Darwin meets RNA viruses

From biomedical/agronomical problems to experimental model systems in evolution

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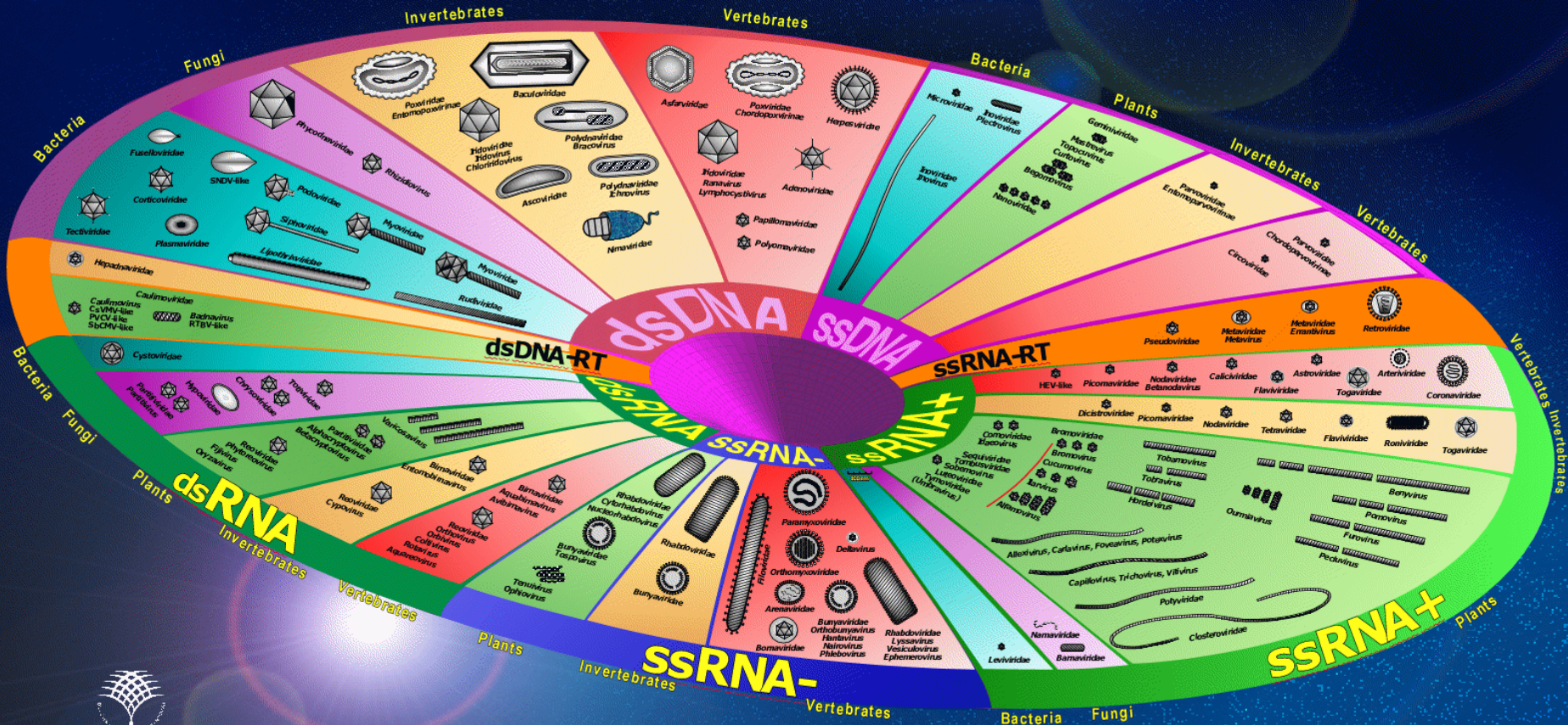
KITP, UCSB, February 16th, 2011

SANTA FE INSTITUTE

Virosphere 2002



DONALD DANFORTH PLANT SCIENCE CENTER

International Committee on Taxonomy of Viruses

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Origin of viruses:

Regressive hypothesis,
Cellular origin or escape hypothesis,
Coevolution or virus-first hypothesis.

Structure:

Helical, a single CP (TEV, TMV, EV),
Icosahedral, several CP forming capsomers (FMDV, PV),
Enveloped (HIV-1, VSV, AdV),
Complex (T4).

Genome:

Nature: DNA, RNA, DNA/RNA,
Shape: linear, circular, segmented,
Strandedness: single, double, mixed.
Sense: positive, negative, ambisense

Replication:

Attachment,
Penetration,
Uncoating,
Replication,
Assembly,
Release.

Advantages of microorganisms for evolution experiments

- They are easy to propagate and enumerate.
- They reproduce quickly, which allows experiments to run for many generations.
- They allow large populations in small spaces, which facilitates experimental replication.
- They can be stored in suspended animation and later revived, which allows the direct comparison of ancestral and evolved types.
- Many microbes reproduce asexually and the resulting clonality enhances the precision of experimental replication.
- Asexuality also maintains linkage between a genetic marker and the genomic background into which it is placed, which facilitates fitness measurements.
- It is easy to manipulate environmental variables, such as resources, as well as the genetic composition of founding populations.
- There are abundant molecular and genomic data for many species, as well as techniques for their precise genetic analysis and manipulation.

Peculiarities of RNA viruses

- High genetic variability. Orders of magnitude greater than for DNA-based organisms.

- High mutation rates: 2.5×10^{-4} m/b/g for VSV, 5×10^{-6} for TEV and 2.5×10^{-3} for CChMVd. Such mutation rates are consequence of the lack of proofreading mechanisms in viral RdRp.

- Compacted genome: 11162 nts for VSV and 9494 for TEV.

- Huge numbers of generations per time unit: $\sim10^{3}$ PFU/cell in 6 - 8 hpi for VSV or $\sim10^{6}$ LFU/g 5 dpi for TEV.

- The variability is a key factor for pathogenicity.

- It is impossible talking about a single defined entity. Instead we shall talk on a distribution of genomes centered around a more frequent one: *Quasispecies*.

- Relatively easy to map genotypes into phenotypic space.

- Viral infectious diseases represent the most important threat to animal and plant health.

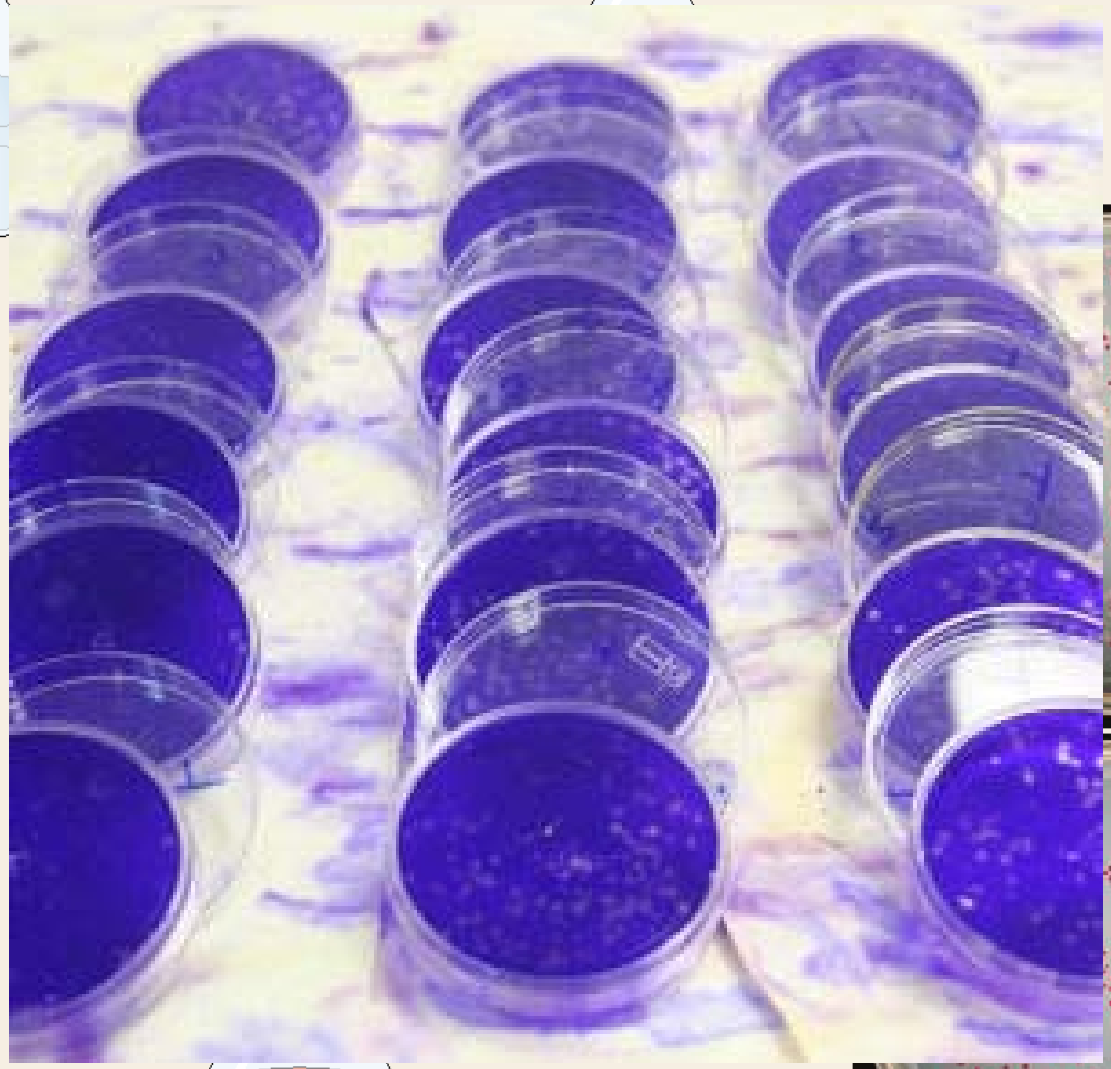
Quantifying the degree of adaptation

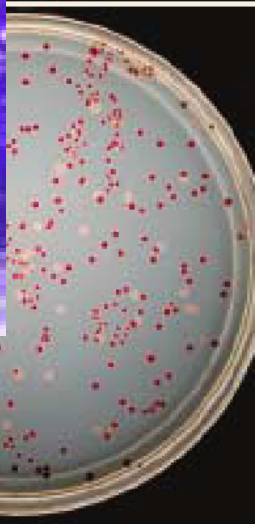
Relative Darwinian fitness: Reproductive ability of a given viral strain in a defined environment. This is a macroscopic property that includes components such as replication, transcription and encapsidation rates as well as virion stability in the environment, resistance to antiviral responses and transmission or adsorption rates.

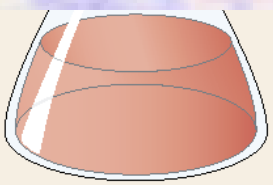
Competition experiments between ancestral and evolved strains.

