Exploring the constraints to increase genome complexity in RNA viruses

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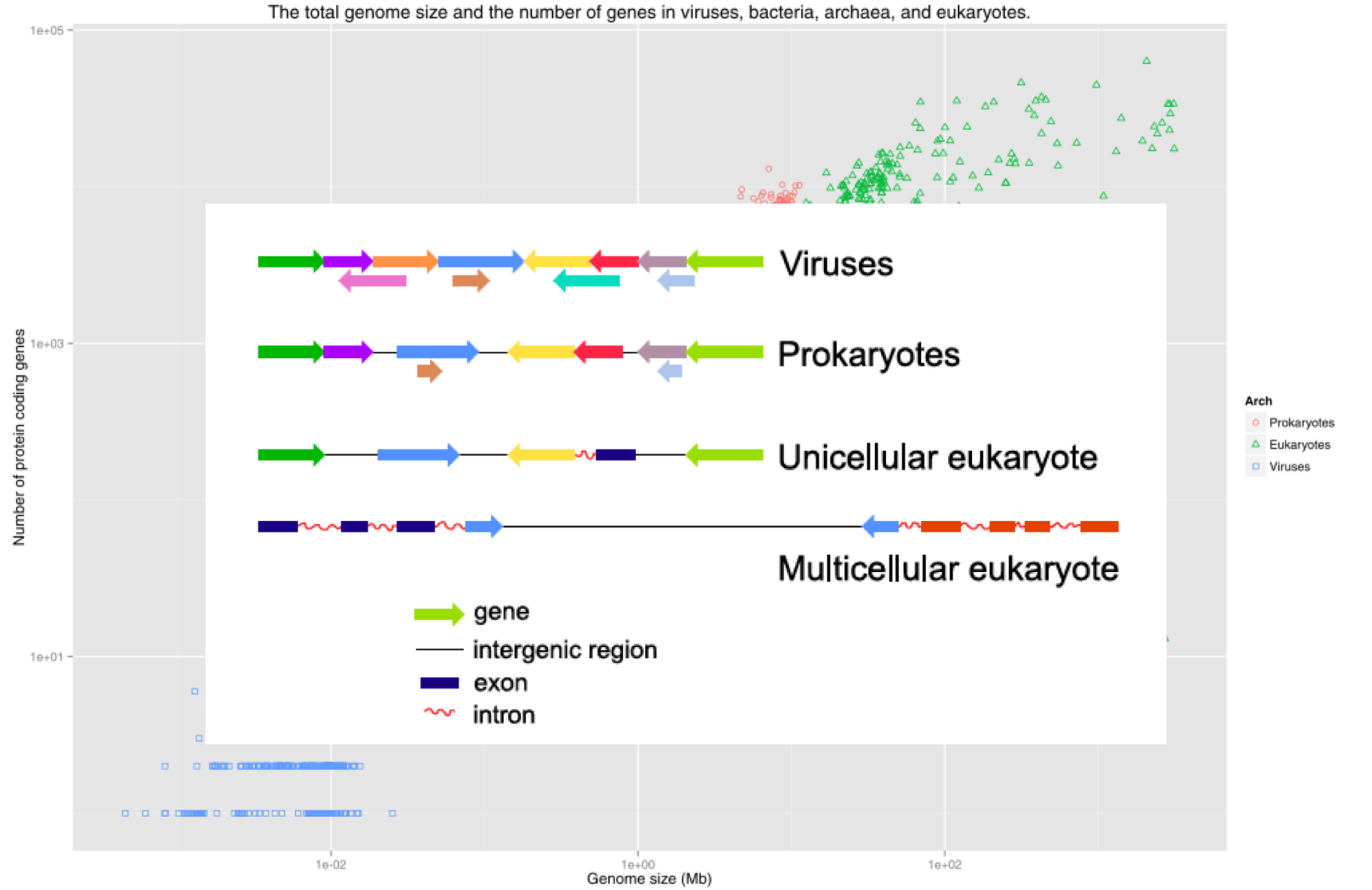
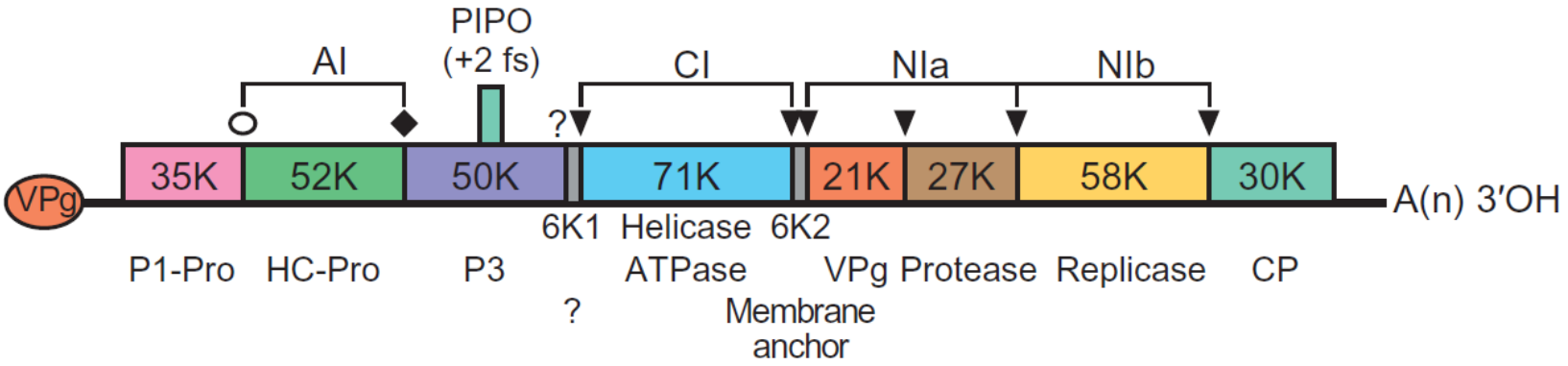
Evolutionary Systems Virology Group



From Koonin EV (2011) *The Logic of Chance*

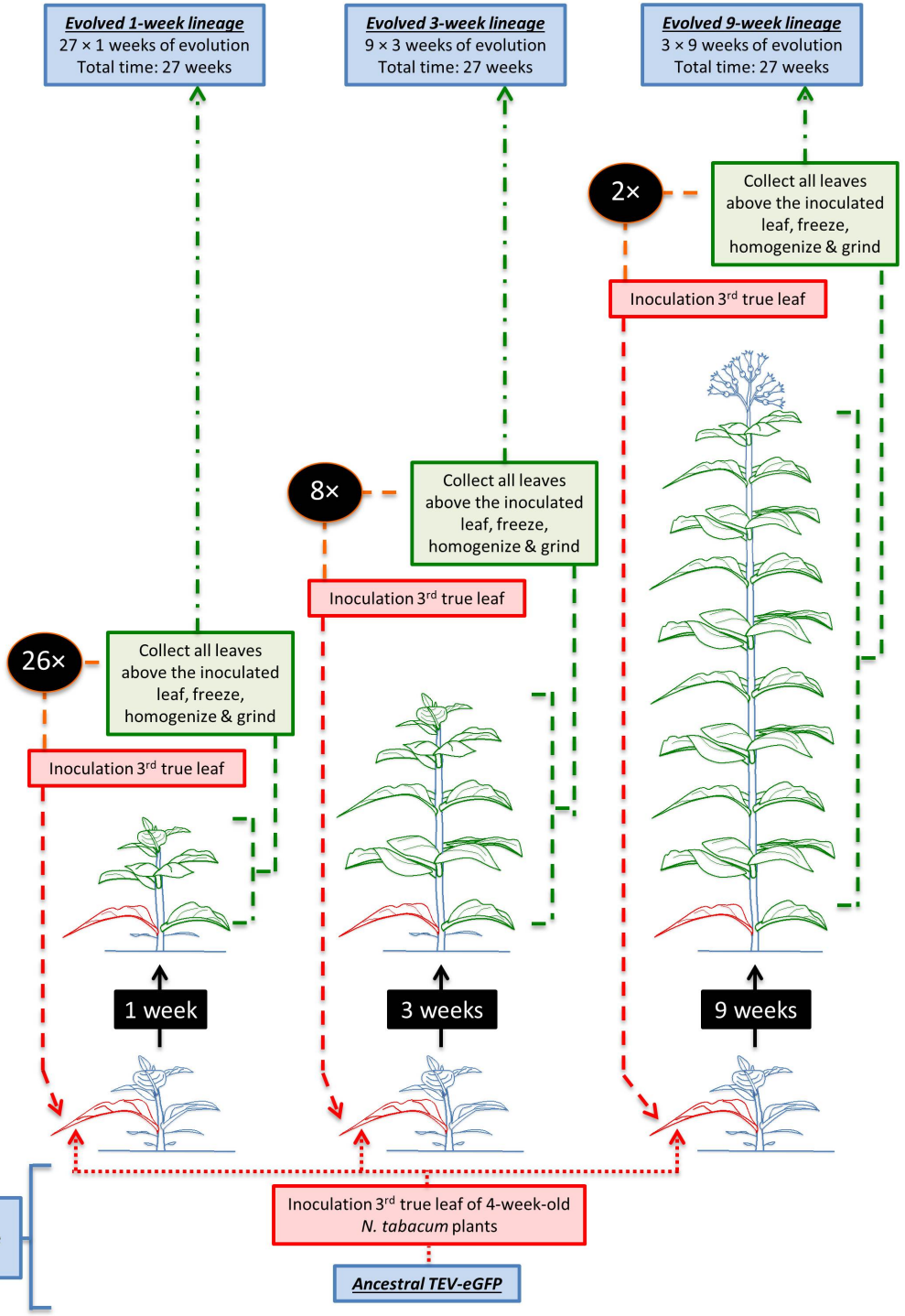
The model system

Tobacco etch potyvirus (TEV)



- ✓ Why did this architecture evolve?
- ✓ Can foreign genes be accommodated in the viral genome?
- ✓ Can viral genes be lost if functions are provided *in trans* by the host?
- ✓ Are there plausible evolutionary trajectories to alternative gene orders?
- ✓ Can genetic and functional redundancy be maintained under certain conditions?
- ✓ What is the evolutionary potential of viruses with altered genomes?

Evolution experiments *in planta*



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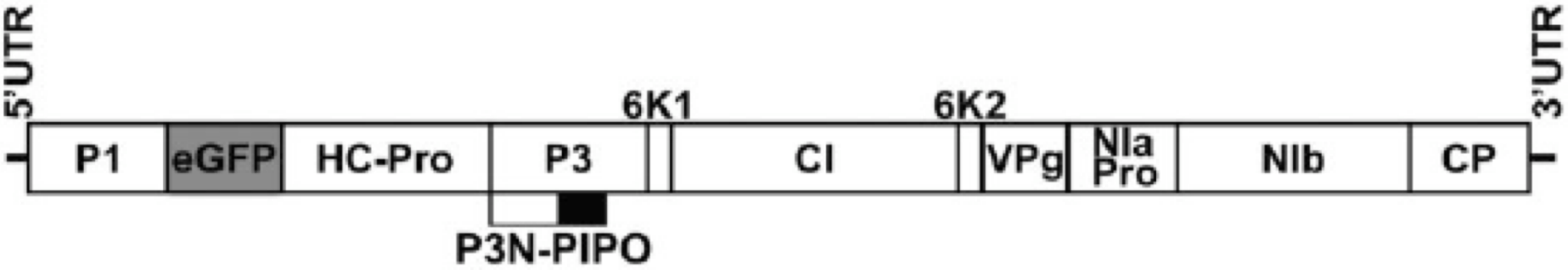
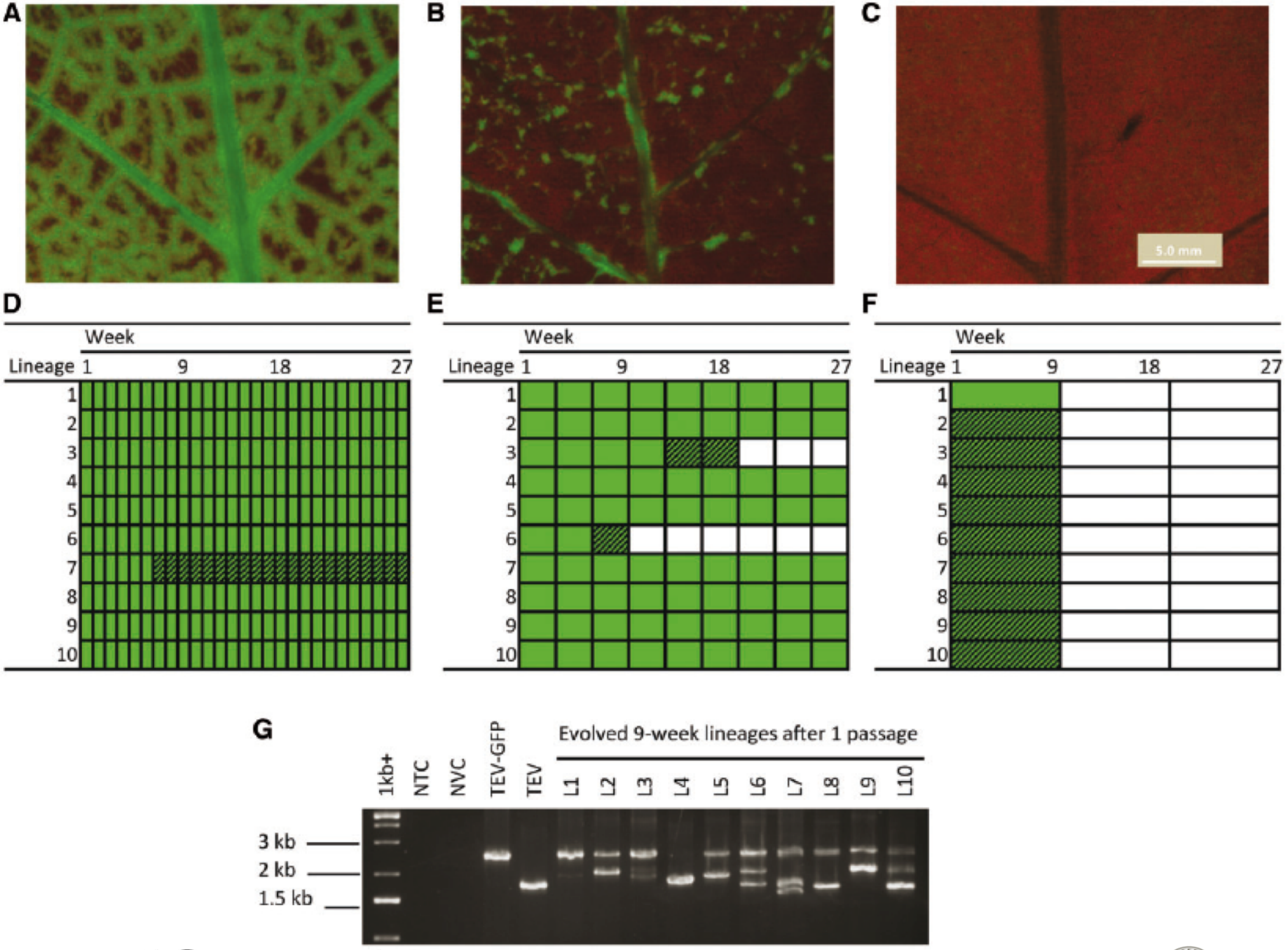
1. Molecular evolution of a foreign nonfunctional gene

