Experimental evolution on microbial fitness landscapes

Arjan de Visser

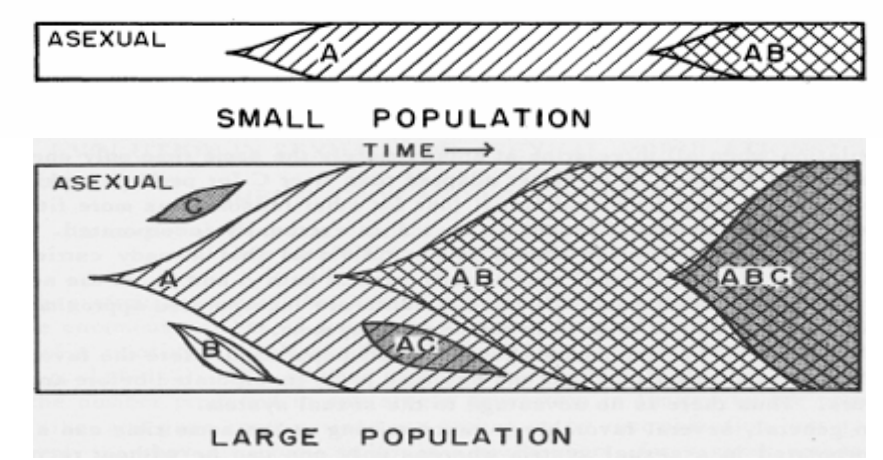
Laboratory of Genetics

WAGENINGEN UNIVERSITY
WAGENINGEN U

Factors determining course of evolution

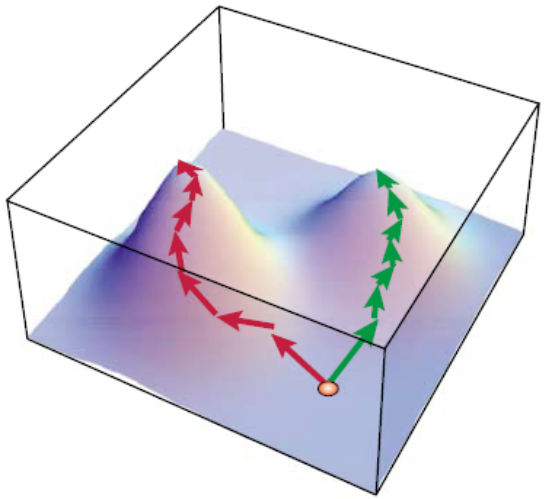
1. Population dynamics

- genetic variation
- population size



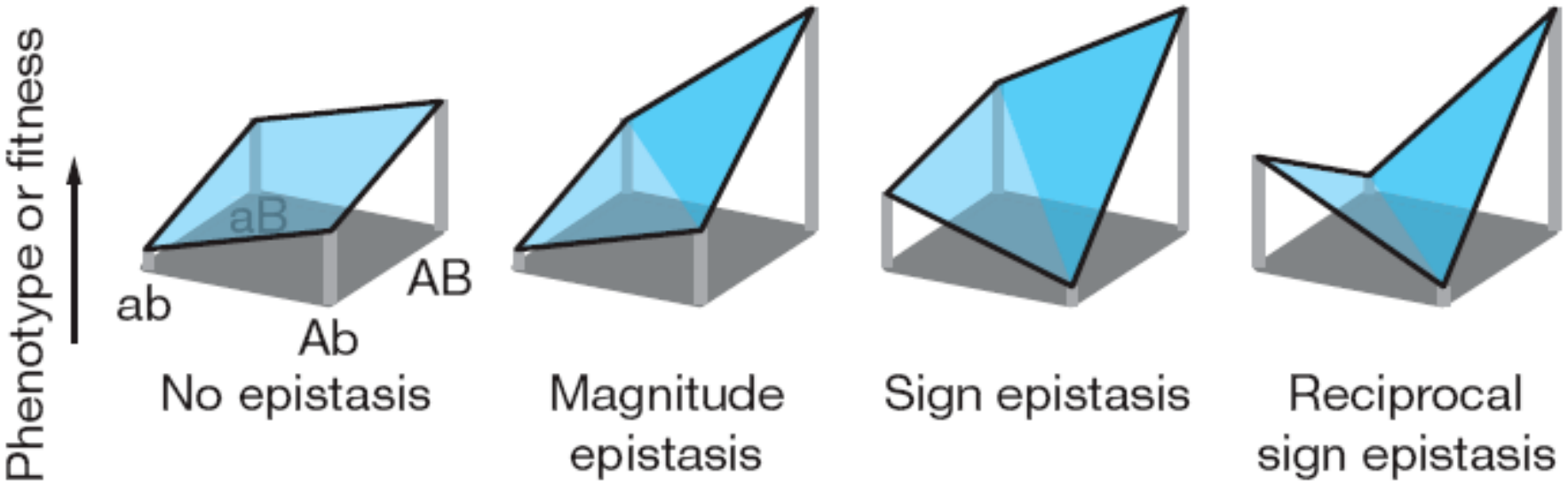
2. Fitness landscape

- environment
- genetic architecture



Fitness landscape topography

- Ultimate cause: largely unknown
- Proximate cause: epistasis



(Poelwijk *et al*. 2007 *Nature*)

Little known about topography of real fitness landscapes

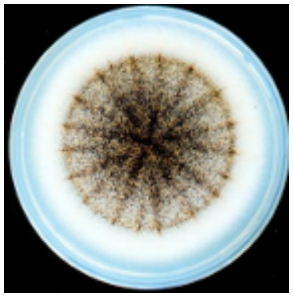
Microbial experimental evolution

- Microbial populations evolve in laboratory for many generations in relatively short time
- Direct observation of evolution under controlled conditions
- Access to intermediates using ‘frozen fossil bed’
- Analyze fitness landscape via construction of mutants

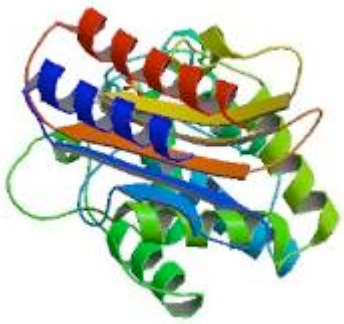
3 studies of empirical fitness landscapes

I. Landscape topography inferred from dynamics of adaptation in bacteria

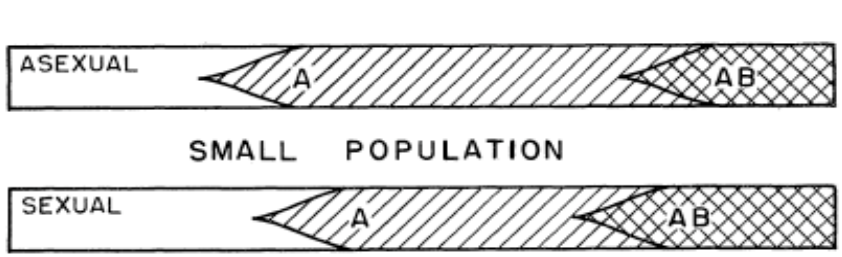
II. Systematic study of landscape involving marker mutations in a fungus



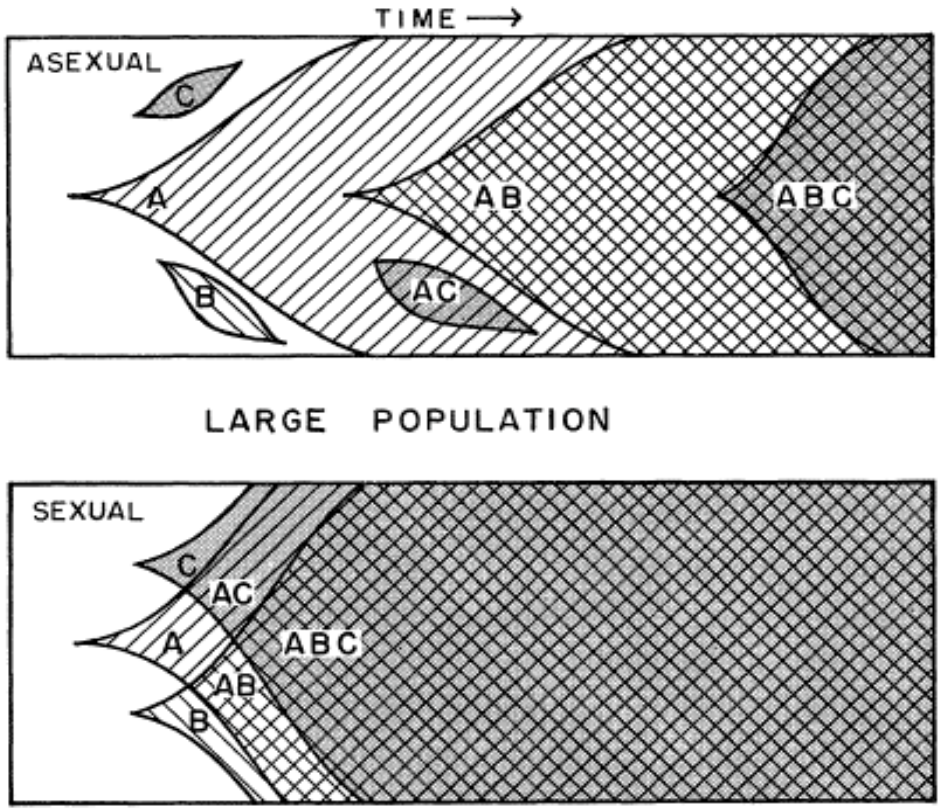
III. Molecular exploration of the landscape of an enzyme



