

Positive selection or free to vary?

Assessing the functional significance of sequence change using
molecular dynamics

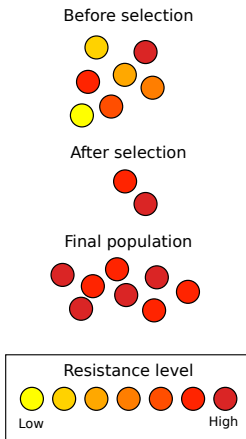
Marcus Lechner

Philipps-University of Marburg

31th TBI Winterseminar in Bled, February 2016

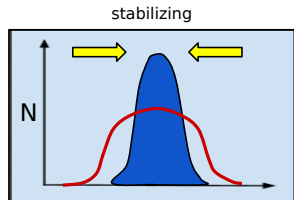
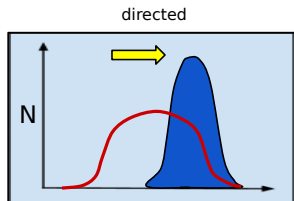
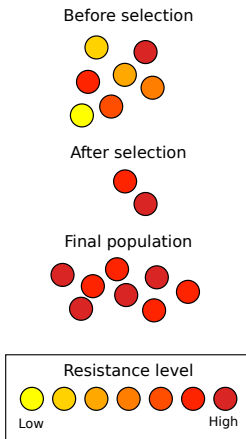
Introduction

- Positive selection: selection for an allele that increases fitness
- Natural variation: variations of an allele that hardly effect fitness

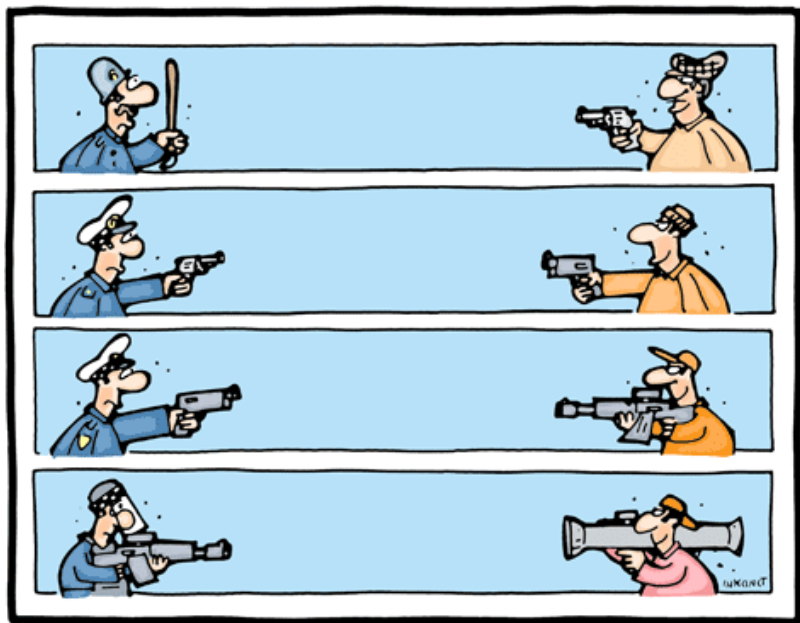


Introduction

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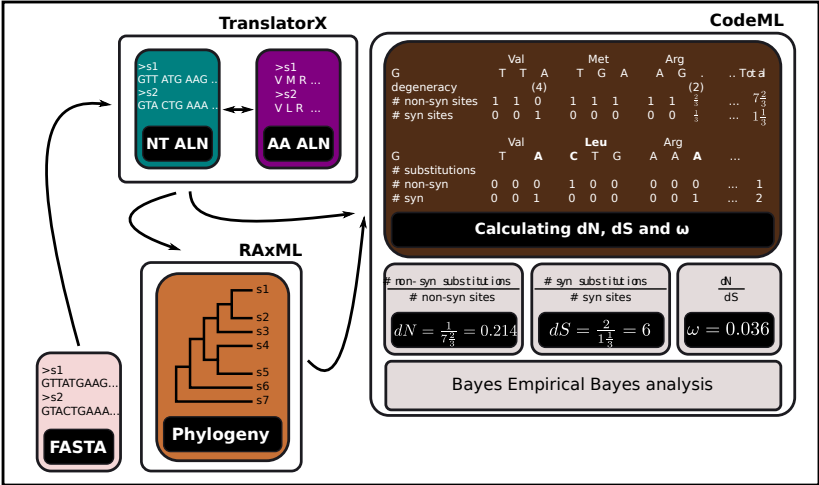