Ancestral

Evolved







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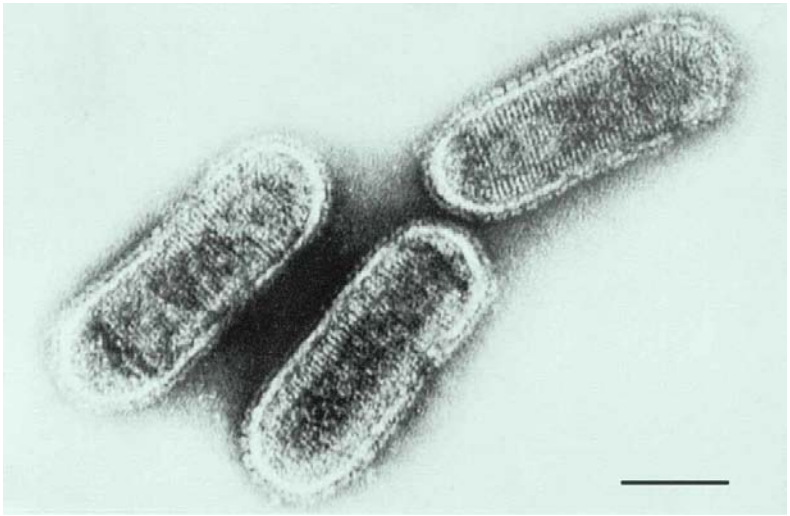
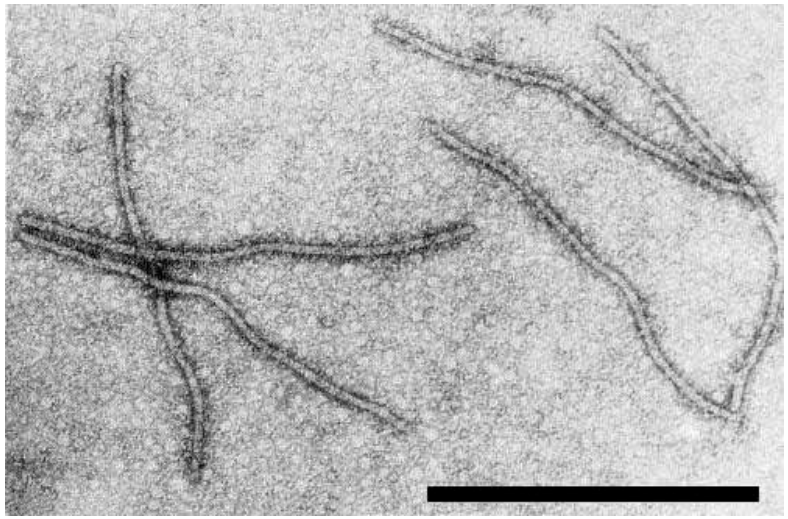
Vesicular stomatitis rhabdovirus

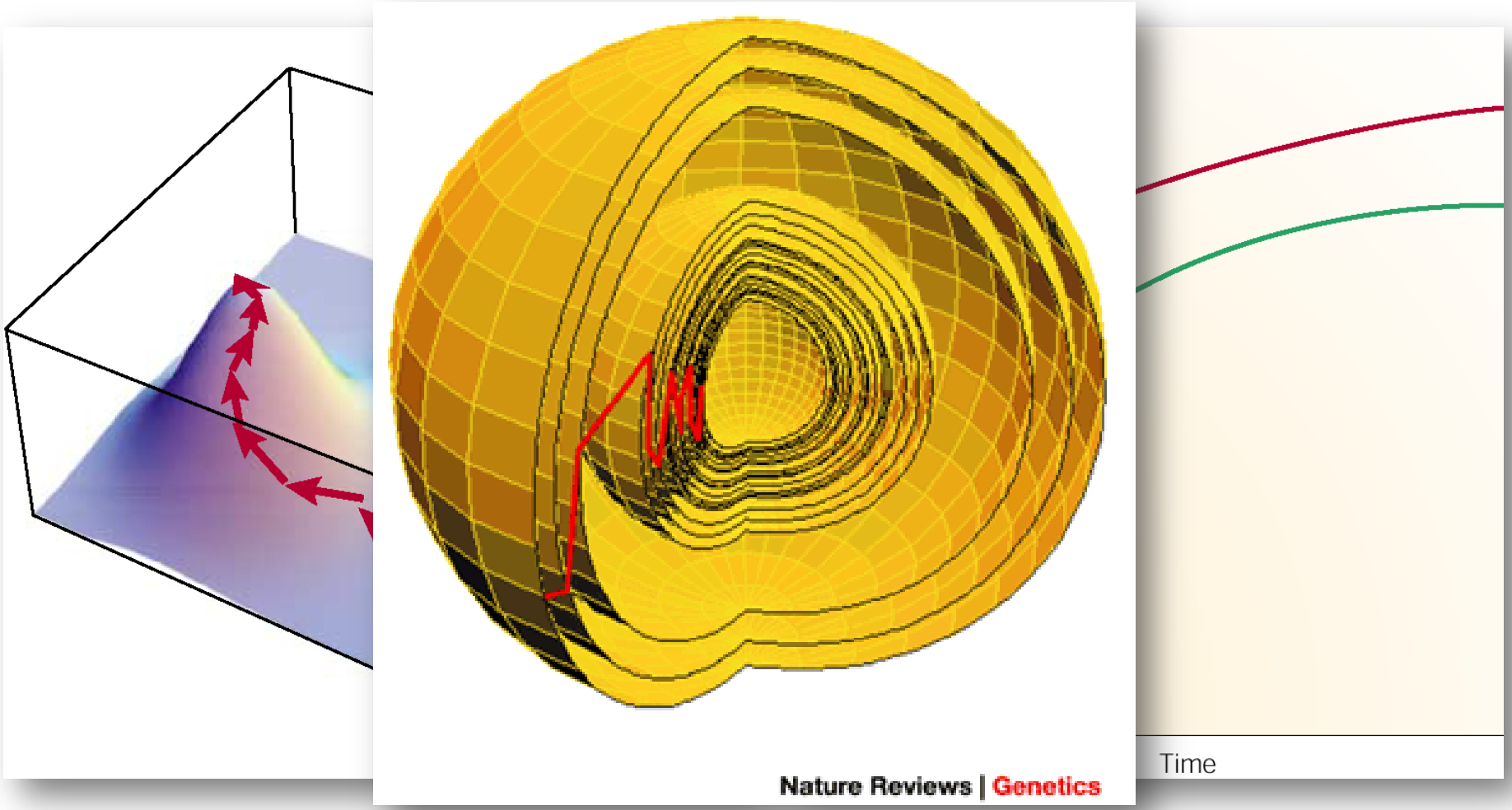


Tobacco etch potyvirus

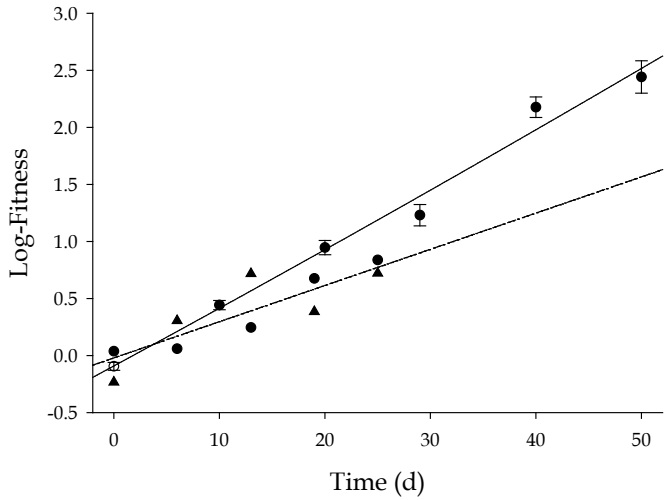


The dynamics of evolutionary adaptation

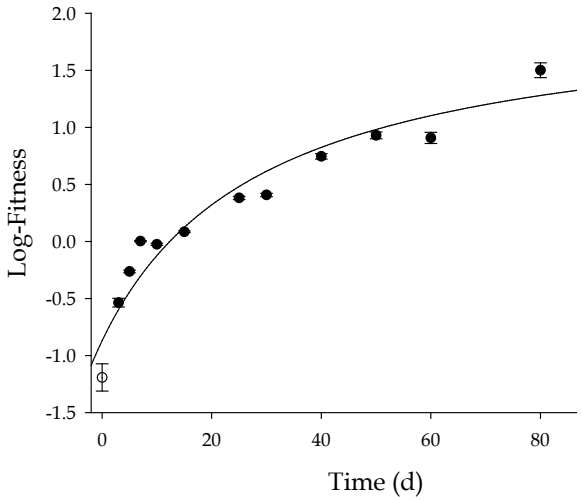
Walking throughout Wright's adaptive landscapes



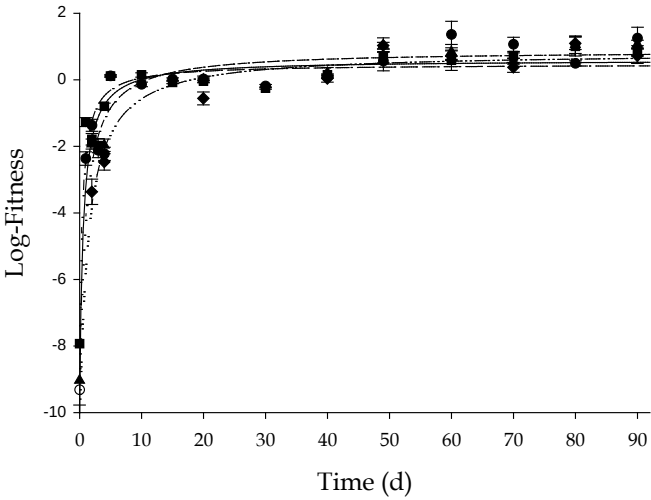
MARM *C*



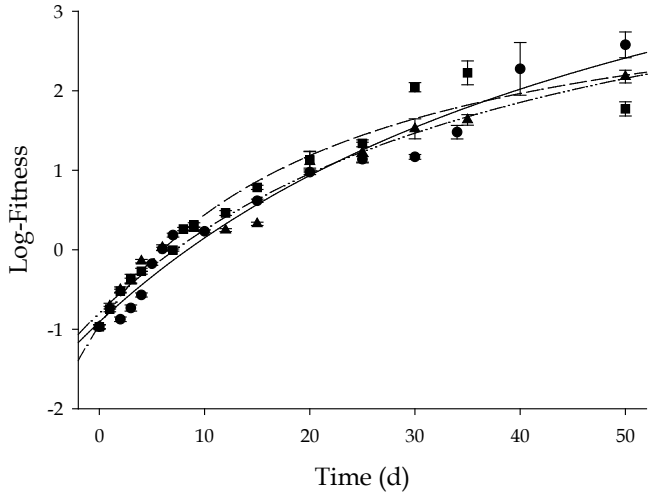
MARM *D*



MARM *F*



MARM *N*



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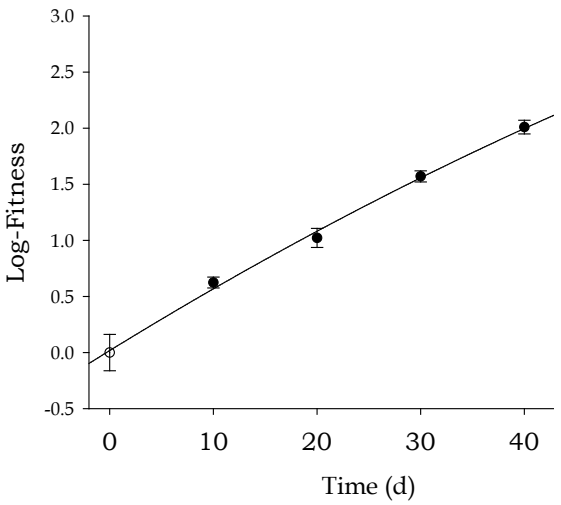
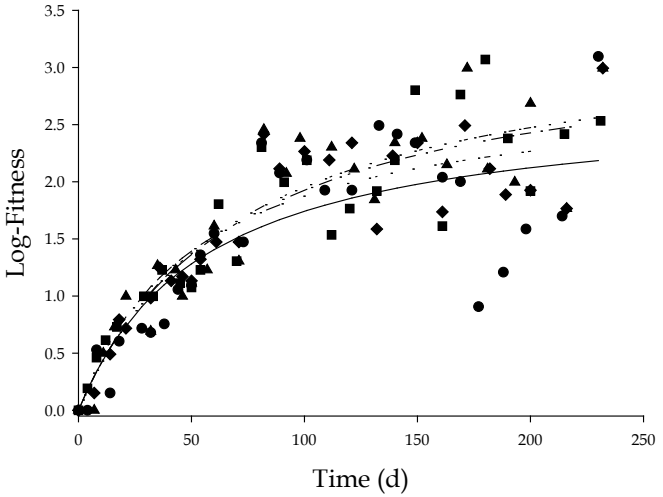