I. Prelude: Adaptation in asexual populations



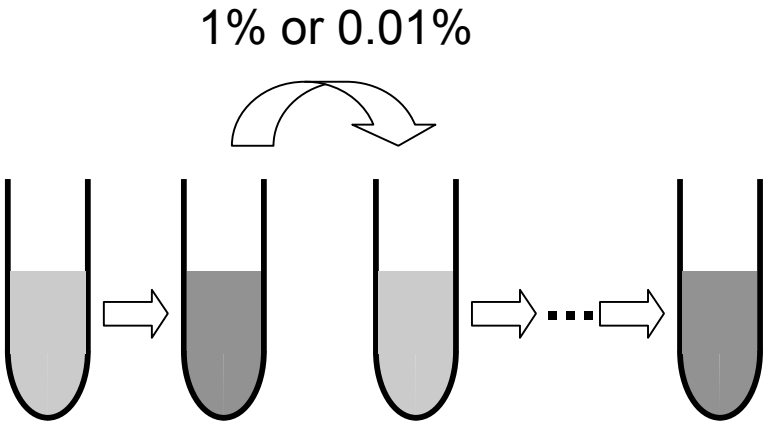
(after H.J. Muller 1932)



Consequences **clonal interference**:

- **Short term**: *slower* substitution of *larger-benefit* mutations
- **Long term**: diminishing returns from mutation supply on rate of adaptation

I. Prelude: Test long-term effects CI in *E. coli*



1,000-generation experiment in minimal glucose medium

Manipulated variables

- Mutation rate:

  wt, *mutY*$^{-}$, *mutS*$^{-}$

- Population size:

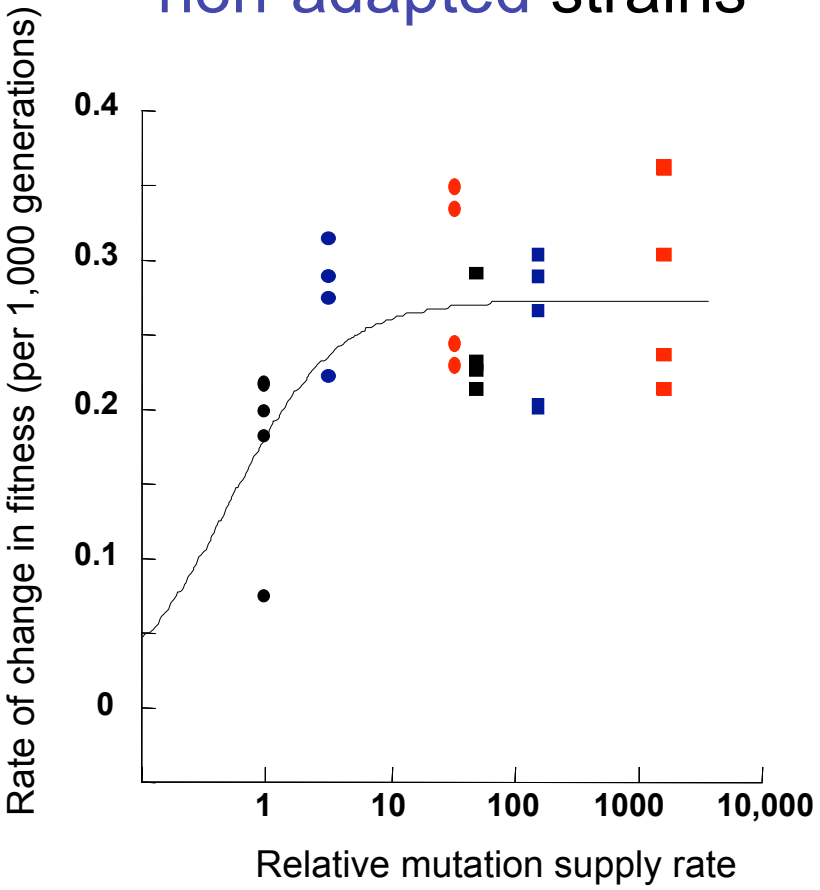
  transfer cell number

- Fraction beneficial mutations:

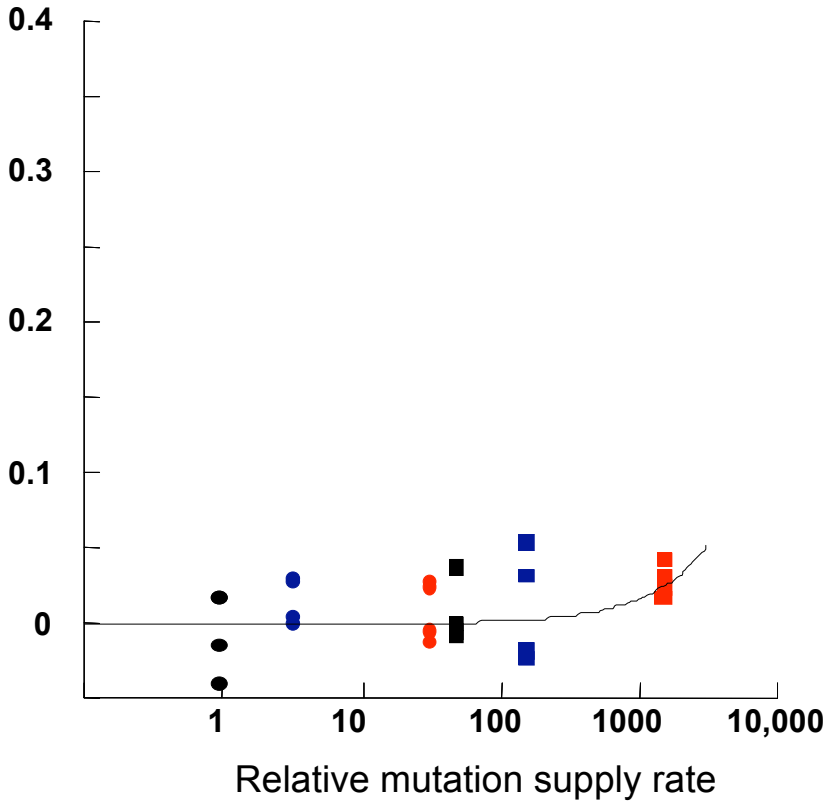
  strain with low and strain with high level of adaptation

I. Prelude: Adaptive benefit from increased mutation supply rate

Populations founded by non-adapted strains



Populations founded by adapted strains



(de Visser, Zeyl, Gerrish, Blanchard & Lenski 1999 *Science*)

I. Fitness landscapes and population size

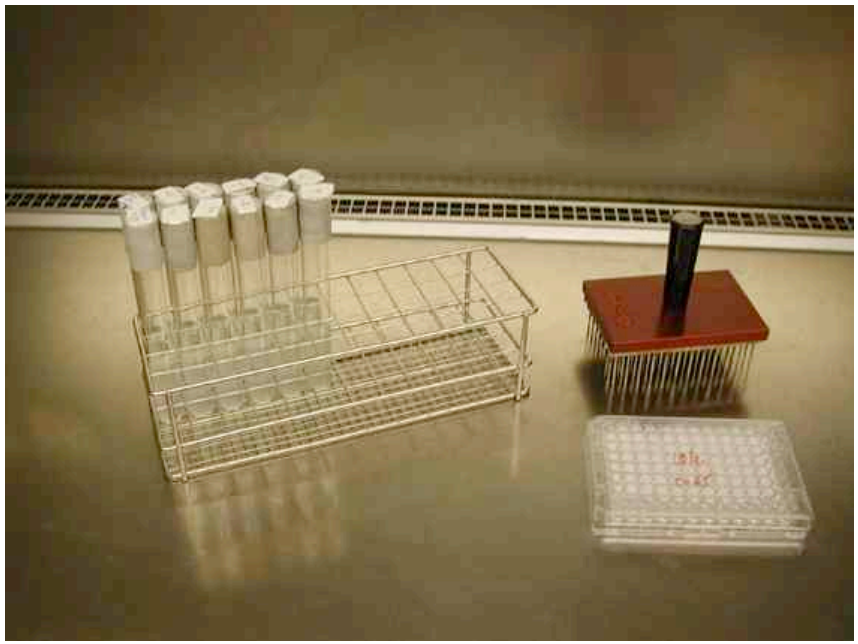
•So far, large populations adapt faster than small populations -- although this advantage may be small due to clonal interference

•However, relationship between rate of adaptation and population size may also depend on fitness landscape

•**Smooth landscape**: *single peak* reached fastest by large populations taking big steps on single-best trajectory

