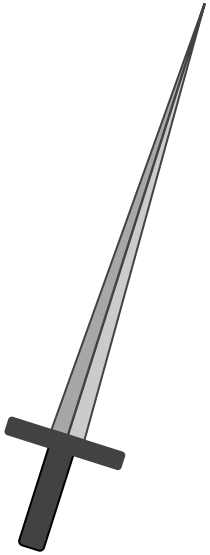
Mutational robustness in RNA virus quasispecies

Santiago F. Elena

Evolutionary Systems Virology Group

IBMCP
Instituto de Biología Molecular y Celular de Plantas
CSIC
SANTA FE INSTITUTE

MUTATION



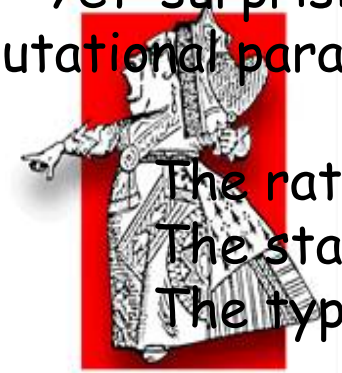
Double-edged sword

→ Preserving information

→ Adaptation

En un lugar de la Mancha, de cuyo nombre...

✓ Yet surprisingly little is known (specially for RNA viruses) on basic mutational parameters such as:



The ratios U_b/U_d and s_b/s_d

The statistical distribution of single nucleotide mutational effects

The type and strength of epistasis among mutant loci

Genomes have to modify themselves to keep being adapted to a changing world

IBMCP Instituto de Biología Molecular y Celular de Plantas

CSIC

SANTA FE INSTITUTE

GENETIC ROBUSTNESS

Ability to preserve fitness despite the presence of mutations in the genome

MEASURING ROBUSTNESS

$W_0 = 1$ W_1

Selection coefficient: $s_1 = W_1 - W_0 = W_1 - 1$

robust: $E(s) \rightarrow 0$
sensitive: $E(s) \rightarrow -1$

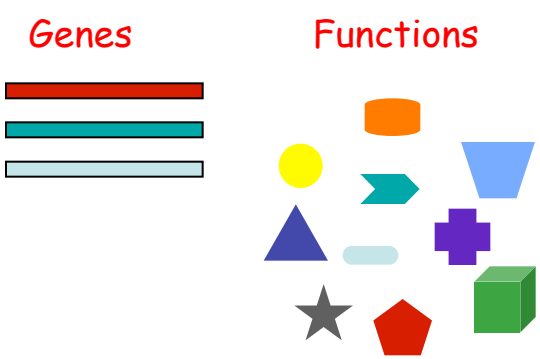
- ✓ Robustness is a selective traits if: heritable variability among individuals that affects fitness exist. The more mutations, the more efficient would be selection.

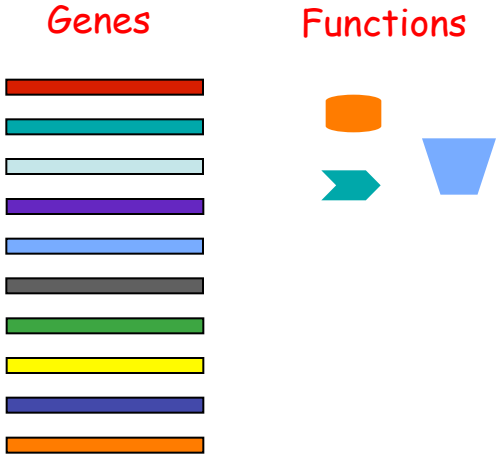
- ✓ Side effect for stabilizing selection on different traits.

- ✓ Given environmental fluctuations, selection would favor mechanisms of environmental robustness, being genetic robustness a side effect: plastogenetic congruence (L.W. Ancel & W. Fontana (2000) *J. Exp. Zool.* **288**:242-83).

- ✓ Problem: buffering the effect of beneficial mutations, including those providing robustness!

How to achieve genetic robustness?
Two opposed strategies





Anti-redundancy

Pleiotropy

Compact genomes – fast replication

High mutation rate ($U > 1$)

Large population sizes

PROKARYOTS

Redundancy

Subfunctionalization

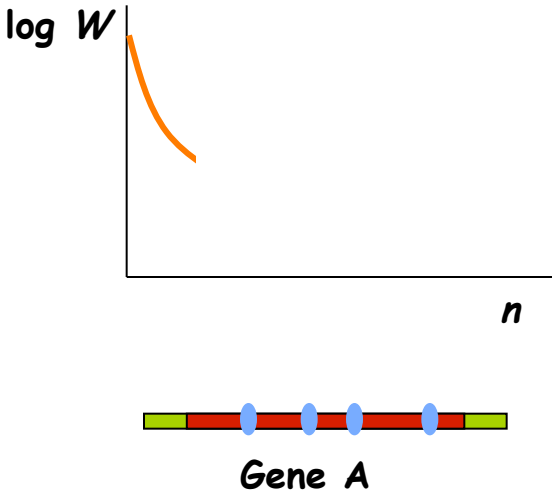
Large genomes – slow replication

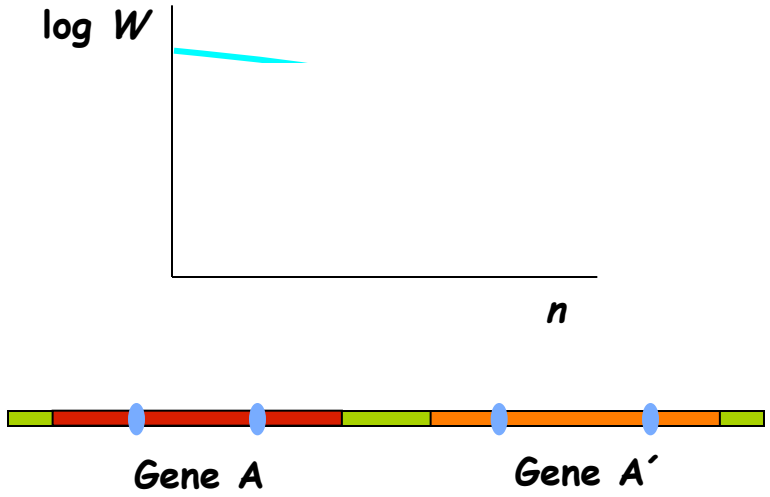
Low mutation rate ($U << 1$)

Small population sizes

COMPLEX EUKARYOTS

Fitness consequences of each strategy





Anti-redundancy

Strong deleterious fitness effects

Antagonistic epistasis

Expected for RNA viruses

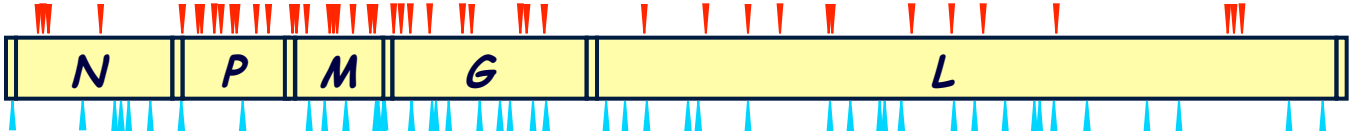
Redundancy

Mild deleterious fitness effects

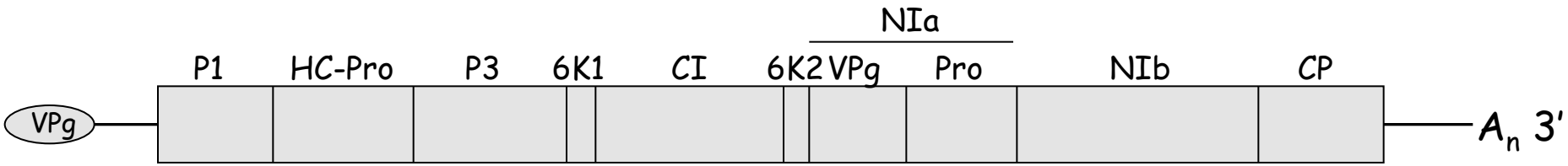
Synergistic epistasis

Expected for complex organisms

Mutational effects for RNA virus



		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



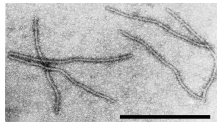
4 6+1 8 13 1 6+1 3 8+1 3

1 3 2 2 3

Nonsynonymous changes + stop codons

Synonymous changes

All randomly chosen

				
	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

R. Sanjuán *et al.* (2004) *Proc. Natl. Acad. Sci. USA* **101**: 8396-401
P. Carrasco *et al.* (2007) *J. Virol.* **81**: 12979-84

Epistasis for RNA virus

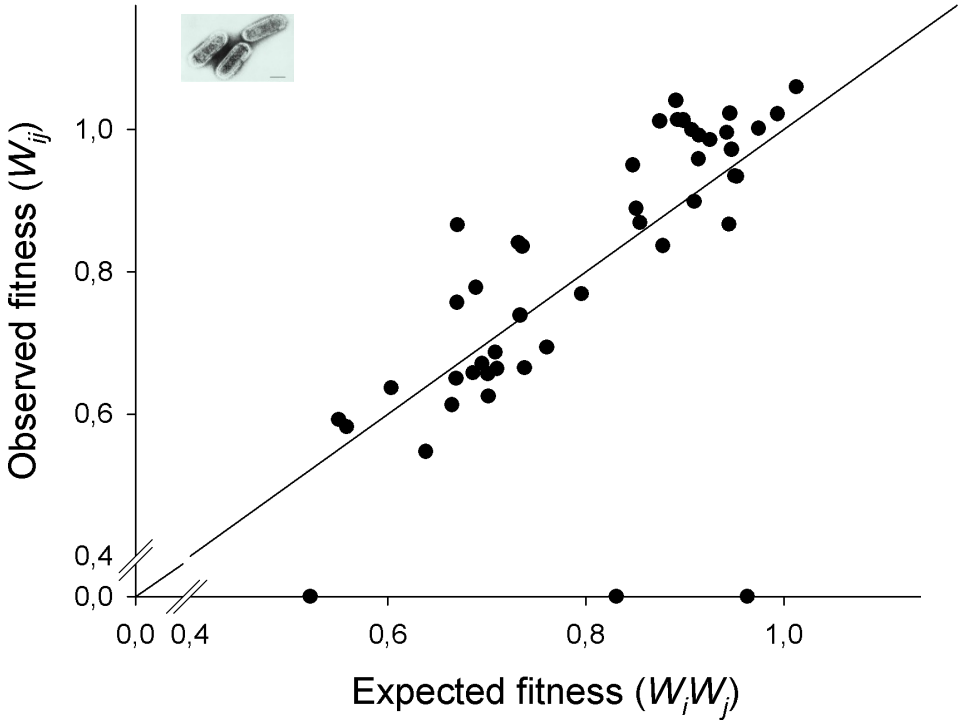
✓ We created collections of mutants carrying two single-nucleotide substitutions of deleterious effect.

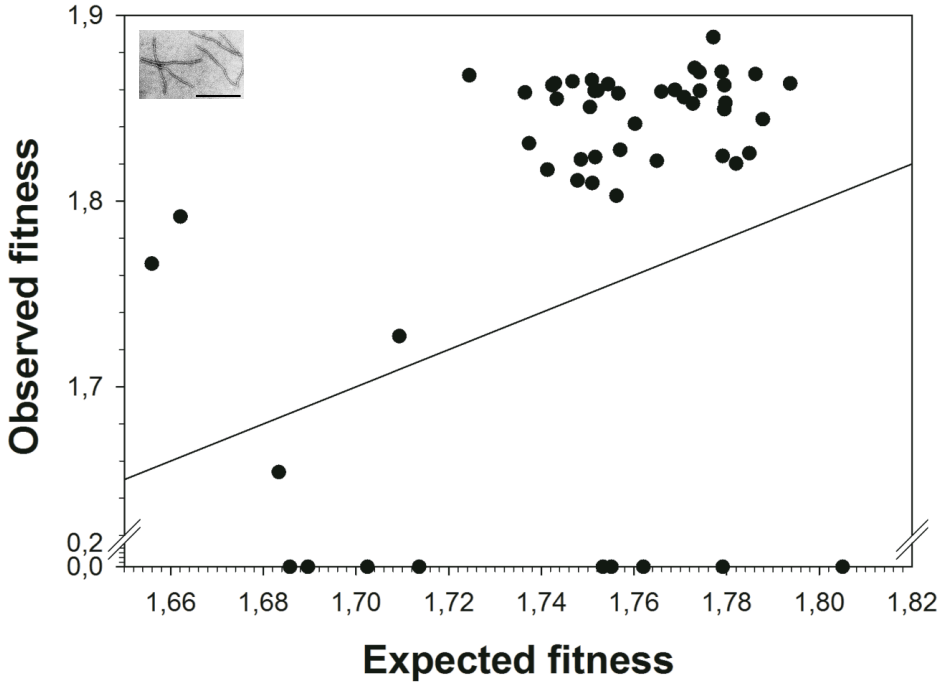
✓ Fitness was determined for each double mutant (W_{ij}) as well as for their corresponding single mutants (W_i and W_j) in paired experiments.

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

$\varepsilon_{ij} < 0 \rightarrow$ synergistic

$\varepsilon_{ij} > 0 \rightarrow$ antagonistic



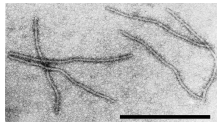


IBMCP Instituto de Biología Molecular y Celular de Plantas

CSIC

R. Sanjuán *et al.* (2004) *Proc. Natl. Acad. Sci. USA* **101**: 15376-9
J. Lalić & S.F. Elena (2012) *Heredity* **109**: 71-7

SANTA FE INSTITUTE

				
	Cases	E(ε)	Cases	E(ε)
multiplicative	31		32	
synergistic	3		1	
synthetic lethals	3		9	
antagonistic	10		11	
Average		0.034±0.010		0.084±0.005

R. Sanjuán *et al.* (2004) *Proc. Natl. Acad. Sci. USA* **101**: 15376-9
J. Lalić & S.F. Elena (2012) *Heredity* **109**: 71-7

Potential mechanisms for viral robustness

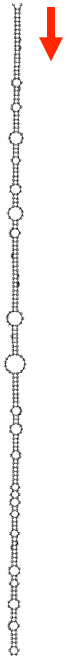
- ✓ Population mechanisms of ***intrinsic*** robustness:
  - ➢ Individual hypersensitivity → High average population fitness.
  - ➢ Quasispecies effect → Drift into neutral networks
  - ➢ Randomly fluctuating ploidy → Complementation
  - ➢ Sex → recombination and segregation
  - ➢ The stamping machine replicator → minimize the accumulation of deleterious mutations and maintains higher population fitness.
- ✓ Mechanisms of ***extrinsic*** robustness:
  - ➢ Cellular chaperones → unspecific masking of mutational effects

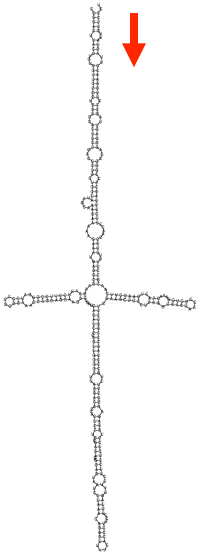
Evidences for genetic robustness in RNA viruses

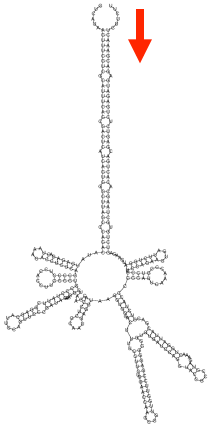
- ✓ From computational studies.

