0.032
	2	1.001±0.014	1.004±0.003	0.991±0.005	1.027±0.009*	1.015±0.011
	3	1.006±0.002*	0.984±0.011	0.992±0.006	1.031±0.008*	1.003±0.008
L*er*-0	1	1.003±0.004	0.980±0.007*	0.953±0.021	1.084±0.038	1.029±0.014
	2	1.001±0.005	1.012±0.004*	1.004±0.024	1.013±0.010	1.014±0.008
	3	0.986±0.004*	0.987±0.051	1.034±0.037	1.028±0.009*	0.978±0.040
St-0	1	0.981±0.005*	0.975±0.016	0.980±0.028	1.027±0.013*	0.995±0.001*
	2	0.998±0.003	0.996±0.008	0.982±0.026	1.057±0.003*	1.021±0.004*
	3	0.992±0.008	0.995±0.007	0.994±0.011	1.136±0.006*	0.991±0.005
Wt-1	1	1.013±0.004*	0.967±0.037	0.997±0.009	0.991±0.036	1.011±0.004*
	2	0.994±0.005	0.999±0.023	0.950±0.014*	1.023±0.010	1.011±0.003*
	3	0.995±0.002*	0.993±0.004	1.004±0.028	1.085±0.019*	1.022±0.005*

The specificity of adaptation





Hillung *et al.* 2014 *Evolution*

The specificity of adaptation

Table 3. GLM analyses of variance for the three traits measured for all evolved lineages across the four new hosts (Di-2, Ei-2, St-0, and Wt-1).

Source of variation	Relative fitness				Infectivity				Virulence			
	Wald's χ^2	d.f.	*P*	η_P^2	Wald's χ^2	d.f.	*P*	η_P^2	Wald's χ^2	d.f.	*P*	η_P^2
Intersection μ	36674175.722	1	< 0.001	1.000	0.000	1	1.000	0.954	6743.107	1	< 0.001	1.000
Local host *LH*	555.603	4	< 0.001	0.318	17.562	4	0.002	0.079	19.098	4	0.001	0.141
Lineage *L*(*LH*)	2667.073	10	< 0.001	0.179	81.834	10	< 0.001	0.667	89.816	10	< 0.001	0.376
Test host *TH*	14629.123	4	< 0.001	0.758	61.624	4	< 0.001	0.550	12.848	4	0.012	0.137
Local host by Test host *LH*×*TH*	4655.737	16	< 0.001	0.278	39.447	16	0.001	0.381	82.540	16	< 0.001	0.357
Test host by Lineage *TH*×*L*(*LH*)	12124.267	40	< 0.001	0.186	86.925	39	< 0.001	1.000	149.934	40	< 0.001	0.161
Biological replicate *R*(*TH*×*L*(*LH*))	53712.912	295	< 0.001	0.979								