WT



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Novella *et al.* 1995. *Proc. Natl. Acad. Sci. USA* **92**, 5841
Elena *et al.* 1998. *Evolution* **52**, 309
Novella *et al.* 1999. *J. Virol.* **73**, 1668

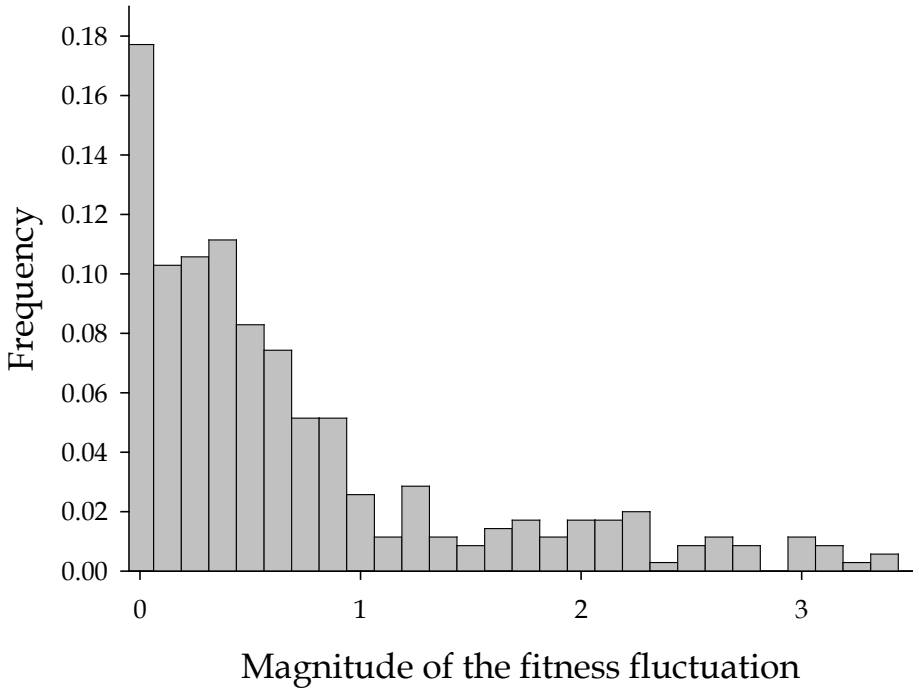
- Under the null hypothesis of random noise, residuals shall distribute Gaussian.

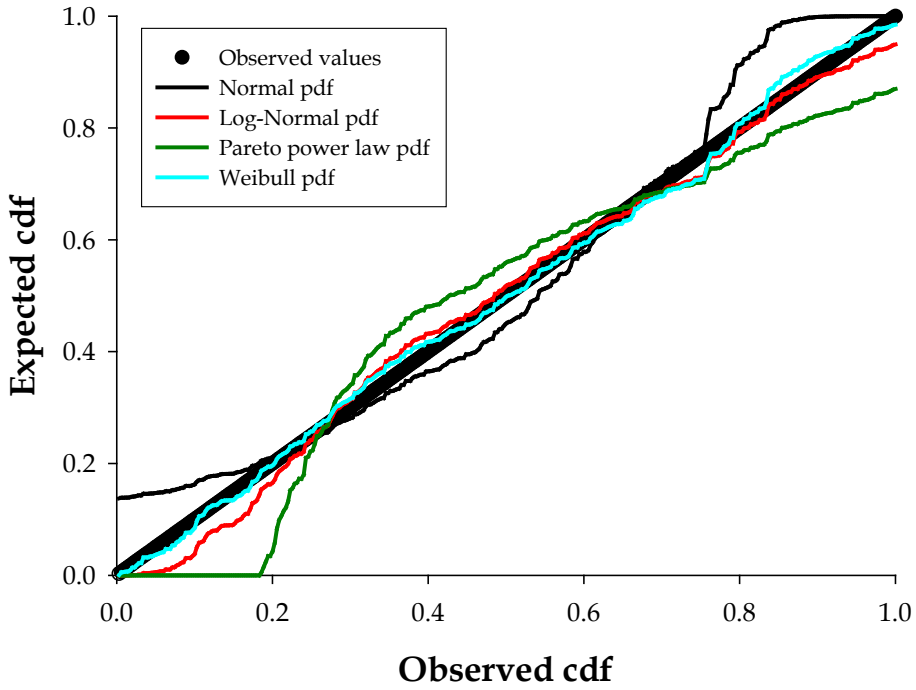
- The presence of non-Gaussian fluctuations in an evolving system may represent the signature of a self-organizing phenomena (Bak *et al.* 1987).

- In particular, the existence of power laws $f(s) = Ks^{-c}$ are indicative of a self-similar behavior, characteristic of fractal objects because changes of scale do not modify the statistical properties of the system.

- Self-organized criticality (SOC) is defined as the spontaneous evolution of large complex systems, formed by many interacting parts, towards a critical state that is robust to perturbations and whose macroscopic behavior is predictable to the extent that it follows a power law.

- RNA viruses have been proposed as paradigms of complex adaptive systems (Solé *et al.* 1999; Domingo 2002).





Critical exponent $c = 0.938$. Values of $0 < c < 2$ are diagnostic for SOC.

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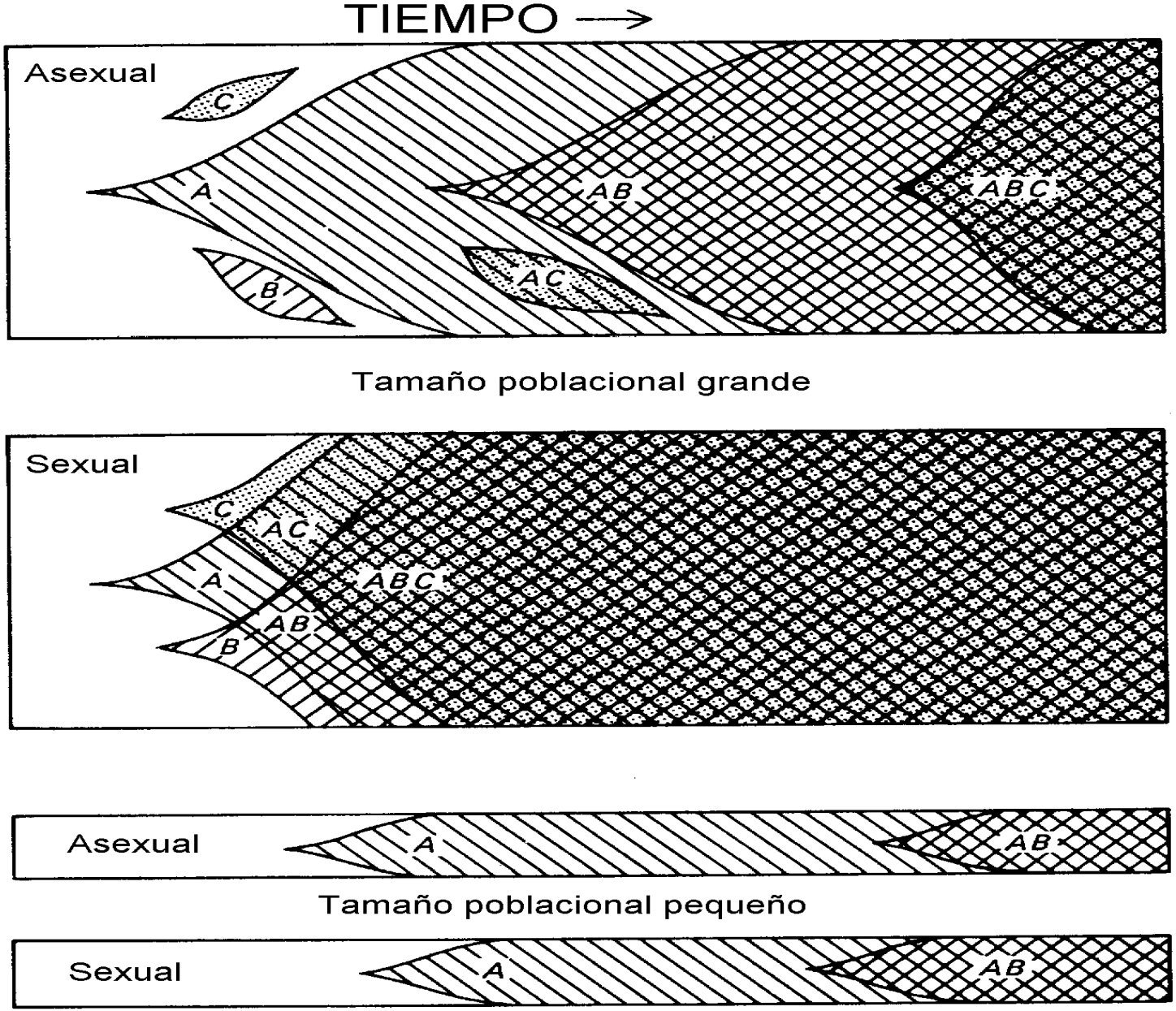
Elena & Sanjuán. 2005. *Biosystems* **81**, 31

- What can generate such critical states?

The first-order phase transition at the error threshold (Eigen 1971): viral populations shall stay centered around the optimum genotype; those going far away are not viable anymore.

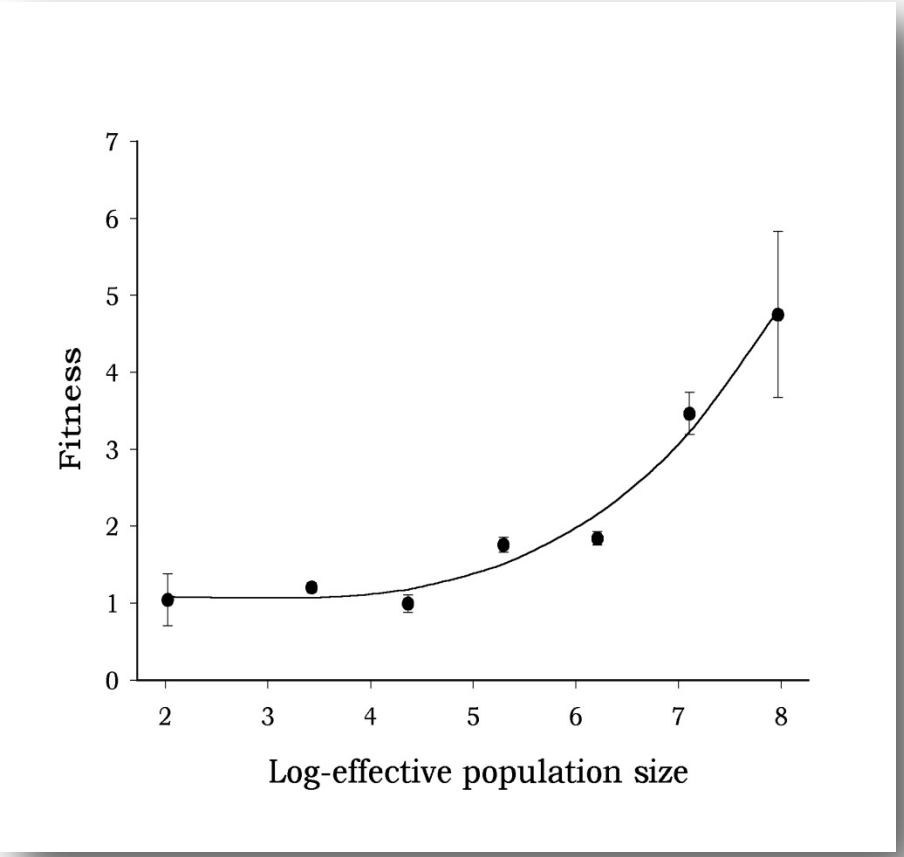
Red Queen coevolutionary dynamics in rugged fitness landscapes (Bak *et al.* 1992): while keeping pace each other, the system is in an unstable equilibrium. If a change in one competitor can not be matched by the other, then the dynamics became chaotic and small fluctuations in frequency can be dramatically amplified and give rise to fixation/elimination of one competitor.