  - A. Wagner & P.F. Stadler (1999) *J. Exp. Zool.* **285**: 119-27: highly conserved RNA secondary structure elements are more robust to nucleotide changes than observed for non-conserved regions (DENV, HCV, HIV-1).

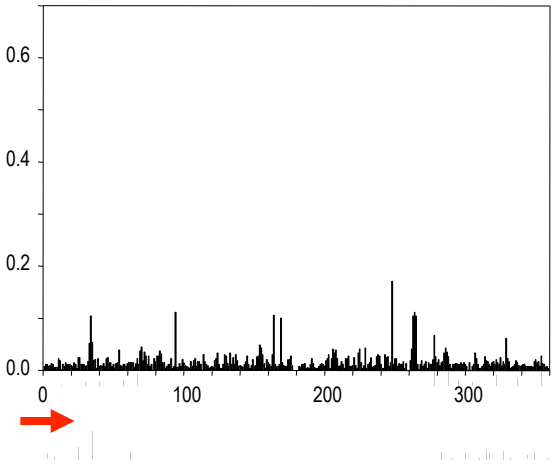
  - R. Sanjuán *et al.* (2006) *Mol. Biol. Evol.* **23**: 1427-36: viroids have evolved different structures. Rod-like are more robust than branched ones.



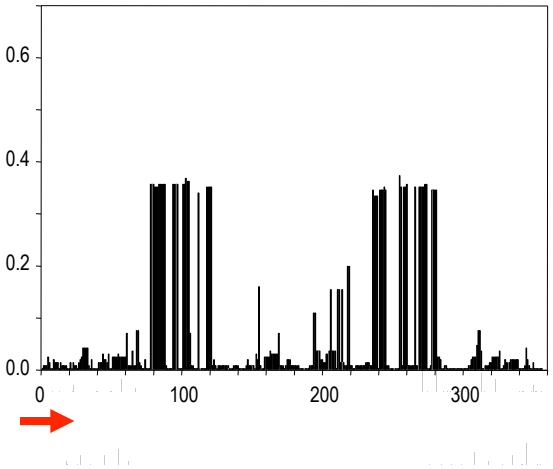




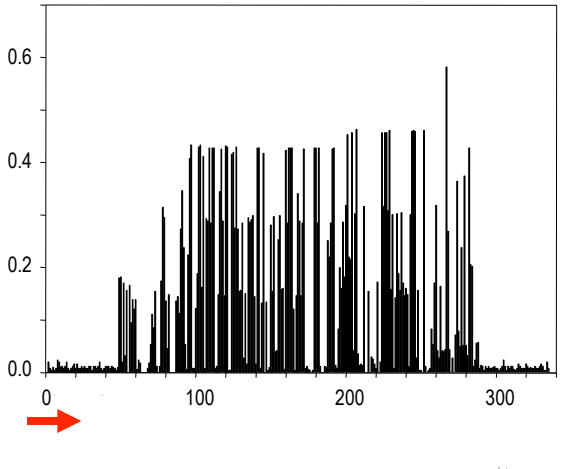
PSTVd (*Pospiviroid*)



CSVd (*Pospiviroid*)



PLMVd (*Pelamoviroid*)



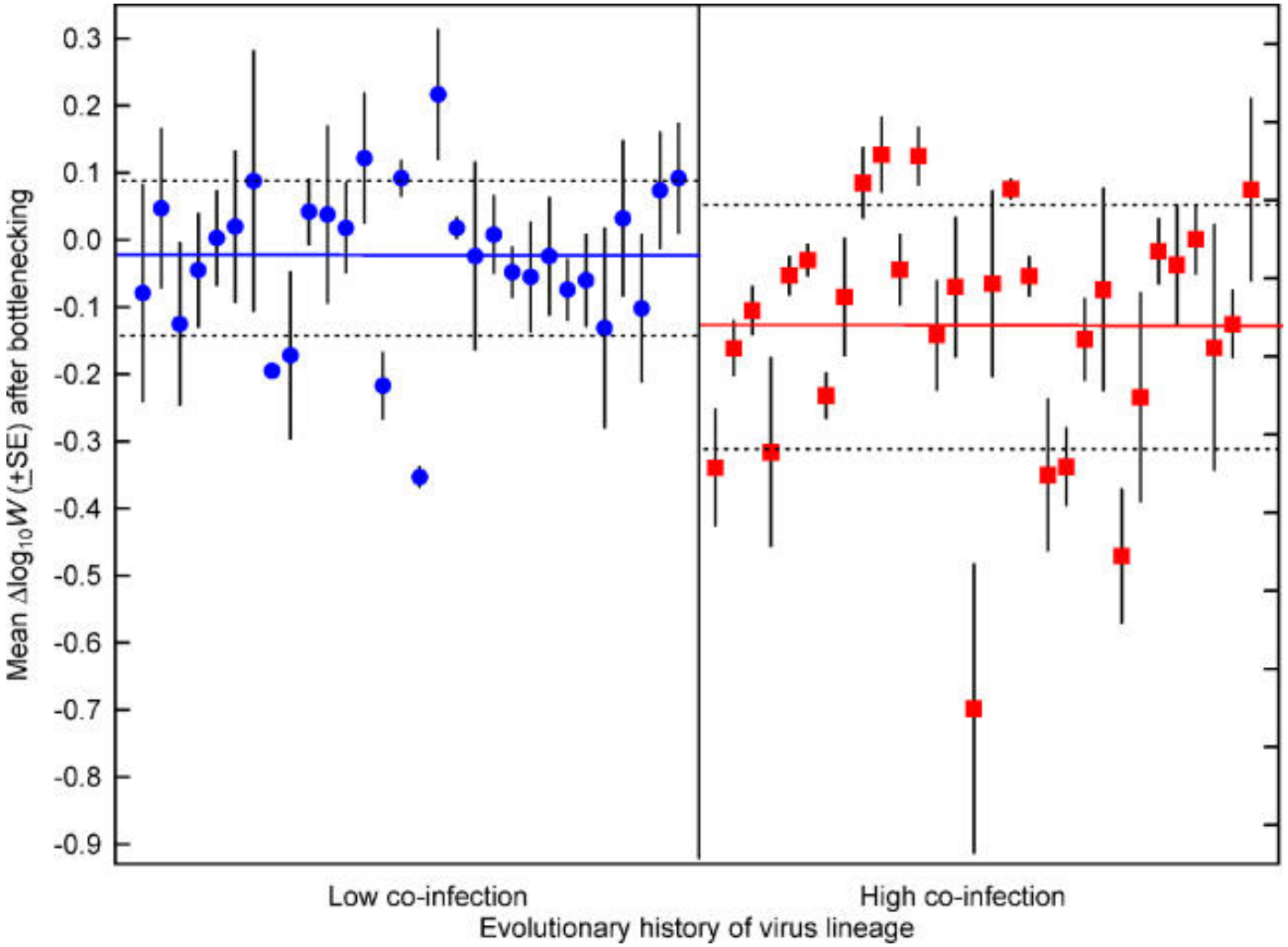
IBMCP Instituto de Biología Molecular y Celular de Plantas

CSIC

R. Sanjuán *et al.* (2006) *Mol. Biol. Evol.* **23**: 1427-36

SANTA FE INSTITUTE

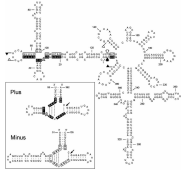
- ✓ From computational studies.
- ✓ From empirical studies.
  - R. Montville *et al.* (2005) *PLoS Biol.* **3**: e381: ϕ6 populations evolved at high MOI experience intense complementation and thus selection for other mechanisms of robustness would be weak Populations evolved at low MOI will evolve alternative mechanisms.



R. Montville *et al.* (2005) *PLoS Biol.* **3**: e281

- ✓ From computational studies.
- ✓ From empirical studies.
  - R. Montville *et al.* (2005) *PLoS Biol.* **3**: e381: ϕ6 populations evolved at high MOI experience intense complementation and thus selection for other mechanisms of robustness would be weak Populations evolved at low MOI will evolve alternative mechanisms.
  - F.M. Codoñer *et al.* (2006) *PLoS Pathog.* **2**: e136: A low replicating but with a wider mutant spectrum viroid is able of outcompeting a fast replicating but with a narrow mutant spectrum when mutation rate was increased: the survival of the flattest.

CSVd



CChMVd

?

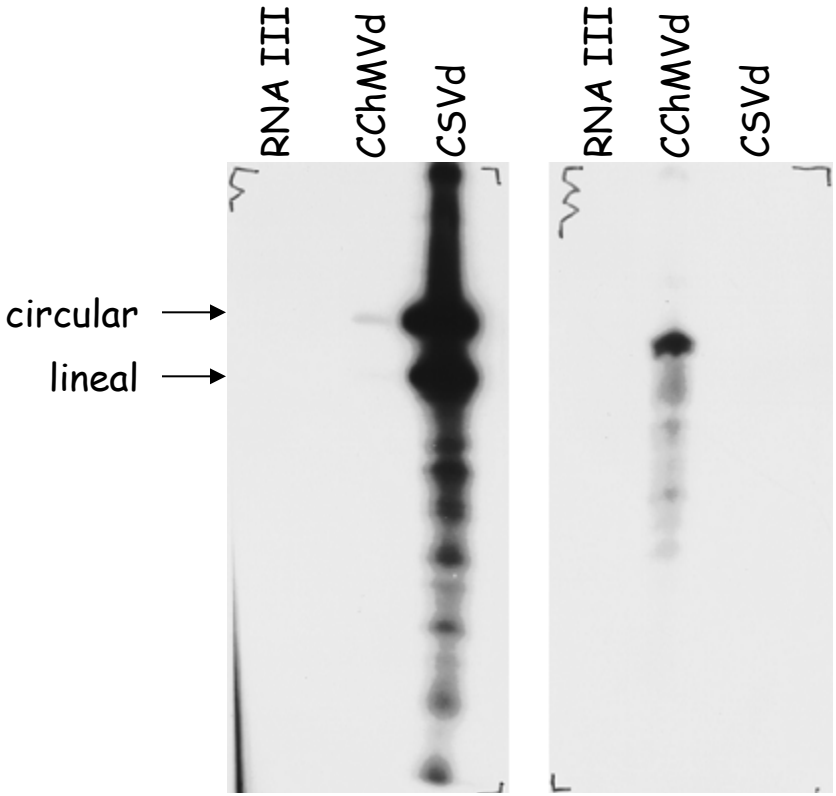
+mutagen

IBMCP
Instituto de Biología Molecular y Celular de Plantas

CSIC

SANTA FE INSTITUTE

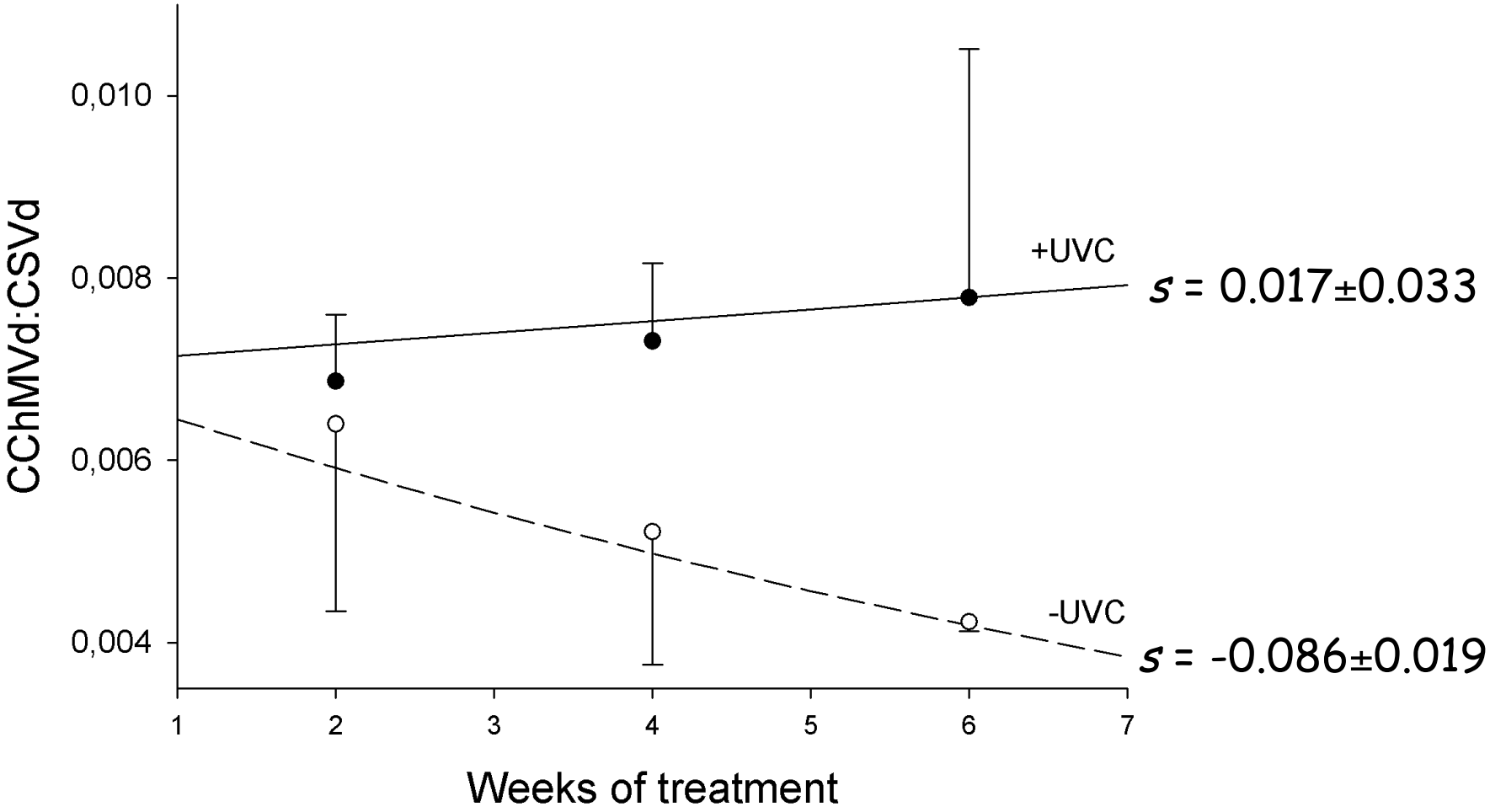
	CSVd (*n* = 11)	CChMVd (*n* = 8)	Ratio
Haplotype diversity (*NHap*)	0.800±0.034	1.000±0.022	1.250
Average number of nucleotide differences (*K*)	1.055±0.014	6.214±0. 038	5.890



Ratio = 0.052

F.M. Codoñer *et al.* (2006) *PLoS Pathog.* **2**: e136

The effect of UVC radiation on the outcome of the competition



$t_4 = 7.667$, $P = 0.002$

- ✓ From computational studies.
- ✓ From empirical studies.
  - R. Montville *et al.* (2005) *PLoS Biol.* **3**: e381: ϕ6 populations evolved at high MOI experience intense complementation and thus selection for other mechanisms of robustness would be weak Populations evolved at low MOI will evolve alternative mechanisms.
  - F.M. Codoñer *et al.* (2006) *PLoS Pathog.* **2**: e136: A low replicating but with a wider mutant spectrum viroid is able of outcompeting a fast replicating but with a narrow mutant spectrum when mutation rate was increased: the survival of the flattest.
  - R. Sanjuán *et al.* (2007) *PLoS Genet.* **3**: e93: A low fitness but diverse VSV population outcompeted a high fitness but less diverse population at increasing concentrations of 5-FU: the survival of the flattest.