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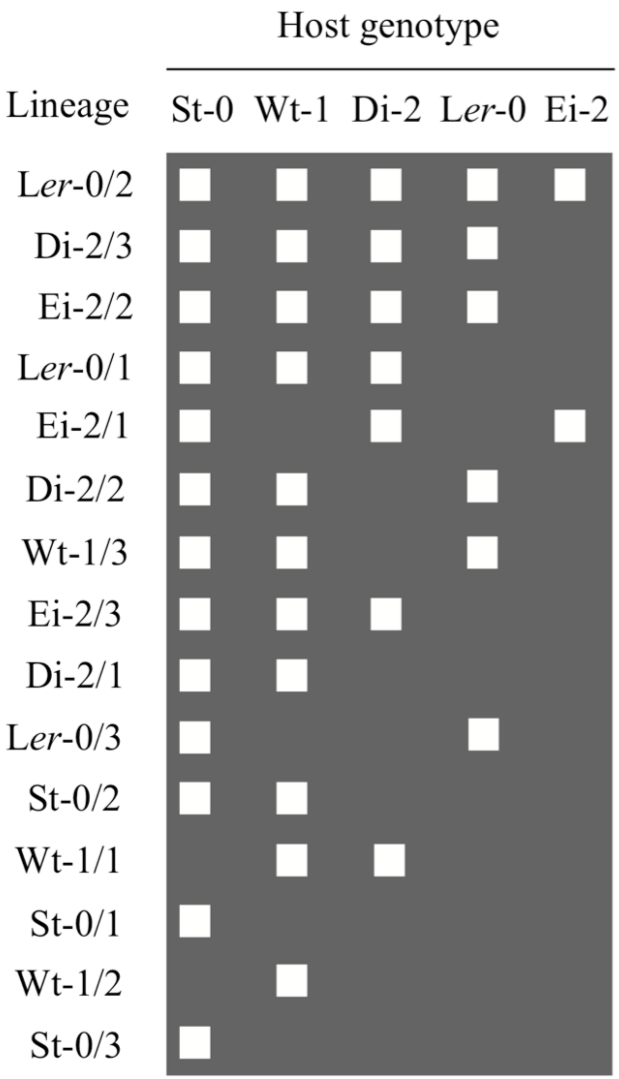
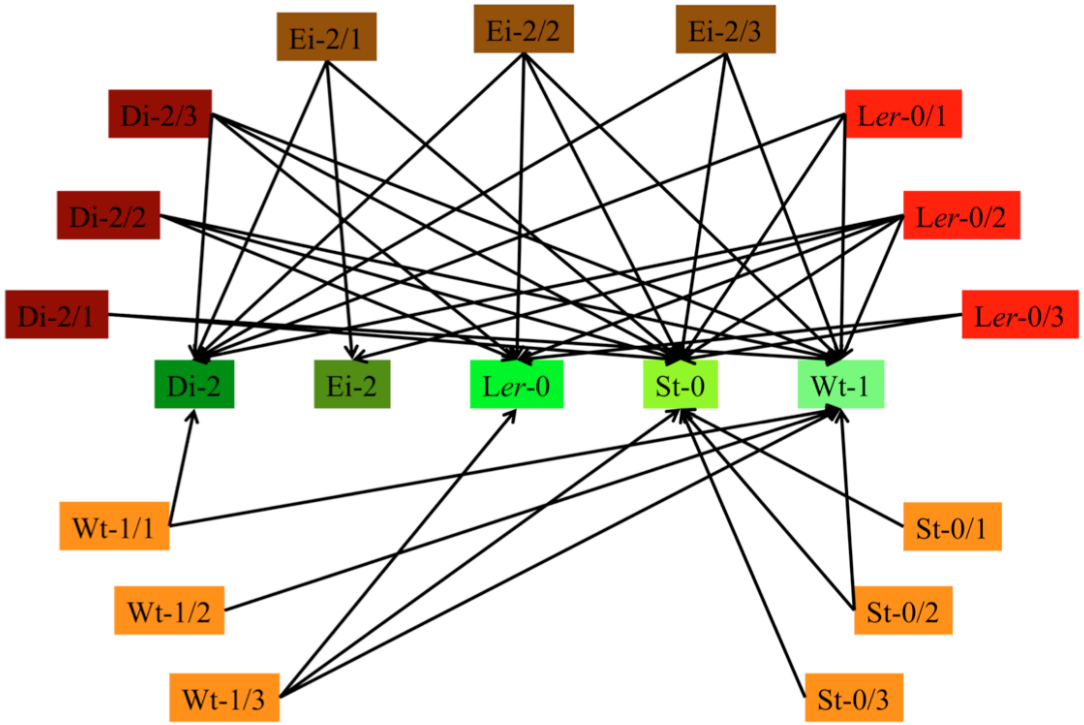
Analysis of the infection network

(A)



(B)



- ✓ Non-modular (P = 0.202).
- ✓ Significantly nested (P = 0.019): generalist viruses infect most ecotypes; very permissive ecotypes are available to all viruses: Gene-for-Gene model.