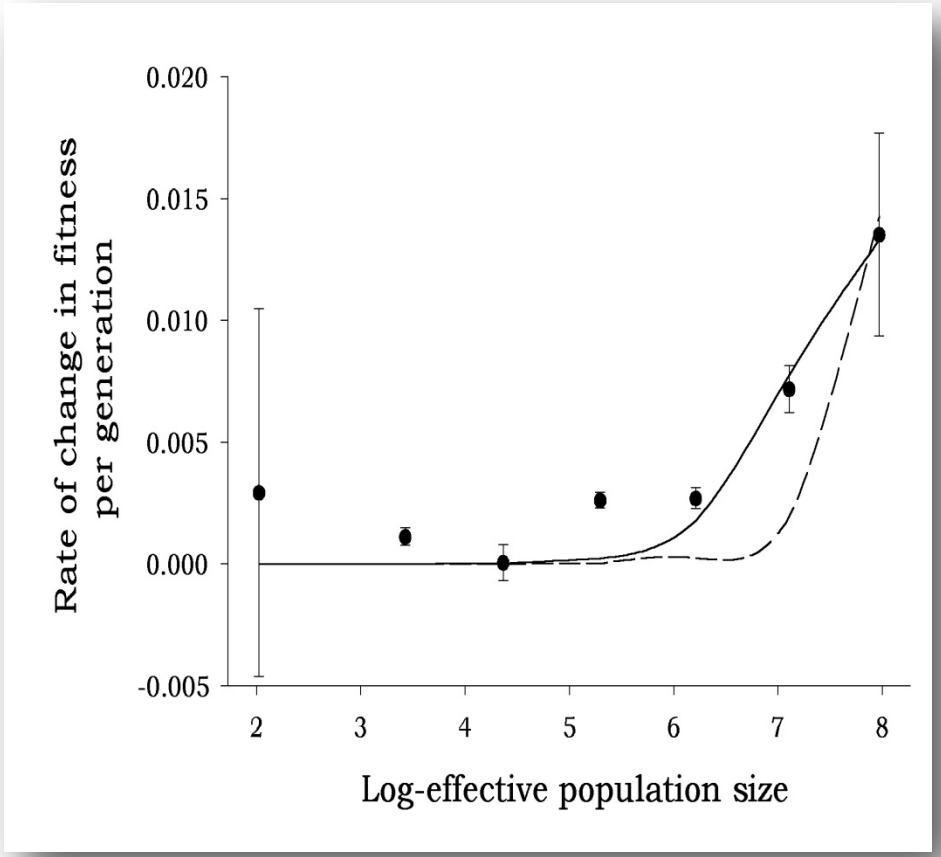
Limits to viral evolution?



1st Prediction: the stronger clonal interference the larger the selective value associated with the mutation that finally becomes fixed.

2nd Prediction: clonal interference imposes a limit to the rate of viral adaptation.





$2.57\times10^{-8} \leq U_b \leq 1.58\times10^{-7}$ $0.24 \leq E(s_b) \leq 0.43$

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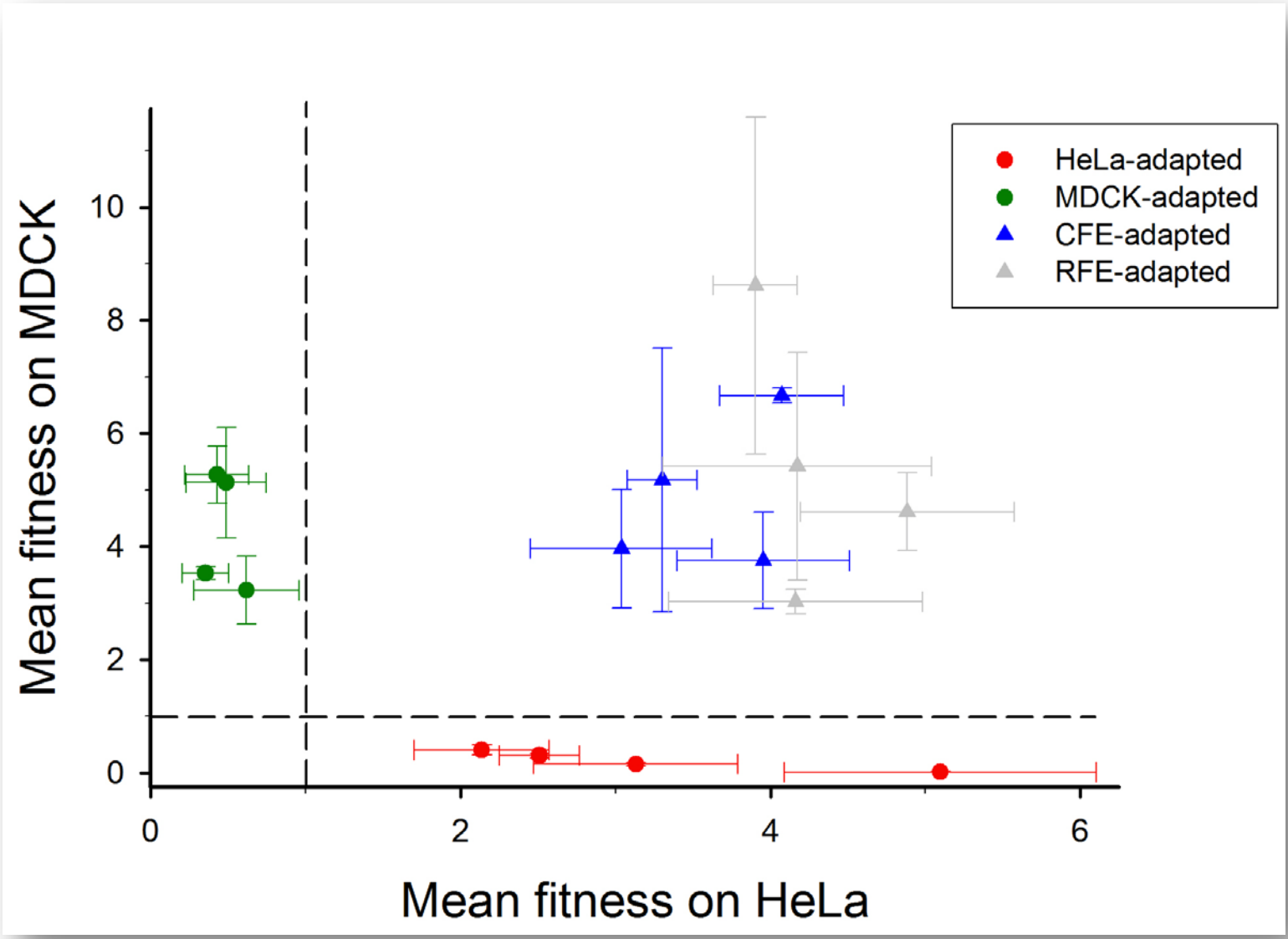
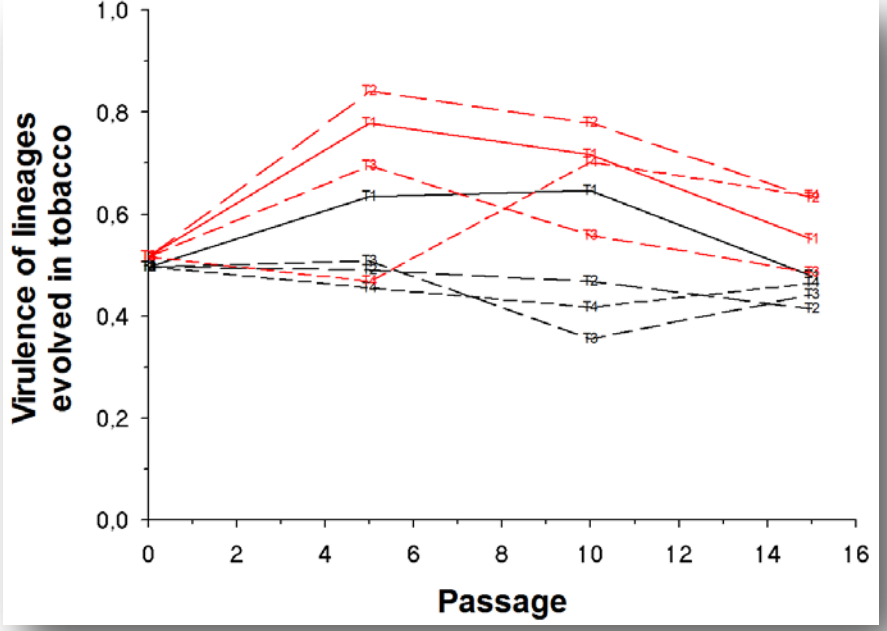
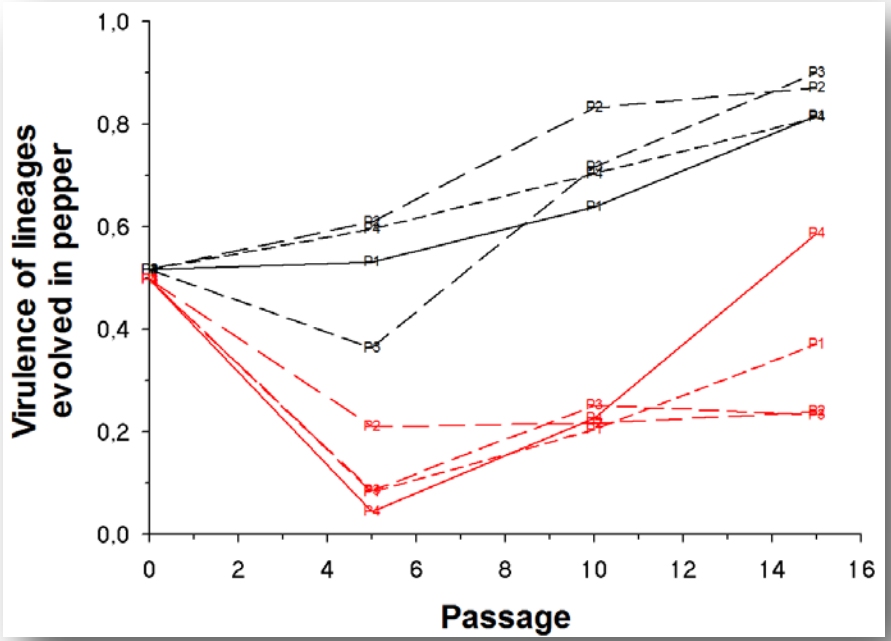
Miralles *et al.* 1999. *Science* **285**, 1745
Miralles *et al.* 2000. *J. Virol.* **74**, 3566