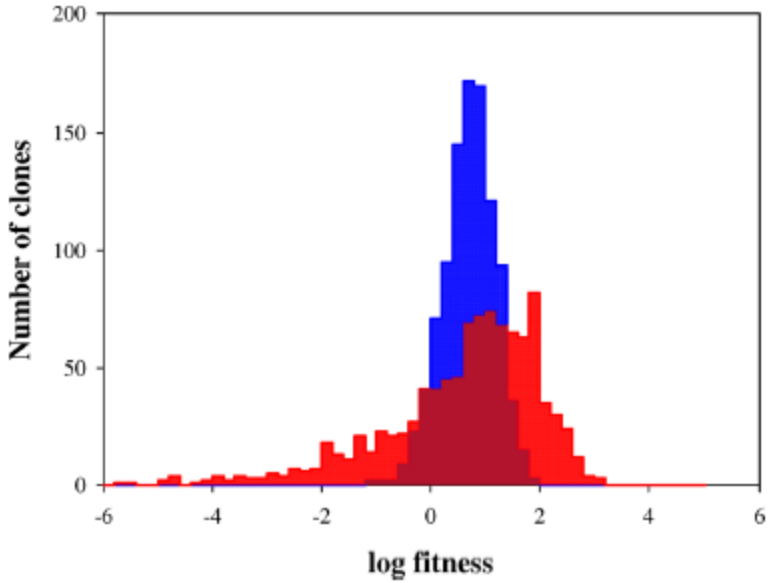
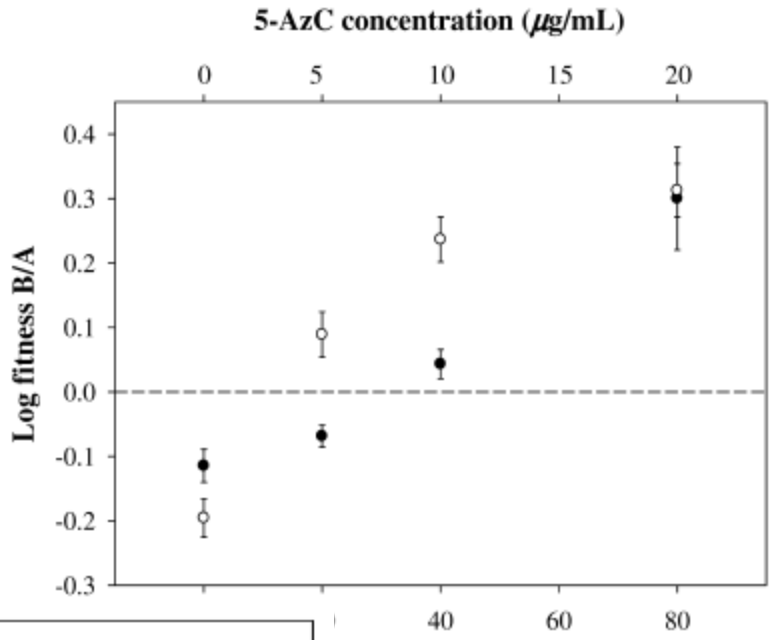


Figure 2. Observed Distribution of 1,000 Fitne
and B Based on Plaque Sizes

Population A is shown in red and population
was 0.386 for population A, with variance 2.0
log-fitness was 0.498, with variance 0.225.
different according to a Mann-Whitney test (
Smirnov test also showed that the two dist
different ($p < 0.001$).

doi:10.1371/journal.pgen.0030093.g002



nents at Varying 5-FU and 5-AzC Doses

les represent 5-FU and 5-AzC values,
f population B relative to population A,
rs indicate 95% confidence intervals.

093.g003

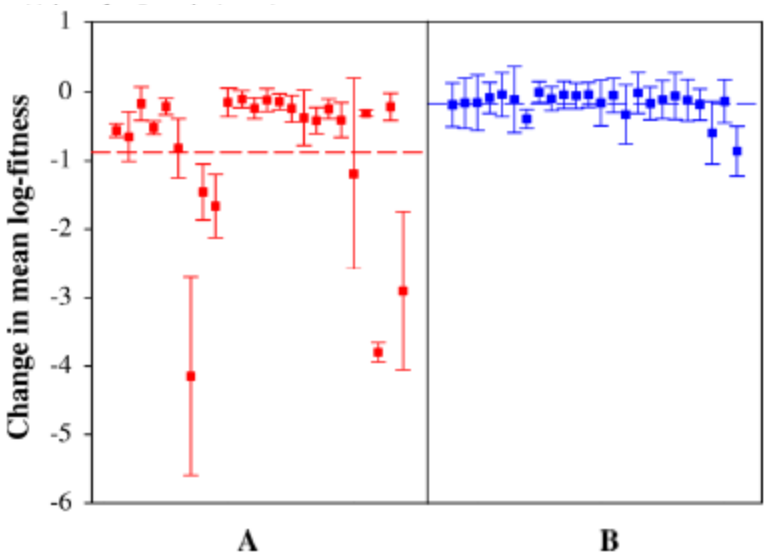


Figure 5. Change in Mean Log-Fitness in Mutation Accumulation Lines Derived from Populations A and B

For A and B, each of the 24 lines is shown. Bars indicate 95% confidence intervals. Horizontal lines indicate the grand mean change in log-fitness.

doi:10.1371/journal.pgen.0030093.g005

- ✓ From computational studies.
- ✓ From empirical studies.
  - R. Montville *et al.* (2005) *PLoS Biol.* **3**: e381: ϕ6 populations evolved at high MOI experience intense complementation and thus selection for other mechanisms of robustness would be weak Populations evolved at low MOI will evolve alternative mechanisms.
  - F.M. Codoñer *et al.* (2006) *PLoS Pathog.* **2**: e136: A low replicating but with a wider mutant spectrum viroid is able of outcompeting a fast replicating but with a narrow mutant spectrum when mutation rate was increased: the survival of the flattest.
  - R. Sanjuán *et al.* (2007) *PLoS Genet.* **3**: e93: A low fitness but diverse VSV population outcompeted a high fitness but less diverse population at increasing concentrations of 5-FU: the survival of the flattest.
  - P. Domingo-Calap *et al.* (2010) *J. Evol. Biol.* **23**: 2453-60: Demonstration of plastogenetic congruence. Selection of thermotolerant Qβ viruses also selects of genetic robustness.

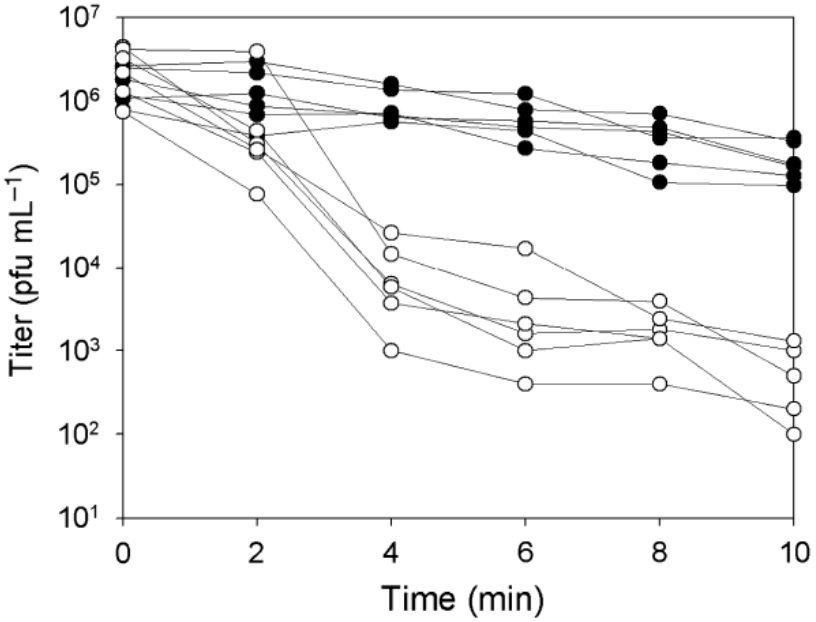


Fig. 1 Heat degradation curve of bacteriophage Qβ-free virions at 52 °C. Black circles correspond to viruses previously passaged six times in the presence of heat shocks (52 °C, 10 min) and white circles to control lines.

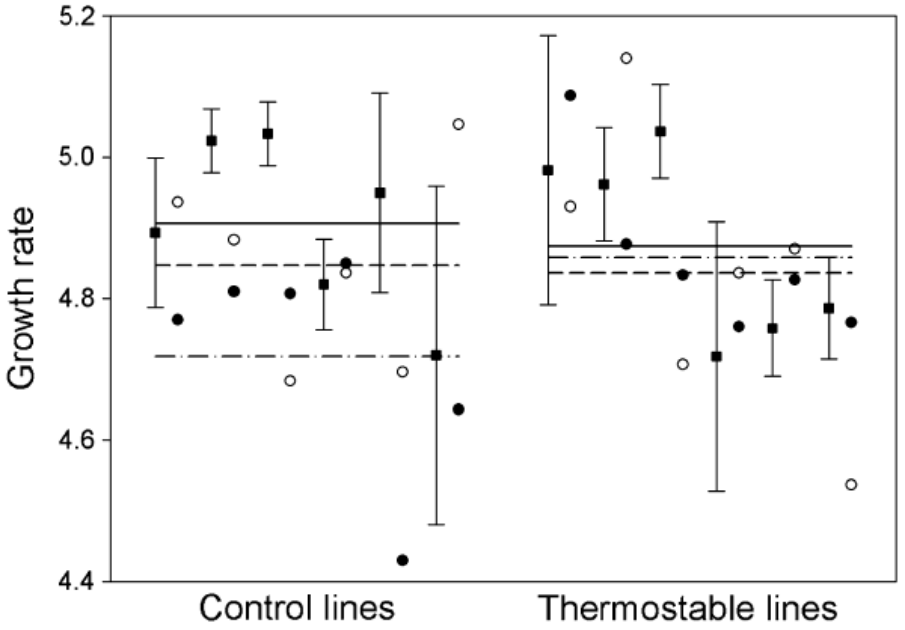


Fig. 4 Changes in growth rate following mutation accumulation in control and thermostable lines. Black squares represent each of the six control/thermostable lines before mutation accumulation. Solid lines indicate the average growth rate of these starting viruses. White circles and dashed lines indicate the individual and mean growth rates for the first replicate of mutagenesis, whereas black circles and dashed-dotted lines correspond to the second replicate.

- ✓ From computational studies.
- ✓ From empirical studies.
  - R. Montville *et al.* (2005) *PLoS Biol.* **3**: e381: ϕ6 populations evolved at high MOI experience intense complementation and thus selection for other mechanisms of robustness would be weak Populations evolved at low MOI will evolve alternative mechanisms.
  - F.M. Codoñer *et al.* (2006) *PLoS Pathog.* **2**: e136: A low replicating but with a wider mutant spectrum viroid is able of outcompeting a fast replicating but with a narrow mutant spectrum when mutation rate was increased: the survival of the flattest.
  - R. Sanjuán *et al.* (2007) *PLoS Genet.* **3**: e93: A low fitness but diverse VSV population outcompeted a high fitness but less diverse population at increasing concentrations of 5-FU: the survival of the flattest.
  - P. Domingo-Calap *et al.* (2010) *J. Evol. Biol.* **23**: 2453-60: Demonstration of plastogenetic congruence. Selection of thermotolerant Qβ viruses also selects of genetic robustness.
  - I. S. Novella *et al.* (2013) *J. Virol.* **87**: 4923-8: Demonstration of plastogenetic congruence. Selection of thermotolerant VSV viruses also selects for genetic robustness.

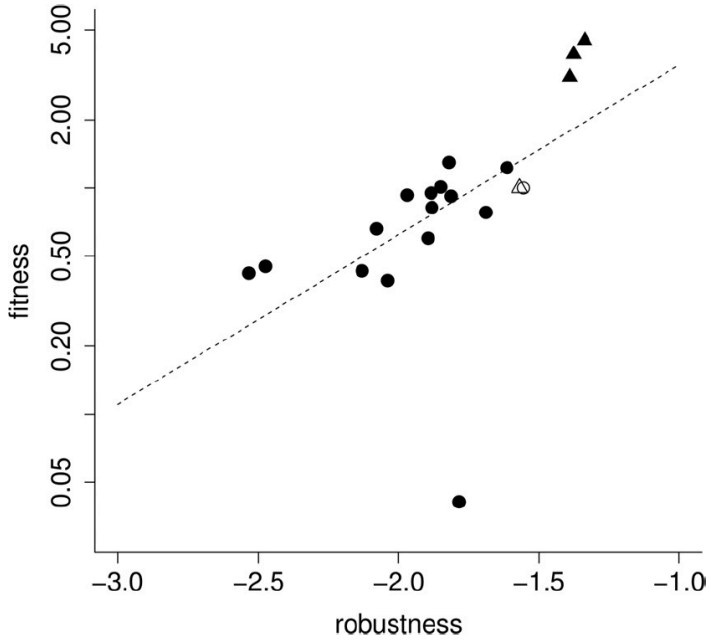


FIG 3 Fitness versus robustness for all strains used in this study. Symbols refer to strains: solid circle, MR strains; open circle, MARM U; solid triangles, adapted wt strains; open triangle, wt. There is a strong positive correlation between log-transformed fitness and robustness (dotted line; $r = 0.57$; $P = 0.009$; without the MRq outlier, $r = 0.85$ and $P = 4.197e^{-06}$).