- ✓ Viruses have evolved highly streamlined genomes and a variety of mechanisms to compress them, suggesting that genome size is under strong selection.
- ✓ HGT has played an important role in virus evolution. However, evolution cannot integrate initially nonfunctional sequences into the viral genome if they are rapidly purged by selection.

TEV-eGFP

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9
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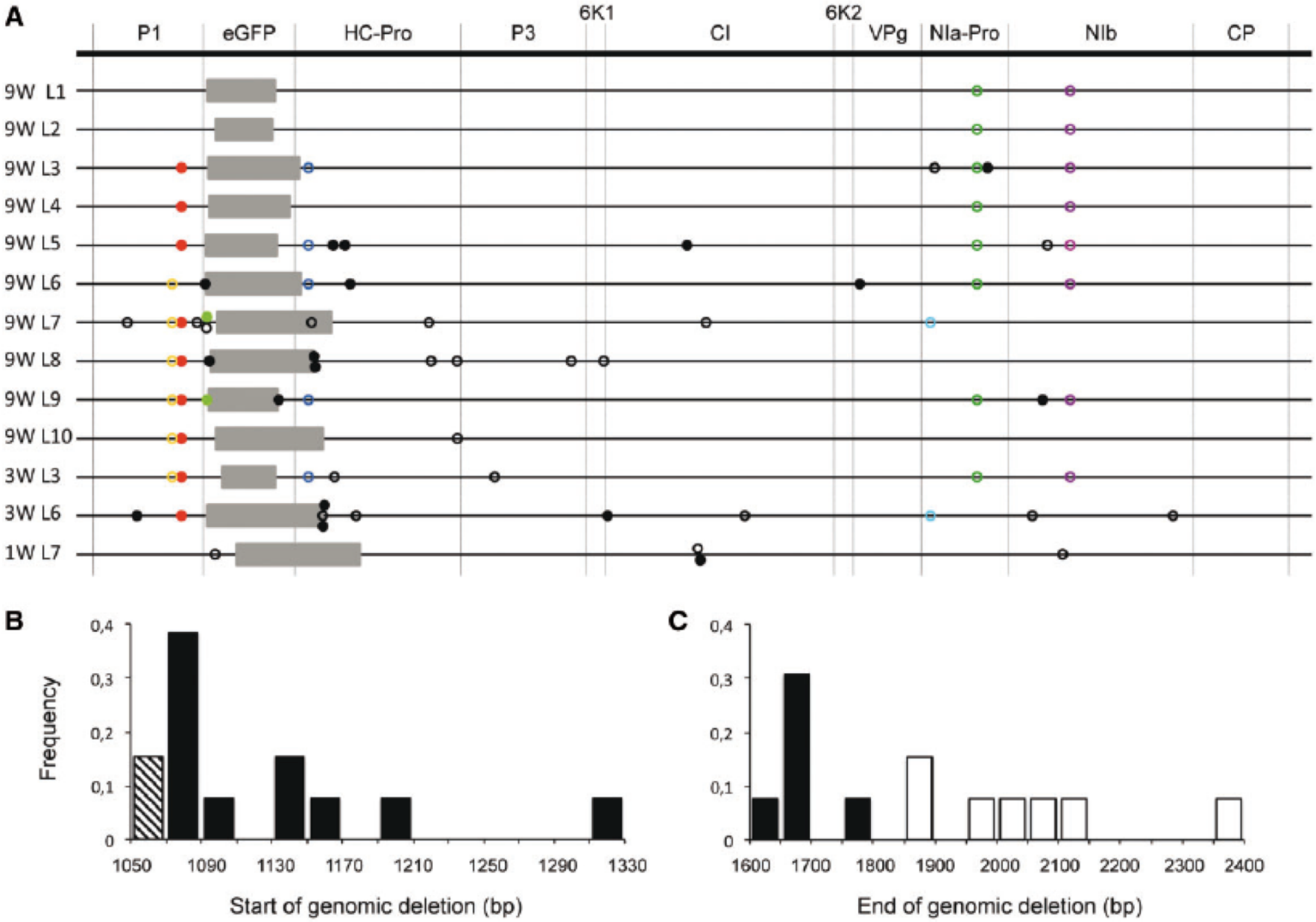
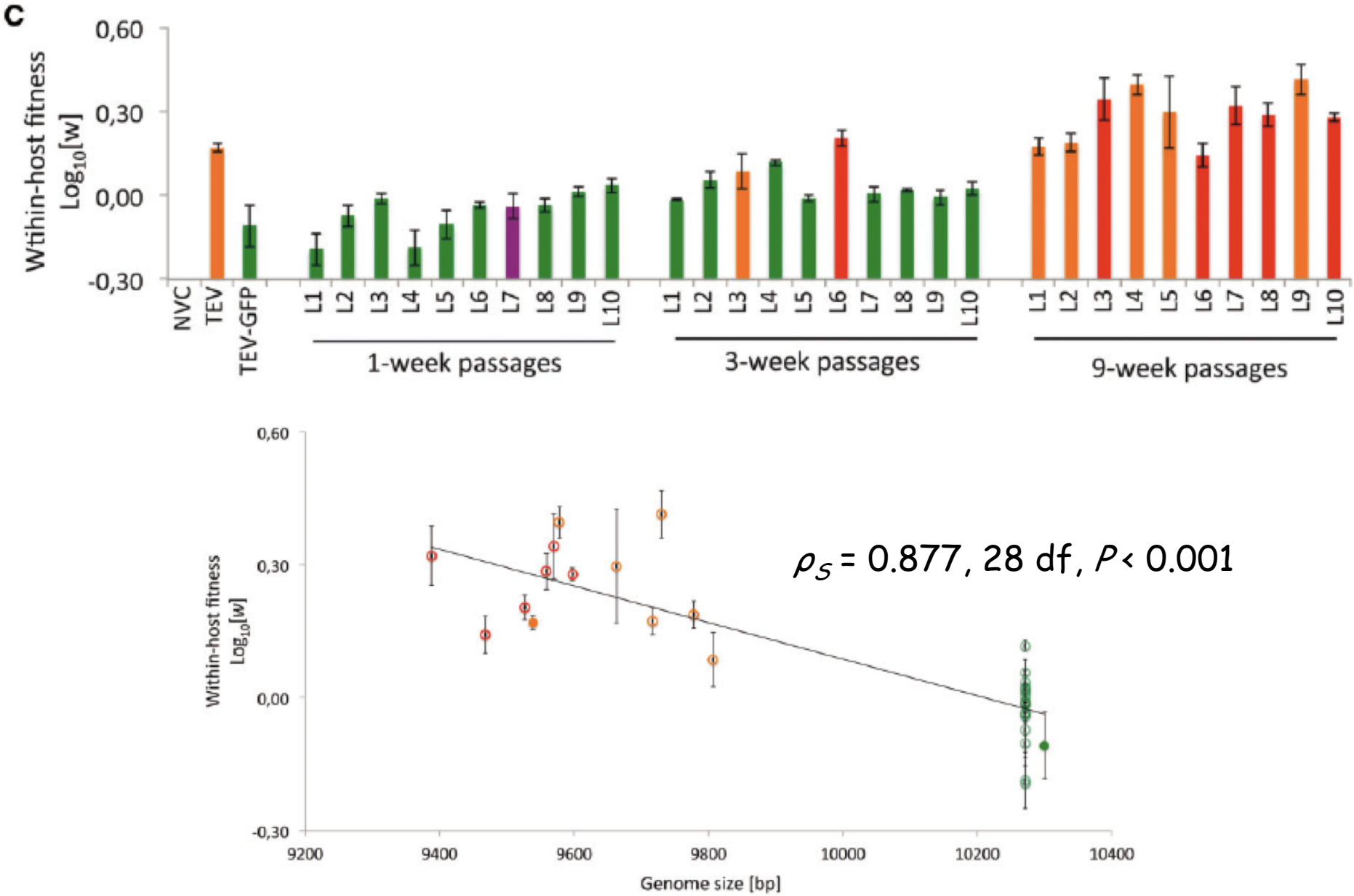
G
Evolved 9-week lineages after 1 passage
1kb+
NTC
NVC
TEV-GFP
TEV
L1
L2
L3
L4
L5
L6
L7
L8
L9
L10
3 kb
2 kb
1.5 kb

Nature of deletions in eGFP



Evolution of phenotypic traits



Conclusions 1

- ✓ When long 9-week passages were performed, the *eGFP* gene was lost in all lineages. Viruses with large genomic deletions were fixed in only 2/10 3-week lineages and none in 1-week lineages.
- ✓ Illumina sequencing revealed considerable convergent evolution in the 9- and 3-week lineages with genomic deletions.
- ✓ Genome size was correlated to within-host fitness. Within-host fitness of the 3-week virus lineages without genomic deletions was higher than for the 1-week lineages.
- ✓ The strength of selection for reduced genome size and the rate of pseudogenization depend on demographic conditions. Results suggest a demographically determined "sweet spot", where heterologous insertions are not immediately lost while evolution can act to integrate them into the viral genome.