Adaptive trade-offs and the specificity of adaptation



Turner & Elena. 2000. *Genetics* **156**, 1465





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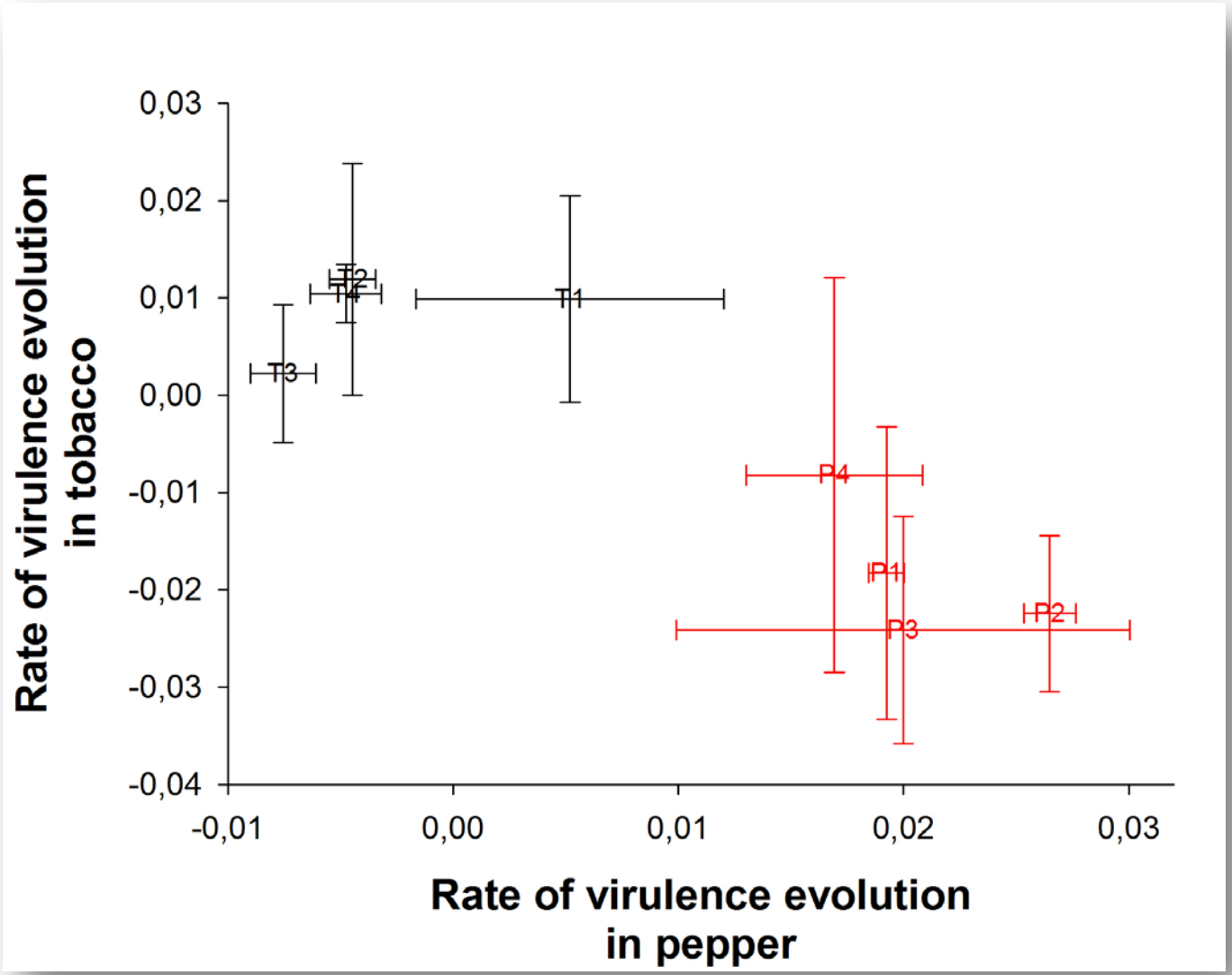
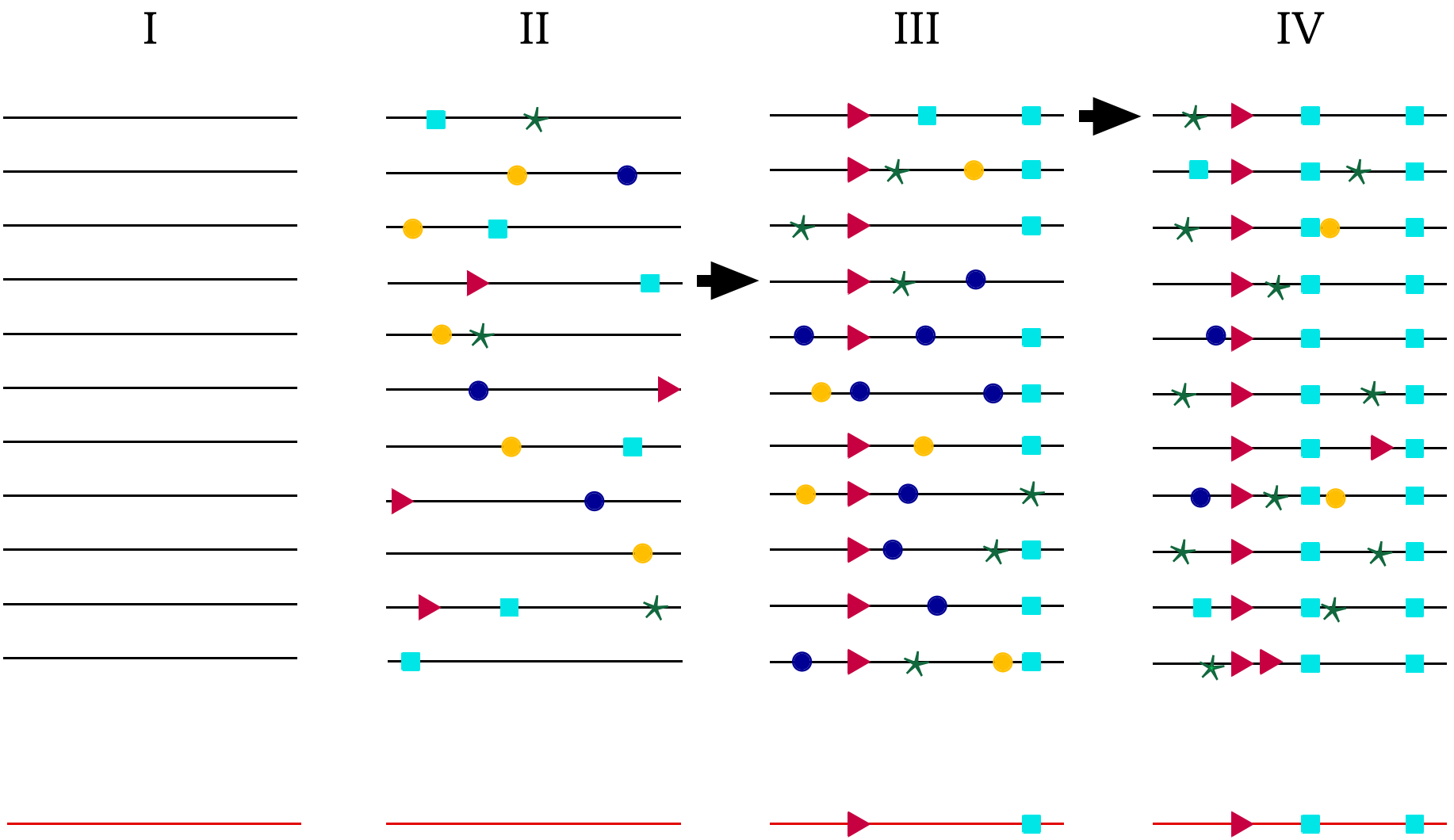
Agudelo-Romero *et al.* 2008. *Infect. Genet. Evol.* **8**, 806

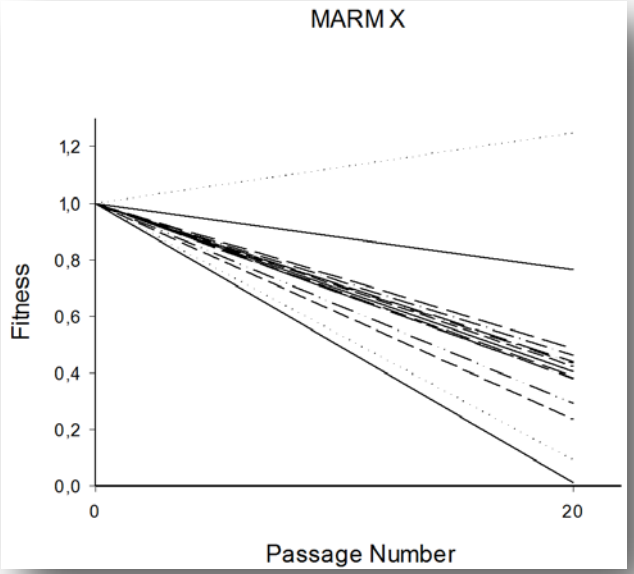


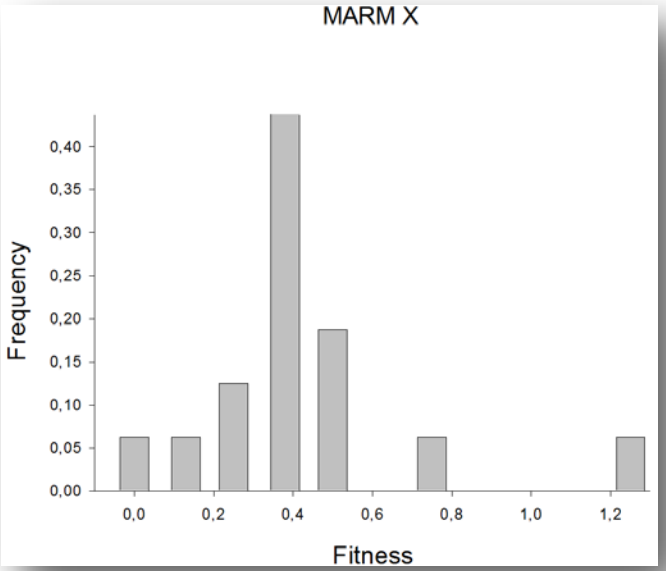
$r = -0.896$, 6 d.f., 1-tailed $P = 0.001$

Agudelo-Romero *et al.* 2008. *Infect. Genet. Evol.* **8**, 806

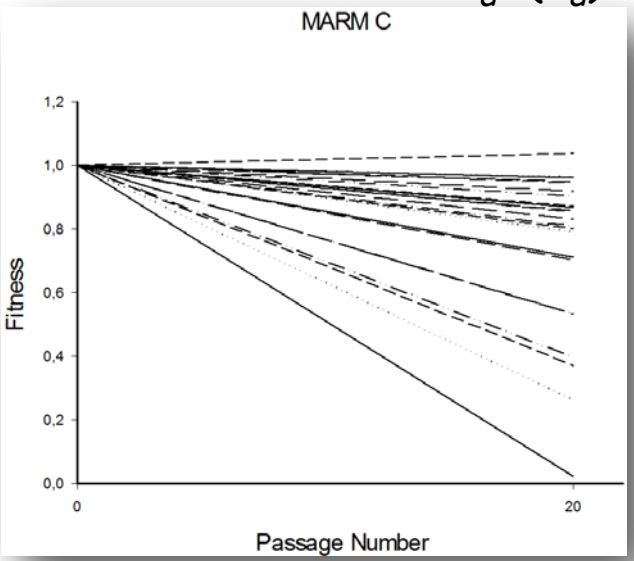
Drift and decay in very small populations