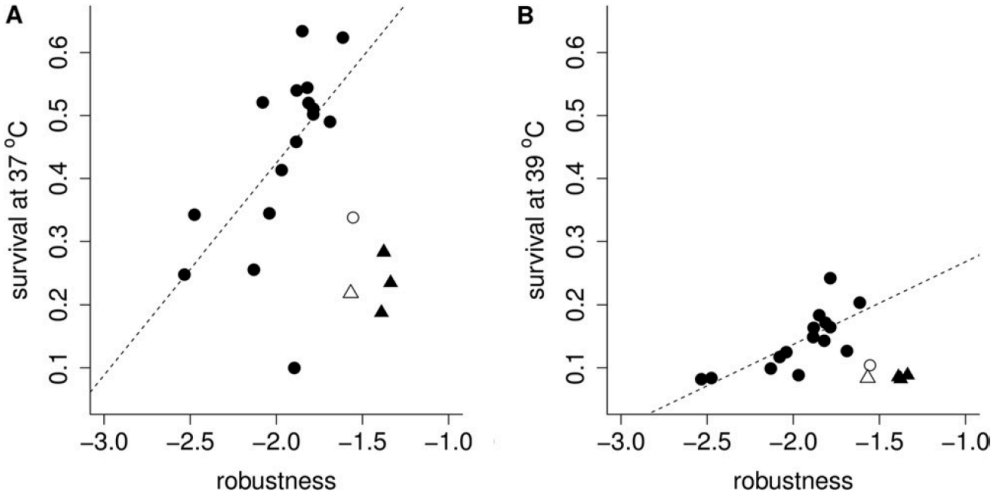
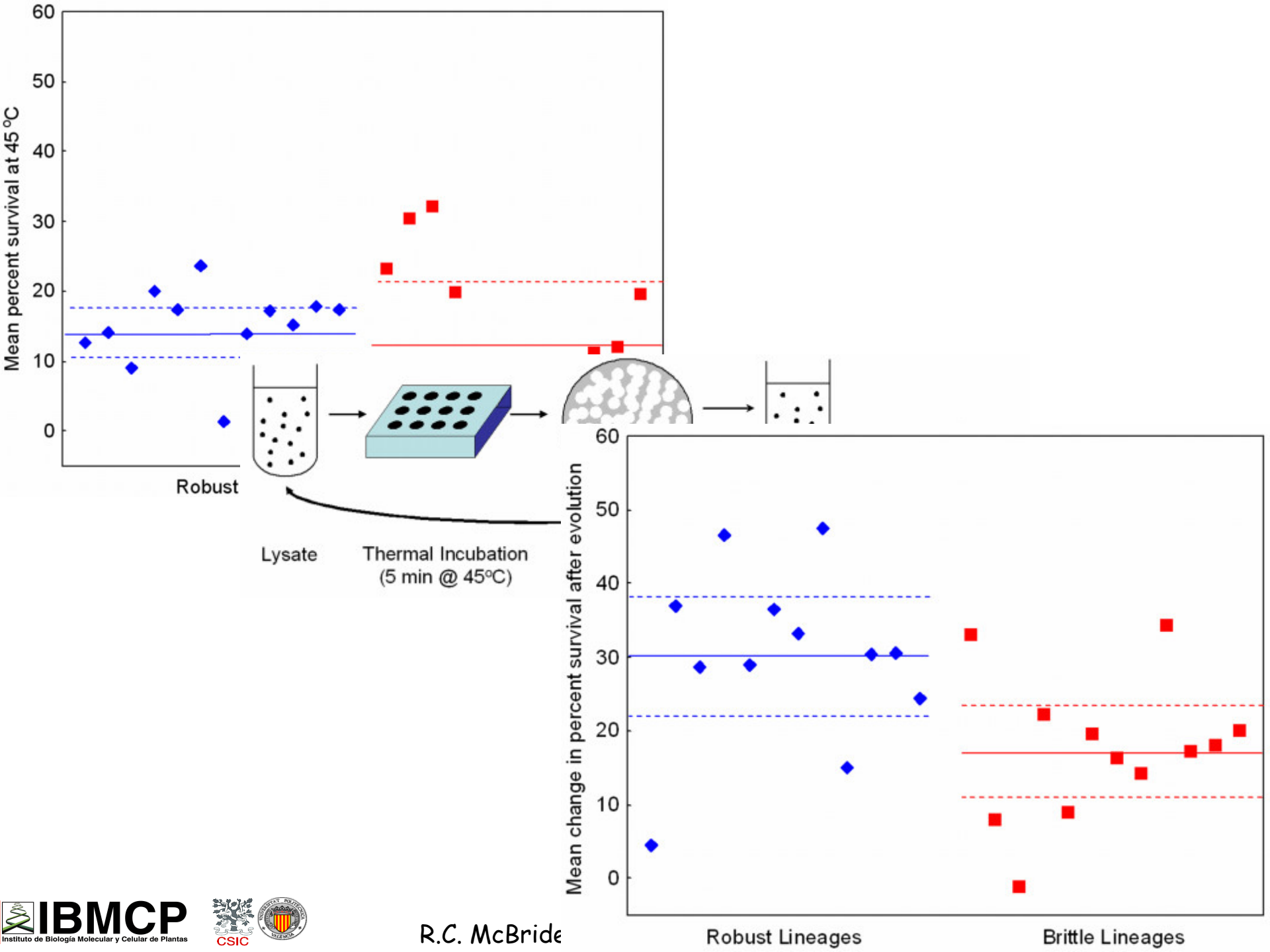


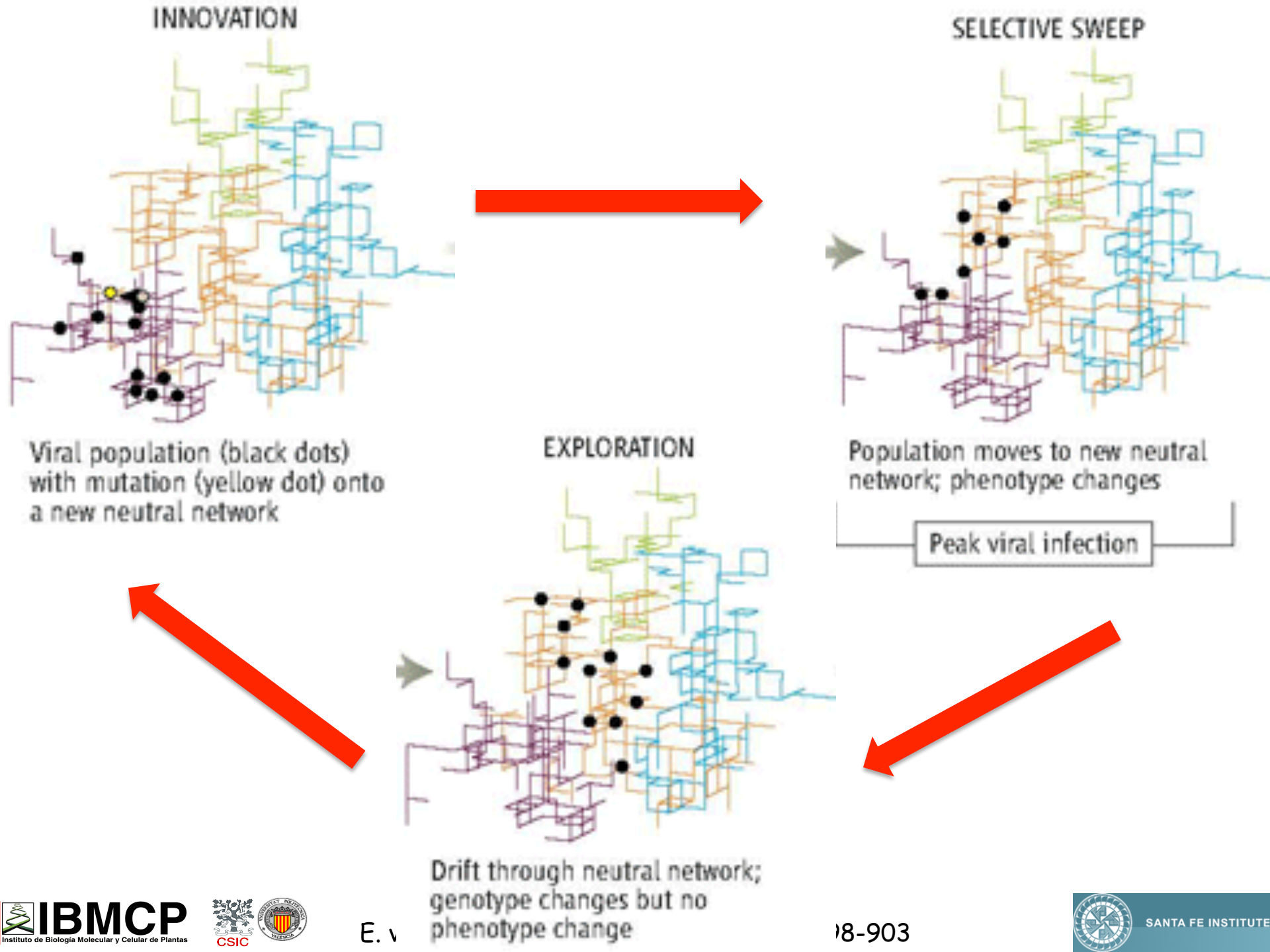
FIG 4 Thermostability versus robustness for all strains used in this study. Symbols refer to strains: solid circle, MR strains; open circle, MARM U; solid triangles, adapted wt strains; open triangle, wt. There is a positive correlation between thermostability and robustness for MR strains at 37°C (dotted line; $r = 0.58$; $P = 0.018$; without the MRi outlier, $r = 0.79$ and $P = 0.0004$) (A) and at 39°C (dotted line; $r = 0.74$; $P = 0.016$) (B).

Consequences of genetic robustness

- ✓ Does genetic robustness promote evolvability?
  - ➢ R.C. McBride *et al.* (2008) *BMC Evol. Biol.* **8**: 231: robust ϕ6 populations adapt faster than to high temperature.


Mean percent survival at 45 °C
60
50
40
30
20
10
0
Robust
Lysate
Thermal Incubation
(5 min @ 45°C)
Mean change in percent survival after evolution
60
50
40
30
20
10
0
Robust Lineages
Brittle Lineages

- ✓ Does genetic robustness promote evolvability?
  - R.C. McBride *et al.* (2008) *BMC Evol. Biol.* **8**: 231: robust ϕ6 populations adapt faster than to high temperature.
  - K. Koelle *et al.* (2006) *Science* 314: 1898-903: Epochal antigenic evolution of IAV/H3N2, alternating periods of stasis punctuated by sudden changes in antigenic phenotypic evolution can be easily explained in terms of neutral networks.


INNOVATION
SELECTIVE SWEEP
Viral population (black dots) with mutation (yellow dot) onto a new neutral network
EXPLORATION
Population moves to new neutral network; phenotype changes
Peak viral infection
Drift through neutral network; genotype changes but no phenotype change

- ✓ Does genetic robustness promote evolvability?
  - R.C. McBride *et al.* (2008) *BMC Evol. Biol.* **8**: 231: robust ϕ6 populations adapt faster than to high temperature.
  - K. Koelle *et al.* (2006) *Science* 314: 1898-903: Epochal antigenic evolution of IAV/H3N2, alternating periods of stasis punctuated by sudden changes in antigenic phenotypic evolution can be easily explained in terms of neutral networks.
  - P.E. Turner *et al.* (2010) *Evolution* **64**: 3273-86: Generalist (environmentally robust) VSV populations are more evolvable than specialists (environmentally brittle) when faced with a new host cell type. Conclusion: generalist show higher mean fitness and less variance across novel hosts.

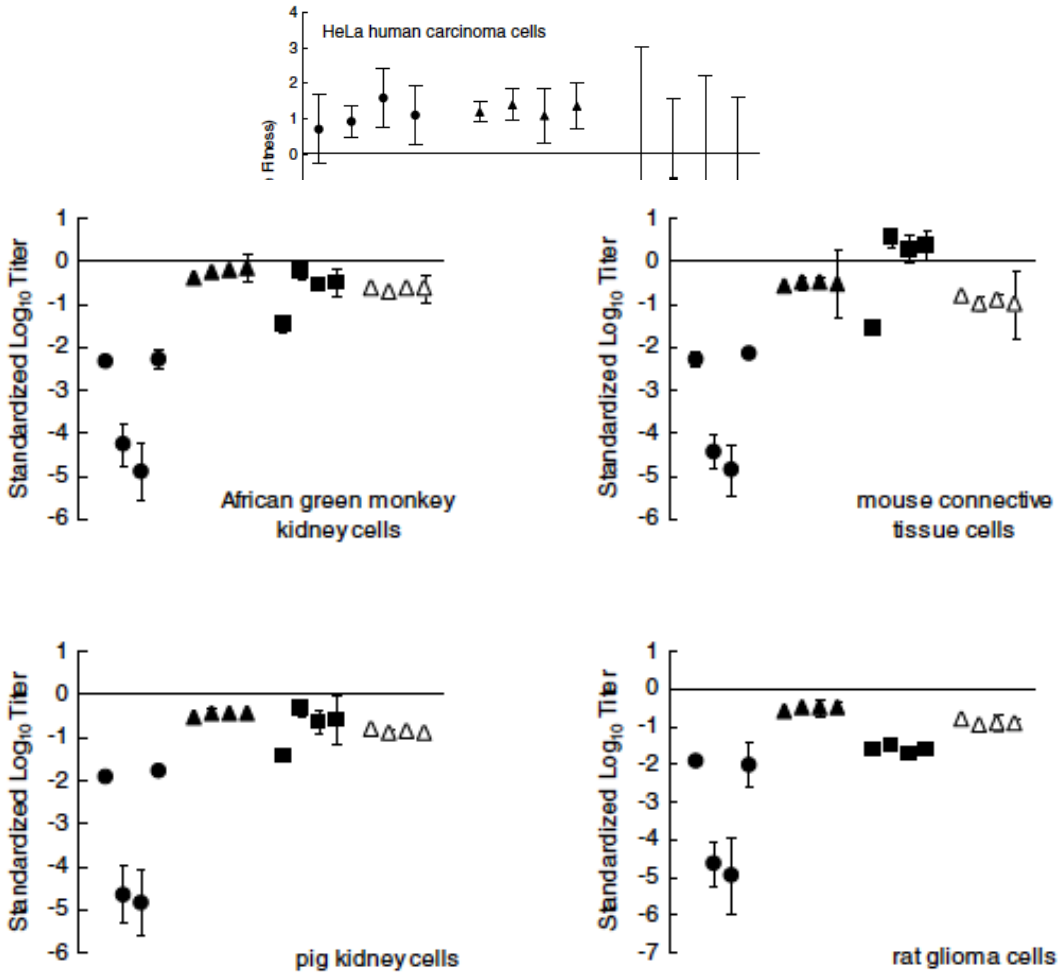


Figure 3. Growth of each evolved VSV population on cells derived from four different novel hosts. Each point is the mean of $\log_{10}$ virus titer (titer in pfu/mL) after 48 h estimated with threefold replication; error bars indicate 95% confidence limits. Growth estimates are standardized by each population's mean titer on its evolved host at 48 h post infection. Standardized virus growth equal to zero indicates the virus grows equally well on the novel host as on its evolved host. Filled circles: HeLa-evolved viruses; filled triangles: alternating-host evolved viruses amplified on HeLa; filled squares: MDCK-evolved viruses; open triangles: alternating-host evolved viruses amplified on MDCK.

experimental evolution on HeLa cells, MDCK cells, or alternating-host passages. Each point is mean log fitness (change in virus titer in pfu/mL after 48 h) measured relative to a common competitor with threefold replication on HeLa, MDCK and BHK (original host) cells. Error bars indicate 95% confidence limits. Filled circles: HeLa-evolved viruses; filled triangles: alternating-host evolved viruses; filled squares: MDCK-evolved viruses

P.E. Turner *et al.* (2010) *Evolution* **64**: 3273-86

IBMCP Instituto de Biología Molecular y Celular de Plantas

CSIC

SANTA FE INSTITUTE

- ✓ Does genetic robustness promote evolvability?
  - R.C. McBride *et al.* (2008) *BMC Evol. Biol.* **8**: 231: robust ϕ6 populations adapt faster than to high temperature.
  - K. Koelle *et al.* (2006) *Science* 314: 1898-903: Epochal antigenic evolution of IAV/H3N2, alternating periods of stasis punctuated by sudden changes in antigenic phenotypic evolution can be easily explained in terms of neutral networks.
  - P.E. Turner *et al.* (2010) *Evolution* **64**: 3273-86: Generalist (environmentally robust) VSV populations are more evolvable than specialists (environmentally brittle) when faced with a new host cell type. Conclusion: generalist show higher mean fitness and less variance across novel hosts.
  - J.M. Cuevas *et al.* (2009) *J. Evol. Biol.* **22**: 2041-8: Found the opposite: brittle VSV adapted faster to a new host cell type than robust.