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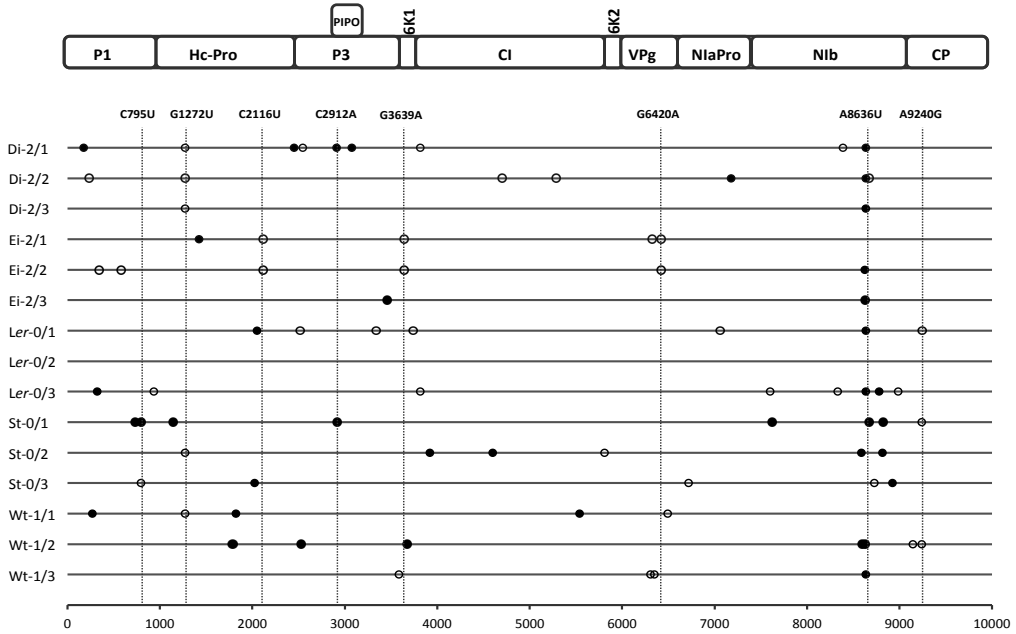
4. The molecular basis of TEV local adaptation

Genomic evolution



- ✓ 79 mutations at 62 sites (2-8 per lineage, except *Ler*-0/2).
- ✓ 42 synonymous, 37 nonsynonymous.
- ✓ 6 convergent synonymous: 3 exclusive of Ei-2 (*C2116U*, *G3639A*, *G6420A*). 1 exclusive of St-0 (*C795U*). *G1272U* shared by Di-2 and St-0 lineages. *A9240G* common to *Ler*-0/1, St-0/1 and Wt-1/2.
- ✓ 3 convergent nonsynonymous: *C2912A* (A923D in *P3* cistron and L923I in *P3N-PIPO*) shared by lineages Di-2/1 and St-0/1. *C8636U* (S2831L in *CP*) shared by all Di-2 and *Ler*-0 and by Wt-1/3. Lineages Ei-2/2 (*C8624U*) and Ei-2/3 (*U8623C*) affect the same *CP* codon but resulting in different amino acid replacements (S2827L and S2827P).

Hillung *et al.* 2014 *Evolution*
Hillung *et al.* 2015 *Phil. Trans. R. Soc. B*

Genomic evolution

- ✓ Treating each lineage as an observation and each host ecotype as a subpopulation, we decomposed nucleotide diversity:

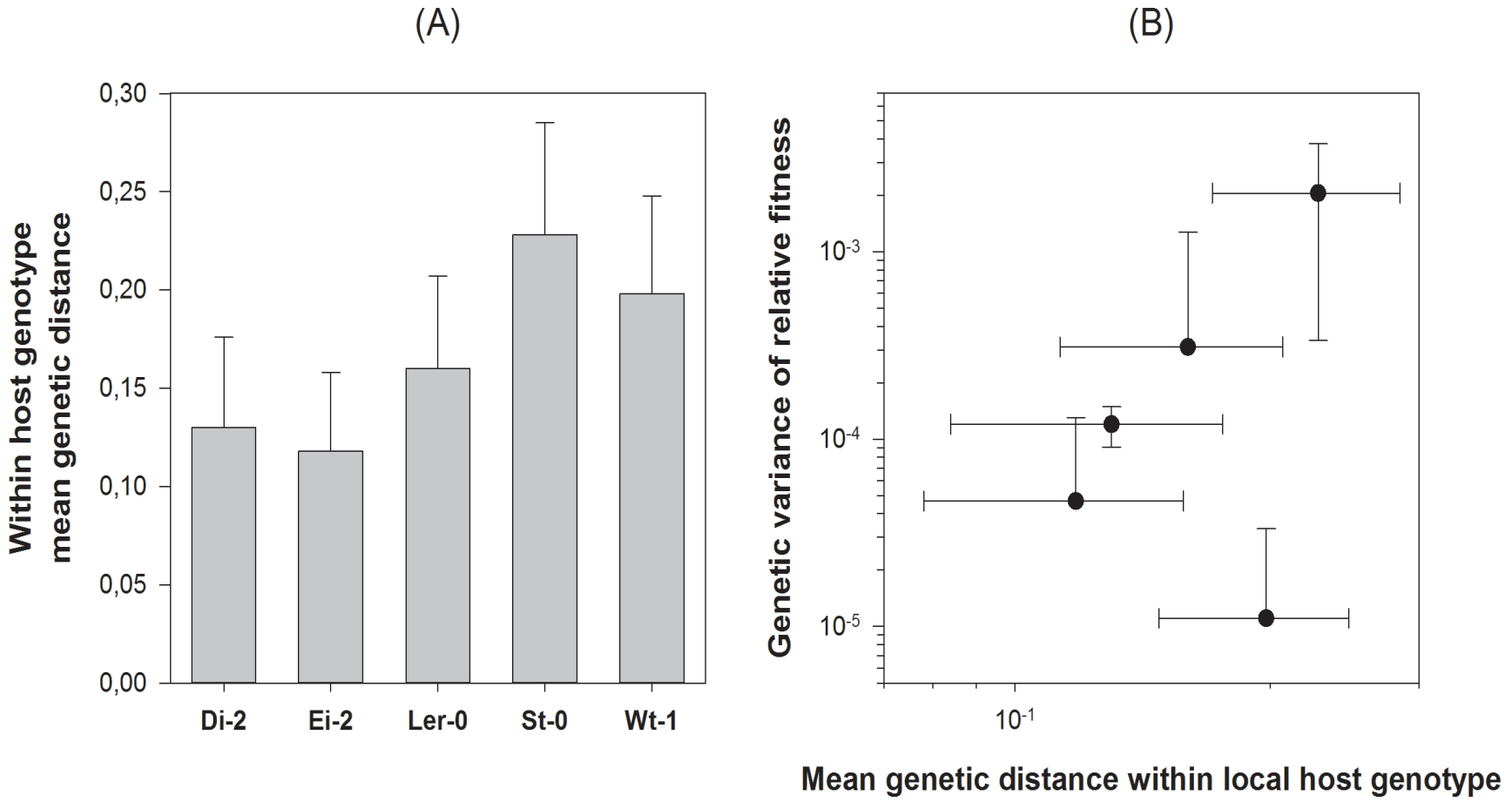
  Within host: π_S = 0.167±0.008.
  Entire sample: π_T = 0.187±0.014.
  Inter-host: $\delta_{ST} = \pi_T - \pi_S$ = 0.019±0.010.

  Coefficient of nucleotide differentiation: $N_{ST} = \delta_{ST}/\pi_T$ = 0.103±0.043 (*z*-test, 1-tail *P* = 0.004).

- ✓ We conclude that minor yet significant genetic differentiation has been generated among viruses replicating in different host genotypes.