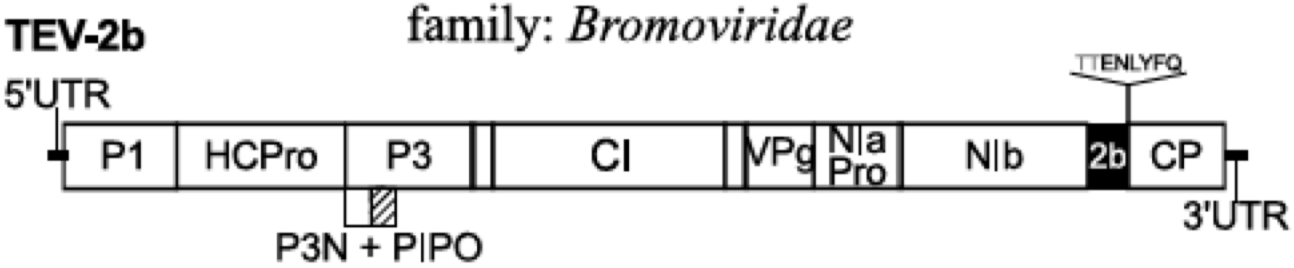
2. Molecular evolution of foreign functional genes

2b gene

Cucumber mosaic virus

genus: *Cucumovirus*

family: *Bromoviridae*



Multifunctional protein: polyprotein cleavage, virulence, viral movement, transmission, VSR

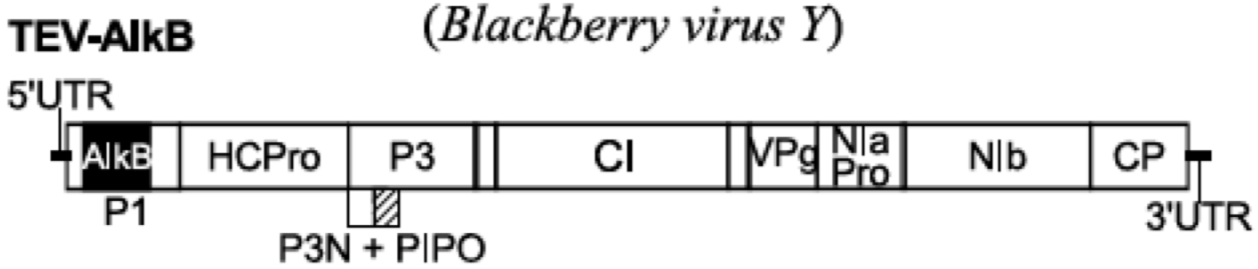
AlkB domain

2OG-Fe(II) oxygenase superfamily

found in *Flexivirdae*

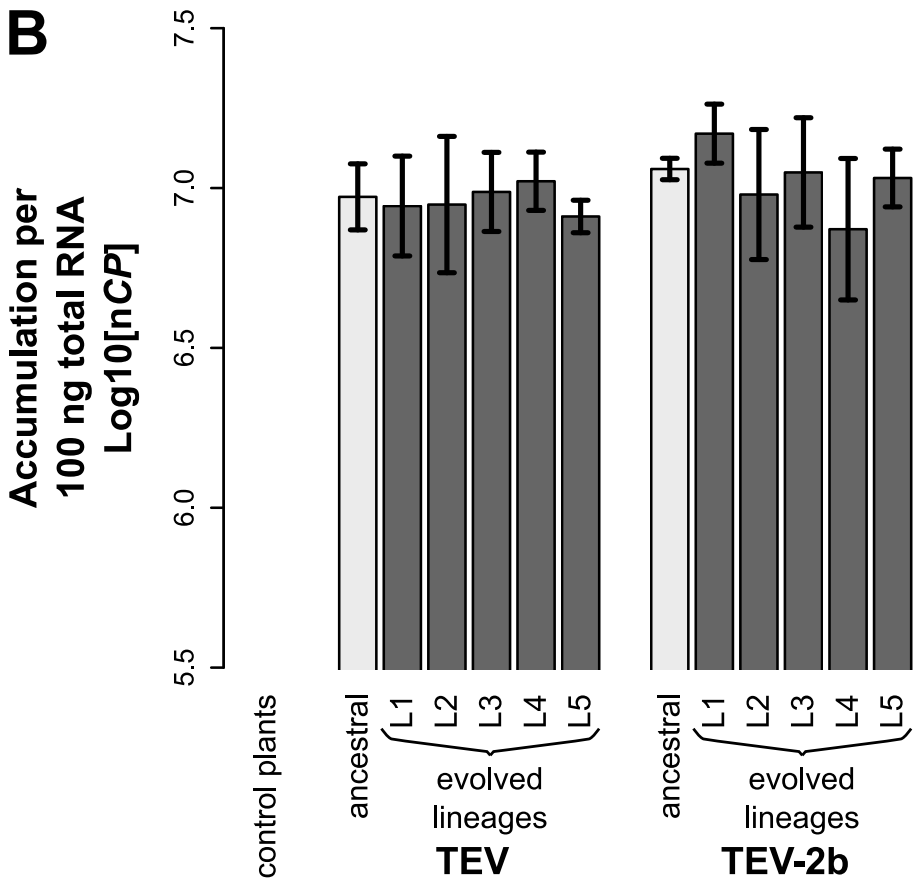
one virus from *Potyviridae*

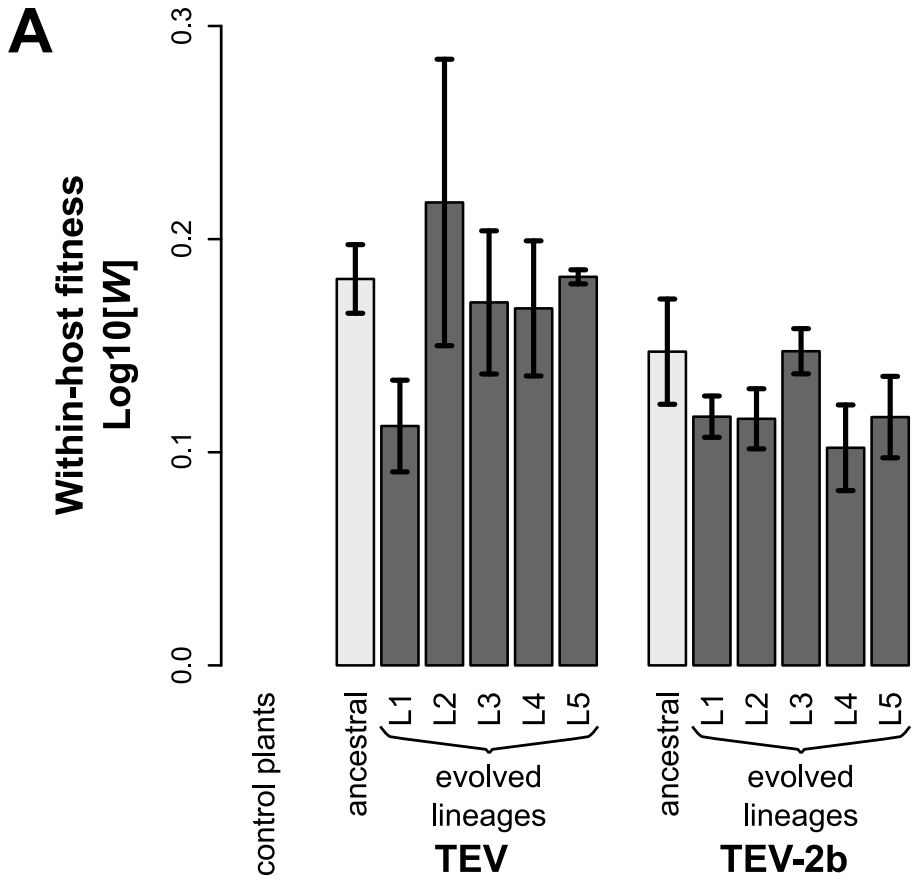
(*Blackberry virus Y*)



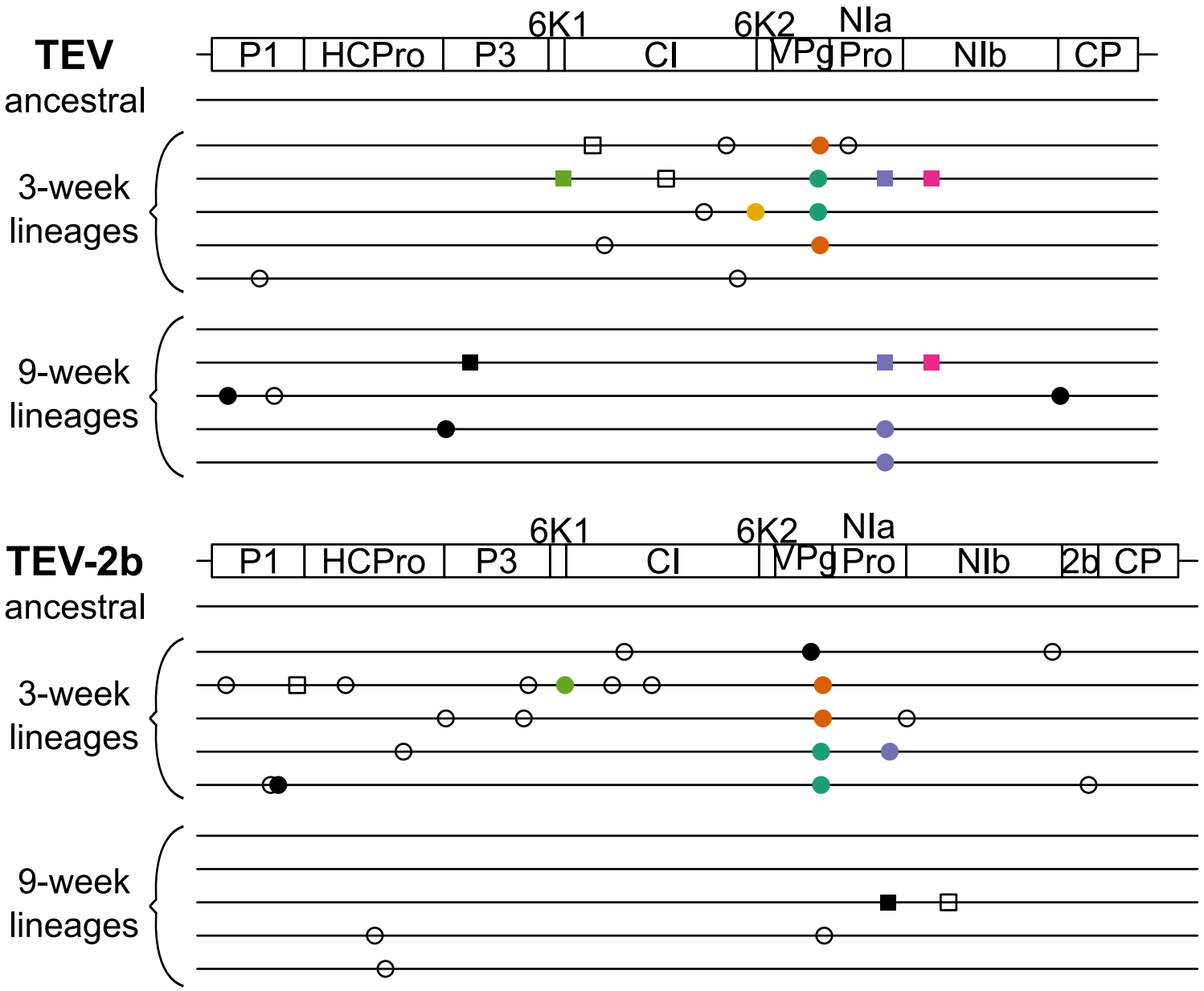
Repair of RNA damages due to alkylation/methylation