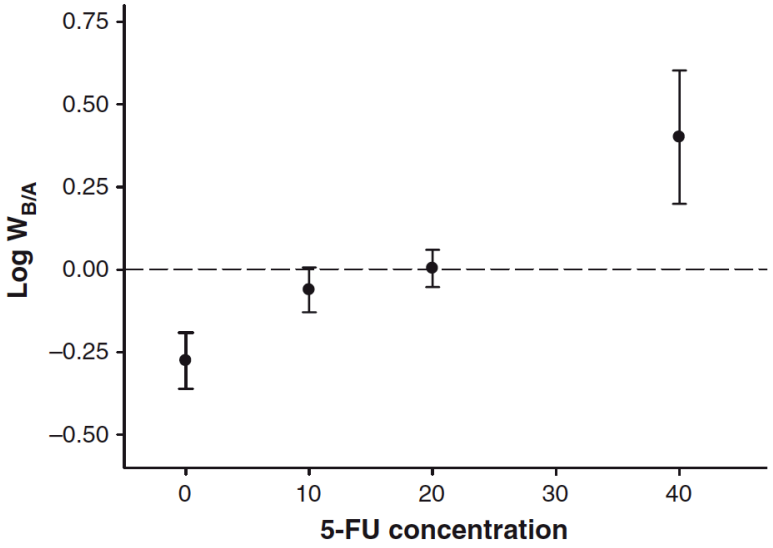


Fig. 1 Log relative fitness of genotype B (log $W_{B/A}$) in MDCK cells as a function of the 5-FU concentration. Error bars indicate the standard error of the mean (SEM).

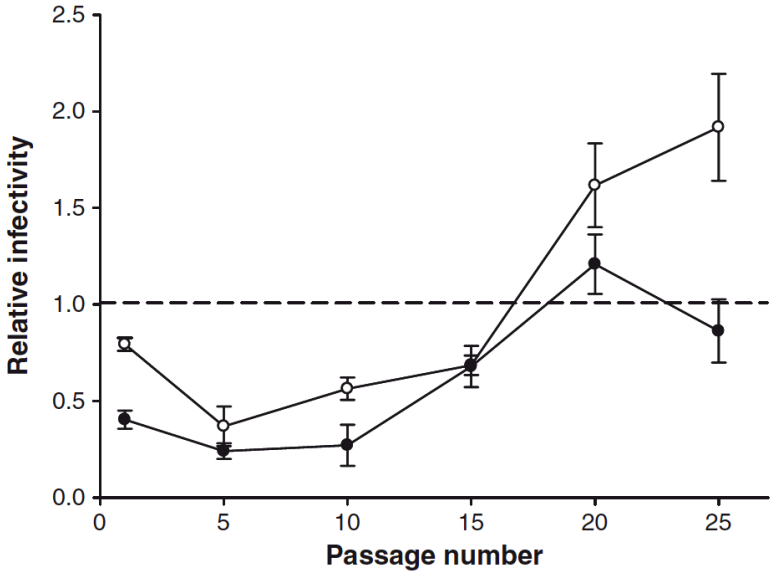


Fig. 2 Change in relative infectivity calculated as the titre in MDCK cells divided by the titre in BHK cells of genotypes A (white circles) B (black circles) during the 25 serial passages in MDCK cells. grand mean of the five lineages is shown for each A and B. Error correspond to the SEM.

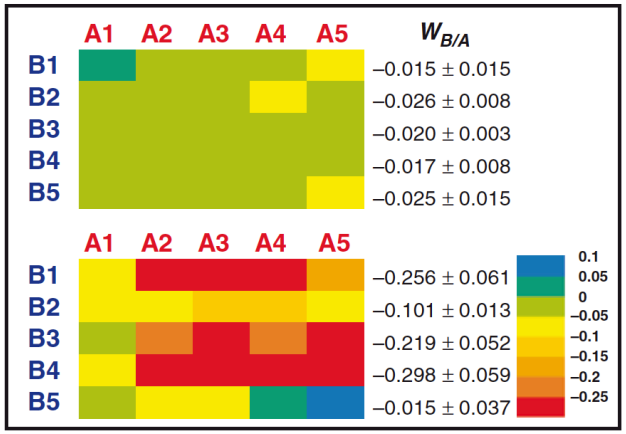


Fig. 3 Log relative fitness of genotype B (log $W_{B/A}$) in MDCK cells for ancestral (upper panel) clones and evolved populations (lower panel). Each grid corresponds to a head-to-head growth assay between a given A-B pair. As there were five independent lineages for each genotype, there were 25 possible such pairs. A colour code (described in the figure) is used to indicate the outcome of each competition. Numbers on the right indicate the average log $W_{B/A}$ ± SEM for each B-derived lineage.

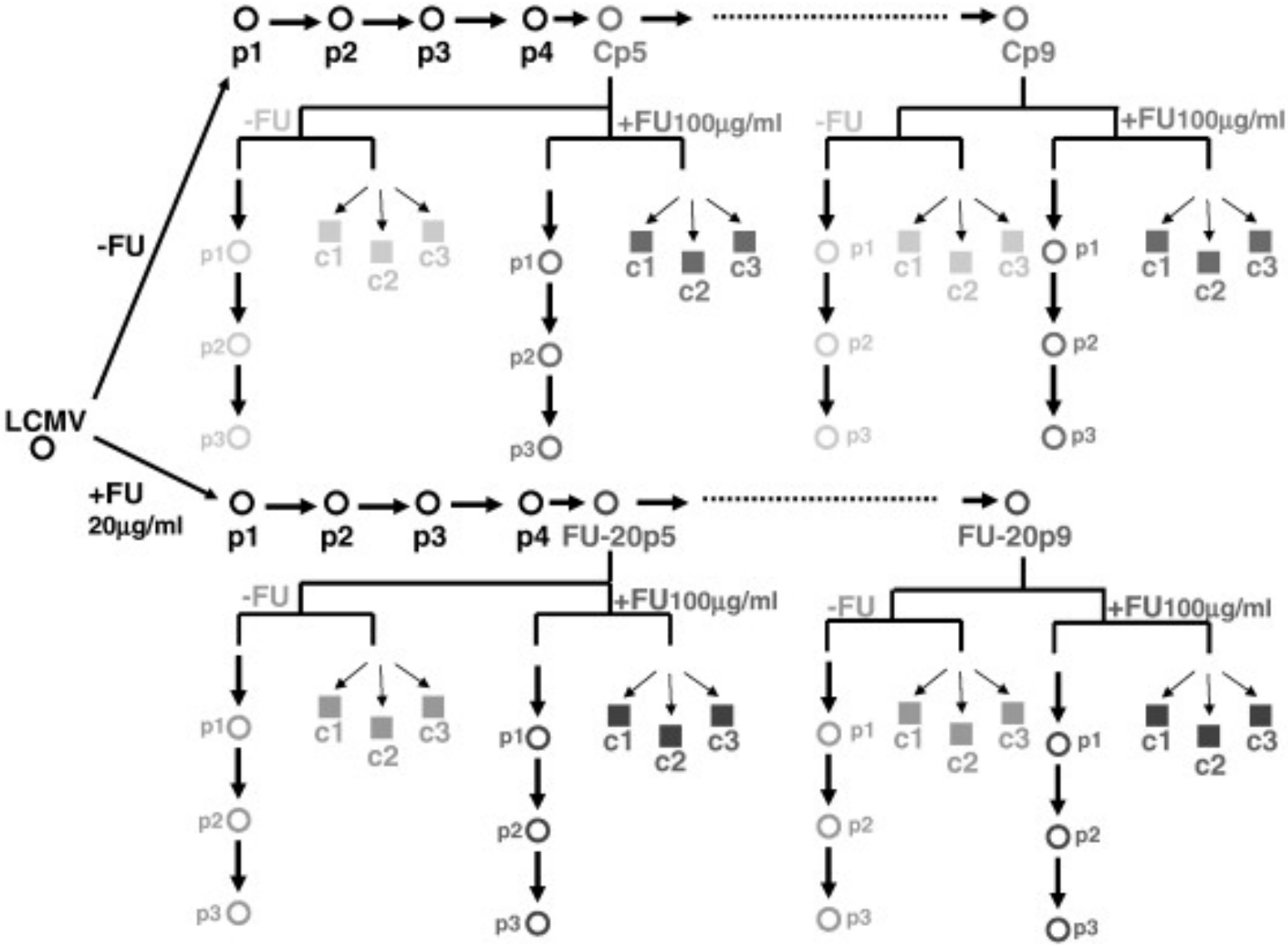
IBMCP Instituto de Biología Molecular y Celular de Plantas

CSIC

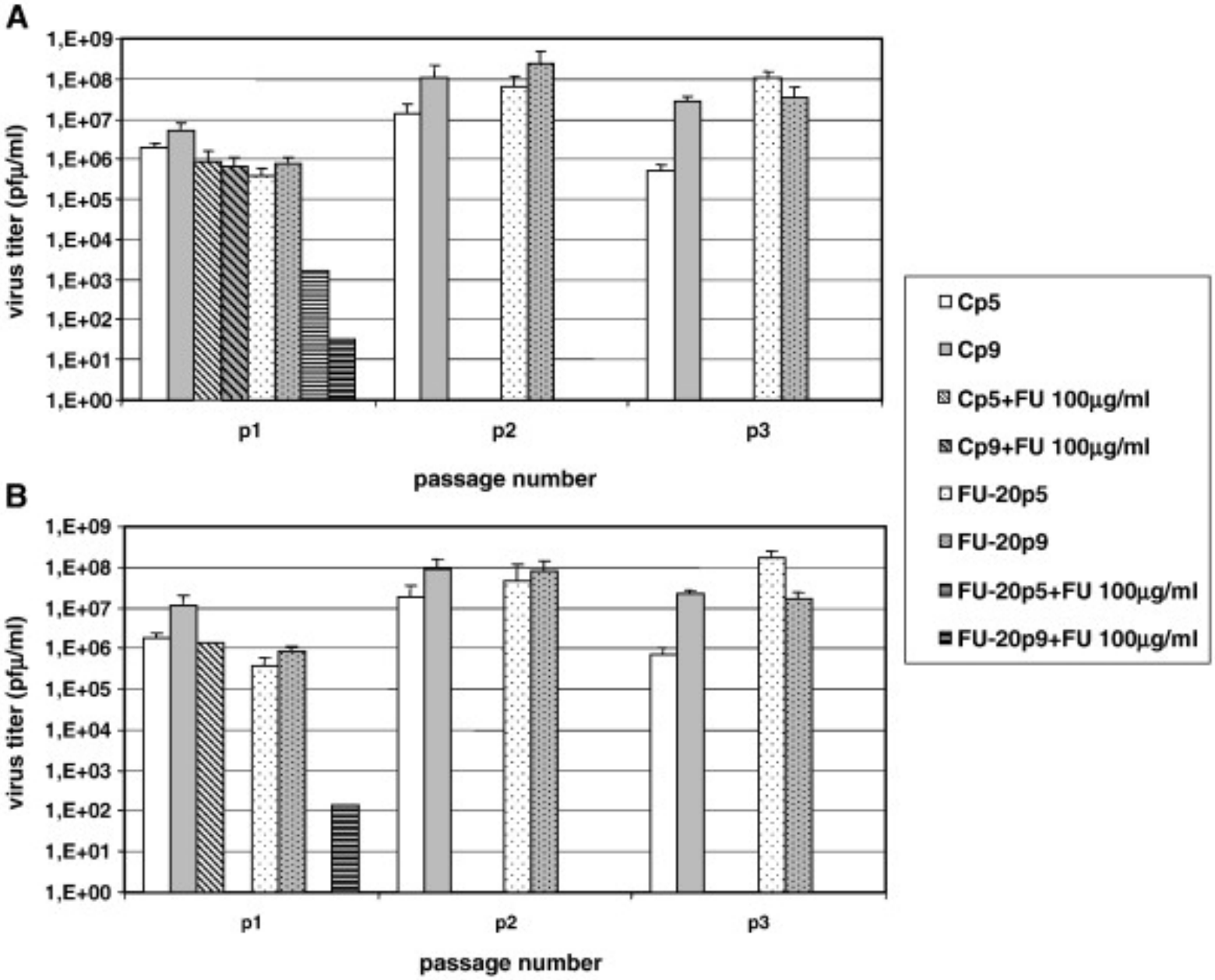
J.M. Cuevas *et al.* (2009) *J. Evol. Biol.* **22**: 2041-8

SANTA FE INSTITUTE

- ✓ Does genetic robustness diminish lethal mutagenesis?
  - ➢ V. Martín *et al.* (2008) *Virology* **378**: 37-4: Evolution of LCMV at subinhibitory concentrations of 5-FU failed to select robust viruses.