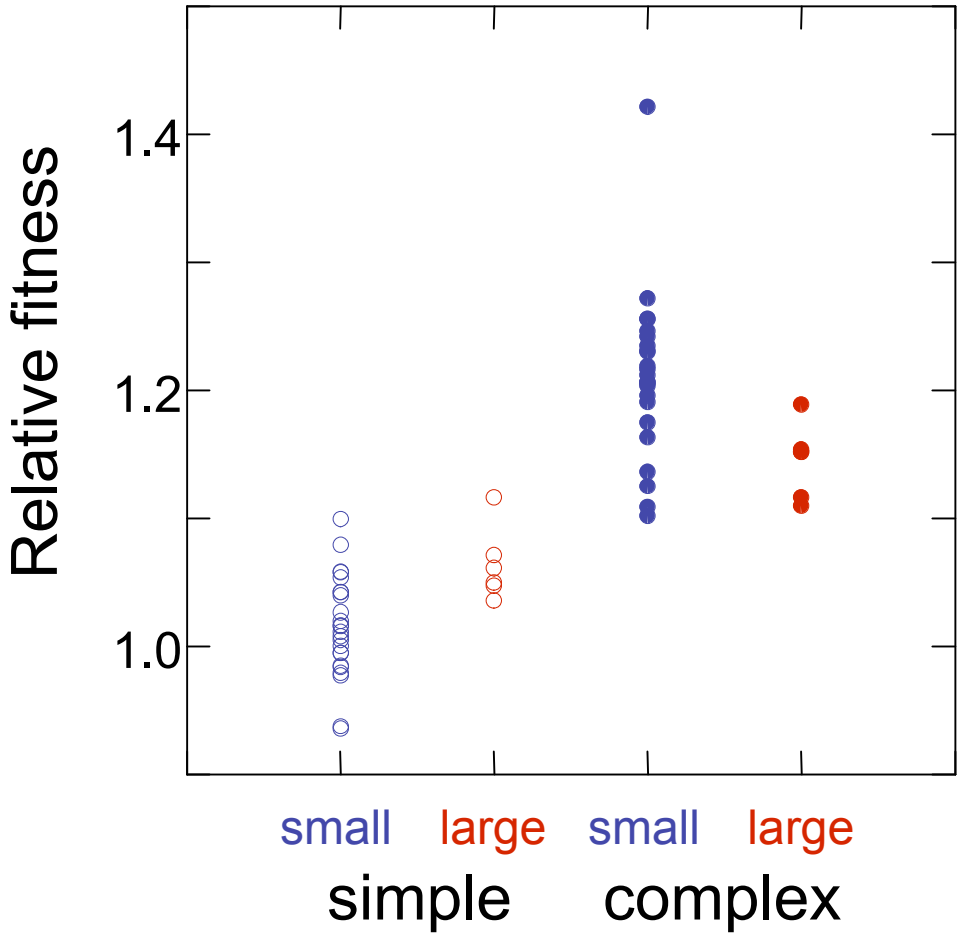
•**Rugged landscape**: *global peak* may be reached faster by smaller populations exploring different trajectories due to greater role drift

I. Test of interaction between population size and environmental complexity



- 24 small (N_e~ 5 x 10^5), 6 large *E. coli* populations (N_e~ 2.5 x 10^7)
- Simple (single C-source) and complex (multiple C-sources) nutrient environment (same N_e)
- 500 generations

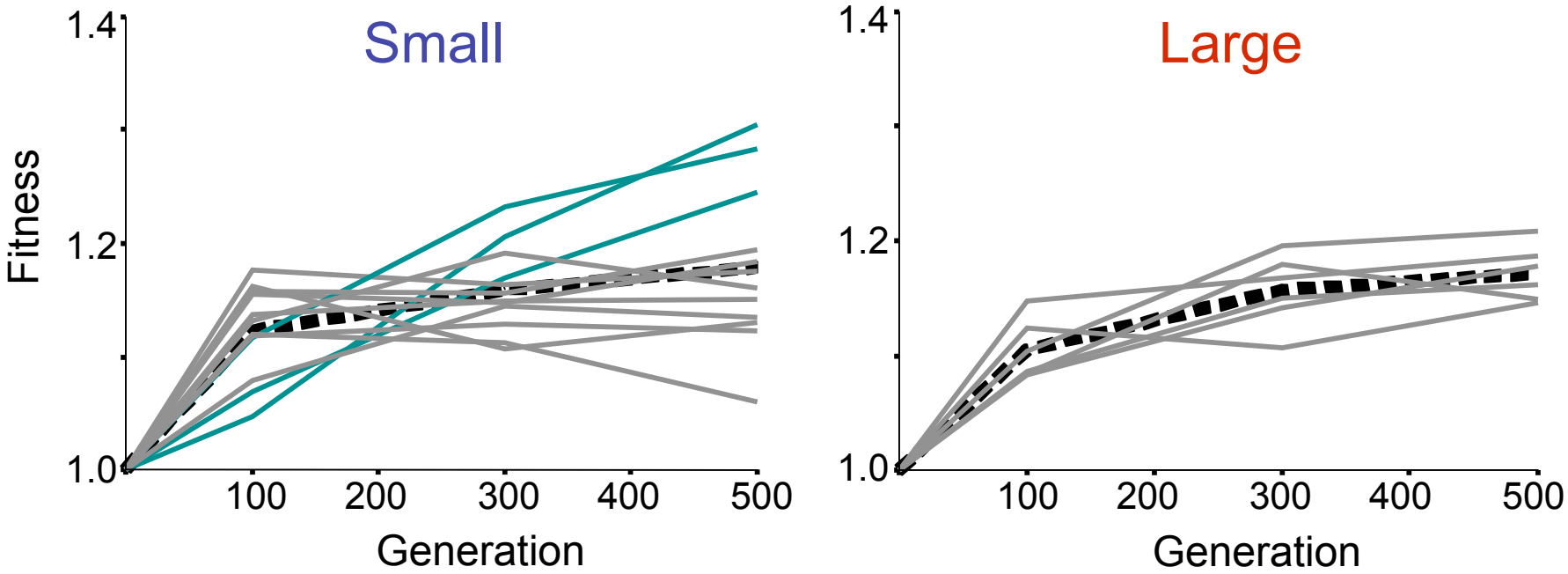
I. Fitness after 500 generations



- Among-population variance only for small *N*: higher adaptive variability small *N*
- *N* x environment interaction: adaptive advantage small *N* in complex environment

(Rozen, Habets, Handel & de Visser 2008 *PLoS One*)

I. Fitness trajectories complex environment

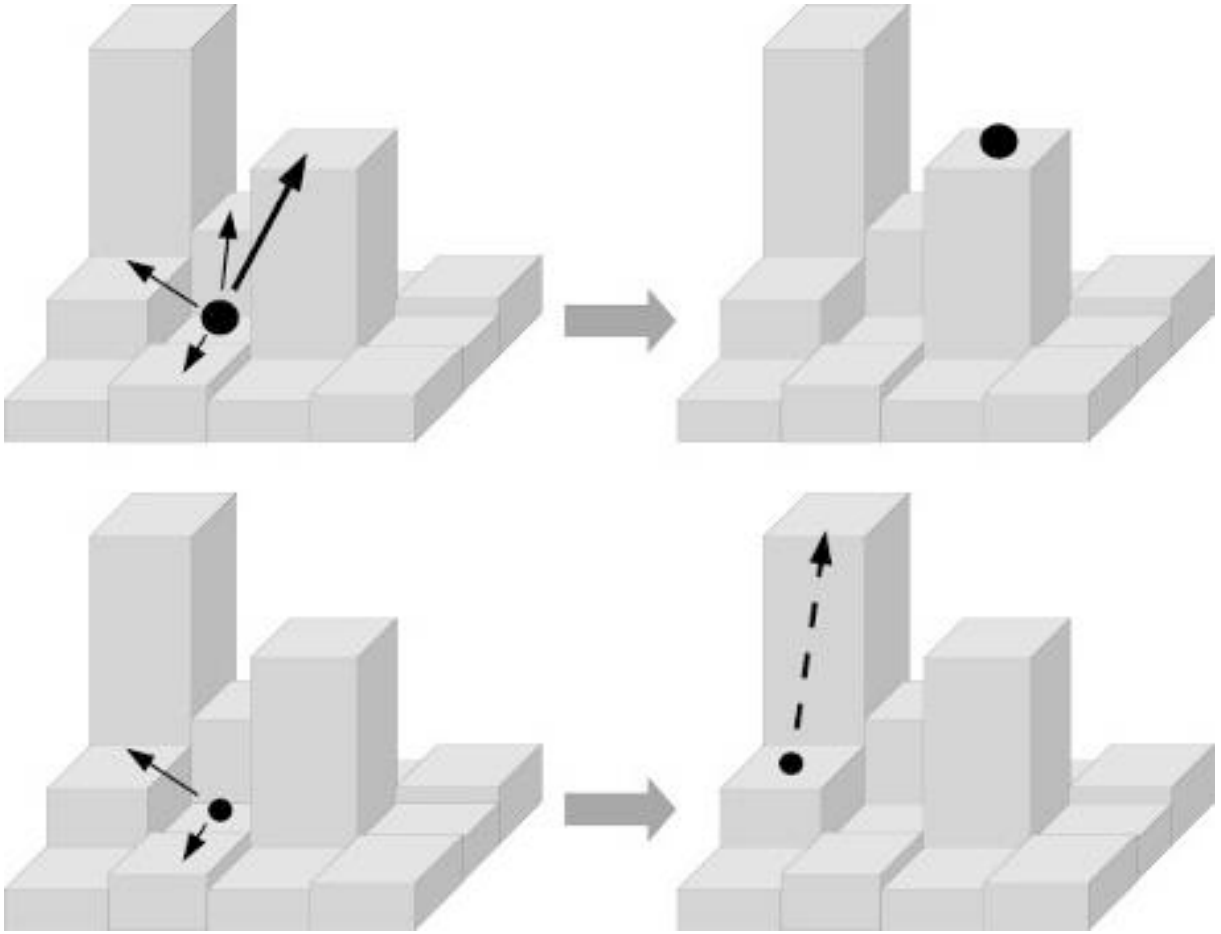


- Greater slope variation in trajectories of small populations
- Highest final fitness reached by initially slowest small populations