2b: Evolution of phenotypic traits

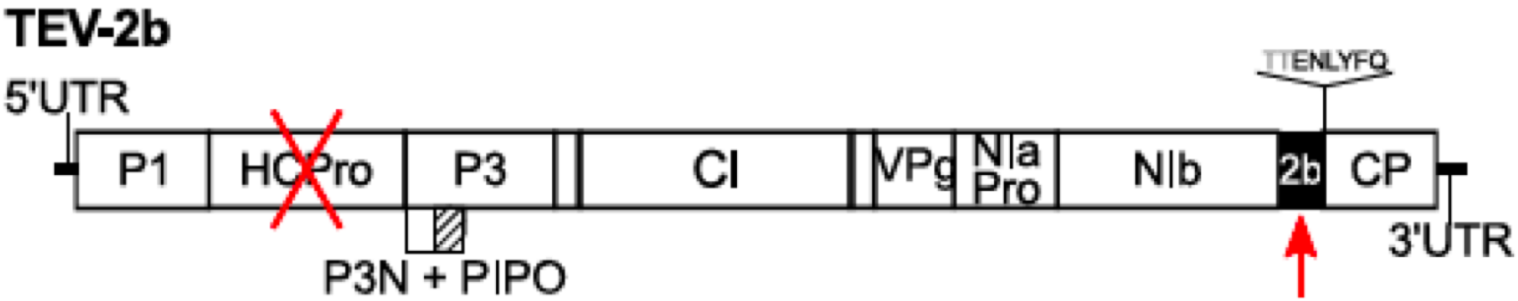




2b: Genomic evolution



2b: A possible beneficial role

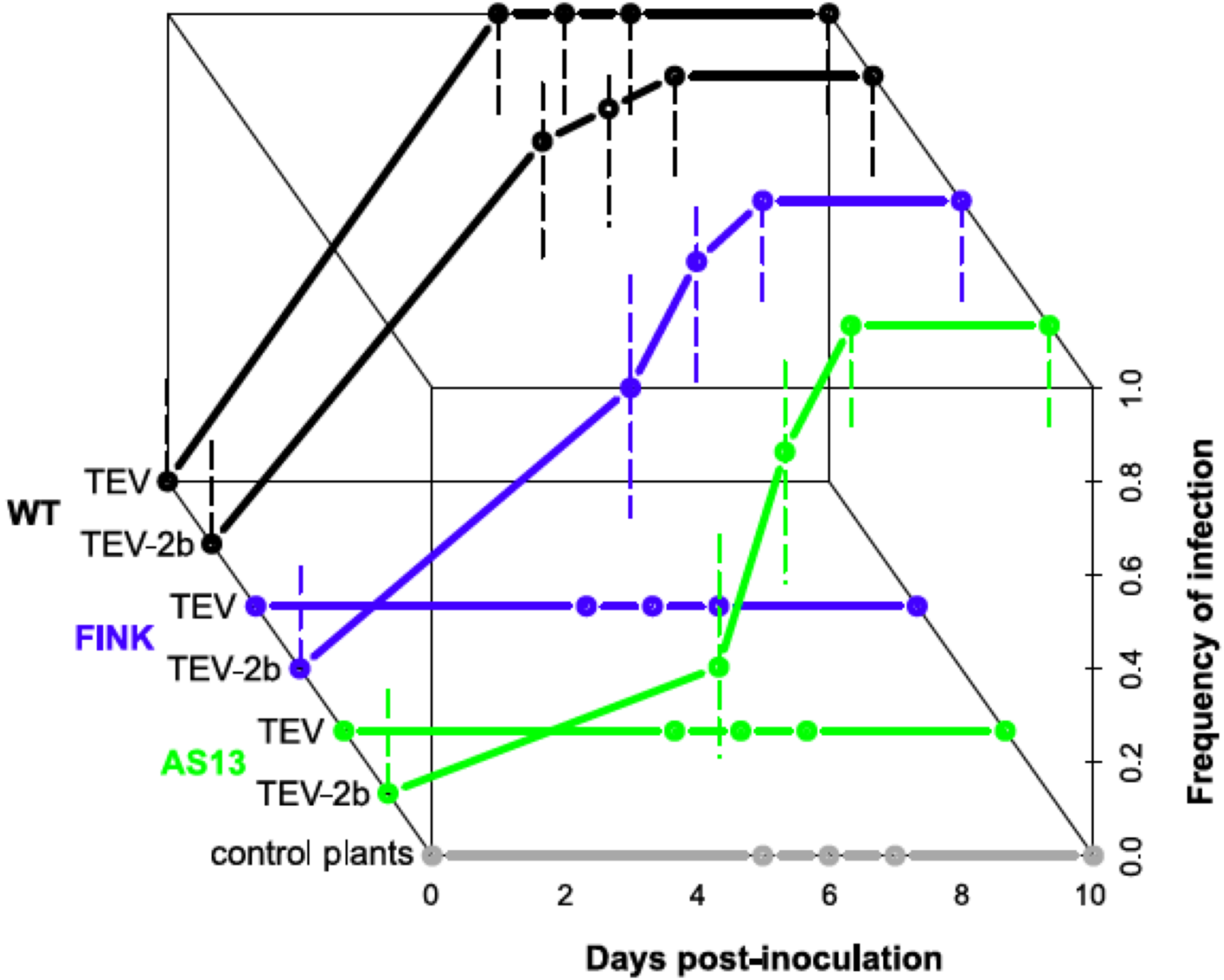


KO mutations of HC-Pro:

R183I in the highly conserved FRNK domain: sRNA binding site for the VSR activity. Asymptomatic infection.

E299A (AS13) in the C-terminal region: protease, VSR, cell-to-cell movement: hypossupresor, asymptomatic infection.

2b: functionally complements HC-Pro



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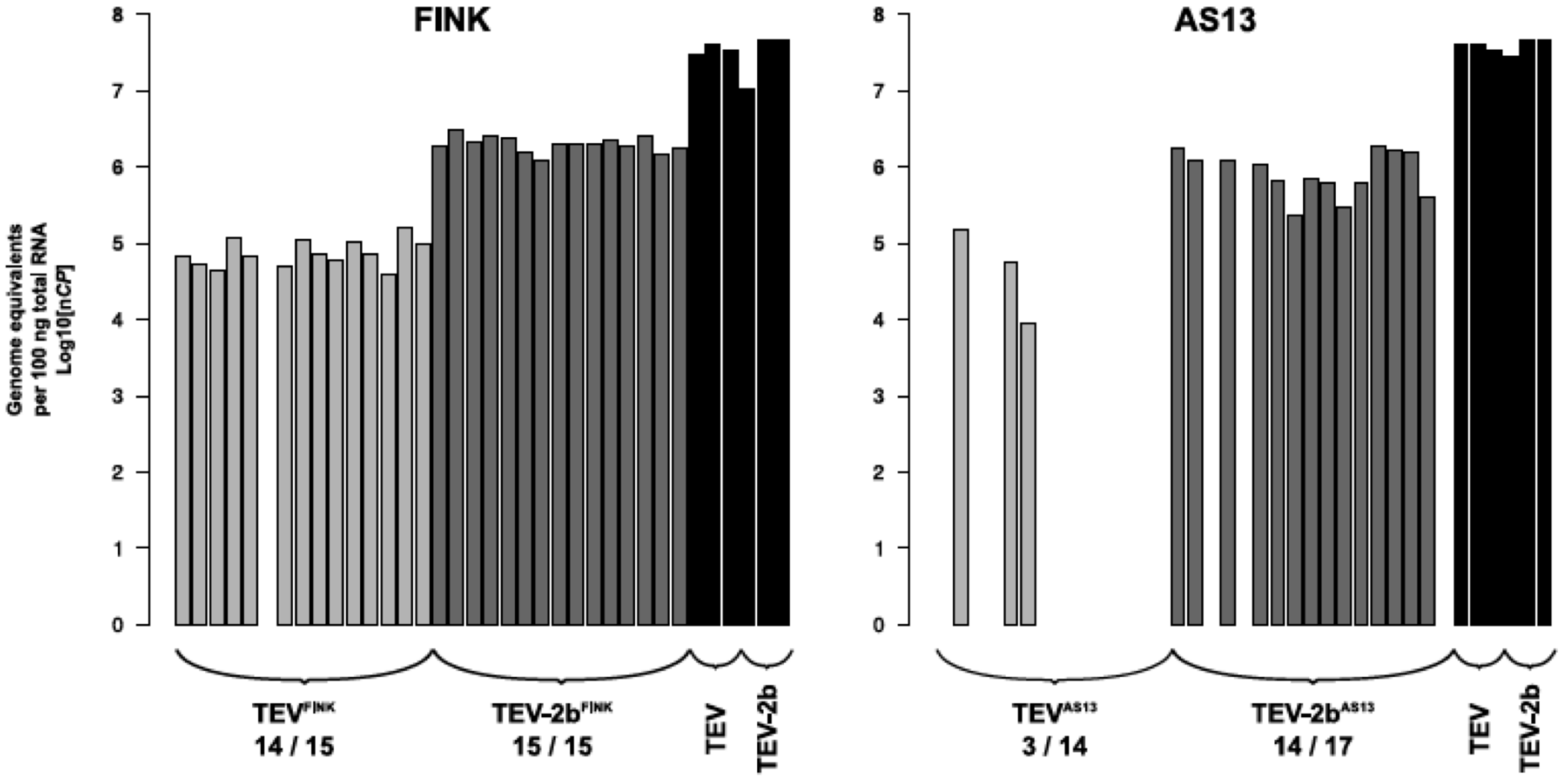
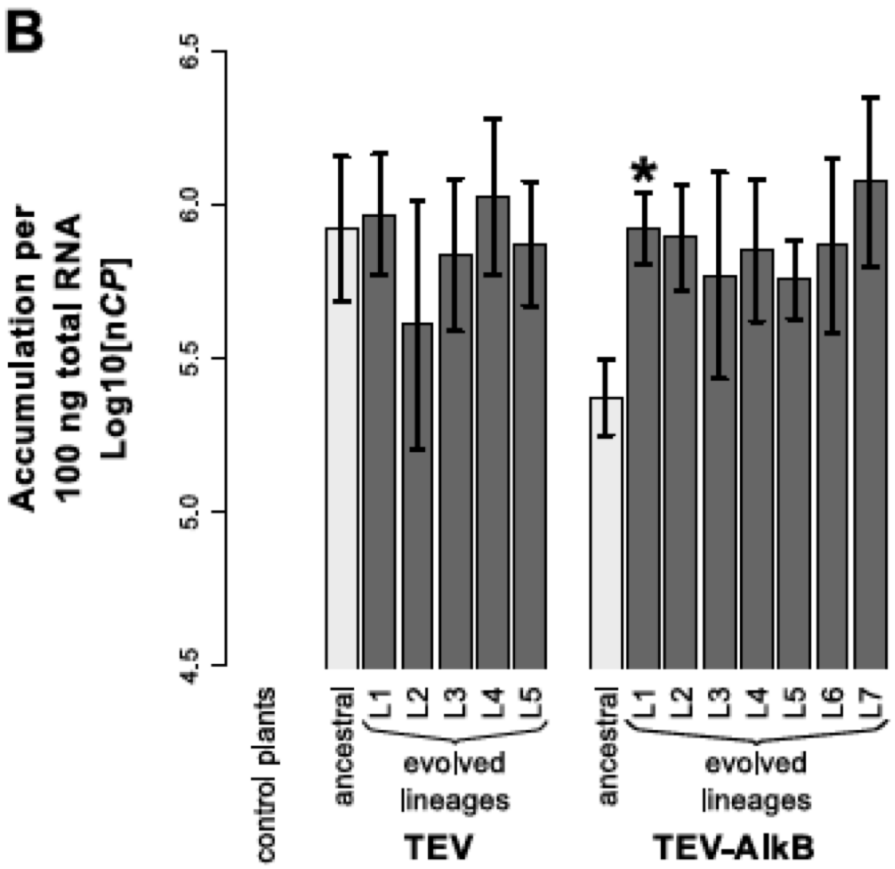
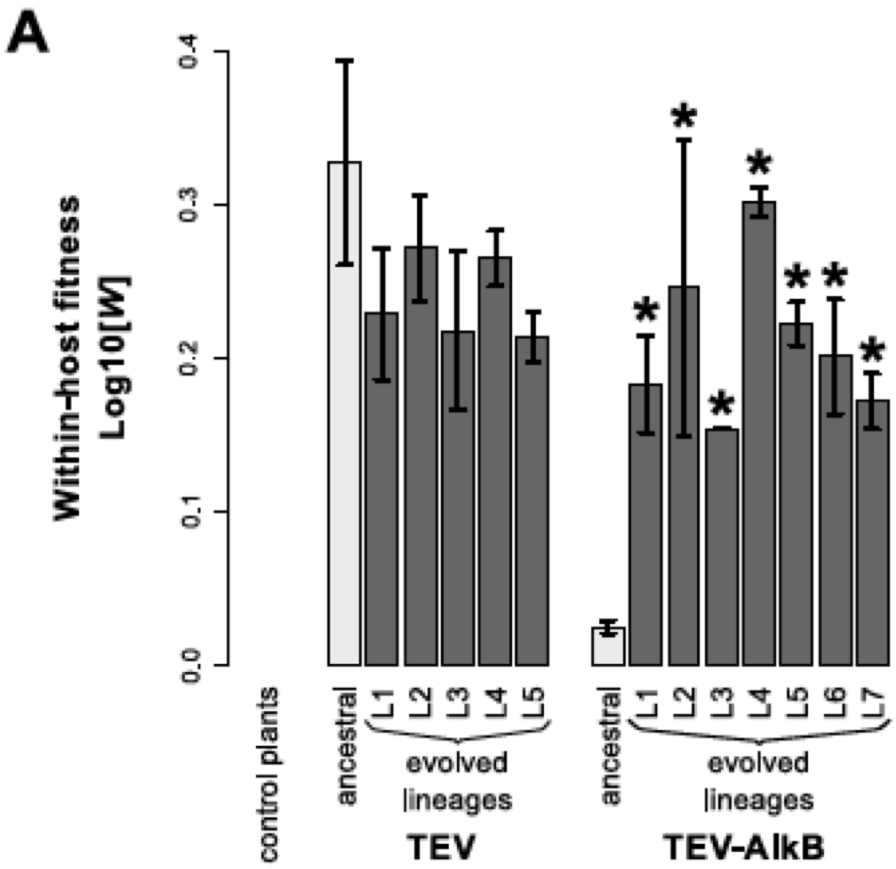