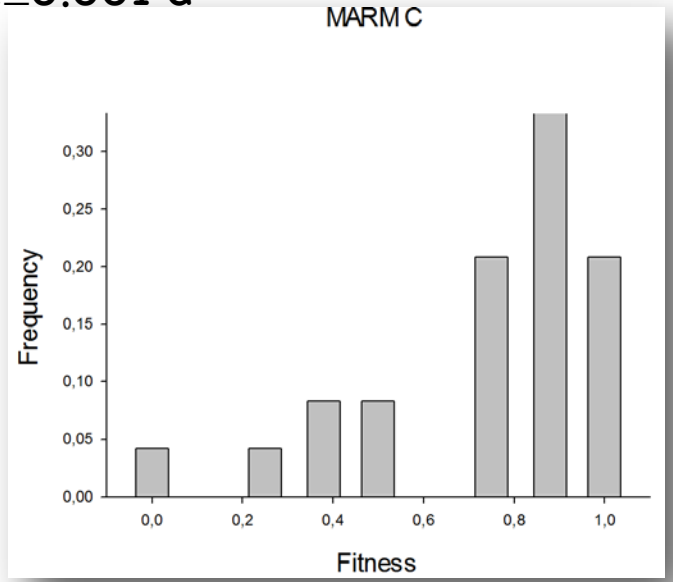

I
II
III
IV





$U_d E(s_d) = -0.012 \pm 0.001\ d^{-1}$





$U_d E(s_d) = -0.008 \pm 0.0001\ d^{-1}$

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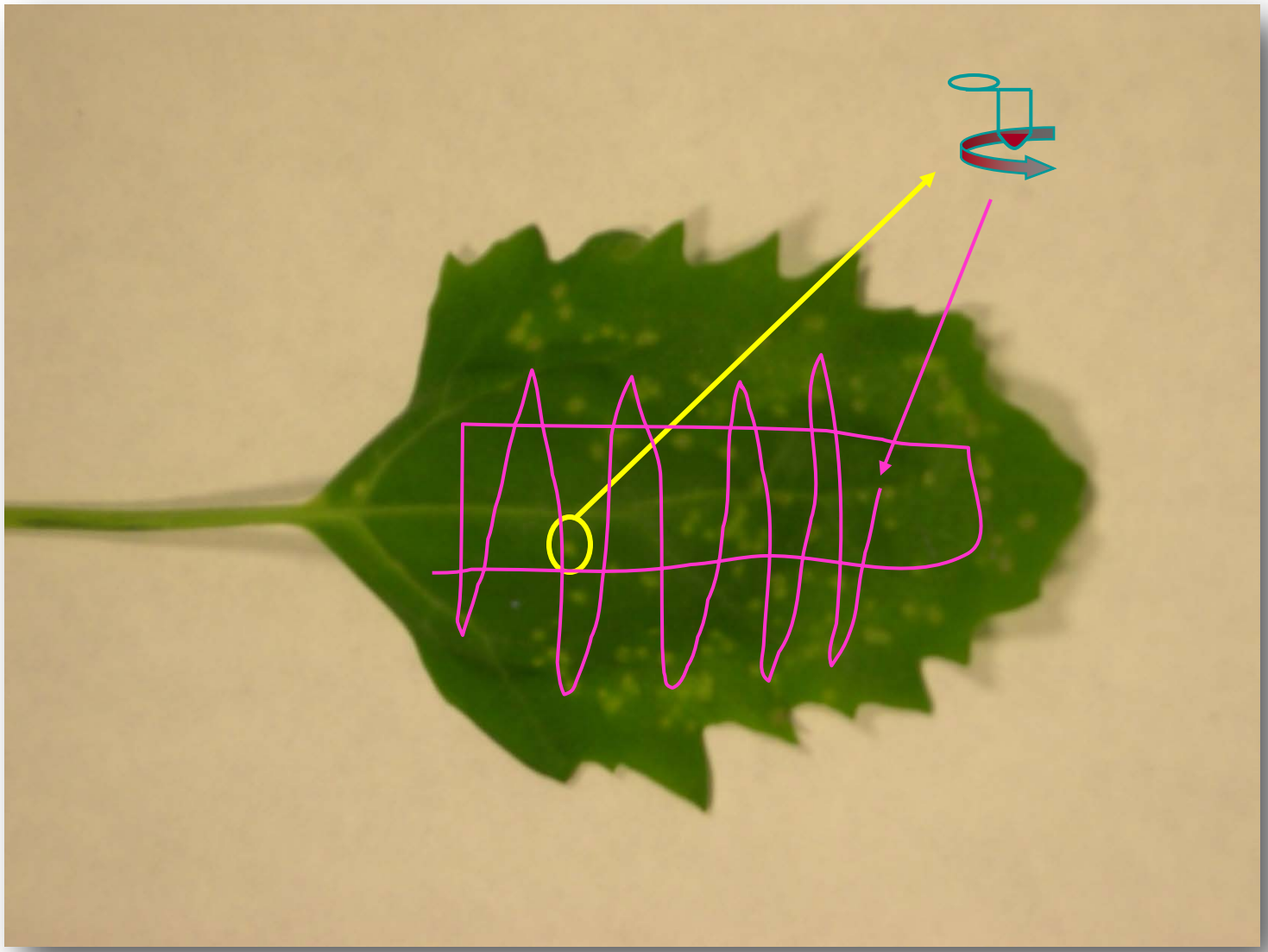
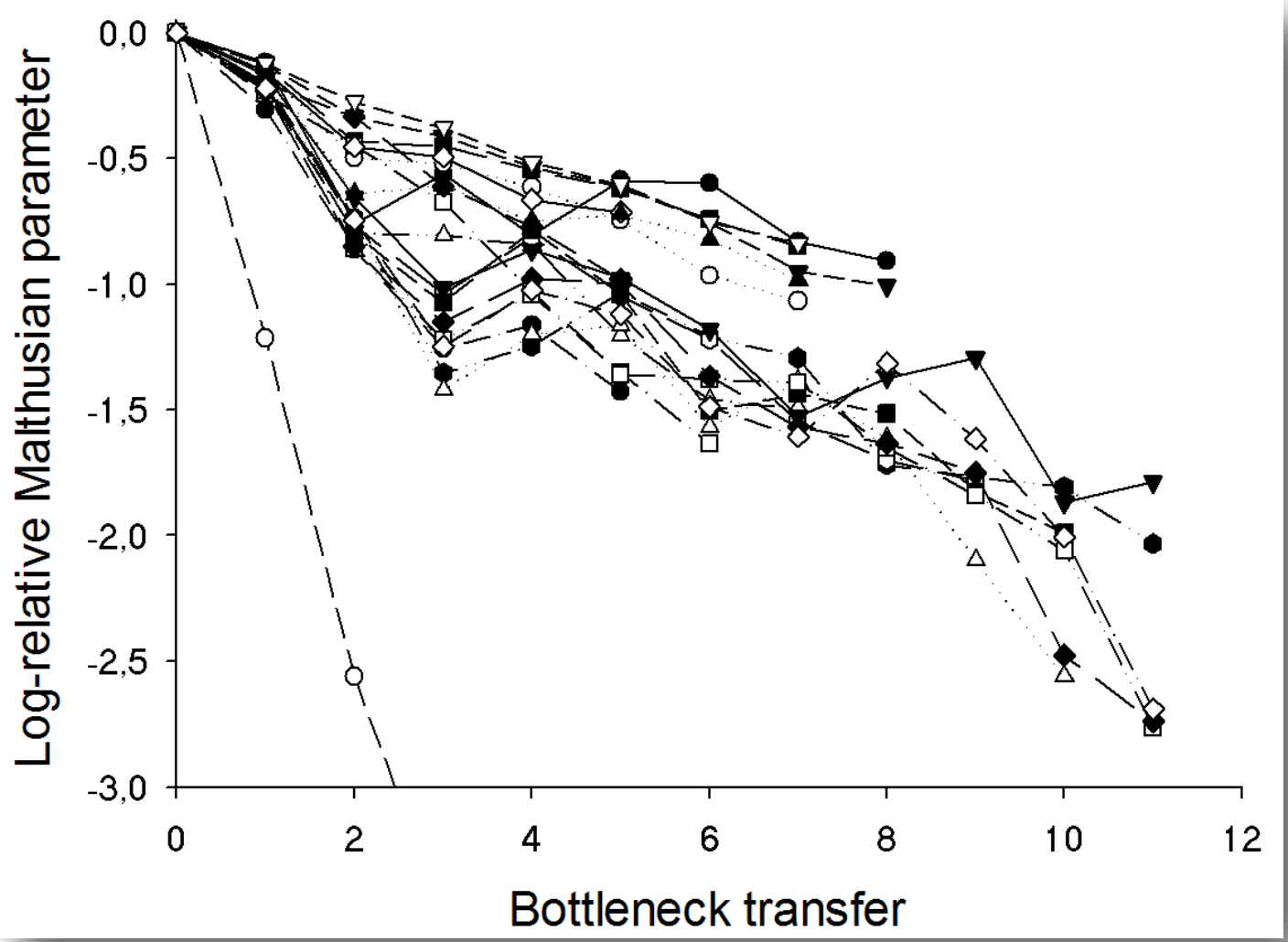
Duarte *et al.* 1992. *Proc. Natl. Acad. Sci. USA* **89**, 6015
Clarke *et al.* 1993. *J. Virol.* **67**, 222
Elena & Moya. 1999. *J. Evol. Biol.* **12**, 1078





$U_dE(s_d) = -0.052 \pm 0.008$ d^{-1}, $t_{19} = 6.500$, 1-tailed $P < 0.001$

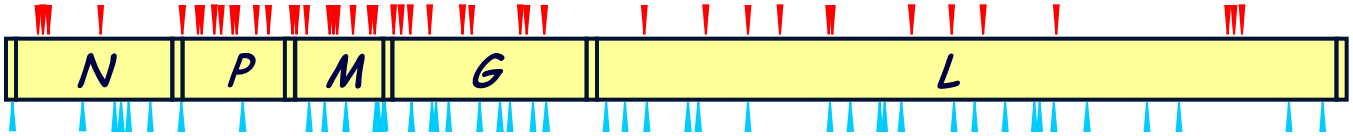
De la Iglesia & Elena. 2007. *J. Virol.* **81**, 4941

- However, MA experiments do not provide any information on the number of mutations fixed nor their molecular nature. Also, they can produce biased estimates due to intra-plaque selection or the existence of robustness mechanisms.

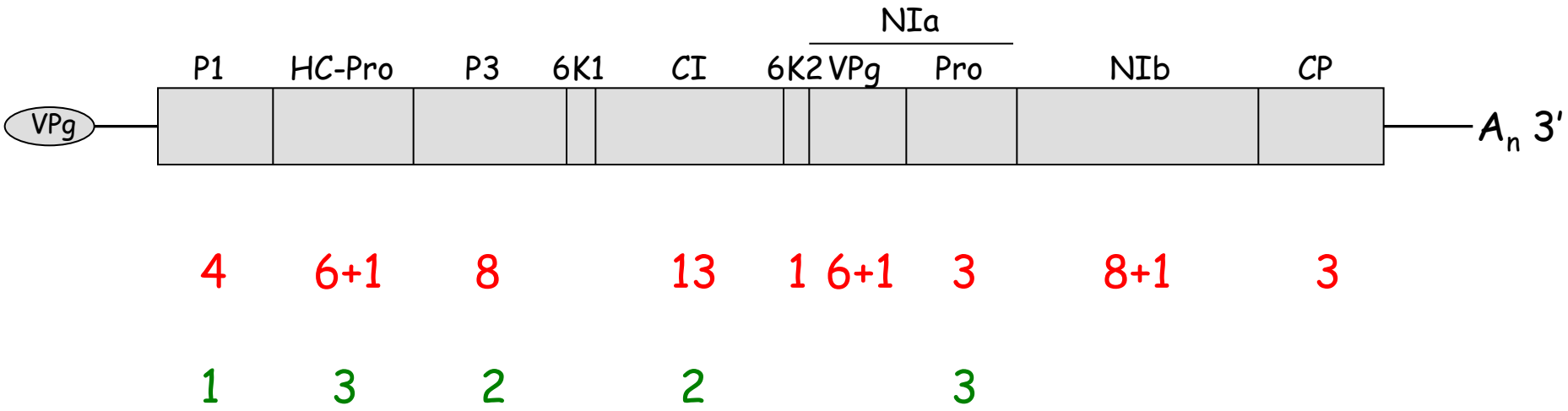
- To avoid this drawback, we took a site-directed mutagenesis approach on a full-length infectious cDNAs (VSV - Dr. G.T.W. Wertz, U. Alabama; TEV – Dr. J.C. Carrington, Oregon State U.).

- For VSV, we created a collection of 91 single-nucleotide substitution mutants.
  - 48 randomly chosen (both site and nt introduced).
  - 43 already described in wild isolates and laboratory populations or clones.

  For TEV, 90 random mutants were created.



		N	P	M		G	L
Random	Synonymous	2		1	2	3	3
	Nonsynonymous	4 + 1	2	2		11	16 + 2
Pre-observed	Nonsynonymous	4	8	10		8	12



Nonsynonymous changes + stop codons

Synonymous changes

All randomly chosen

- However, MA experiments do not provide any information on the number of mutations fixed nor their molecular nature. Also, they can produce biased estimates due to intra-plaque selection or the existence of robustness mechanisms.