I. The perils of greed

large *N*

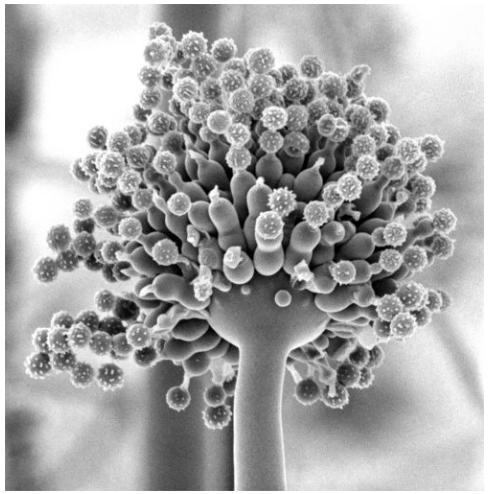
small *N*



(Handel & Rozen 2009 *BMC Evol. Biol.*)

II. Adaptation on empirical fungal fitness landscapes

with **Su-Chan Park** and **Joachim Krug** (University of Cologne)



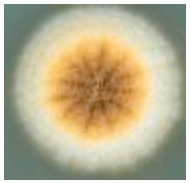
Aspergillus niger (courtesy of Mycology Online & Maren Klich)

- Construct all possible 2^8 combinations of mutations at 8 loci, and measure fitness
- Simulate adaptation with and without recombination on this landscape

II. *Aspergillus niger* strain construction

Heterozygous diploid strain

fwn	*arg*$^-$	*pyr*$^-$	*leu*$^-$	*phe*$^-$	*lys*$^-$	*oli*^{R}	*crn*^{R}
olv	+	+	+	+	+	+	+

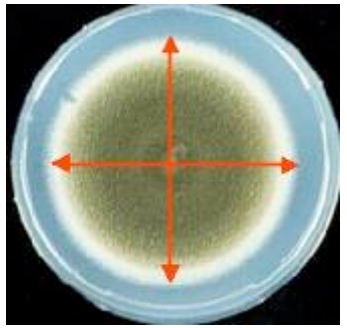


- 2^8 = 256 possible haploid segregants carrying different combinations of mutations
- Among ~2,500 segregants tested, 186 found

Among 186 strains isolated,

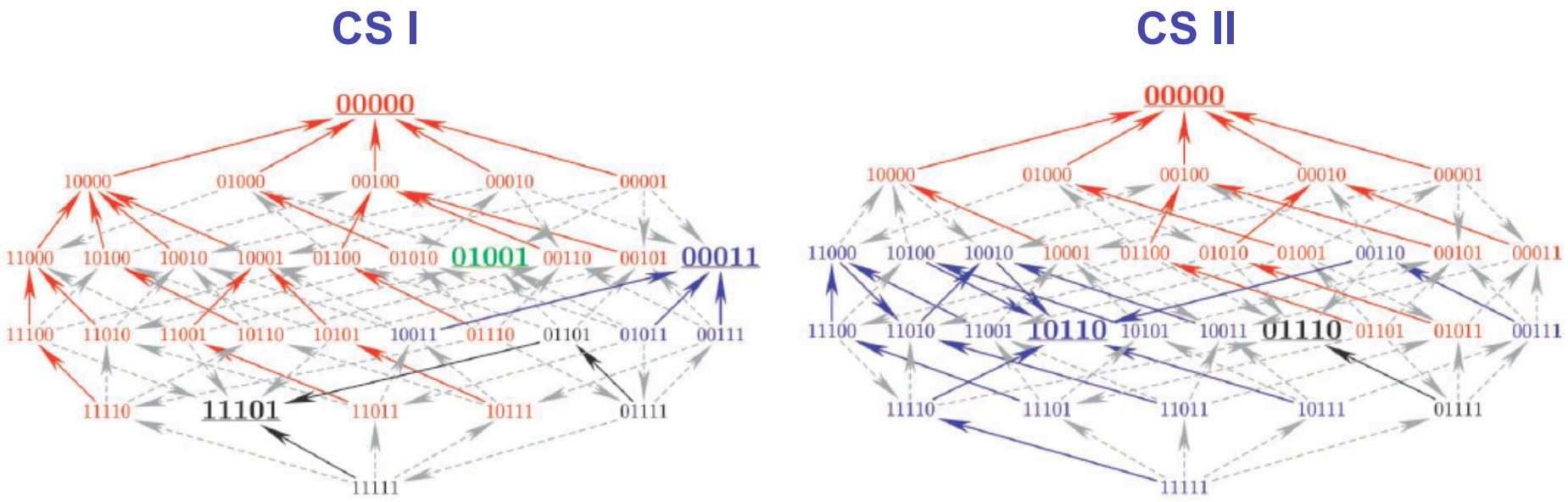
2 Complete Subsets (CS) of strains involving all 2^5 = 32 combinations of 5 individually deleterious mutations:

- **CS I**: *arg*$^-$, *pyr*$^-$, *leu*$^-$, *oli*^{R}, *crn*^{R}
- **CS II**: *arg*$^-$, *pyr*$^-$, *leu*$^-$, *phe*$^-$, *oli*^{R}



Fitness: growth rate on supplemented medium

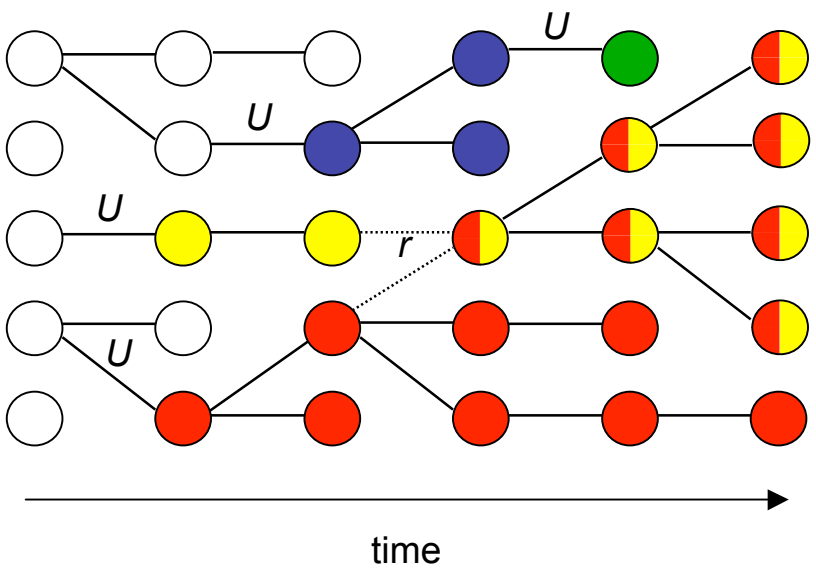
II. Arrow plots of *A. niger* landscapes



(de Visser, Park & Krug 2009 *Am. Nat.*)

- Ruggedness: several local maxima (underlined)
- Of 120 possible pathways from 11111 to 00000, only ~20% are accessible by natural selection
- Basins of attraction under 'greedy walk' dynamics shown in color

II. Simulations using Wright-Fisher model

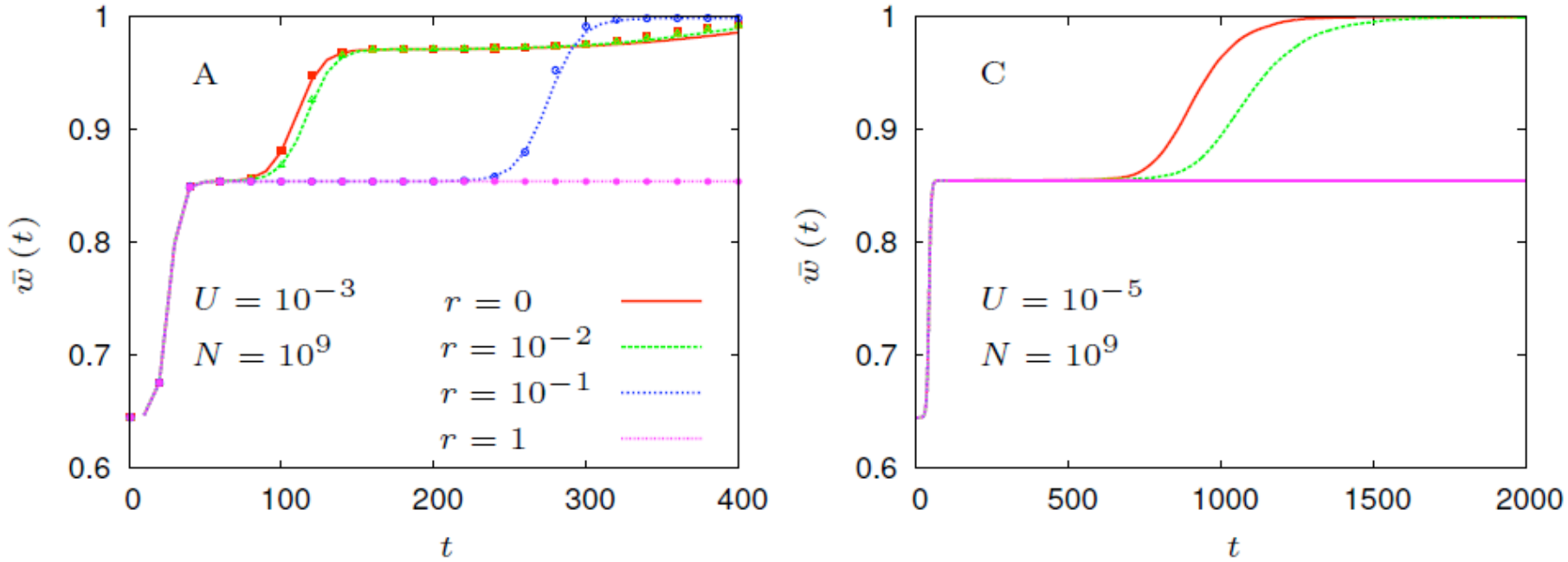


- Constant haploid population size *N*
- Reproduction with probability proportional to genotype's fitness *w*
- Mutation with probability *U* per genome (*L* = 5) per generation, changes into neighboring genotype
- Free recombination with probability *r* per pair of individuals per generation

Does sex facilitate adaptation on the *A. niger* landscapes?