Molecular arms race



2008-275 © INKCINCT Cartoons www.inkcinct.com.au

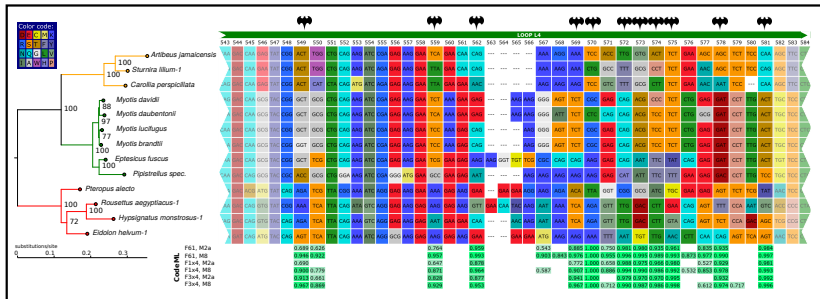
Determination of sites under positive selection (recap)



Martin Hölzner, Mx1 of bats

Determination of sites under positive selection (recap)

- Positive selection: excess of non-synonymous nucleotide substitutions relative to synonymous substitutions
- Tools: CodeML (PAML), Fixed Effects Likelihood (FEL, HyPhy)

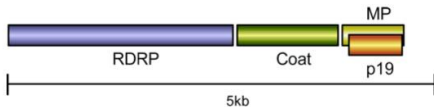
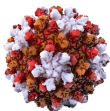


Martin Hölzner, Mx1 of bats

Tombusvirus - overprinted gene set



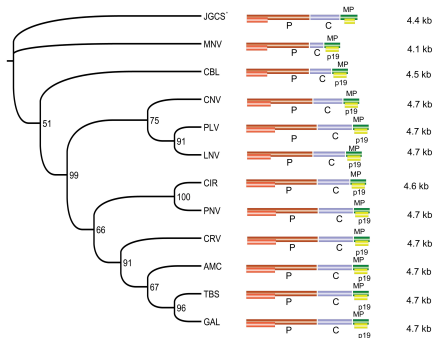
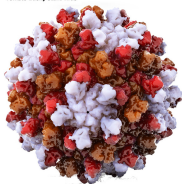
Tombusvirus - overprinted gene set



Tombusvirus

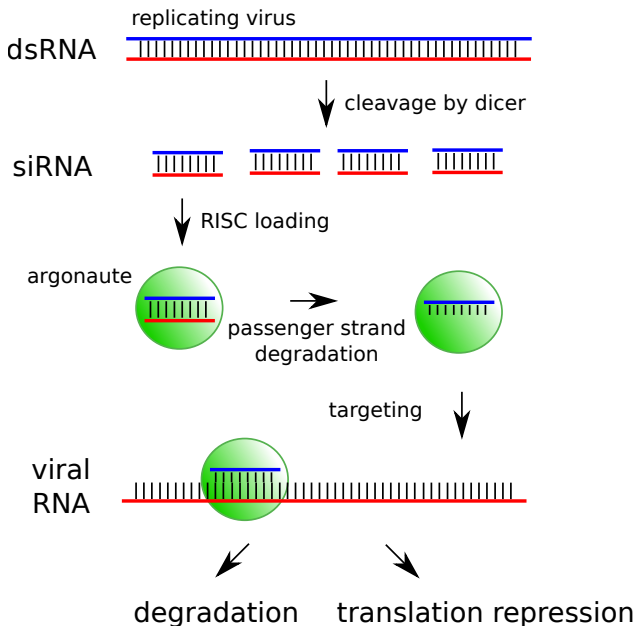
- +ssRNA genome, *Tombusviridae* family
- 17 species known, 11 sequenced
- host: plants (e.g. tomato, eggplant, tobacco)
- cause stunting of growth, leaf mottling, deformed or absent fruit