AlkB: Evolution of phenotypic traits





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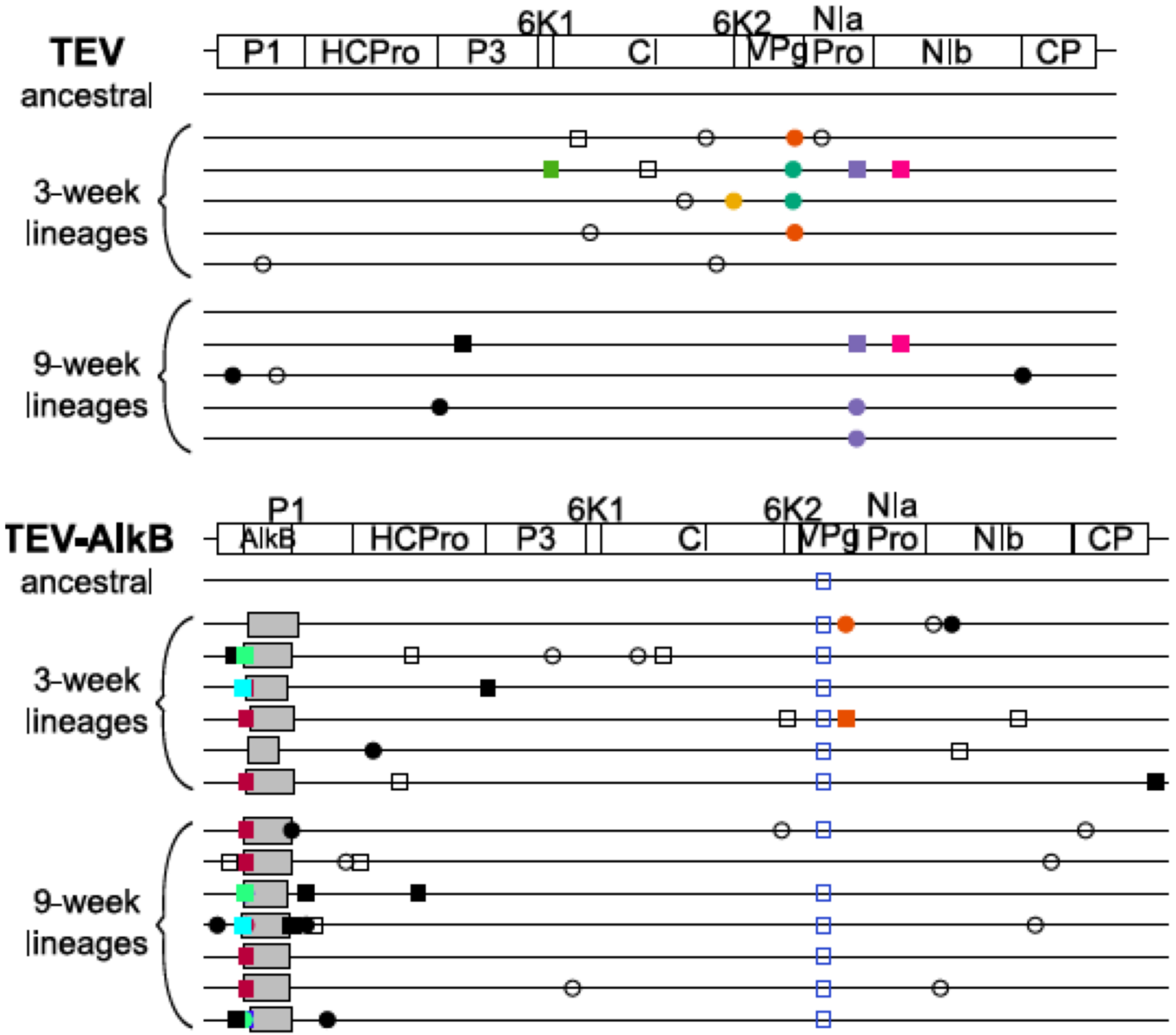


Conclusions 2

- ✓ HGT of *2b* cistron into TEV genome is stable and functional. A promising event for future evolution experiments.

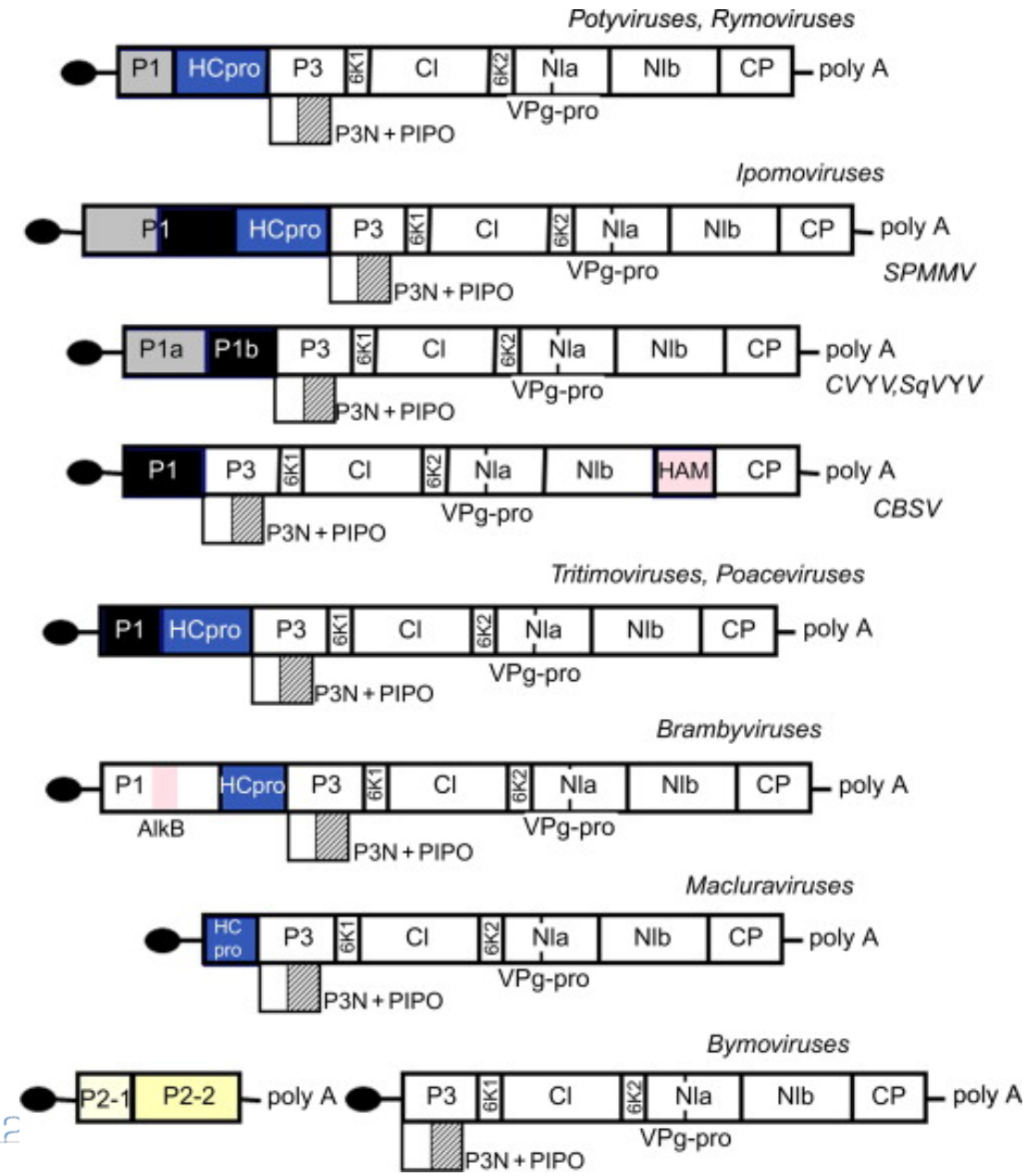
- ✓ HGT of the conserved AlkB domain from a plant is not very likely.

- ✓ Because of orthologous AlkB domains exist in flexiviruses, it is still likely that BVY obtained it by recombination with one of these viruses during coinfection.

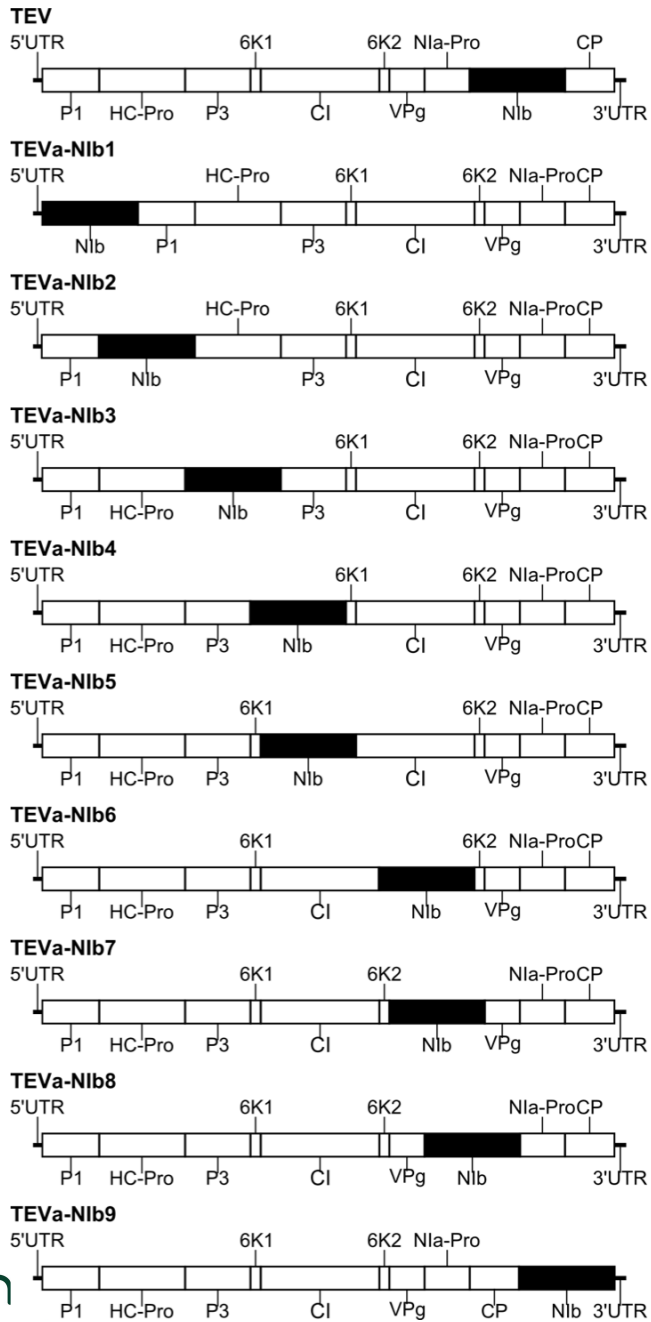
3. Barriers to the evolution of alternative gene orders

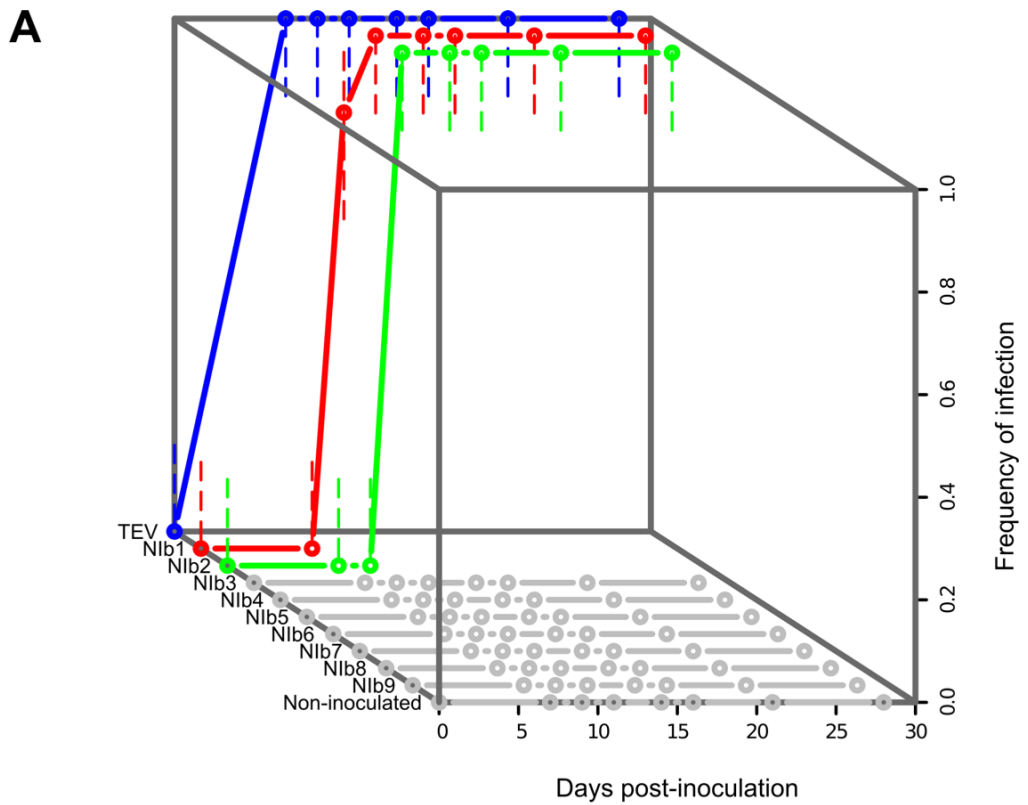
Majer *et al.* (2014) *J. Virol.* **88:** 4586
Willemsen *et al.* (2016) *Genetics* **202**: 1503