- ✓ To assess whether selection played a role in genetic differentiation among host genotypes, we performed a Tajima's *D* test and found that it was significantly negative (*D* = −2.172, *P* = 0.015). The existence of 55 singletones suggests than population expansion, rather than purifying selection, explains *D* < 0.

Association between molecular diversity and genetic variance for fitness



Discarding Wt-1: r_S = 1.000, 2 d.f., 1-tail P < 0.001

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Selection for translational efficiency at synonymous sites

- ✓ A possible explanation for convergence at synonymous sites is that selection for translational efficiency results in the replacement of poorly used codons by synonymous ones for which the host cell has a large pool of tRNAs.

- ✓ For each of the 62 mutations, we computed the relative change in usage between the evolved and the ancestral codons $C = f_{evolved} / f_{ancestral} - 1$. The hypothesis being true $C > 0$ for convergent synonymous mutations and significantly larger than for all other types of mutations.

- ✓ For convergent synonymous mutations $\langle C \rangle = 0.494 \pm 0.351$ (±1 SEM); for the rest of mutations $\langle C \rangle = -0.009 \pm 0.066$ (2-samples *t*-test, 1-tail $P = 0.016$).

- ✓ We conclude that convergent synonymous mutations fixed during evolution resulted in codons that were ~50% more used by the *A. thaliana* translational machinery.

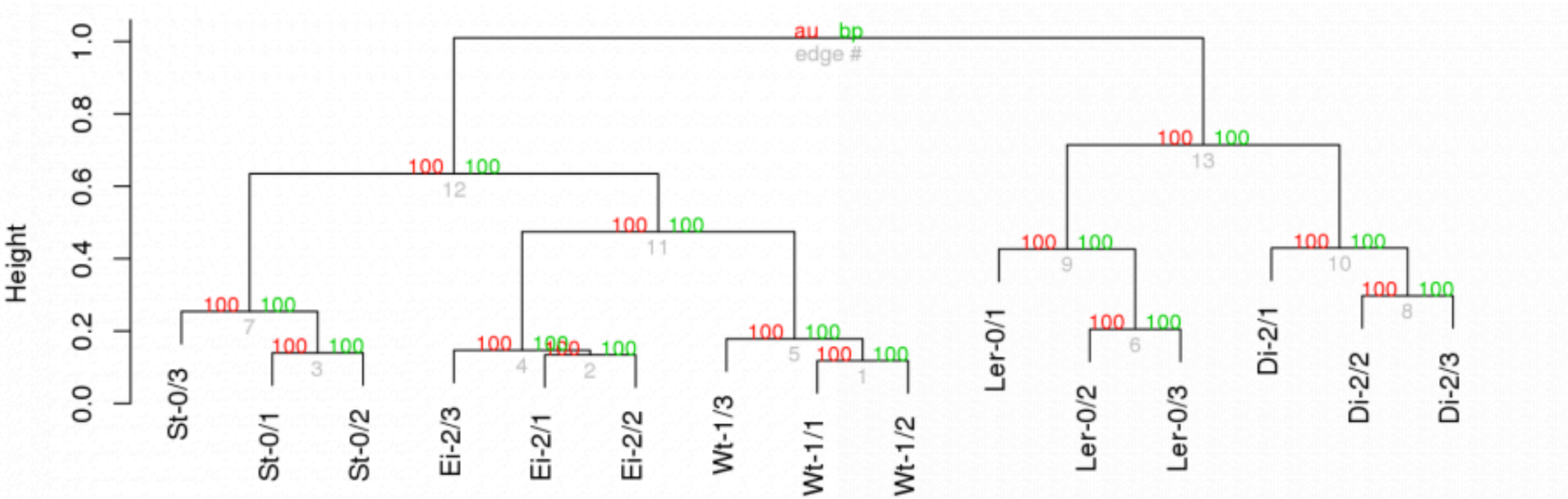
- ✓ Transcriptomic profiles of infected and control plants from each ecotype (Agilent 4 × 44).