TBSV
Tomato bushy stunt virus

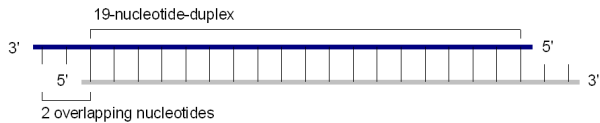


RDRP: RNA-dependent RNA-polymerase
Coat: coat protein
M: movement protein
p19: suppressor of RNAi

RNA-induced silencing complex in plants (RISC)

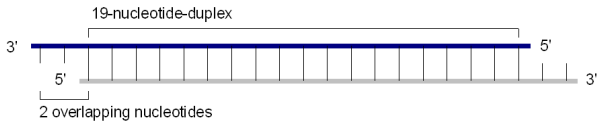


p19 suppressor of RNA interference

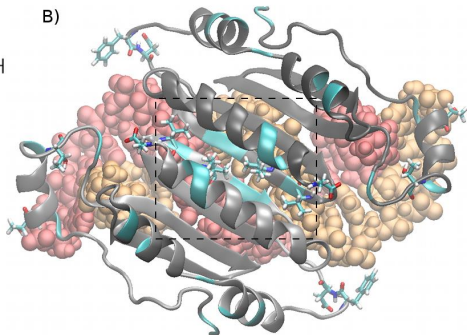
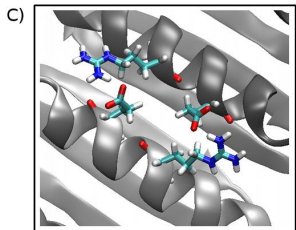
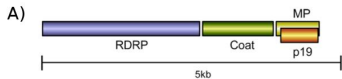