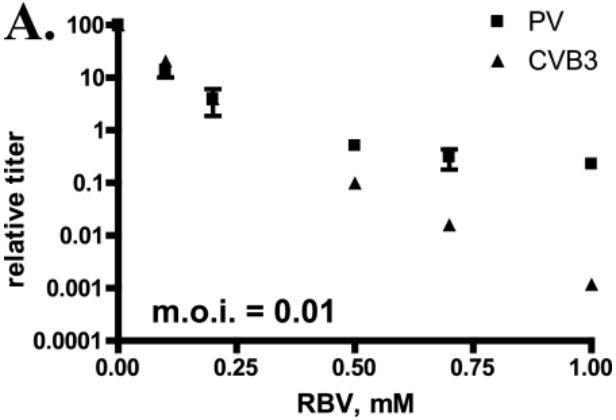
V. Martin *et al.* (2008) *Virology* **378**: 37-47

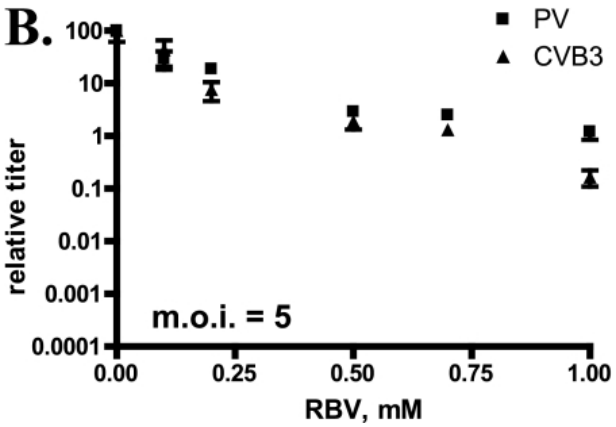

A
virus titer (pfu/ml)
1,E+09
1,E+08
1,E+07
1,E+06
1,E+05
1,E+04
1,E+03
1,E+02
1,E+01
1,E+00
p1
p2
p3
passage number
B
virus titer (pfu/ml)
1,E+09
1,E+08
1,E+07
1,E+06
1,E+05
1,E+04
1,E+03
1,E+02
1,E+01
1,E+00
p1
p2
p3
passage number
Cp5
Cp9
Cp5+FU 100µg/ml
Cp9+FU 100µg/ml
FU-20p5
FU-20p9
FU-20p5+FU 100µg/ml
FU-20p9+FU 100µg/ml

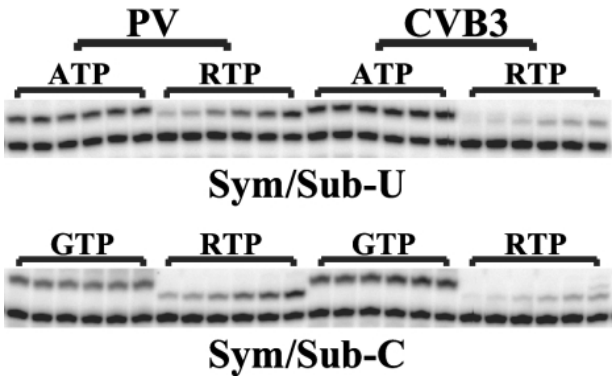
✓ Does genetic robustness diminish lethal mutagenesis?

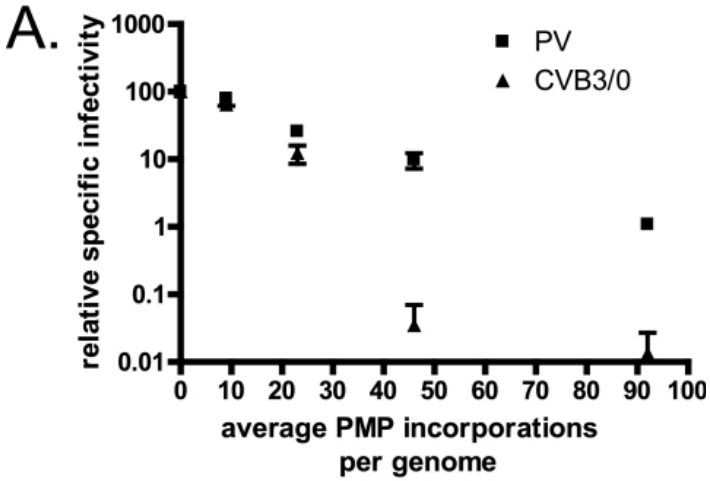
- V. Martín *et al.* (2008) *Virology* **378**: 37-4: Evolution of LCMV at subinhibitory concentrations of 5-FU failed to select robust viruses.

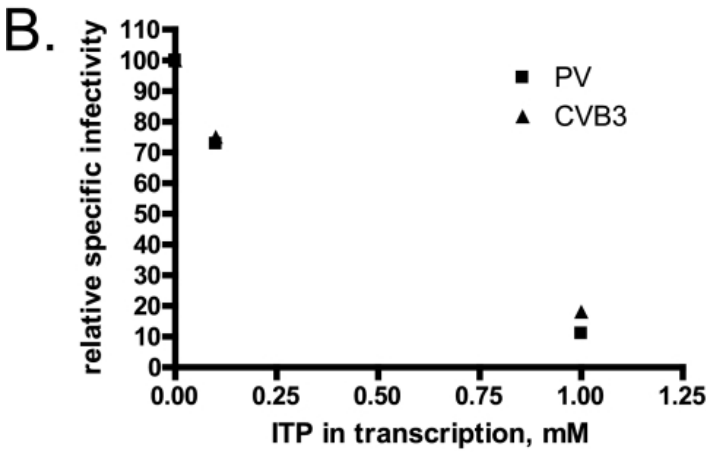
- J.D. Graci *et al.* (2012) *J. Virol.* **86**: 2869-73: CVB3 is less robust than PV and more sensitive to the mutagenic action of ribavirin.

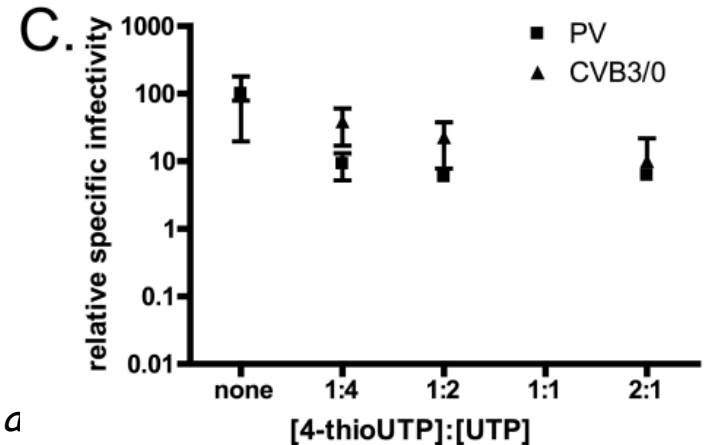












IBMCP Instituto de Biología Molecular y Celular de Plantas

CSIC

J.D. Graci *et a*

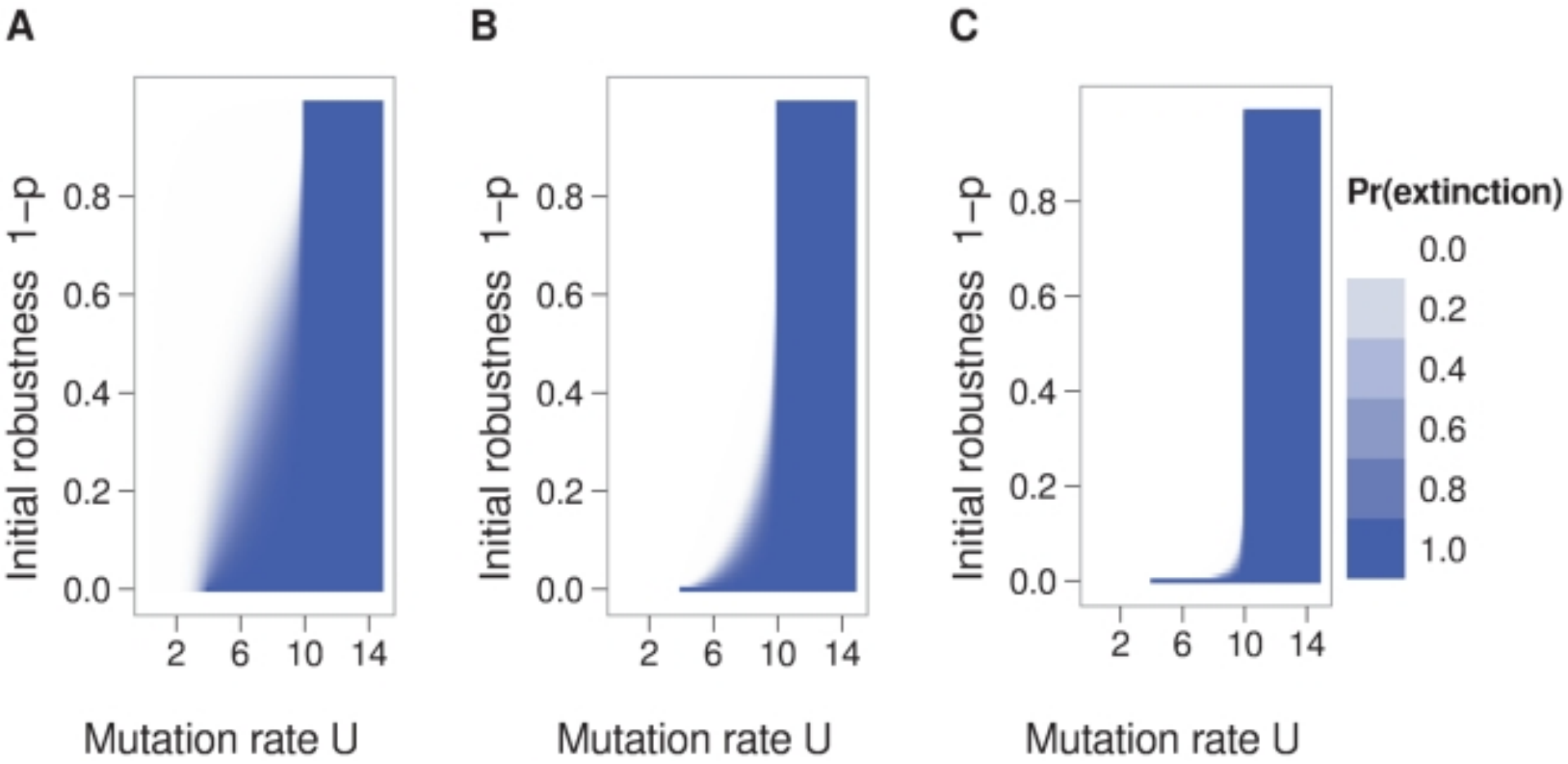
SANTA FE INSTITUTE

✓ Does genetic robustness diminish lethal mutagenesis?

- V. Martín *et al.* (2008) *Virology* **378**: 37-4: Evolution of LCMV at subinhibitory concentrations of 5-FU failed to select robust viruses.

- J.D. Graci *et al.* (2012) *J. Virol.* **86**: 2869-73: CVB3 is less robust than PV and more sensitive to the mutagenic action of ribavirin.

- E.B. O'Dea *et al.* (2010) *PLoS Comput. Biol.* **6**: e1000811: Theoretical work shows that robustness matters only when initial viral population sizes are small and deleterious mutation rates are only slightly above the level at the critical mutation rate.


A
B
C
Initial robustness 1−p
0.0
0.2
0.4
0.6
0.8
2
6
10
14
Mutation rate U
Pr(extinction)
0.0
0.2
0.4
0.6
0.8
1.0

✓ Further readings:

EMBO *reports*

review

Mechanisms of genetic robustness in RNA viruses

Santiago F. Elena[a], *Purificación Carrasco, José-Antonio Daròs & Rafael Sanjuán*
Instituto de Biología Molecular y Celular de Plantas (CSIC-UPV), València, Spain

Two key features of RNA viruses are their compacted genomes and their high mutation rate. Accordingly, deleterious mutations are common and have an enormous impact on viral fitness. In their multicellular hosts, robustness can be achieved by genomic redundancy, including gene duplication, diploidy, alternative metabolic pathways and biochemical buffering mechanisms. However, here we review evidence suggesting that during RNA virus evolution, alternative robustness mechanisms may have been selected. After briefly describing how genetic robustness can be quantified, we discuss mechanisms of intrinsic robustness arising as consequences of RNA-genome architecture, replication peculiarities and quasi-species population dynamics. These intrinsic robustness mechanisms operate efficiently at the population level, despite the mutational sensitivity shown by individual genomes. Finally, we discuss the possibility that viruses might exploit cellular buffering mechanisms for their own benefit, producing a sort of extrinsic robustness.

Keywords: fitness; deleterious mutations; quasi-species; genetic robustness; virus evolution
EMBO reports (2006) 7, 168–173. doi:10.1038/sj.embor.7400636

Introduction

RNA viruses have the highest mutation rate among living species (that is, between 10^{-3} and 10^{-5} errors per nucleotide and replication cycle), very small and compacted genomes, short generation times and extremely large populations (Domingo & Holland, 1997). This might be beneficial in the long-term, as it allows viral populations to quickly explore genotypic space and find beneficial mutations. However, it is clearly detrimental in the short-term as most mutations have deleterious fitness effects. The balance between the continuous generation of mutants and the action of selection leads to a dynamic population structure, known as 'quasi-species' (Domingo & Holland, 1997).

In recent years, the interest of evolutionary biologists in the mechanisms, consequences and evolution of genetic robustness has been revitalized by new and powerful techniques that allow the tracking and manipulation of genotypes (de Visser *et al*, 2003). Robustness is defined as a reduced sensitivity to perturbations affecting phenotypic expression. If perturbations are inheritable, then we talk about genetic robustness; if they are not (for example, changes in physical and chemical parameters, or developmental noise), then we talk about environmental robustness. Robustness should occur when there are several copies of a single gene, when several genes contribute to the same function or through biochemical buffering mechanisms. This includes gene duplication, polyploidy, alternative metabolic pathways or chaperone proteins. As illustrated in Fig 1A, a lack of robustness is expected in haploid genomes that have no duplications, overlapping gene functions, repair systems and arepleiotropic. A small number of mutations can produce a strong effect, but as mutations accumulate, they affect the same function with increasing probability and, thus, their marginal contribution to fitness diminishes. Hence, the observed fitness is above the expected multiplicative value or, in other words, epistasis is antagonistic (Wolf *et al*, 2000). By contrast, in the presence of redundancy and buffering mechanisms, the fitness of genomes is only mildly affected; however, as the mutation load increases, these mechanisms ultimately collapse. Fitness will therefore be lower than the expected multiplicative value, which means that there will be synergistic epistasis (Fig 1B).

In principle, genetic robustness might evolve for one of the following reasons. First, as long as robustness has a heritable basis, shows variability among individuals and affects the probability of survival, it can be a target for selection and evolutionary optimization (Wilke & Adami, 2003). The selection pressure for increasing robustness depends on the occurrence of mutations. The more frequent mutations are, the more efficient selection will be at promoting the evolution of robustness. Second, it might evolve because buffering is a necessary consequence of character adaptation; that is, robustness is a side-effect of stabilizing selection acting on different traits (Meiklejohn & Hartl, 2002). Third, given that environmental fluctuations often have a strong impact on fitness, selection would efficiently favour mechanisms of environmental robustness. On the basis of theoretical arguments and RNA folding simulations, some authors have predicted that genetic robustness should be intrinsically correlated to environmental robustness and, thus, that the former could evolve as a correlated response to selection favouring the latter (Ancel & Fontana, 2000; Wagner *et al*, 1997). This is an appealing hypothesis because, during their life cycle, RNA viruses must cope not only with the deleterious effect of mutations but also with dramatic and fast fluctuations in their environments such as alternating among host species, tissue- and organ-specific microenvironments or the presence of antiviral agents.