- ✓ Comparisons:
  1. Similarities/differences among replicated lineages in their host ecotypes.
  2. Drivers of local *vs* universal adaptation.
  3. Differences among the most specialist and the most generalist lineages.

- ✓ Similarities among pairs of transcriptomic profiles evaluated using Pearson's correlation coefficients.
  Gene set analysis (GSA) was carried out for identifying significant GO terms using a logistic regression model (LRpath).

Transcriptomes cluster according to local host ecotype

$$EV\downarrow h,l,k - C\downarrow k\uparrow[2]$$

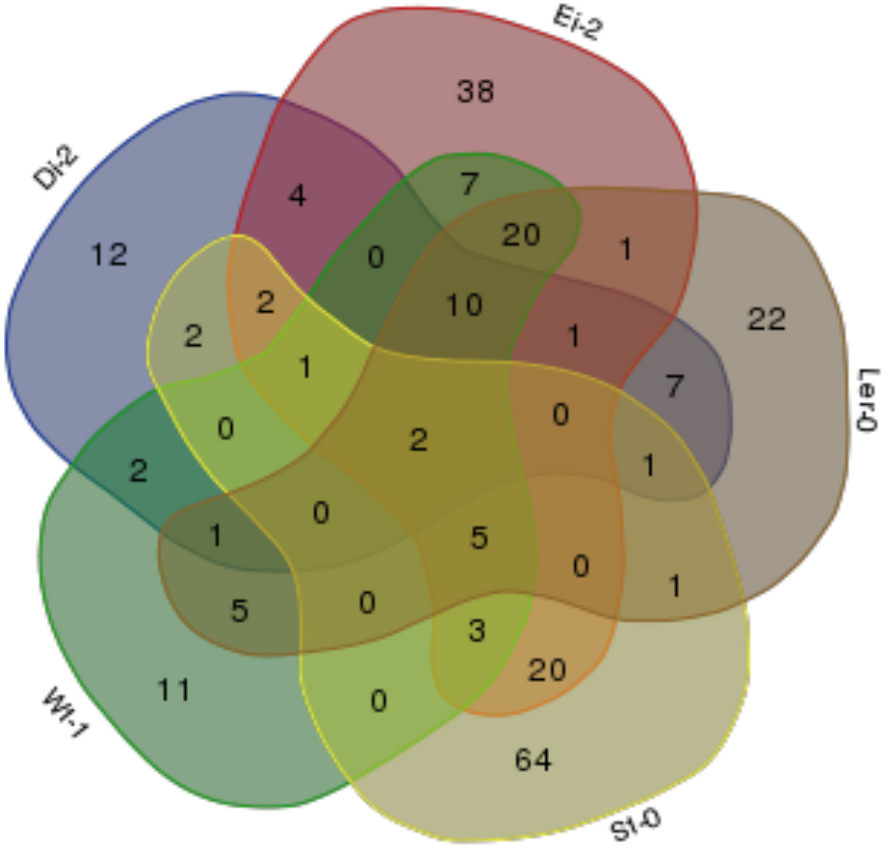


Hillung *et al.* 2016 *Sci. Rep.*

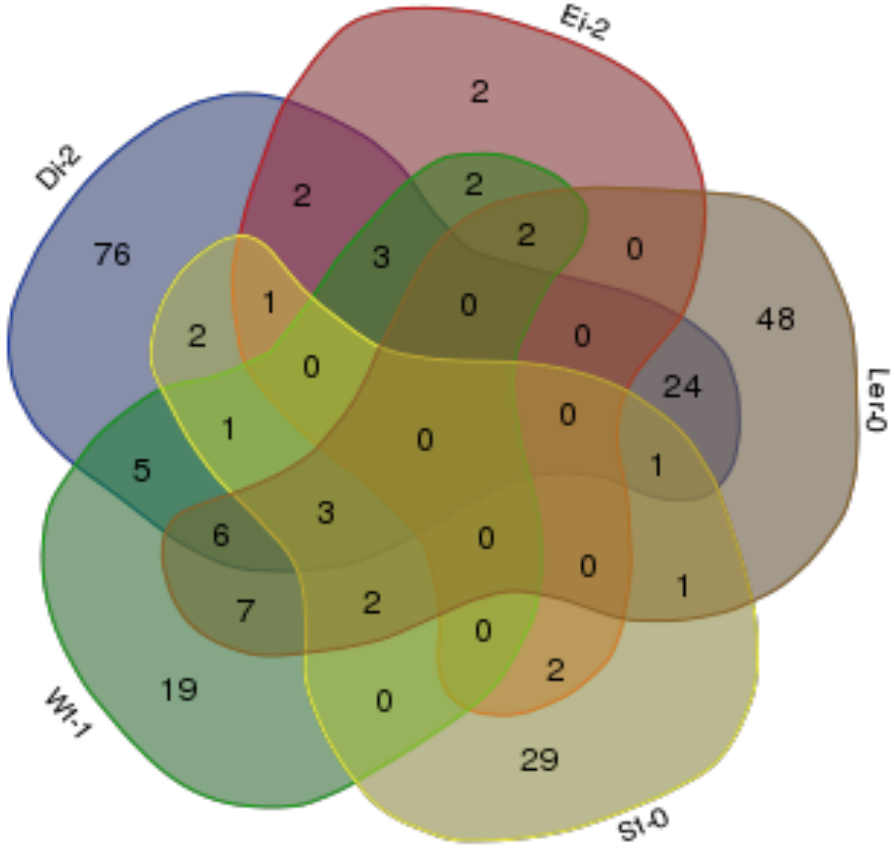
Ecotype-specific *vs* universal drivers of virus adaptation

$$(EV{\downarrow}h,l,k - C{\downarrow}k{\uparrow}[2]) - (AV{\downarrow}k - C{\downarrow}k{\uparrow}[1])$$