← siRNA

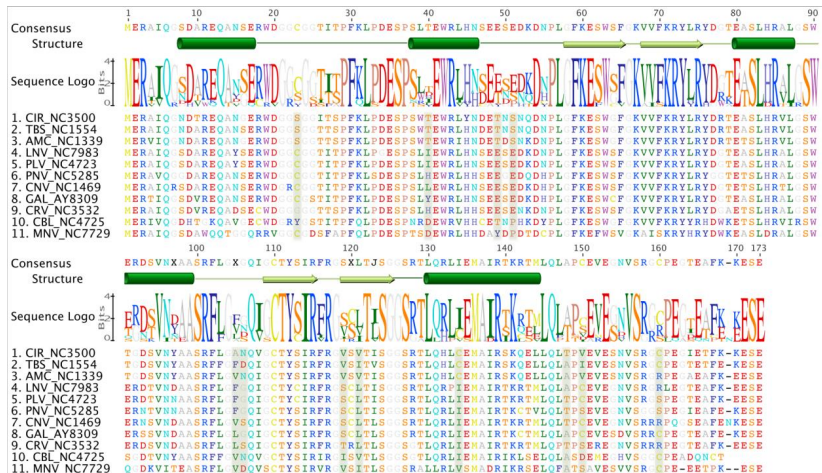
p19 suppressor of RNA interference



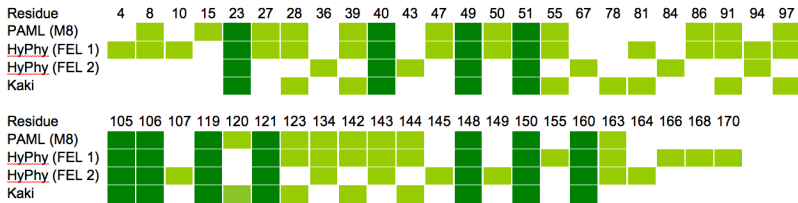
← siRNA ↓



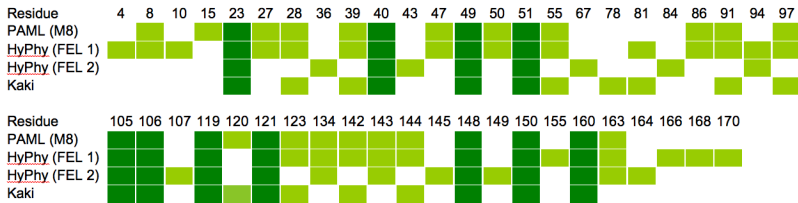
Sequence and structure conservation



Sites under positive selection

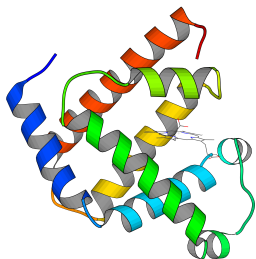


Sites under positive selection

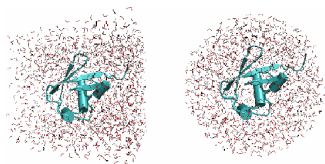


Molecular dynamics (MD)

Structure

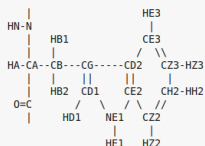


Water box/sphere



Force field

RESI TRP		0.00			
GROUP					
ATOM N	NH1	-0.47	!		
ATOM HN	H	0.31	!	HN-N	
ATOM CA	CT1	0.07	!		HE3
ATOM HA	HB	0.09	!		CE3
GROUP				HA-CA	CB
ATOM CB	CT2	-0.18	!		CG
ATOM HB1	HA	0.09	!		CD2
ATOM HB2	HA	0.09	!		CZ3-HZ3
GROUP					
ATOM CG	CY	-0.03	!		
ATOM CD1	CA	0.035	!		
ATOM HD1	HP	0.115			
ATOM NE1	NY	-0.61			
ATOM HE1	H	0.38			
ATOM CE2	CPT	0.13			
ATOM CD2	CPT	-0.02			



Basic workflow

- derive topology
- equilibrate
- solvate
- neutralize
- equilibrate
- heat
- simulate

Molecular dynamics (MD)

Tools

- NAMD (1 month / 100 ns @ 64 cores)
- Gromacs (4 months / 100 ns @ 64 cores)
- VMD (structure and trajectory viewer)

Measures

- RMSD (root-mean-square deviation of atomic positions)
How much does the molecule move?
- RMSF (average atomic mobility)
Where are highly fluctuating areas?
- sasa (solvent accessible surface area)
- structure (folding, refolding)
- interacting sites (h-bonds, salt-bridges)
- energy contributions
- ...

What we tested

200 ns

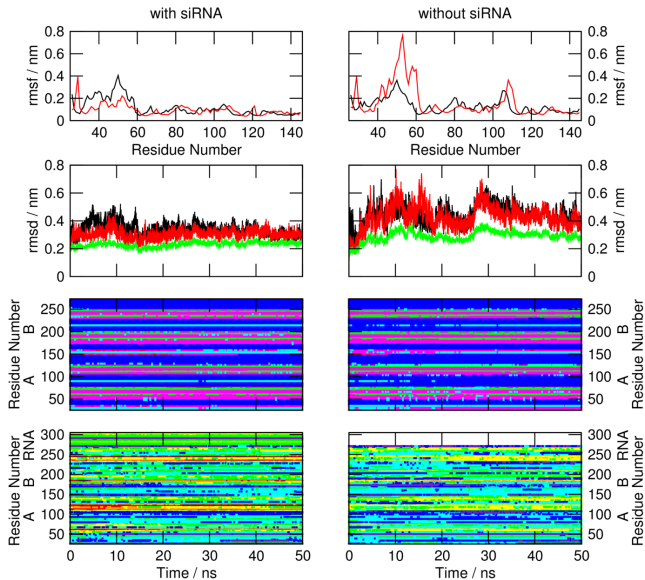
- 1x tomato bushy stunt (TBS) virus p19 dimer (PDB ID 1R9F)
- 2x permissible mutants at pos 139 (conserved)
- 6x permissible mutants at pos 143 (positive selection)

50 ns

- 10x homology modeled p19 dimer from the genus

→ 19x with RNA + 19x without RNA

MD observations, WT example



black: monomer 1, red: monomer 2, green dimer (monomer 1+2); blue: more accessible