Instituto de Biología Molecular y Celular de Plantas (CSIC-UPV), Avenida de los Naranjos s/n, 46022 València, Spain
[a]Corresponding author. Tel: +34 963 877 895; Fax: +34 963 877 859;
E-mail: sfelena@ibmcp.upv.es

Submitted 29 August 2005; accepted 30 November 2005

Available online at www.sciencedirect.com
SciVerse ScienceDirect
Current Opinion in Virology
ELSEVIER

RNA virus genetic robustness: possible causes and some consequences

Santiago F Elena[1,2]

In general terms, robustness is the capacity of biological systems to function in spite of genetic or environmental perturbations. The small and compacted genomes and high mutation rates of RNA viruses, as well as the ever-changing environments wherein they replicate, create the conditions for robustness to be advantageous. In this review, I will enumerate possible mechanisms by which viral populations may acquire robustness, distinguishing between mechanisms that are inherent to virus replication and population dynamics and those that result from the interaction with host factors. Then, I will move to review some evidences that RNA virus populations are robust indeed. Finally, I will comment on the implications of robustness for virus evolvability, the emergence of new viruses and the efficiency of lethal mutagenesis as an antiviral strategy.

Addresses
[1] Instituto de Biología Molecular y Celular de Plantas (CSIC-UPV), Campus UPV CPI 8E, Ingeniero Fausto Elio s/n, 46022 València, Spain
[2] The Santa Fe Institute, 1399 Hyde Park Road, Santa Fe, NM 87501, USA

Corresponding author: Elena, Santiago F (santiago.elena@csic.es, sfelena@ibmcp.upv.es)

Current Opinion in Virology 2012, **2**:525–530
This review comes from a themed issue on **Virus evolution**
Edited by **Raul Andino** and **Marco Vignuzzi**
For a complete overview see the Issue and the Editorial
Available online 19th July 2012
1879-6257/$ – see front matter,
http://dx.doi.org/10.1016/j.coviro.2012.06.008

RNA viruses are the most successful parasites on Earth, infecting hosts from all biological kingdoms, including other parasites. This success results from their evolutionary plasticity (i.e. evolvability): a combination of short generation times, huge population sizes and high mutation rates [1–3]. Alas, these properties come along with some costs. First, fast replication requires that genomes must be kept small, with overlapping reading frames and encoding multifunctional proteins [4,5]. Second, high mutation rates limit the length of the genome that can be transmitted without incurring in too many errors [6]. High mutation rates may be favored in stressful situations where the input of beneficial mutations allows for escape and survival (e.g. changing cell types, tissues and hosts or the presence of antiviral responses or drugs). However, in all situations deleterious and lethal mutations represent the larger fraction of all possible mutations [7], thus jeopardizing viral fitness [8,9]. How do RNA viruses maintain their functionality in this scenario? Are they robust to the accumulation of deleterious mutations? In this review I try to answer these questions and look beyond to the consequences of RNA virus robustness.

What is robustness and how can it be measured?

In a hallmark article, De Visser *et al*. [10••] reviewed the notion of robustness and explored its causes and consequences. *Robustness is the preservation of the phenotype in the face of perturbations*. The robustness of phenotypes appears at various levels of organization: from gene expression, protein folding, metabolic flux, physiological homeostasis, and development, to fitness. From an evolutionary standpoint, fitness is the most relevant level. Phenotypes can be robust either against mutations or environmental perturbations.

Three reasons may account for the evolution of genetic robustness (GR). First, as long as it is heritable, shows variability among individuals and affects fitness, GR can be a target for selection [11]. The more frequent mutations are, the more efficient selection will be at promoting the evolution of GR. Second, GR is a side effect of stabilizing selection acting on different traits [12]. Third, given that environmental fluctuations often have strong impact on fitness, selection would favor mechanisms of environmental robustness (ER), emerging GR as a correlated response (plastogenetic congruence) [13,14]. This is particularly appealing in the case of RNA viruses because they must cope not only with deleterious mutations but also with dramatic and fast fluctuations in their environments.

Keeping in mind the definition of GR, a way of estimating it is to evaluate the effect of large collections of individual point mutations on viral fitness. If a point mutation *i* reduces the fitness of a genotype with respect to that of the wild-type in an amount s_i, then the average effect $\bar{s}$ across the collection of point mutations can be seen as a measure of mutational sensitivity and, henceforth, as an inverse of GR. In other words, if the average effect of mutations on a virus is small, we conclude it is robust. By contrast, if the average effect is large, we conclude the virus is brittle.

Potential mechanisms for viral GR

In a previous review, we elaborated on possible mechanisms by which RNA viruses may attain GR [15••]. We

The role of mutational robustness in RNA virus evolution

Adam S. Lauring[1,2], *Judith Frydman*[3] *and Raul Andino*[4]

Abstract | RNA viruses face dynamic environments and are masters at adaptation. During their short 'lifespans', they must surmount multiple physical, anatomical and immunological challenges. Central to their adaptative capacity is the enormous genetic diversity that characterizes RNA virus populations. Although genetic diversity increases the rate of adaptive evolution, low replication fidelity can present a risk because excess mutations can lead to population extinction. In this Review, we discuss the strategies used by RNA viruses to deal with the increased mutational load and consider how this mutational robustness might influence viral evolution and pathogenesis.

Fitness
The ability of an entity to survive and reproduce. In experimental virology, replicative efficiency is often used as a surrogate for fitness. In this Review, we define viral fitness as the capacity of a virus to generate infectious progeny.

[1]Division of Infectious Diseases, Department of Internal Medicine, University of Michigan Medical School, [2]Department of Microbiology and Immunology, University of Michigan Medical School, 5510B MSRB I, SPC 5620, 1150 West Medical Center Drive, Ann Arbor, Michigan 48109-5620, USA. [3]Department of Biology, Stanford University, Clark Center E200, 318 Campus Drive, Stanford, California 94305, USA. [4]Department of Microbiology and Immunology, University of California, 600 16th Street, GH-S572, UCSF Box 2280, San Francisco, California 94143-2280, USA. Correspondence to A.S.L. and R.A.
e-mails:
alauring@med.umich.edu; raul.andino@ucsf.edu
doi:10.1038/nrmicro3003
Published online
25 March 2013

RNA viruses exhibit extremely high mutation rates, orders of magnitude greater than those of most DNA-based life forms[1] (BOX 1). Although the measurement of viral mutation rates is a complex issue in itself, the studies carried out to date suggest that many RNA viruses generate 10^{-4} to 10^{-6} errors per nucleotide, which is equivalent to approximately one mutation per genome, per replication cycle[2]. Given the large population sizes observed in both experimental and natural infections with these viruses, every possible point mutation and many double-mutation combinations could theoretically be generated during each replication cycle within a population. Even a defined molecular clone quickly transforms into a collection of related sequences when introduced into cells[3]. This low replicative fidelity ensures that viral populations can generate and maintain mutations that allow them to quickly adapt to changes in the environment. The mutability and fleeting existence of each viral genome means that RNA virus populations exist as dynamic mutant networks in which sequences are continuously diversified and regenerated by mutation of related sequences (FIG. 1). The low replicative fidelity seems to be crucial for viral survival in the host ecosystem, as variants with abnormally low mutation rates are attenuated *in vivo*[4,5].

The focus on mutation as a driving force in viral evolution has tended to overlook the tremendous cost of low replicative fidelity. Most mutations have deleterious effects on viral fitness. In vesicular stomatitis virus (VSV), more than 90% of random single-nucleotide mutations reduce replicative fitness, and 40% are lethal[6]. Similar trends have been found in tobacco etch virus and the phages ΦX174 and Qβ[7]. Furthermore, increasing error rates pharmacologically, with mutagenic nucleoside drugs[8,9], or genetically, through the use of variant RNA-dependent RNA polymerases[10–12], leads to viral extinction. These studies indicate that the mutation rate in RNA virus populations is perilously close to the maximum tolerable error rate. The mutational tolerance of a virus will determine the type (for example, variation in structural or non-structural proteins) and extent of genetic diversity that can be maintained in the population. Thus, viral population diversity results from both the generation of and the tolerance to mutations; these two factors together drive adaptation and viral evolution.

It has long been recognized that not all genotypic changes are expressed as alterations in phenotype, and in population genetics, this buffering of mutational effects is termed genetic robustness. Early work on genetic robustness was largely based on theory (reviewed in REF. 13), but a number of experimental studies over the past 10 years have established and extended the concept of genetic robustness and shown that this buffering allows a viral population to increase its genetic diversity without a dramatic alteration in phenotype. Importantly, these experimental systems have also begun to elucidate the molecular underpinnings of mutational tolerance and to identify the conditions in which genetic robustness is adaptive. Recent studies further suggest that the relationship between robustness and evolvability might be particularly important for viral pathogenesis[14].

As a result of this recent work, we now have a clearer picture of how robustness influences the short- and long-term evolution of RNA viruses. In this Review, we begin by defining genetic robustness and how it can be measured, before considering how genetic robustness influences the composition of viral populations. We then

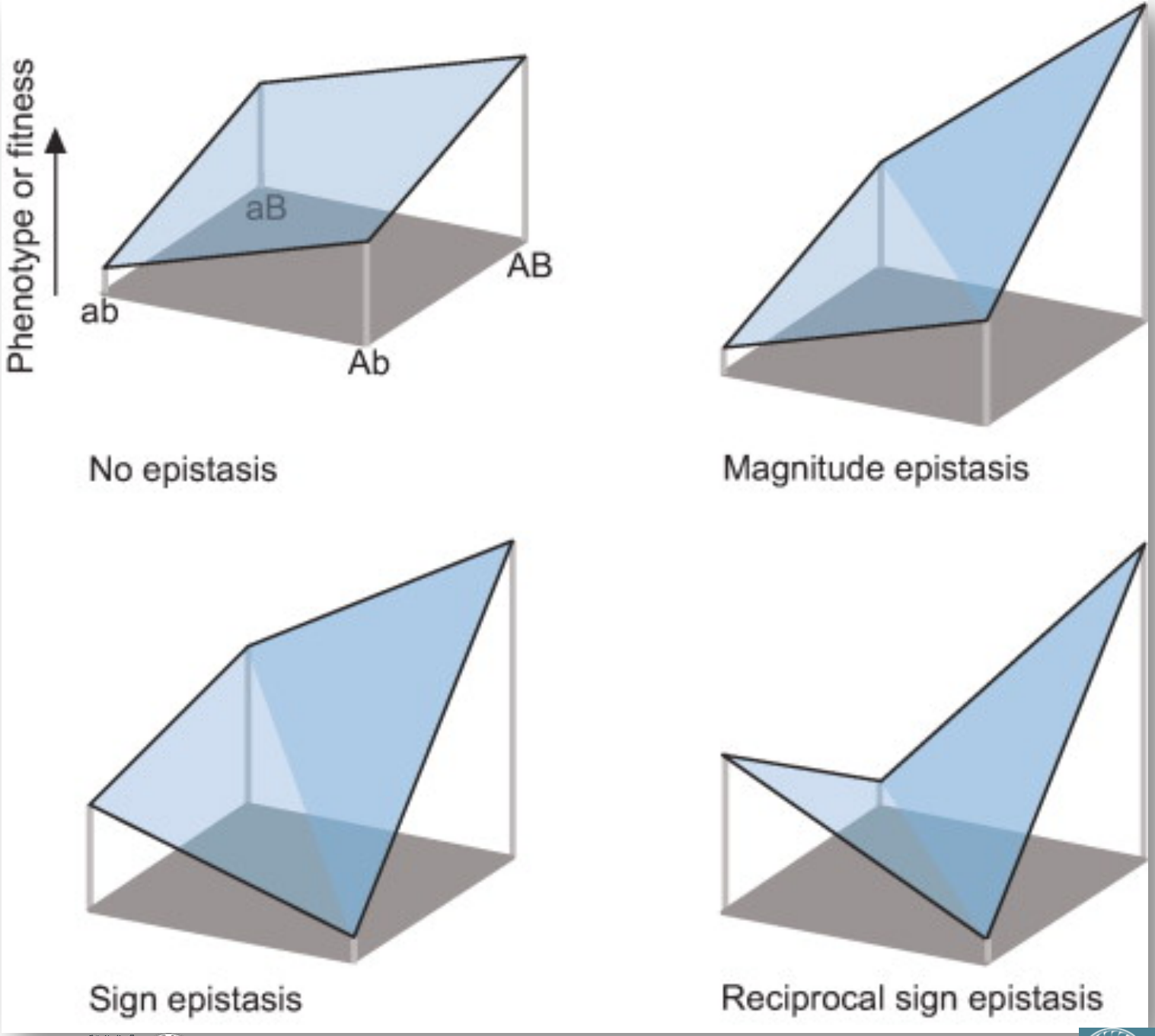
IBMCP Instituto de Biología Molecular y Celular de Plantas
CSIC
SANTA FE INSTITUTE

More on epistasis

The distribution of G×G in the primary host

- ✓ G×G aka epistasis, is the interaction between genes or mutations in determining phenotypes.

- ✓ The direction, magnitude and prevalence of epistasis is central to theories seeking to explain the origin of genetic systems, such as sex and recombination, dominance, ploidy, phenotypic plasticity, or robustness, the ruggedness of adaptive landscapes, or attempting to mechanistically explain dynamical biological processes such as the accumulation of mutations in finite populations or speciation by reproductive isolation.


Phenotype or fitness
aB
AB
ab
Ab
No epistasis
Magnitude epistasis
Sign epistasis
Reciprocal sign epistasis

- ✓ We generated a collection of 53 double mutants by combining 20 individual mutations whose deleterious fitness effect had been previously quantified.