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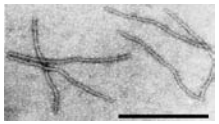
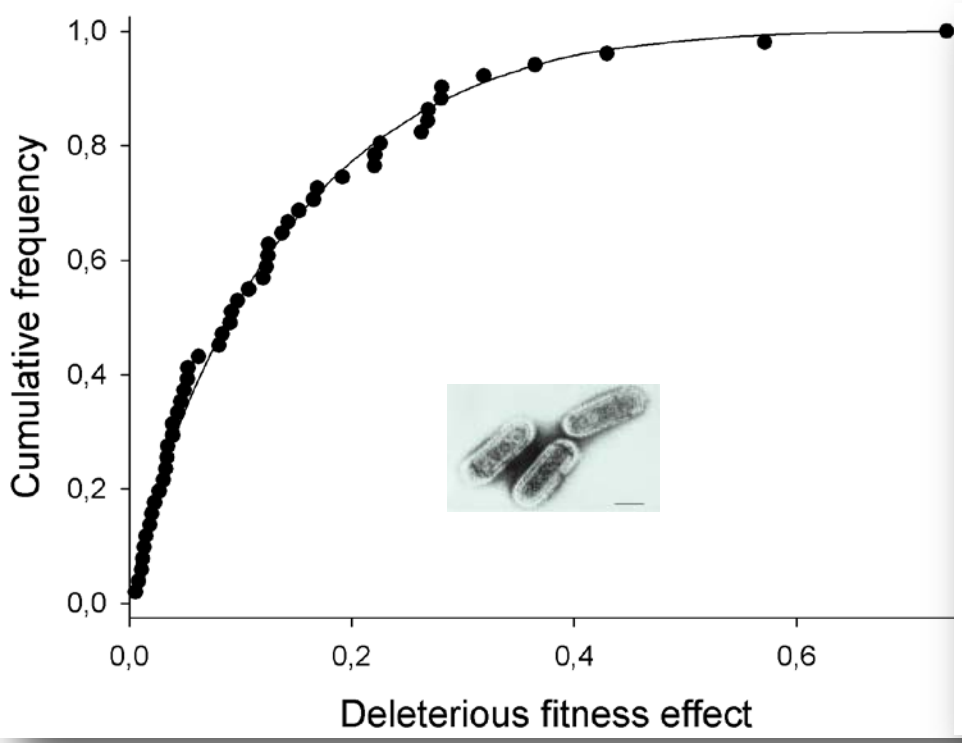
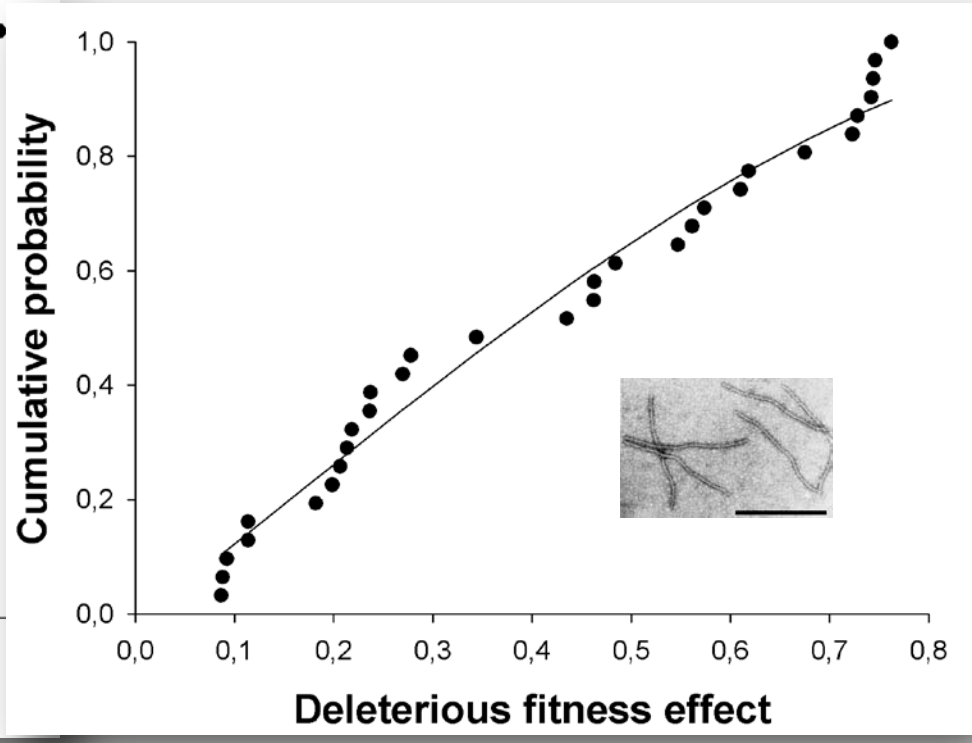
- For VSV, we created a collection of 91 single-nucleotide substitution mutants.
  48 randomly chosen (both site and nt introduced).
  43 already described in wild isolates and laboratory populations or clones.

  For TEV, 90 random mutants were created.

- The fitness of each mutant genotype, relative to its non-mutated ancestor, was measured by head-to-head competition experiments.

				
	Proportion	E(*s*)	Proportion	E(*s*)
Lethal	39.6%	–1	40.9%	–1
Deleterious	29.2%	–0.244	36.4%	–0.490
Neutral	27.1%	0	22.7%	0
Beneficial	4.2%	0.042	0.0%	-
Total	100% (48)	–0.476	100% (66)	–0.491





$E(s_d) = -15.4\%$

$E(s_d) = -41.1\%$

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Sanjuán *et al.* 2004. *Proc. Natl. Acad. Sci. USA* **101**, 8396
Carrasco *et al.* 2007. *J. Virol.* **81**, 12979

The effect of epistasis on fitness and evolvability

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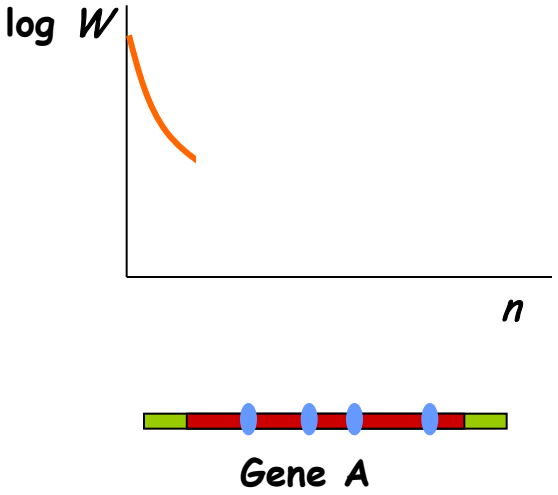
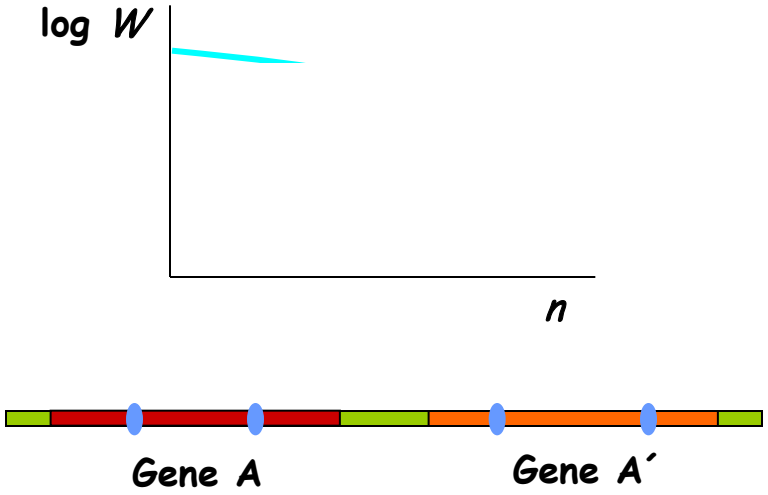
Genome complexity and epistasis





Anti-redundancy

Strong deleterious fitness effects

Antagonistic epistasis

Expected for RNA viruses

Redundancy

Mild deleterious fitness effects

Synergistic epistasis

Expected for complex organisms

Krakauer & Plotkin. 2002. *Proc. Natl. Acad. Sci. USA* **99**, 1405
Sanjuán & Elena. 2006. *Proc. Natl. Acad. Sci. USA* **103**, 14402

- Previous information only drawn from MA experiments: Elena (1999) found no average epistasis; Burch & Chao (2004) and Bonhoeffer *et al.* (2004) found a dominance of antagonistic epistasis. Again, MA experiments do not provide any information on the number and nature of mutations accumulated.

- We created a collection of 47 mutants carrying two single-nucleotide substitutions of deleterious effect and 15 genomes carrying two beneficial mutations.

- Fitness was determined for each double mutant (W_{ij}) as well as for their corresponding single mutants (W_i and W_j) in paired experiments.

- The strength and sign of epistasis was estimated as $\varepsilon_{ij} = W_{ij} - W_i W_j$.

  For deleterious mutations ($W_i < 1$)

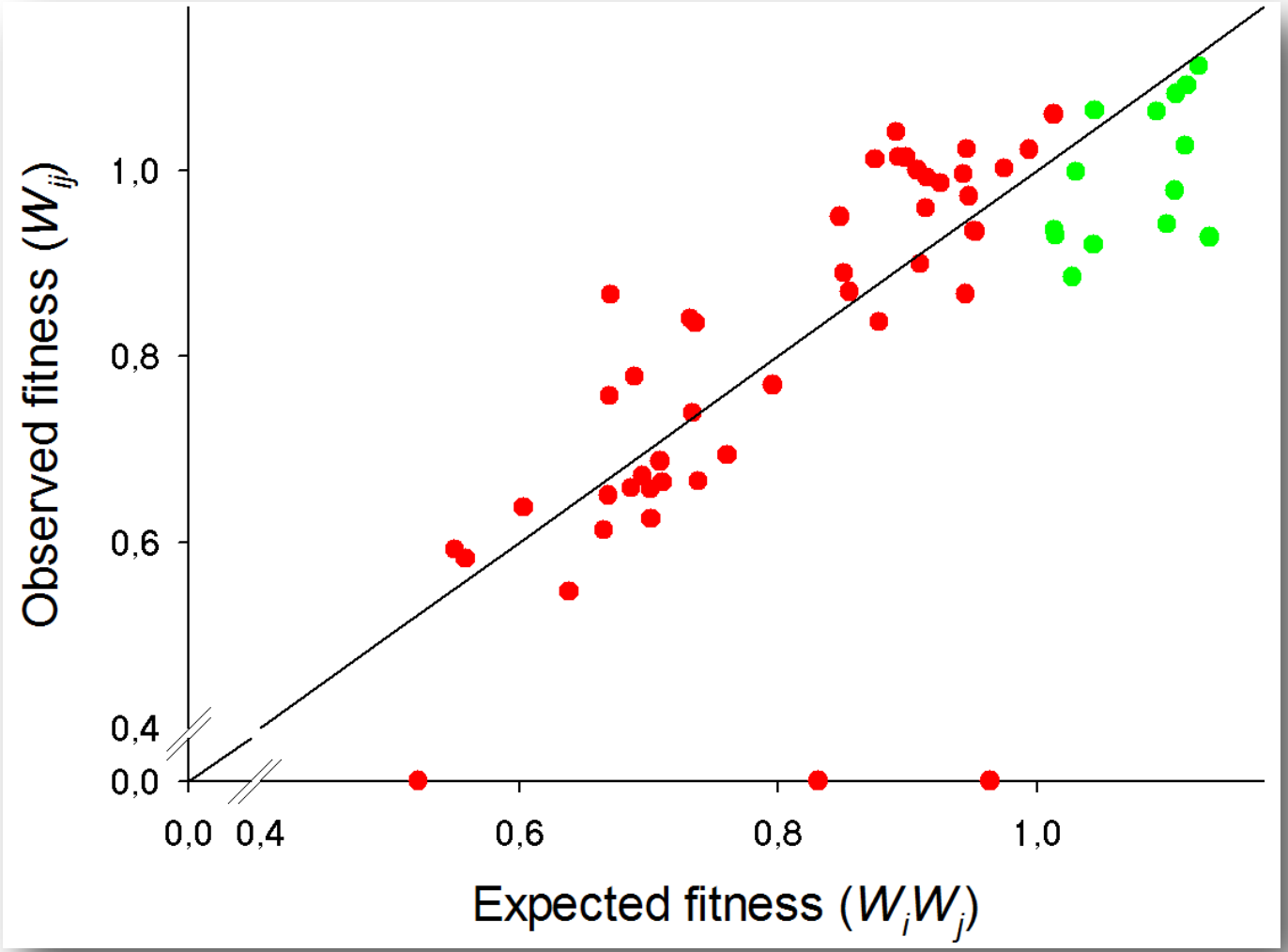
  synergistic epistasis if $\varepsilon < 0$ and

  antagonistic epistasis if $\varepsilon > 0$.