Conserved gene order in the *Potyviridae*

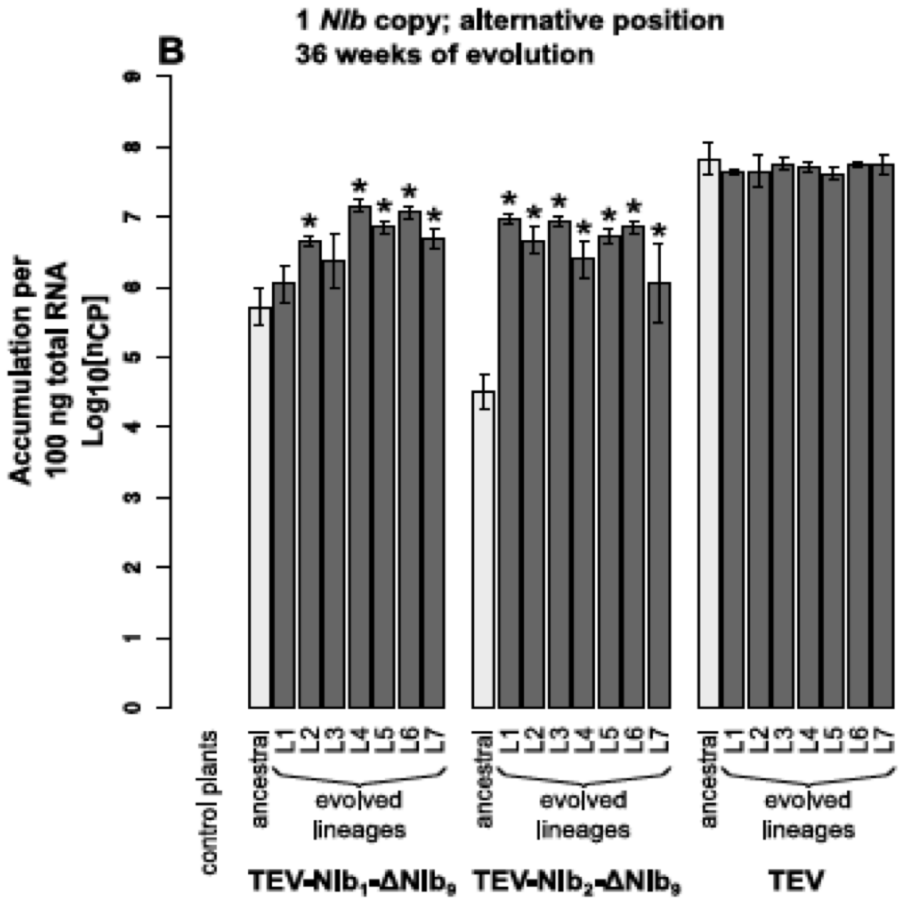


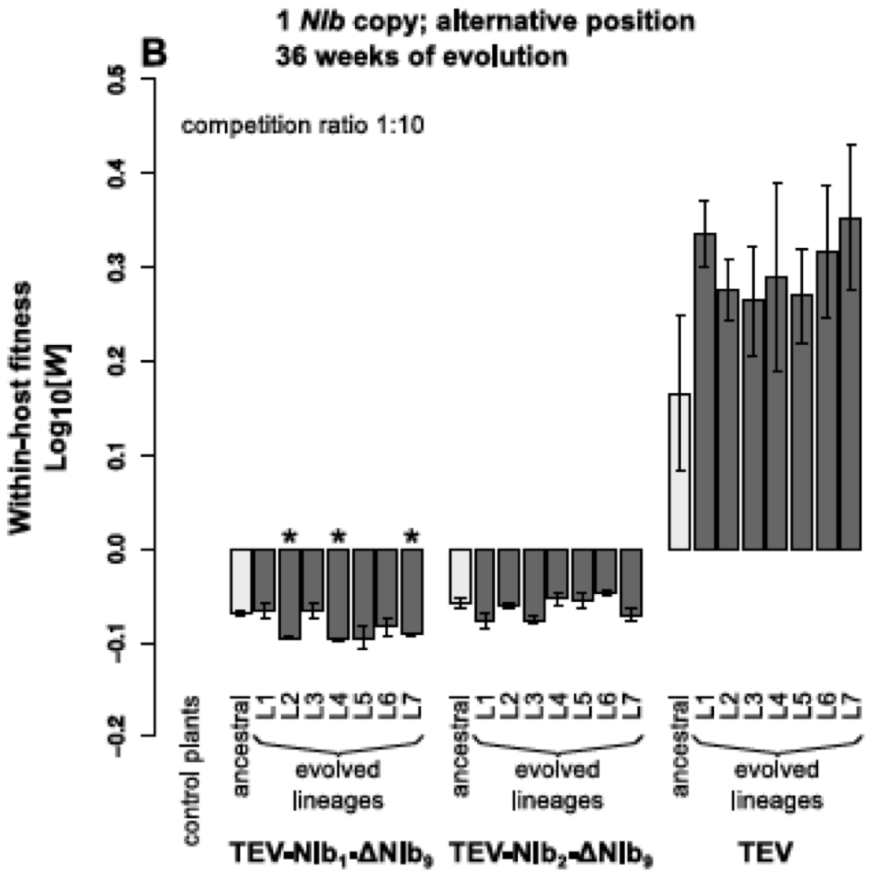
Engineered architectures



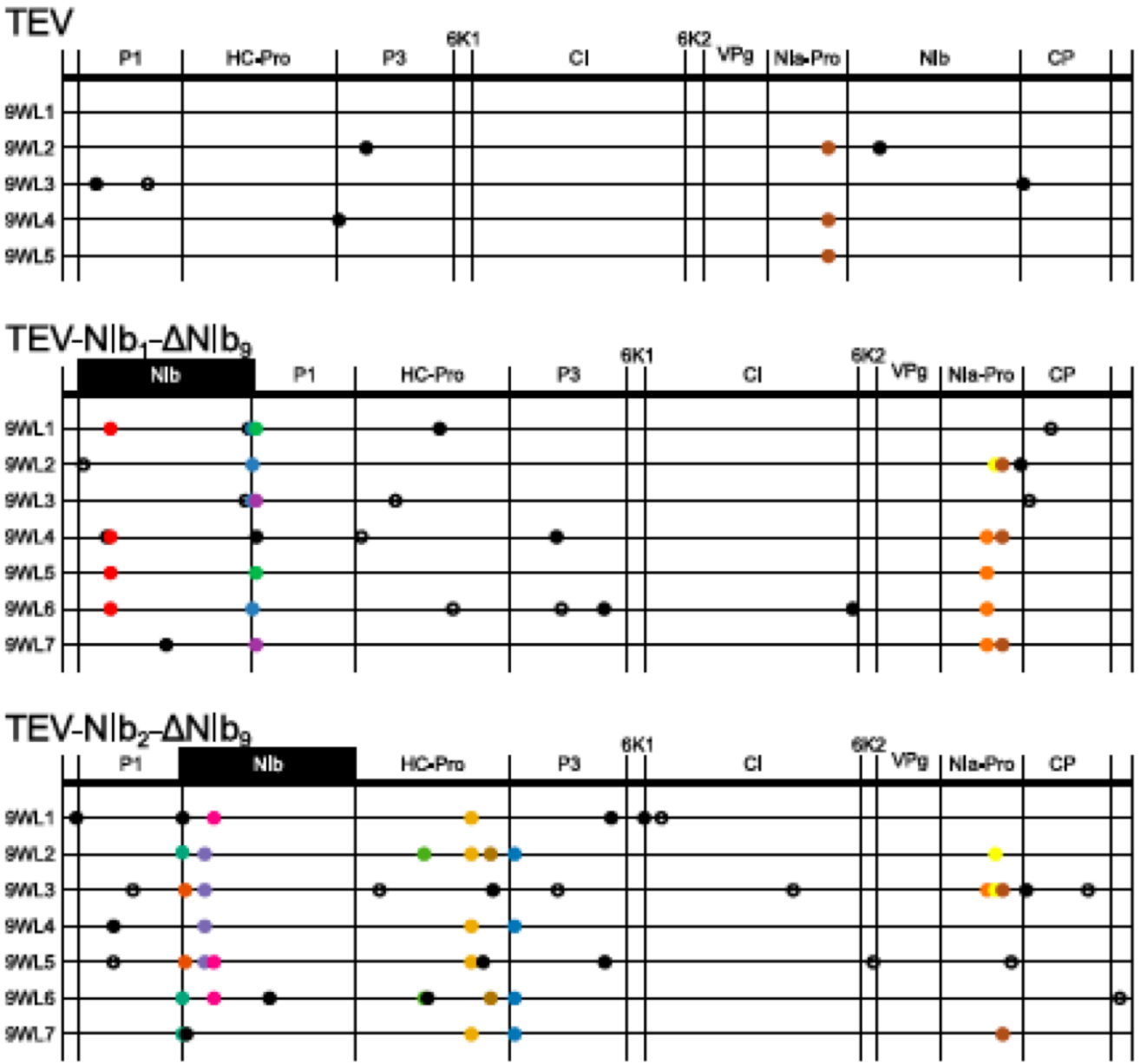


Evolution of phenotypic traits





Genomic evolution

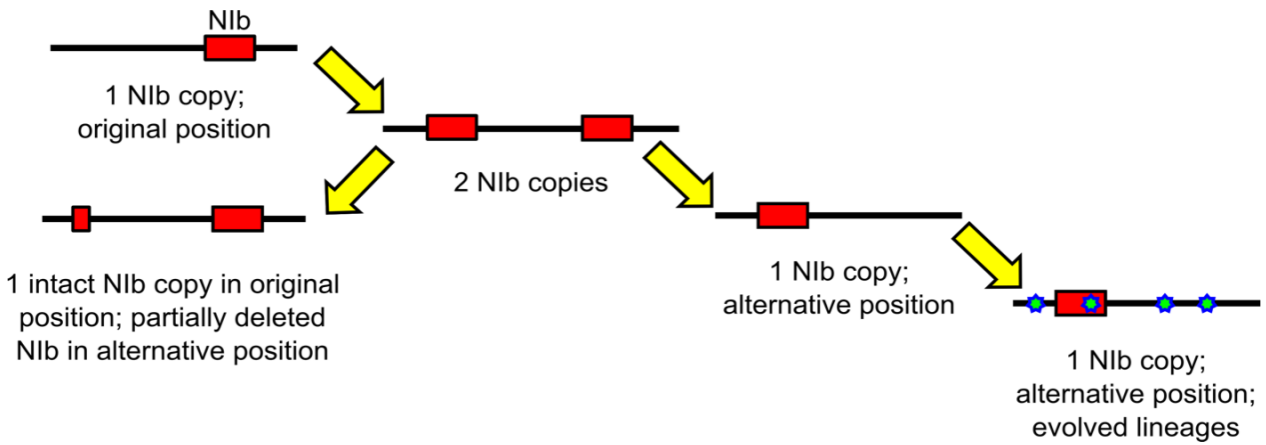


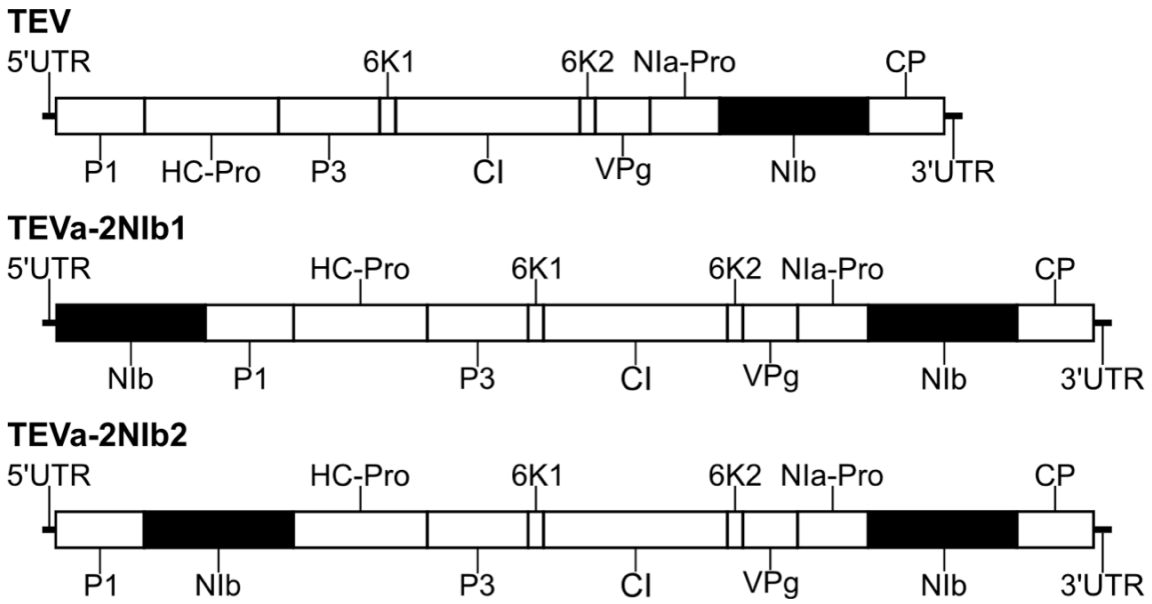
Conclusions 3

- ✓ Limited number of viable positions for reordering.
- ✓ Even if a reordered virus evolves in isolation and under optimal conditions, its subsequent evolutionary trajectory is slow and appears to select for improved accumulation. Therefore it will probably still be displaced from the population if must compete directly with the wildtype virus.
- ✓ By showing these different barriers to alteration of gene order, we make a strong case for why gene order has been so well conserved among the potyviruses.