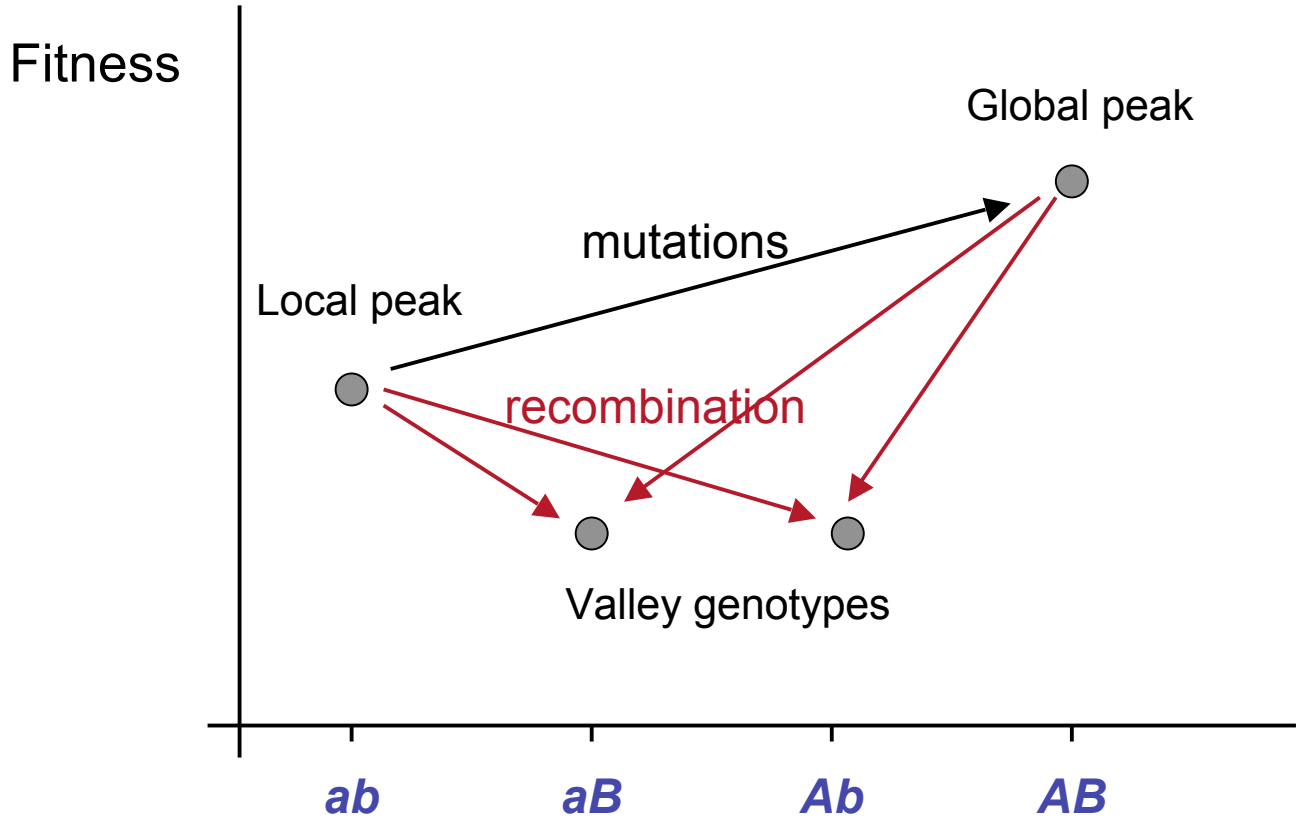
II. Adaptation on *A. niger* landscapes

CS I



- Sex generally slows down adaptation
- Only for intermediate ***U*** and ***r*** and on one landscape (CS I), sex accelerates escape from local maximum

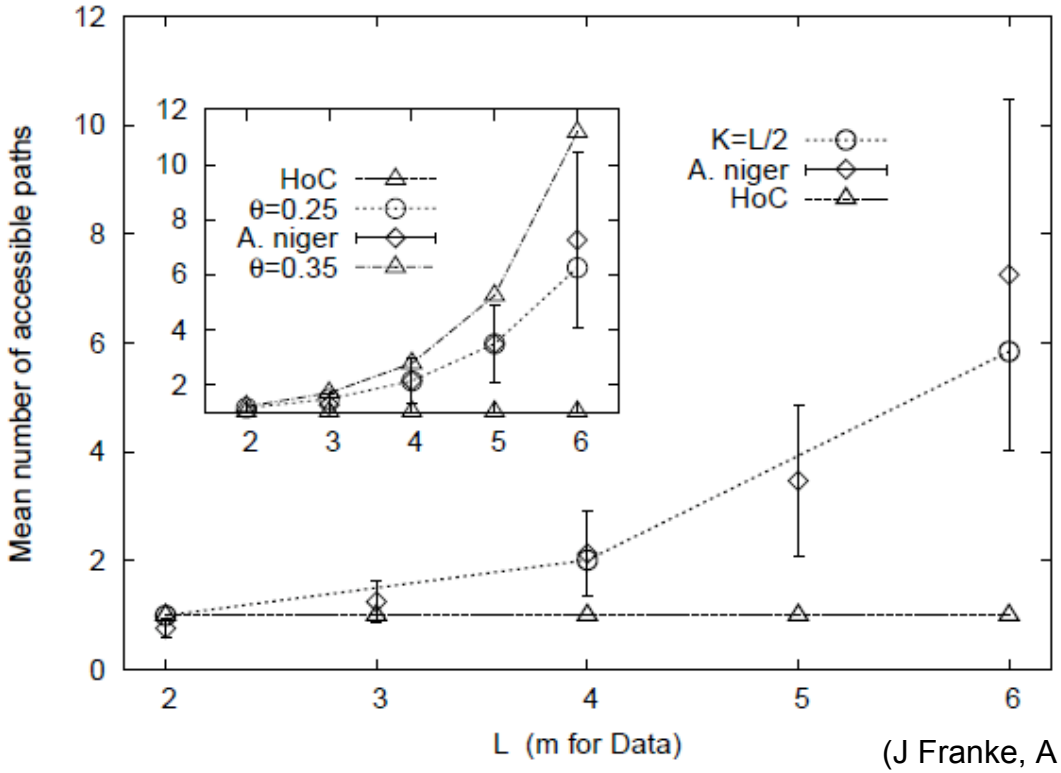
II. Sex breaks down escape genotypes



How reconcile these results with wide-spread sex?

- *A. niger* data not representative
- accessibility global peak may increase with number of loci

II. Accessibility increases with *L*



Comparison 8-locus *A. niger* data with 3 landscape models:

(i) House-of-Cards (max rugged)

(ii) Rough Mount Fuji

(iii) Kauffman's LK model

(J Franke, A Klözer, JAGM de Visser & J Krug, unpublished)

•Except for HoC model, accessibility increases with *L*: decreased accessibility per pathway overwhelmed by (factorial) increase in path number