  For beneficial mutations ($W_i > 1$)

  synergistic epistasis if $\varepsilon > 0$ and

  antagonistic epistasis if $\varepsilon < 0$.



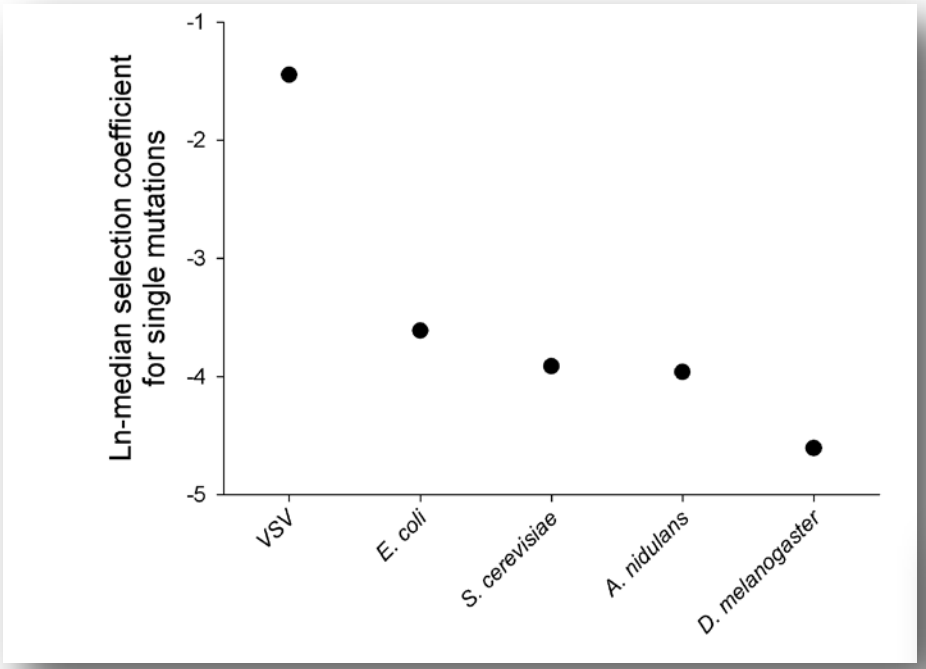
Among beneficial pairs:

- 8 significant cases of antagonistic epistasis,
- including 5 cases of extreme sickness by epistasis
- $\langle \varepsilon \rangle = -0.047 \pm 0.007$ (*t*-test, $P = 0.0021$)

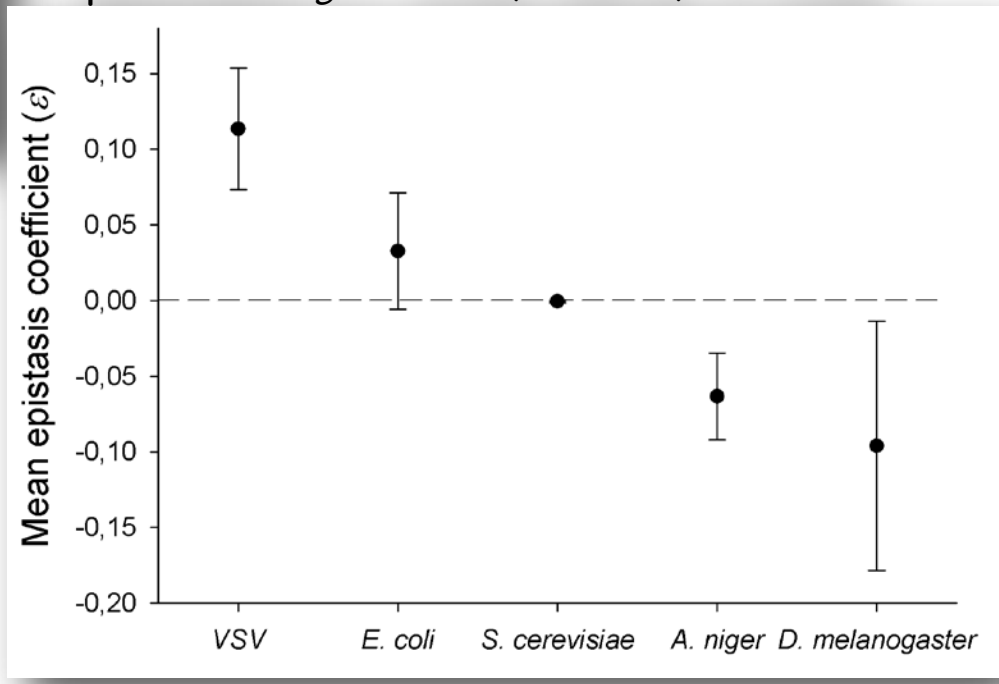
Sanjuán *et al.* 2004. *Proc. Natl. Acad. Sci. USA* **101**, 15376

Comparison with more complex organisms

Spearman's r_S = –1,000, 4 df, P < 0.001



Spearman's r_S = –0.315, 249 df, P < 0.001



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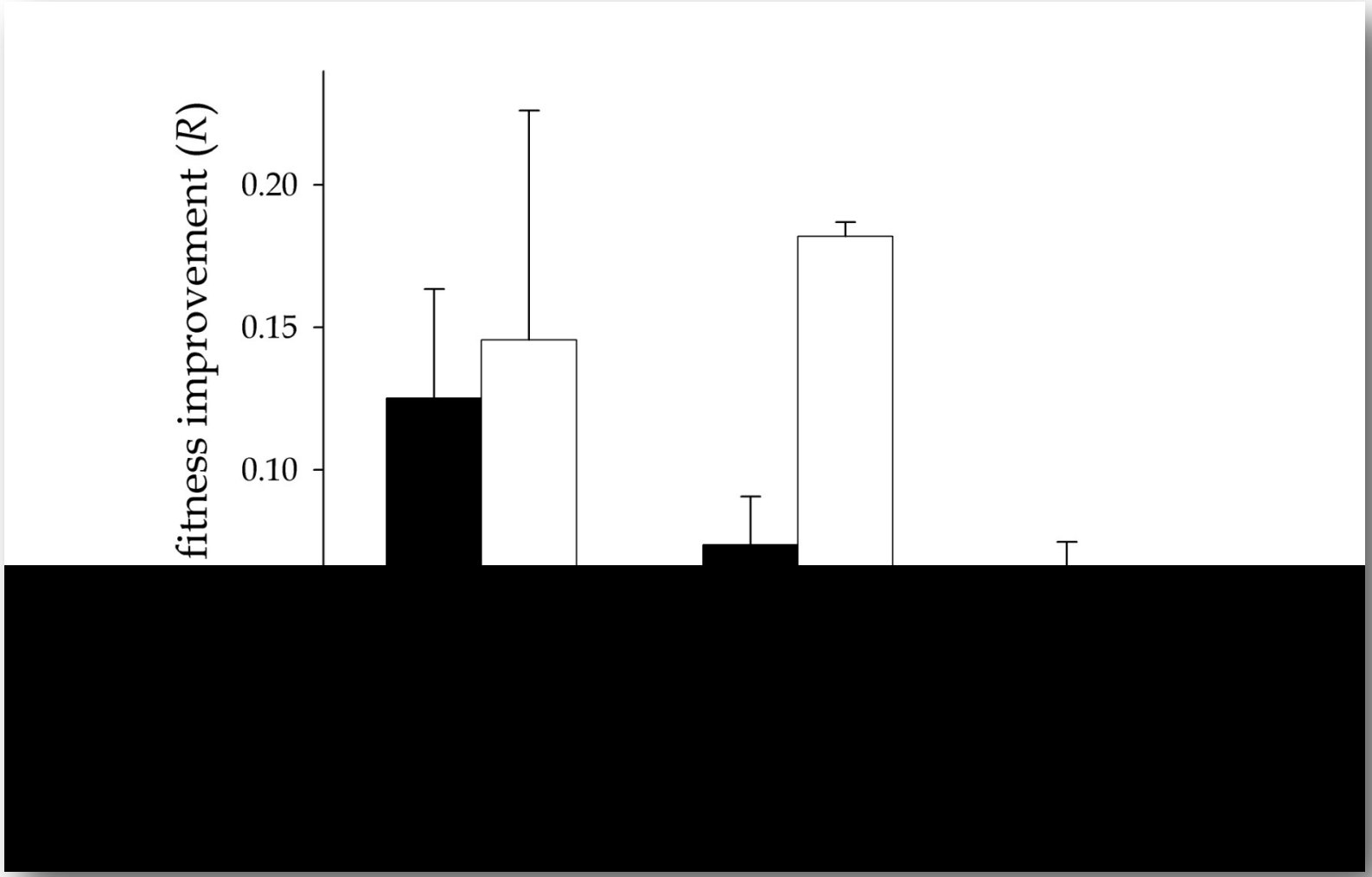
Sanjuán & Elena. 2006. *Proc. Natl. Acad. Sci. USA* **103**, 14402

The effect of epistasis in the rate of adaptation



Friedman's test: $\chi^2 = 5.828$, 1 d.f., $P = 0.016$

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Sanjuán *et al.* 2005. *Genetics* **170**, 1001

Implications for the evolution of RNA viruses (and beyond)

- With an excess of antagonistic epistasis among beneficial mutations, Fisher-Muller's type of arguments for the advantage of sex and recombination do not work. In the extreme case of decompensatory epistasis, both beneficial alleles can not reach fixation at the same time since both together are less fit than each one separately. Clonal interference would be more intense.

- Still, sex might be beneficial purging deleterious mutations, but an excess of synergistic epistasis is required, which is not the case. Indeed, the existence of variability among loci in the sign and strength of epistasis decreases the parameter space over which sex may evolve.

- On average, epistasis among pairs of mutations were ***antagonistic***, as expected for a anti-redundant genome.

Well studied topics in virus evolution

- ✓ Dynamics of adaptation (including escape mutants).
- ✓ Host-range
- ✓ Social life – Prisoner's dilemma – cooperation – synergism
- ✓ Genome contamination in the absence of selection (Müller's ratchet and mutational meltdown).
- ✓ Distribution of deleterious fitness effects.

Less-well studied topics in virus evolution

- ✓ Distribution of epistasis and its effect on the rate of adaptation.
- ✓ Distribution of beneficial effects.
- ✓ Mechanisms of mutational robustness.
- ✓ Lethal mutagenesis.
- ✓ The role of space.
- ✓ G×E and emerging viruses.
- ✓ MOI, N_e (within host and during horizontal/vertical transmission).

Very poorly studied topics in virus evolution

- ✓ Evolution of host's regulatory networks manipulation.
- ✓ Evolution of genome architectures (segmentation, gene order, …).
- ✓ MOI and the effect of *trans* complementation.

- ✓ What parameters are more relevant for modeling purposes? Cross-talk experimentalist-theoreticians to create biologically inspired and relevant models.
- ✓ Definition of viral fitness (R_0, malthusian, W).
- ✓ Empirical fitness landscapes.
- ✓ Ultra-deep sequencing.

Acknowledgments

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