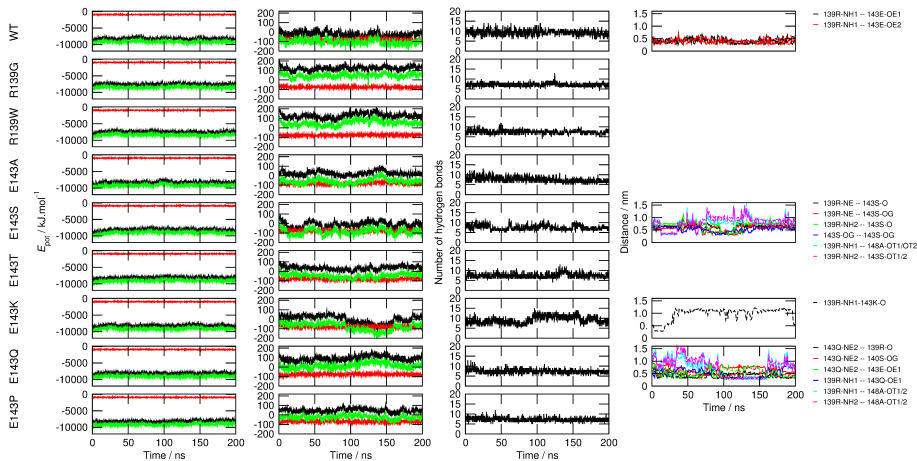
MD observations combined

total energy

interaction energy

hydrogen bonds

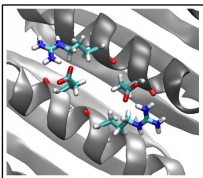
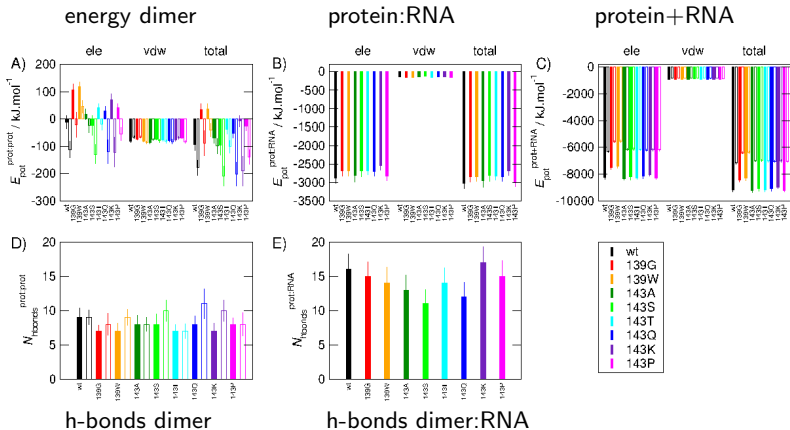
salt-bridges



loss of salt bridge Glu143 – Arg139

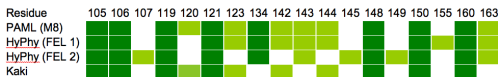
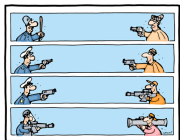
black: Coulombic, red: van der Waals, green: total

MD summary



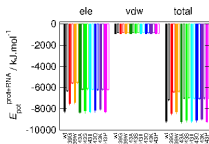
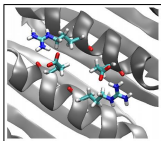
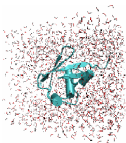
- loss of key salt bridge → least energetically favorable
- permissible changes to Arg139 (conserved) have greater effect than changes to residue 143 (predicted as positively selected)
- RNA stabilizes dimerization

Take home



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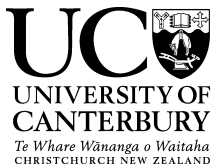
- positive selection: excess of non-synonymous nucleotide substitutions relative to synonymous substitutions
- additional constraints might apply (overprinting, codon usage, ...)
- natural variation often misinterpreted as positive selection
- MD simulations to assess impact on structure & interactions



- p19 dimer robust to disruptive changes at the dimer interface
- not predicted to be under positive selection when constraints are used

Thank you

- Anthony Poole (Christchurch)
- Jane Allison (Auckland)
- Marc Hoepfner (Kiel)



Biomolecular
Interaction Centre