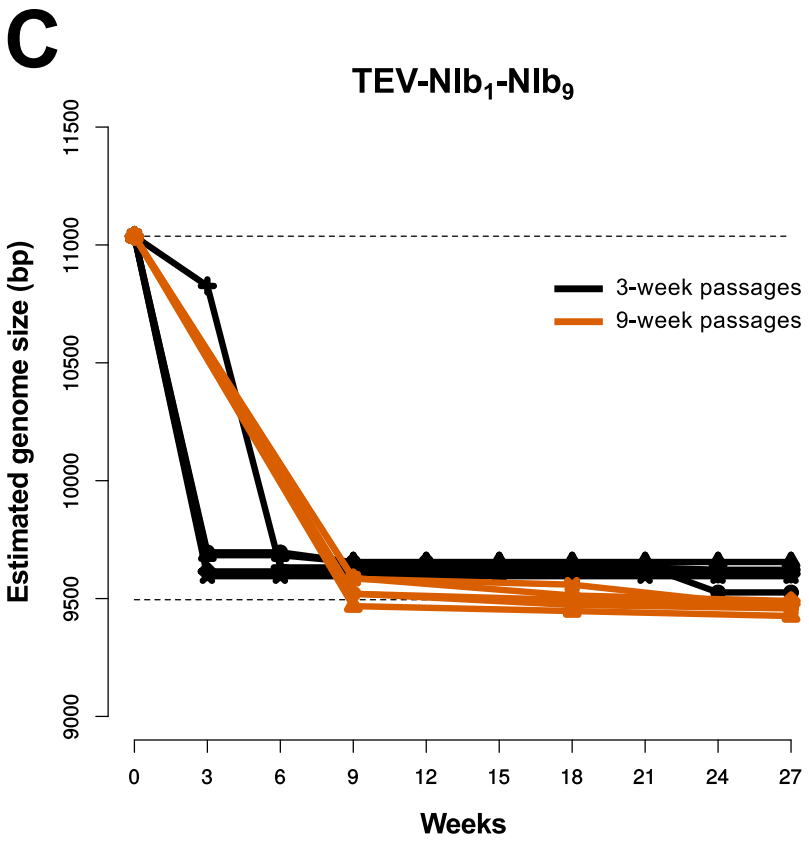
4. Molecular evolution of redundant genomes

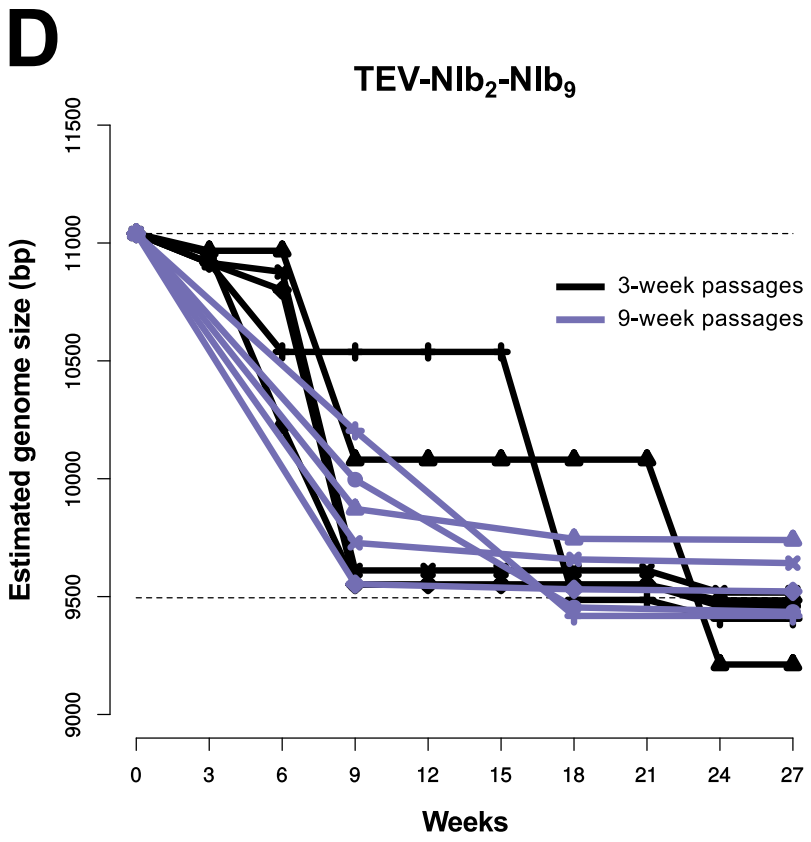
Exploring reorganization through duplication



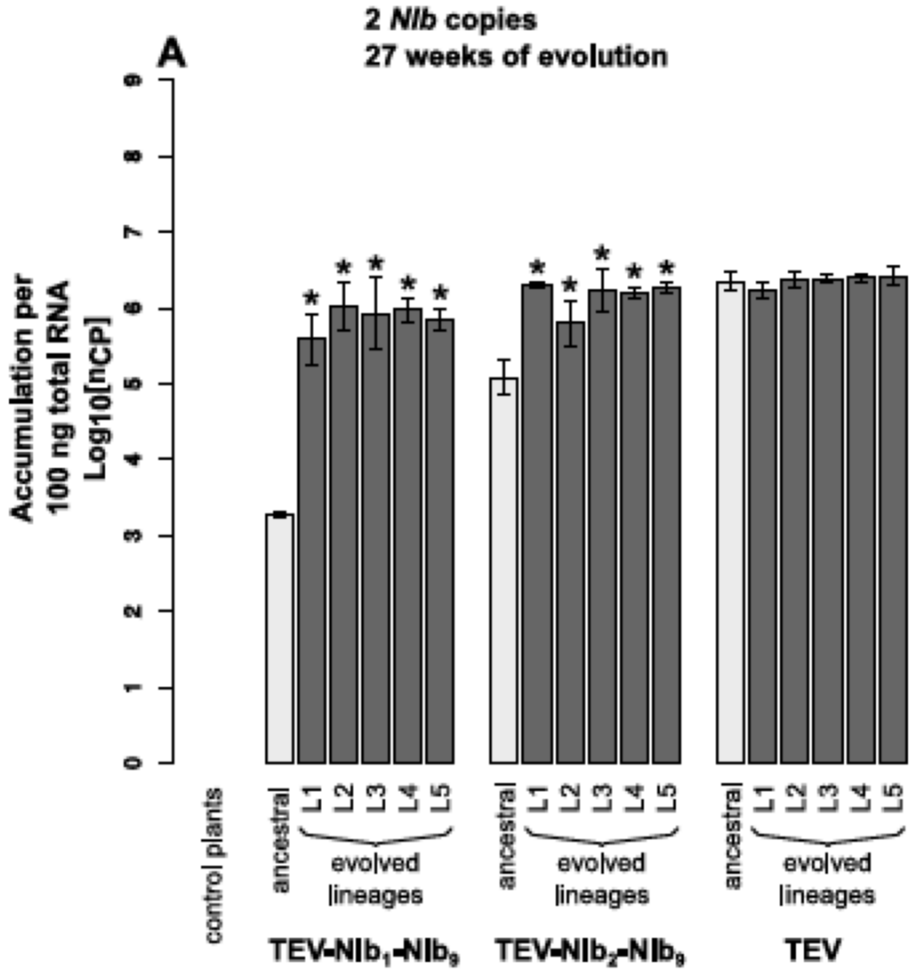


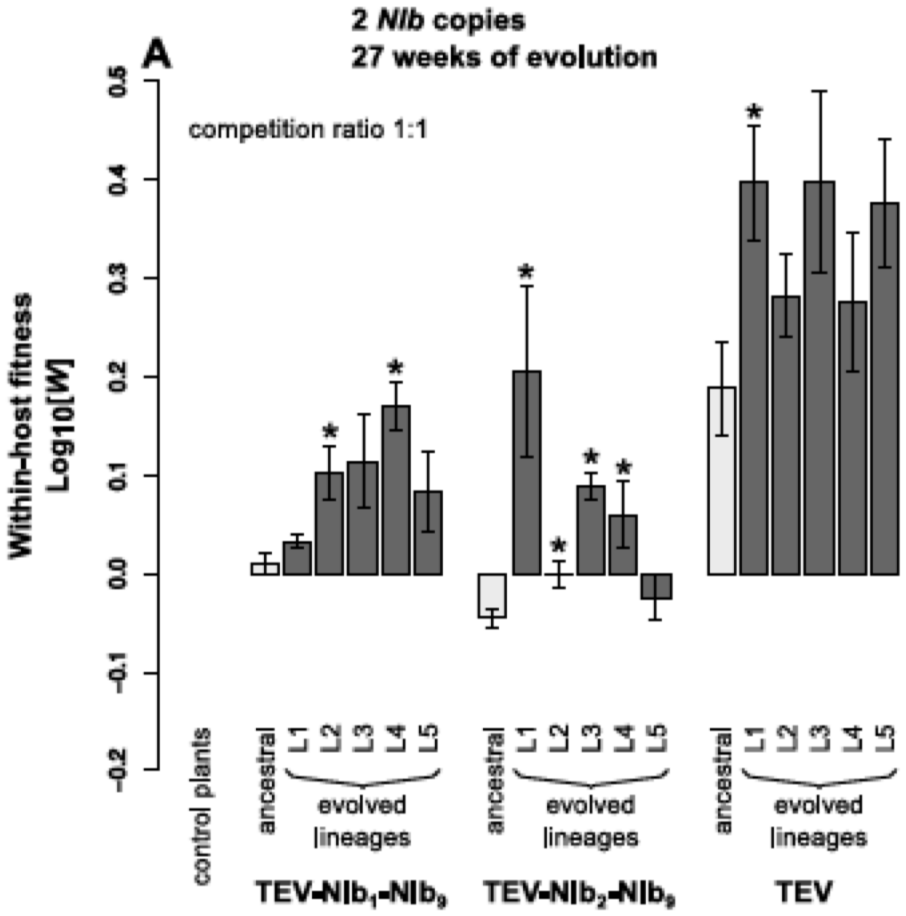
Additional copy is eroded at different rates



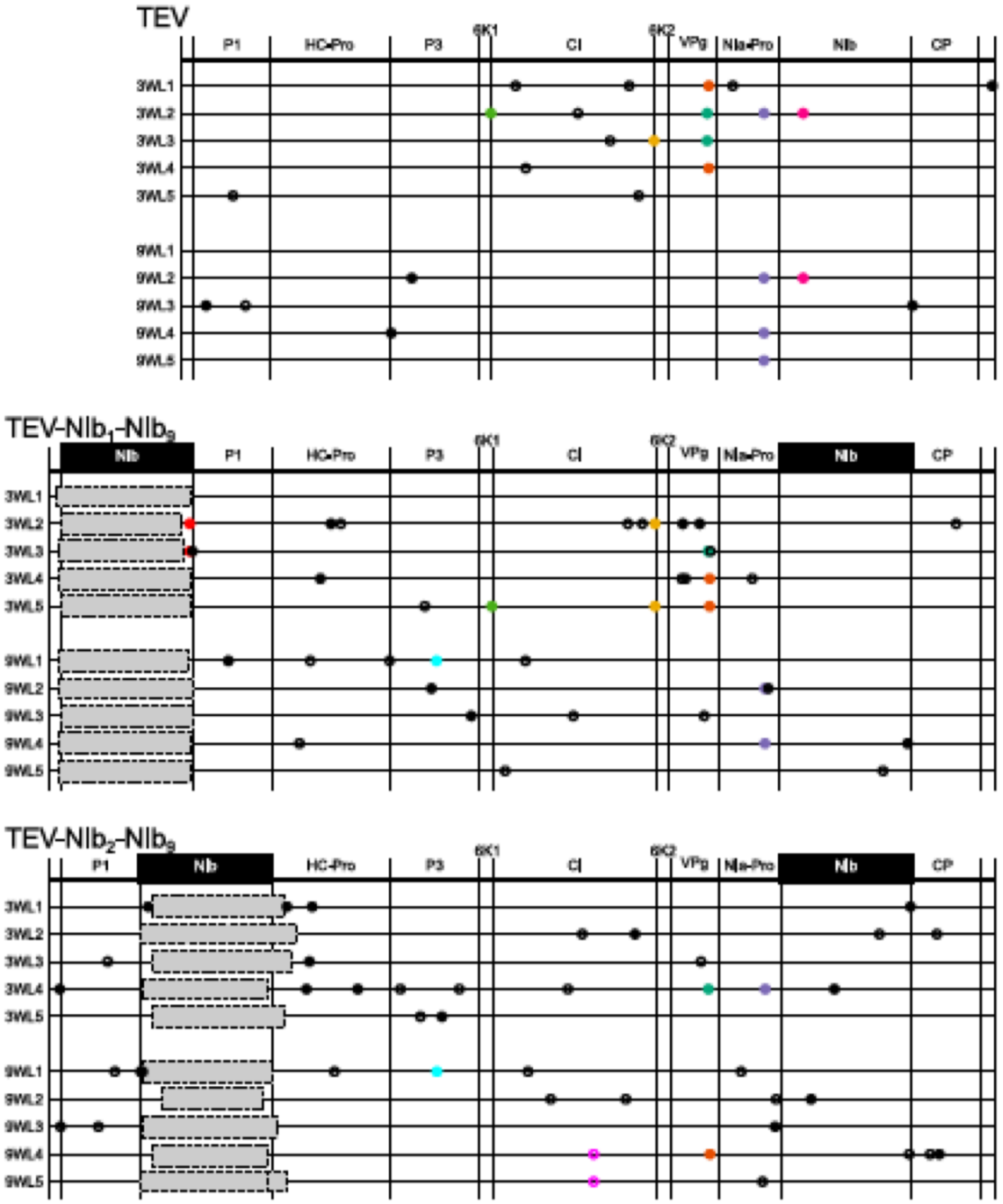


Evolution of phenotypic traits





Genomic evolution



Conclusions 4

- ✓ Strong fitness cost of gene duplication: viruses with duplicated genomes are always worse competitors than wildtype viruses.