•*A. niger* landscape fits models with intermediate ruggedness

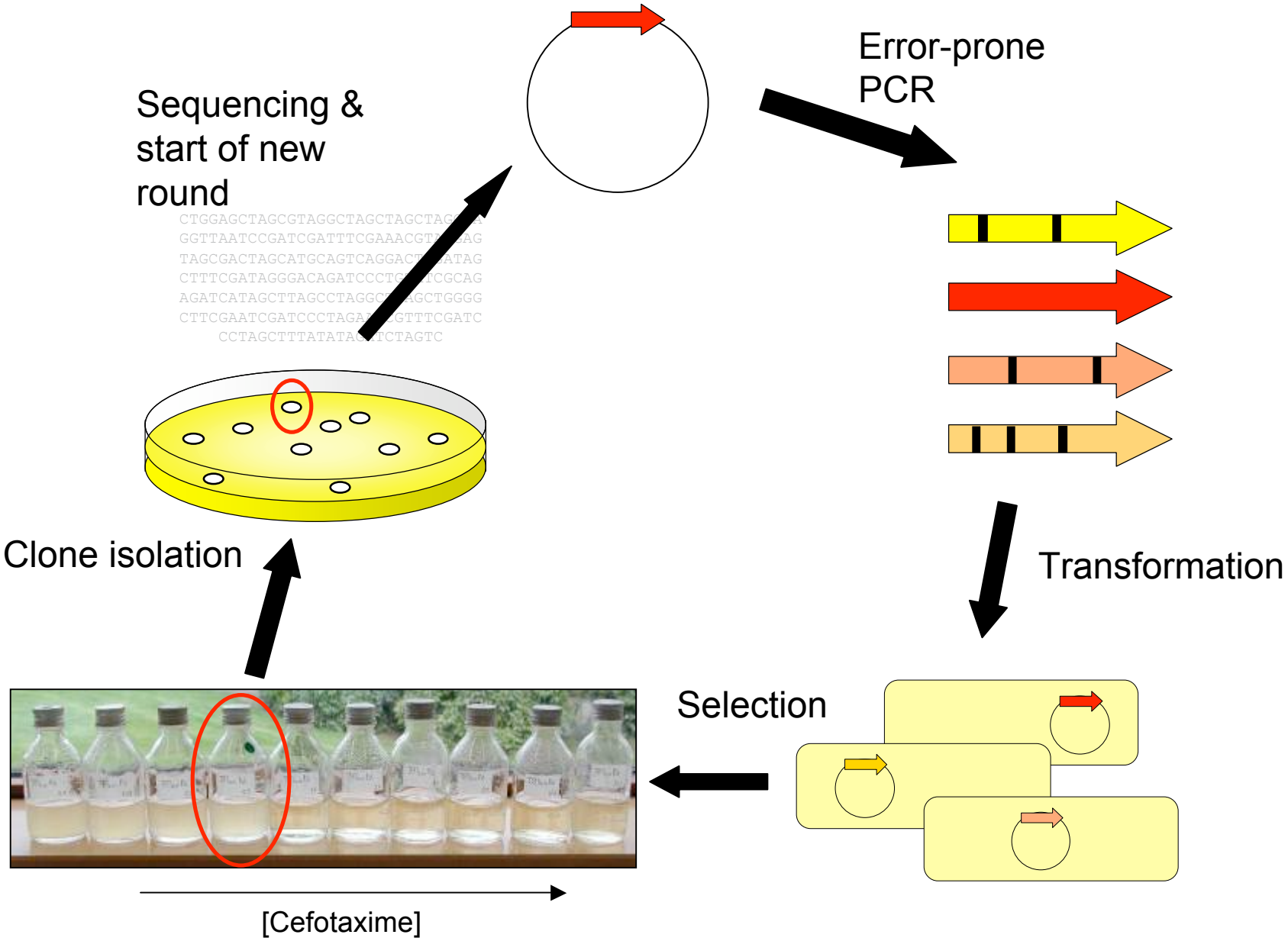
III. Exploring the fitness landscape of an antibiotic resistance enzyme

work by **Merijn Salverda**

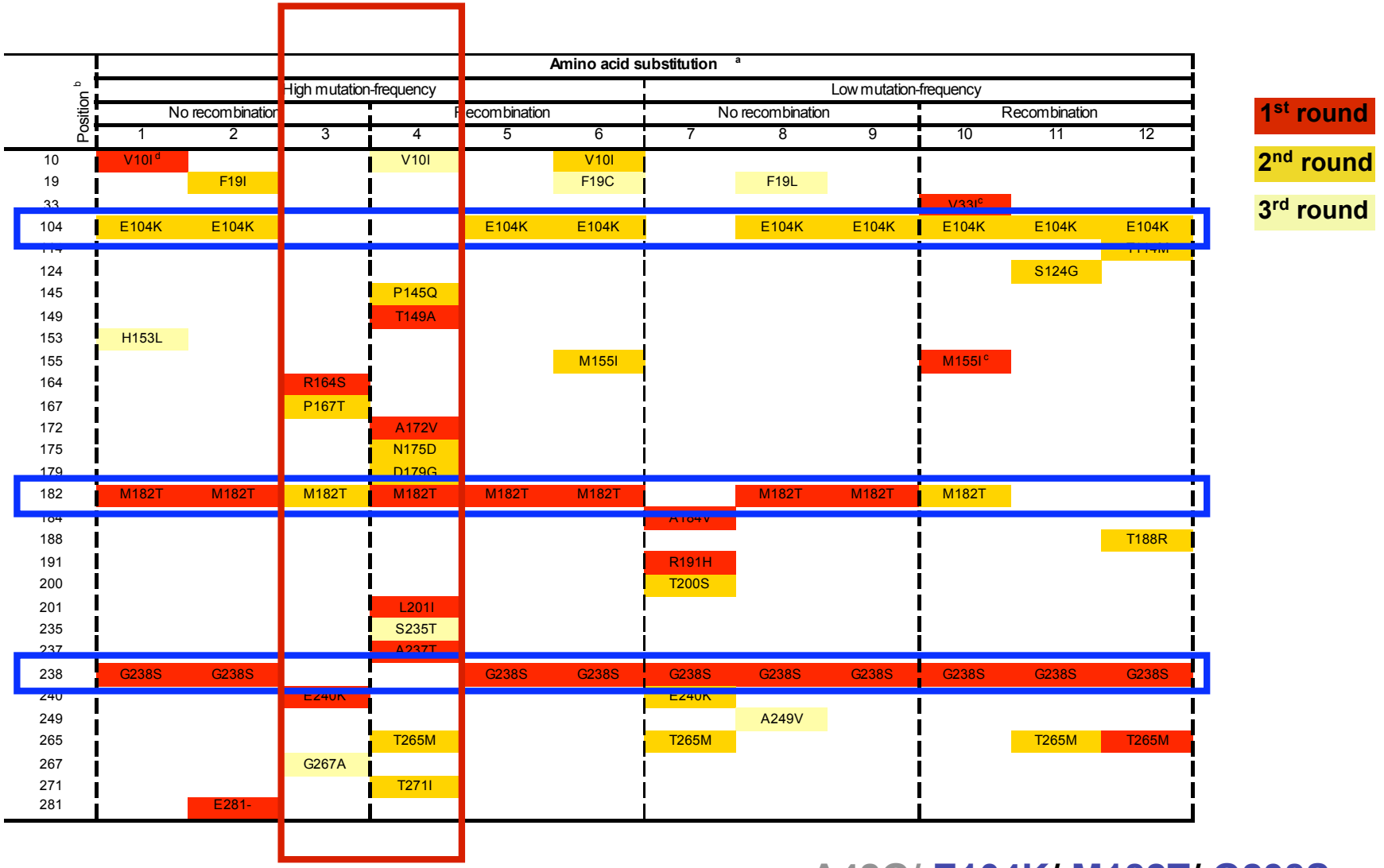
TEM-1 ß-lactamase

- Breaks down ß-lactam antibiotics (penicillins, cephalosporins)
- Low resistance to cefotaxime (Ctx)
- Five point mutations can jointly increase Ctx-resistance ~100,000-fold:
  g4205a / A42G / E104K / M182T / G238S
  (Stemmer 1994, Hall 2002, Weinreich *et al*. 2006)

III. *In vitro* evolution



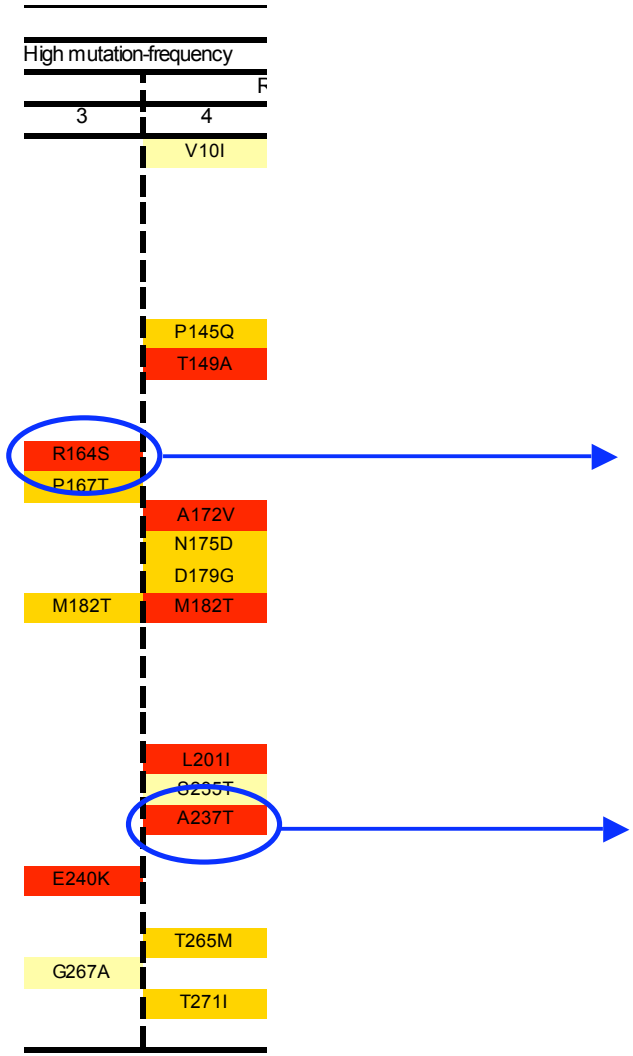
III. Amino acid substitutions 1st experiment



A42G/ E104K/ M182T/ G238S

(Salverda, Dellus, Gorter, Debets, van der Oost, Hoekstra, Tawfik & de Visser 2011 *PLoS Genet*)