- ✓ Mathematical definition of magnitude epistasis:

$$\varepsilon_{xy} = W_{00} W_{xy} - W_{x0} W_{0y}$$

$\varepsilon_{xy} > 0$ positive (antagonistic) epistasis
$\varepsilon_{xy} < 0$ negative (synergistic) epistasis
$\varepsilon_{xy} = 0$ no epistasis (additive)

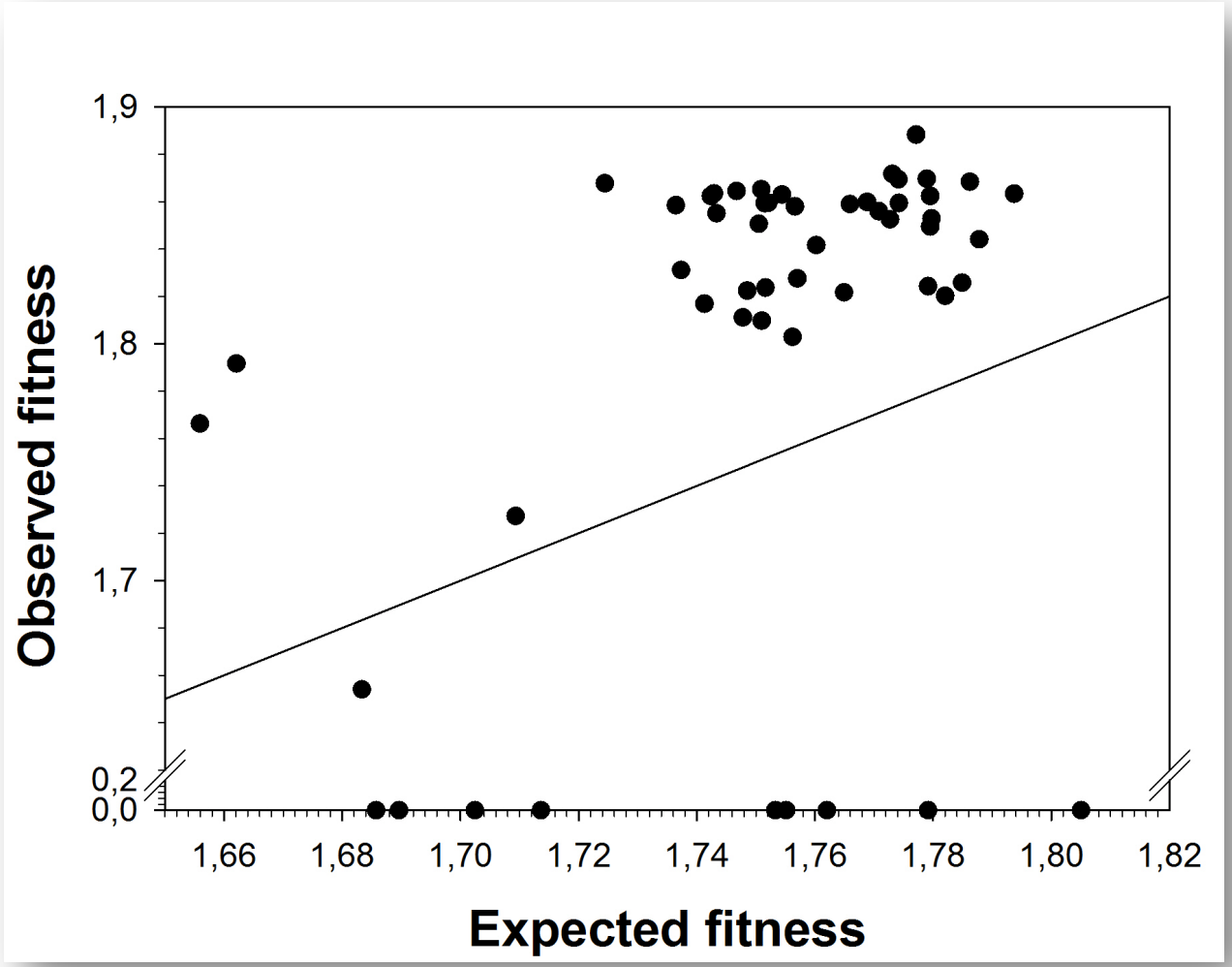
- ✓ Mathematical condition for sign epistasis (Poelwijk *et al.* 2011):

$$|W_{x0} - W_{00} + W_{xy} - W_{0y}| < |W_{x0} - W_{00}| + |W_{xy} - W_{0y}|$$

- ✓ Additional mathematical condition for reciprocal sign epistasis (Poelwijk *et al.* 2011):

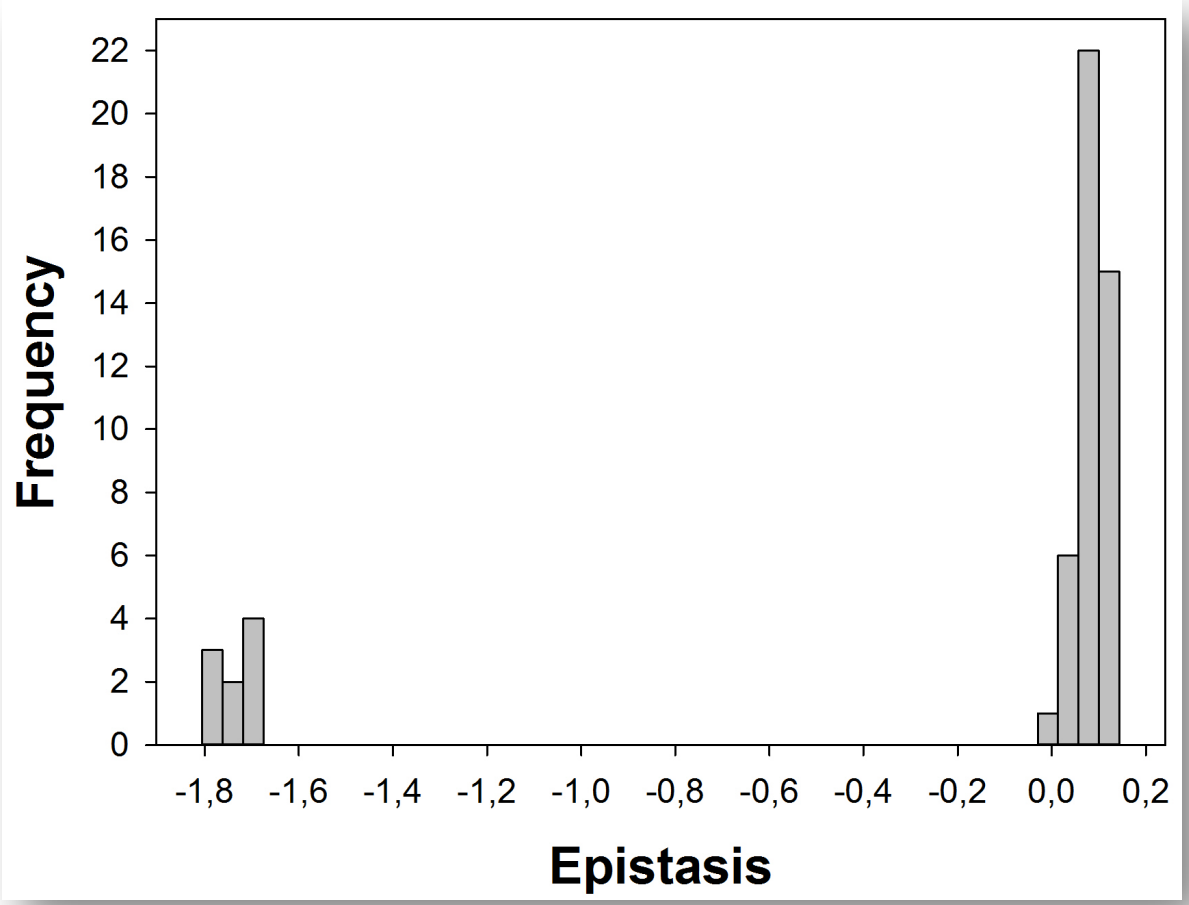
$$|W_{0y} - W_{00} + W_{xy} - W_{x0}| < |W_{0y} - W_{00}| + |W_{xy} - W_{x0}|$$

Epistasis among pairs of deleterious mutations



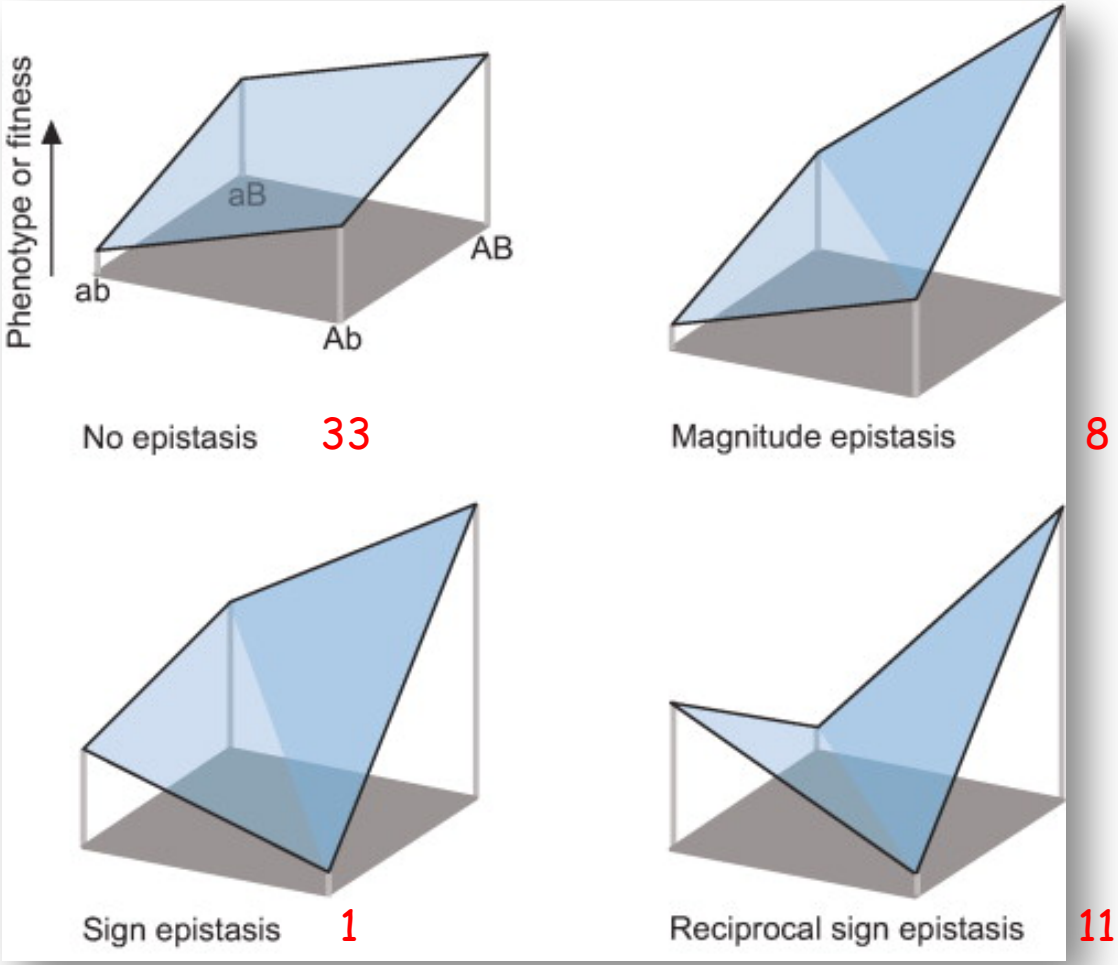
- ✓ 20 significant deviations from the additive expectation (*t*-test, $P < 0.049$).
  - 9 cases of synthetic lethals (negative epistasis).
  - 11 cases of positive epistasis.

Statistical properties of the epistasis distribution



- ✓ Without synthetic (at least, $\langle\varepsilon\rangle = -0.028 \pm 0.005$ (t-test, $P < 0.001$).
- ✓ Significant negative skewness ($g_1 = -1.856 \pm 0.328$; $P < 0.001$).
- ✓ Significantly leptokurtic ($g_2 = 2.346 \pm 0.642$, $P = 0.002$).

Pervasive reciprocal sign epistasis



- ✓ 33% less cases of magnitude than of sign epistasis (Binomial test, 1-tailed $P = 0.032$).
- ✓ Over-representation of reciprocal sign epistasis among cases of sign epistasis (Binomial test, $P < 0.001$).

Epistasis determines the rate of adaptation

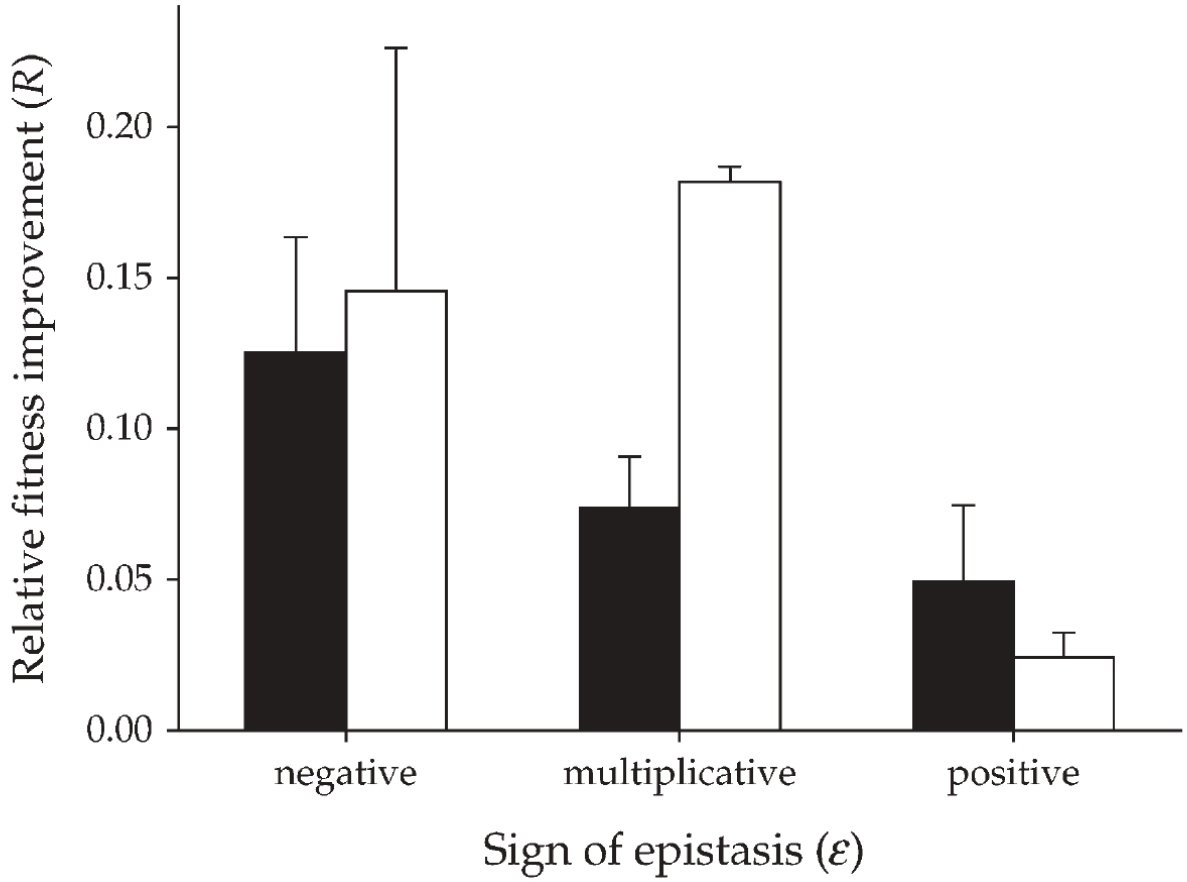


FIGURE 3.—Average fitness improvement as a function of the type of epistasis characteristic of the two mutations carried by the double mutants, for the two effective population sizes. Solid bars show $N_e = 2 \times 10^2$ PFU and open bars show $N_e = 2 \times 10^4$ PFU. Error bars show standard errors of the means.