- ✓ After duplication, the duplicated gene copy is always removed.

5. Molecular evolution of a viral gene in presence of an homologous NIRV

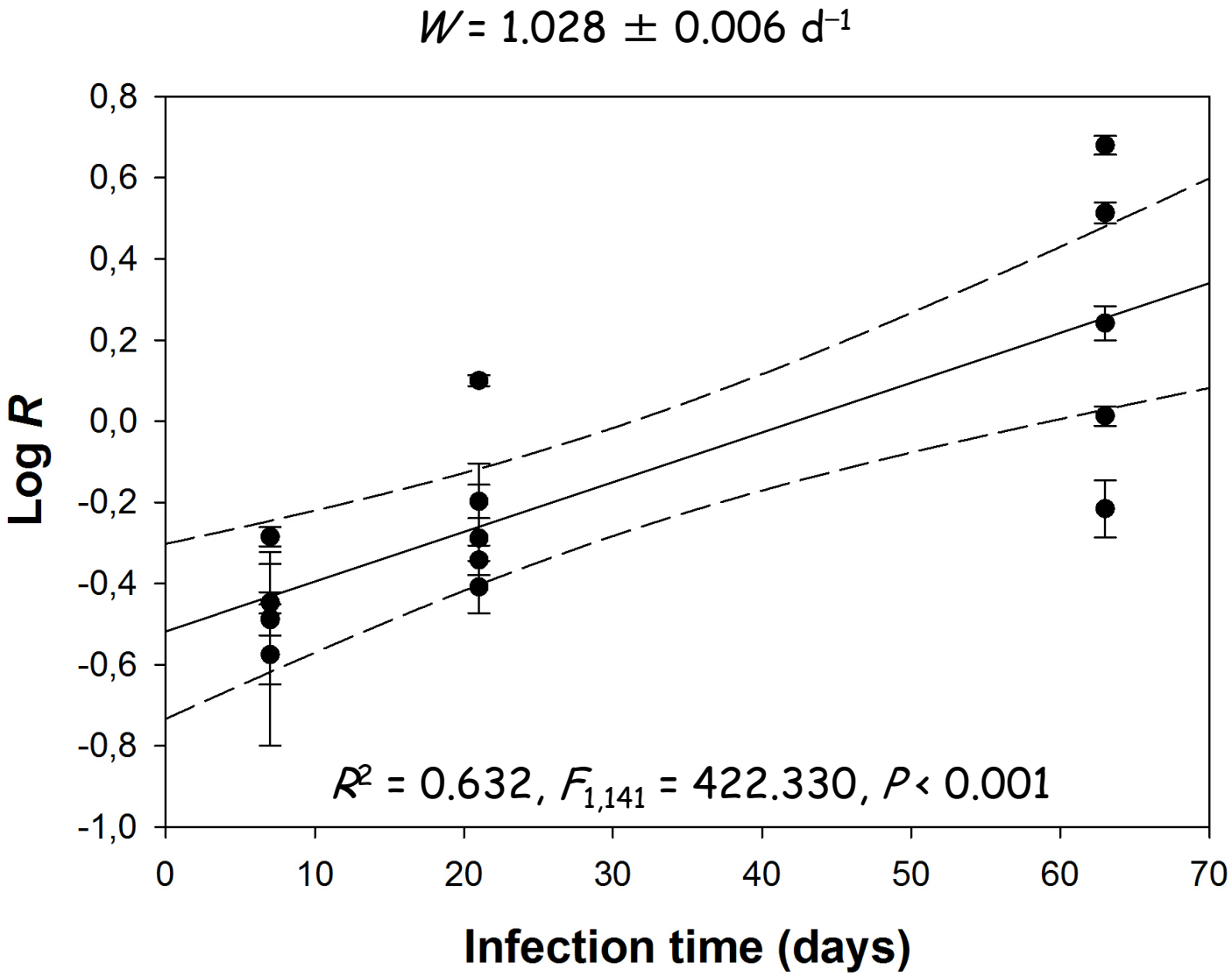
- ✓ Nonretroviral integrated RNA viruses (NIRVs) are genes of nonretroviral RNA viruses found in the genomes of many eukaryotic organisms.

- ✓ NIRVs are thought to sometimes confer resistance to virus infection, meaning that they could impact spread of the virus in the host population.

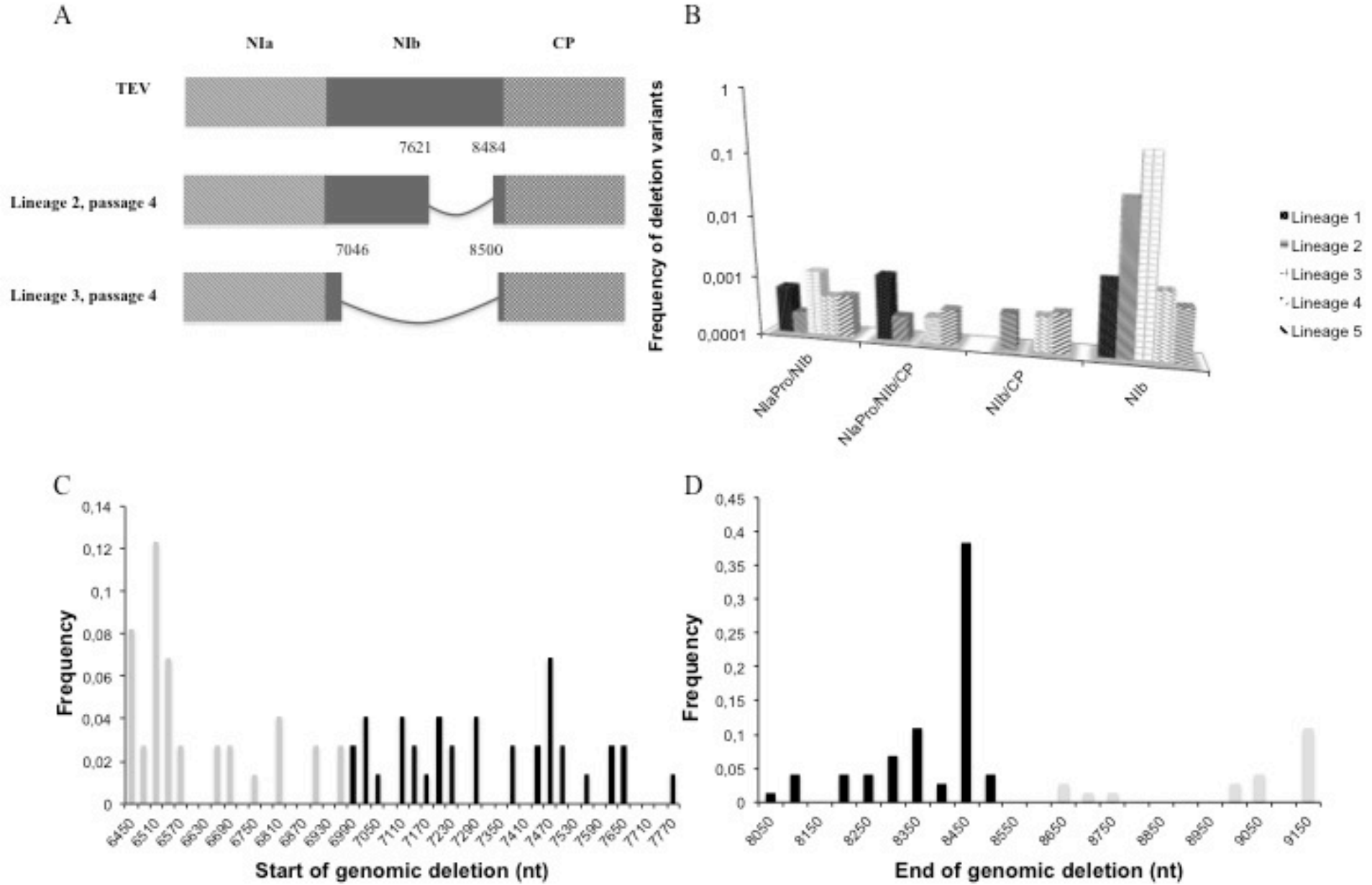
- ✓ However, a NIRV that is expressed may also impact the evolution of virus populations within host organisms.

- ✓ Experimental system: wildtype TEV and TEV-Δ*NIb* evolving (3 weeks passages) in transgenic *Nicotiana tabacum* 35S::*NIb*. TEV *NIb* transgene is a NIRV and generates genetic and functional redundancy.

TEV-Δ*NIb* has a fitness advantage in presence of NIRV

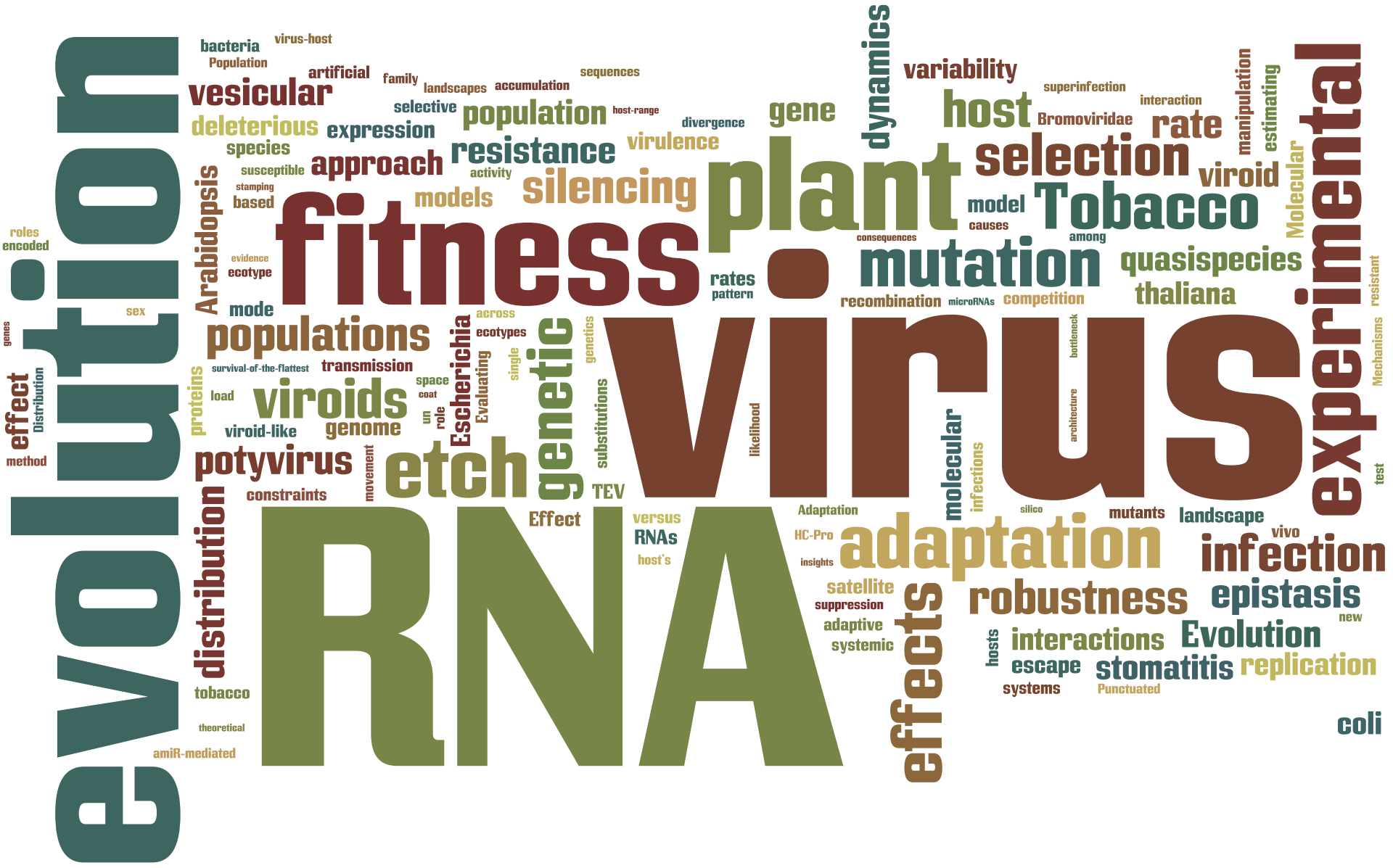


Nature of deletions in viral *NIb* cistron



Conclusions 5

- ✓ We found that TEV-Δ*NIb*, which is incapable of autonomous replication in wildtype plants, thought it had fitness higher than the full-length TEV in the transgenic plants.
- ✓ Moreover, when the full-length TEV was evolved in transgenic plants, we observed genomic deletions within *NIb* and in some cases the adjacent cistrons.
- ✓ When we passaged TEV and TEV-Δ*NIb* in transgenic plants, we found mutations in proteolytic sites, suggesting the adaptation of polyprotein processing to altered NIb expression.
- ✓ These results raise the possibility that NIRV expression can favor the deletion of the corresponding genes in the viral genome, resulting in the formation of viruses that are replication defective in hosts that do not express the same NIRV.
- ✓ Moreover, virus genome evolution was contingent upon the deletion of the viral *NIb*, suggesting NIRV expression could also alter patterns of virus evolution.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