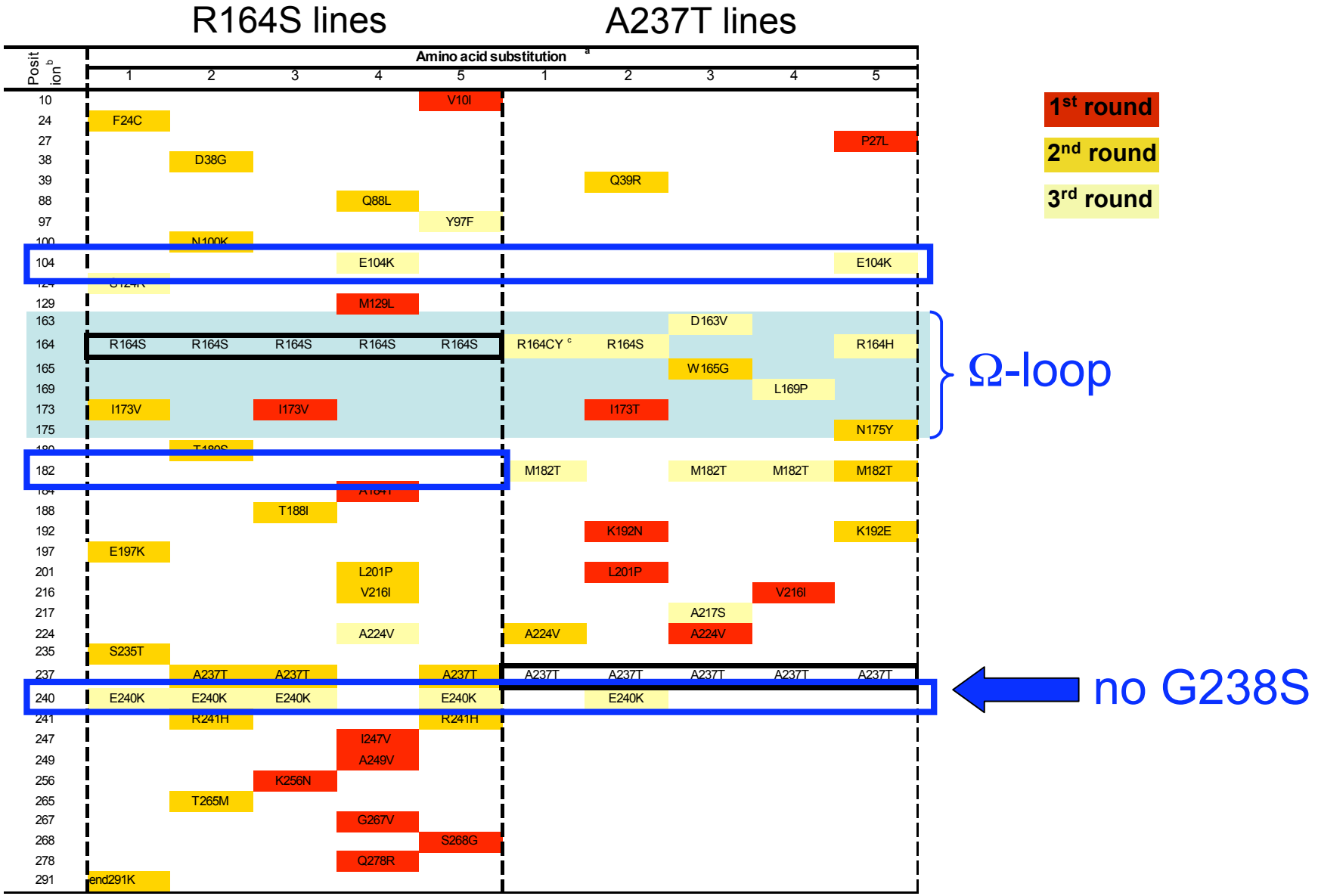
III. Candidate early epistatic mutations in two lines lacking large-effect mutation G238S



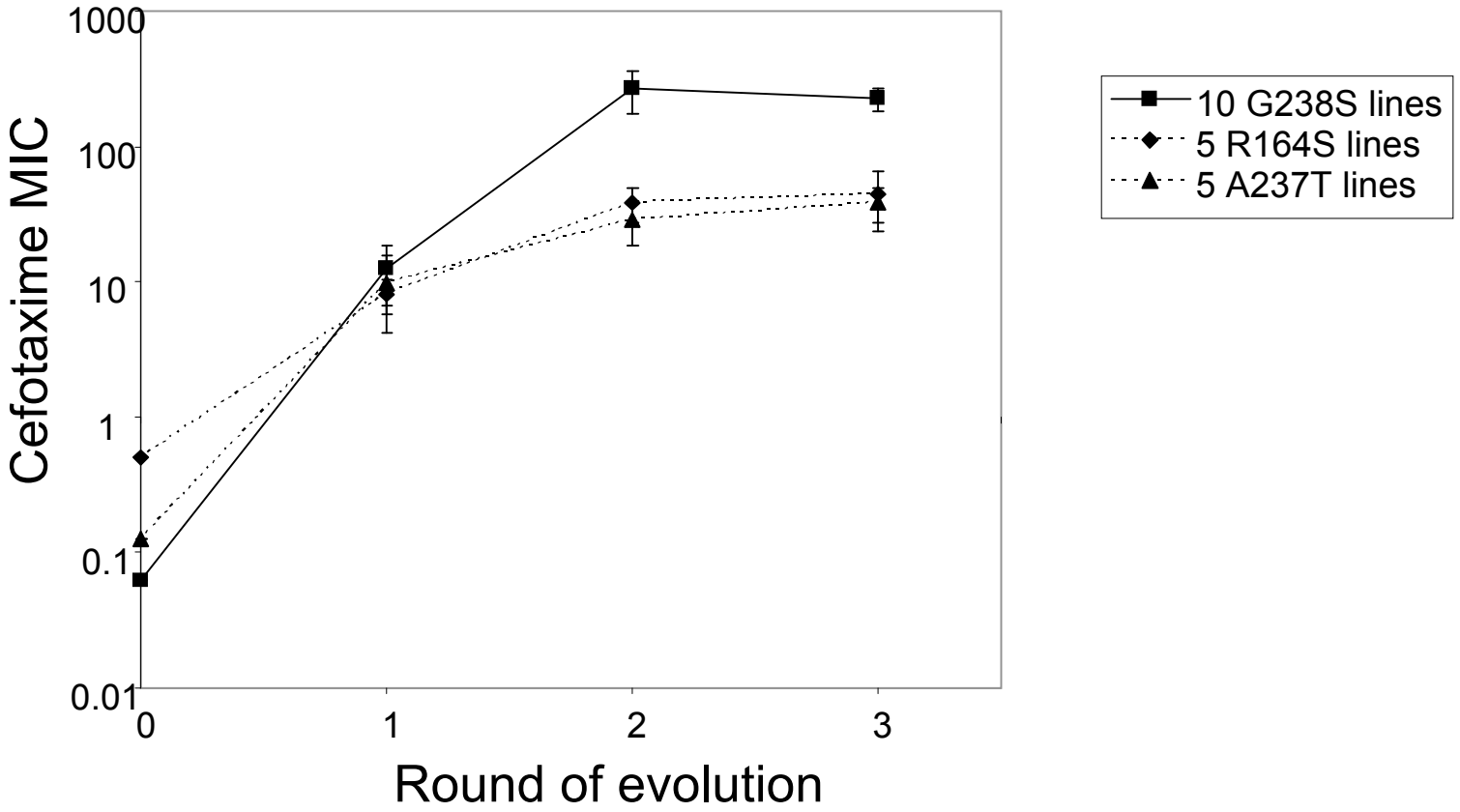
Increases Ctx-resistance and has negative epistatic interaction with G238S (Giakkoupi *et al.* 2000)

Frequently found in clinical isolates, never in combination with G238S

III. Amino acid substitutions 2nd experiment



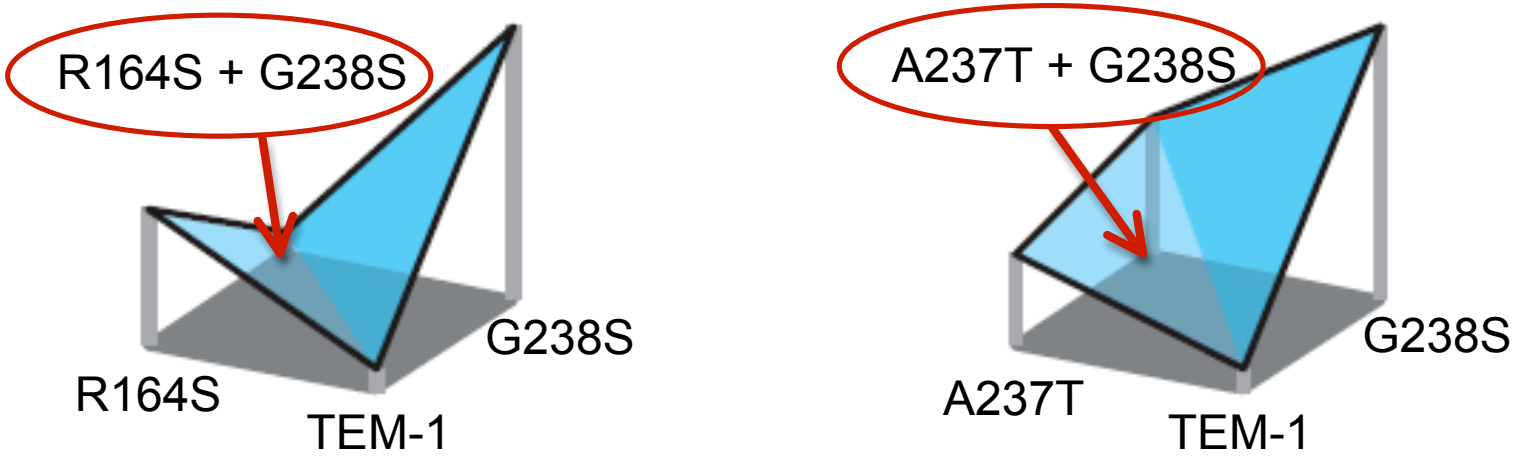
III. Alternative pathways approach lower adaptive peaks