Up-regulated functional categories



Down-regulated functional categories



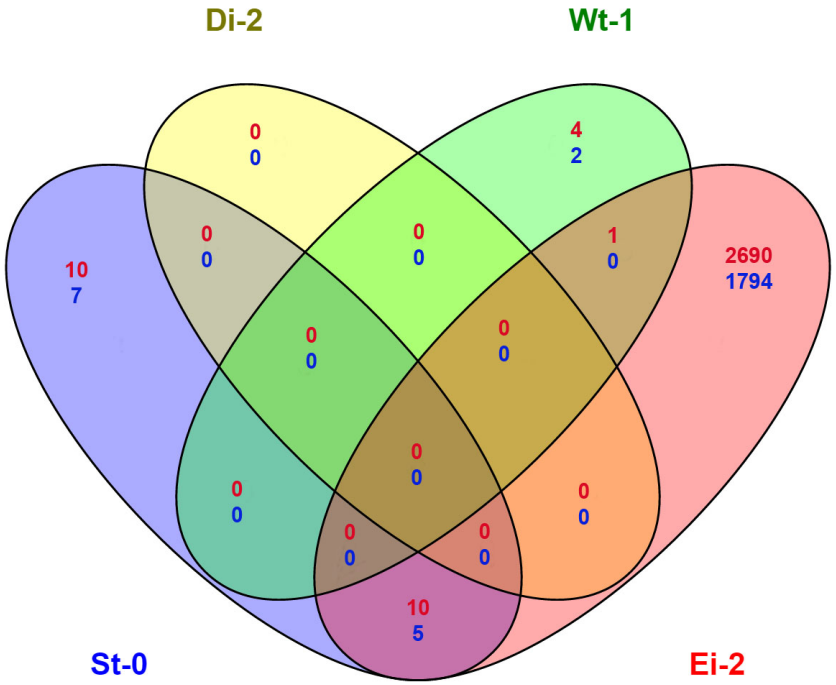
Hillung *et al.* 2016 *Sci. Rep.*

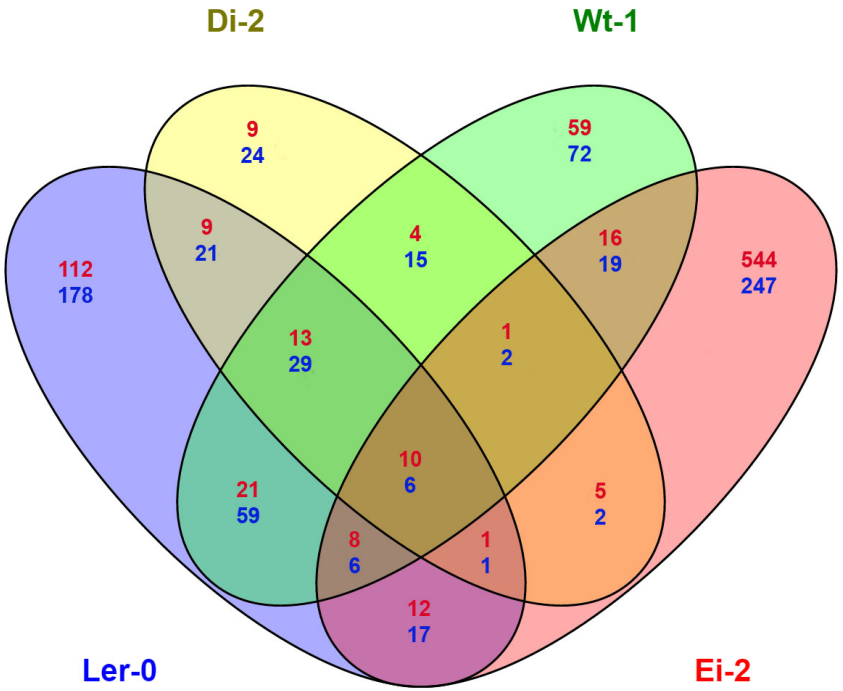
Generalists alter a similar set of genes across host ecotypes whereas specialist do not

Most generalist Ler-0/2

Most specialist St-0/3

$(EV{\downarrow}\mathrm{Ler}{-}0,2,k-C{\downarrow}k{\uparrow}[2])-(EV{\downarrow}\mathrm{Ler}{-}0,2,\mathrm{Ler}{-}0$ $(EV{\downarrow}\mathrm{St}{-}0,3,k-C{\downarrow}k{\uparrow}[2])-(EV{\downarrow}\mathrm{St}{-}0,3,\mathrm{St}{-}0$ $-C{\downarrow}\mathrm{Ler}{-}0{\uparrow}[2])$





Hillung *et al.* 2016 *Sci. Rep.*

Take-home messages

- ✓ Adaptation to a new host species involves dramatic changes in the way host and virus interact at the transcriptomic level.
- ✓ Adaptation to a susceptible host ecotypes may facilitate access to previously resistant genotypes.
- ✓ More permissive ecotypes (G2) selected for specialist viruses while restrictive (G1) ecotypes selected for generalist viruses.
- ✓ No evolved virus was superior to all other in every ecotype and no ecotype was resistant against all evolved virus. Such nestedness of the infection matrix is compatible with a Gene-for-Gene model.
- ✓ Similarities and differences between transcriptomic profiles of infected plants depend on the local host ecotype in which a virus has evolved: ecotype-specific drivers of adaptation.
- ✓ Generalist viruses affect in a similar way the transcriptomes of different host ecotypes, whereas specialist viruses do quite differently.

Acknowlegements

Dr. Patricia Agudelo-Romero, Paula Agudo, Dr. Silvia Ambrós, Anamarija Butković, Dr. José L. Carrasco, Dr. Purificación Carrasco, Dr. Javier Carrera, Héctor Cervera, Dr. José M. Cuevas, Francisca de la Iglesia, Dr. Joaquín Dopazo (CIPF), Francisco García-García (CIPF), Rubén González, Dr. Julia Hillung, Dr. Jasna Lalić, Dr. Fernando Martínez, Rebeca Navarro, Àngels Pròsper, and Dr. Guillermo Rodrigo

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