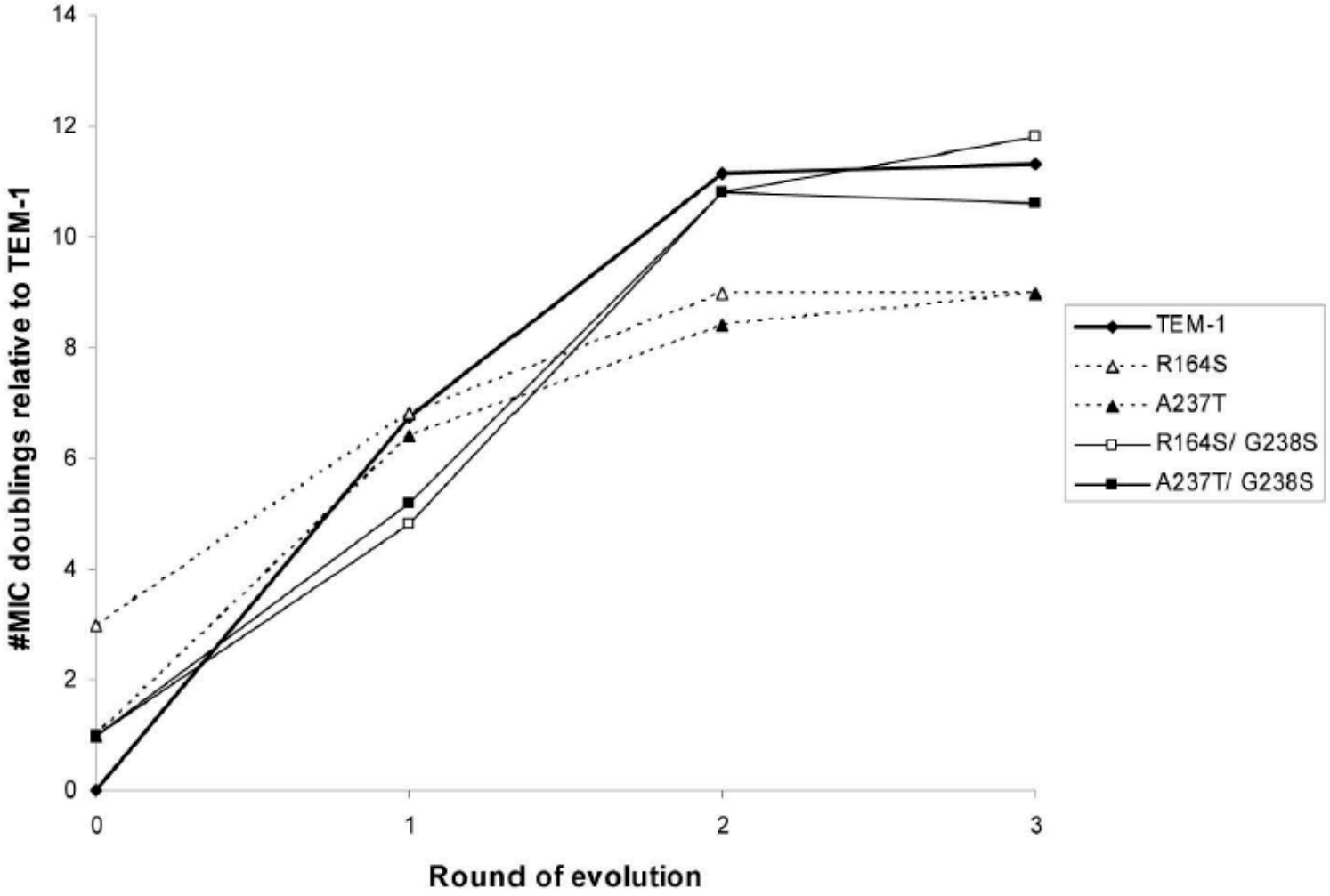
- Fitness landscape TEM-1 for ctx^R contains multiple adaptive peaks
- Initial mutations can determine which peak is approached

III. Constraints from epistatic pairs

•Do pairs of negatively interacting mutations cause adaptive constraints: is only solution reversion, or can novel mutations neutralize negative interaction?



III. MIC trajectories 3rd experiment



III. Amino acid substitutions 3rd experiment

	G238S/R164S lines					G238S/A237T lines				
position	L1	L2	L3	L4	L5	L1	L2	L3	L4	L5
4						S4N				
7				H7Q						
16	F16L									
17		A17S								
21	L21H									
24							F24S			
27						P27S				
38					D38V					
47	I47V									
56				I56T						
60		F60Y								
92		G92D					G92D			
104		E104V	E104K	E104Q	E104K	E104K		E104K	E104K	E104K
115						D115E				
120										R120K
124										S124N
153					H153R				H153R	
164	S164R	S164R	S164R	S164R	S164R		R164G			
174							P174R			
182	M182T			M182T		M182T	M182T			
184	A184V									
188			T188K							
192	K192N									
197				E197K						
198					L198V			L198I		
208	I208K									
224							A224V	A224V		
235							S235T			
237						T237A	A237T	A237T	A237T	A237T
238	G238S	G238S	G238S	G238S	G238S	G238S	G238S	G238S	G238S	G238S
240	E240K									
268								S268G		
275			R275Q						R275Q	R275L
289						H289Y				
MIC1	2	4	1	2	1	4	4	4	1	2
MIC2	64	64	128	128	256	512	32	128	64	64
MIC3	512	64	128	512	256	512	32	128	64	64

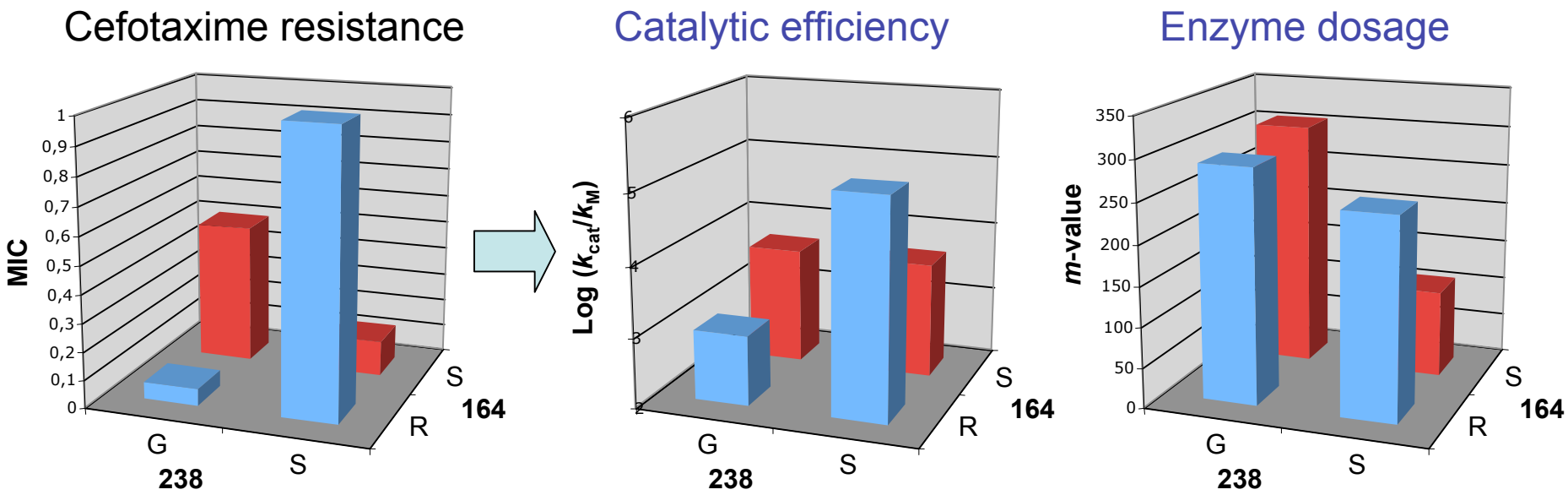
1st round

2nd round

3rd round

- Adaptive constraints from sign epistasis can be alleviated both by reversion and novel mutations
- Strength of constraint varies and correlates with strength epistasis

III. Biochemical basis epistatic interaction



- Sign epistasis between beneficial mutations G238S and R164S results from combined epistatic effects at level of enzyme activity and dosage

Summary and outlook

- Fitness landscapes can be studied empirically, either by studying dynamics and repeatability of phenotypic and molecular changes *or* by constructing mutants carrying all possible mutations of sets of mutations
- These studies increasingly show that real fitness landscapes are substantially rugged
- Future questions:
  - How does topography affect evolutionary predictability?
  - What are the evolutionary causes of different topographies?

Acknowledgments

1st study

- Danny Rozen
- Michelle Habets
- Cliff Zeyl
- Phil Gerrish
- Jeff Blanchard
- Rich Lenski
- Andreas Handel

2nd study

- Joachim Krug
- Su-Chan Park
- Jasper Franke
- Andreas Klözer

3rd study

- Merijn Salverda
- Eynat Dellus
- Florien Gorter
- John van der Oost
- Dan Tawfik
- Fons Debets
- Rolf Hoekstra