

**Sweet are the uses of adversity:**

Which, like the toad, ugly and venomous,  
Wears yet a precious jewel in his head;  
And this our life, exempt from public haunt,  
Finds tongues in trees, books in the running brooks,  
Sermons in stones, and good in every thing.

*As You Like It*

Act 2, scene 1, 12–17

**Sweet are the uses of adversity:**

Insights into adaptation and speciation  
using experimental evolution

# Rosenzweig lab Experimental evolution

Adaptation NIH

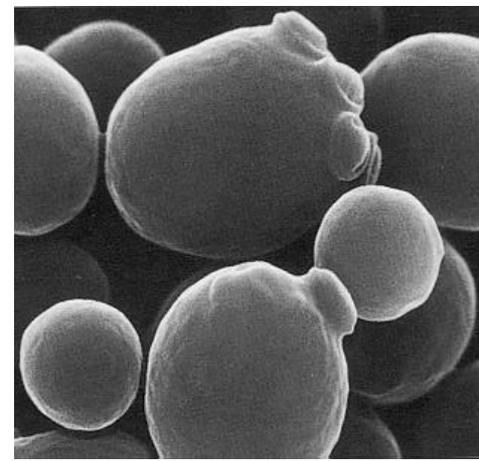
Speciation NIH, NASA

Multicellularity TEMPLETON, NASA, NSF

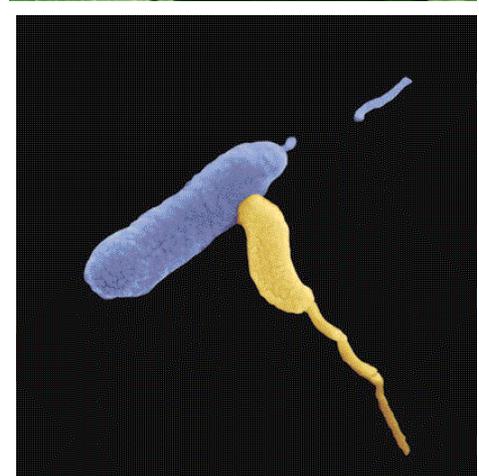
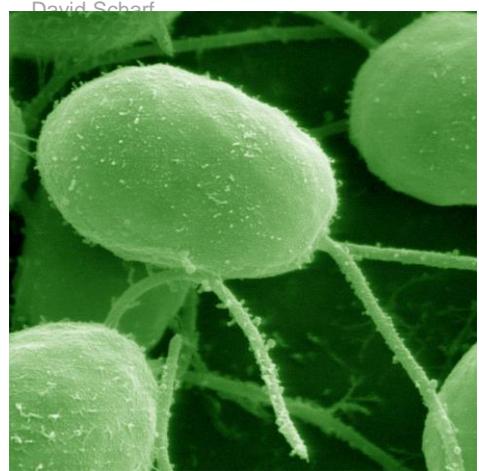
Origins of biocomplexity NIH, NASA

Origins of organelles and symbionts NASA

Chronological aging NIH



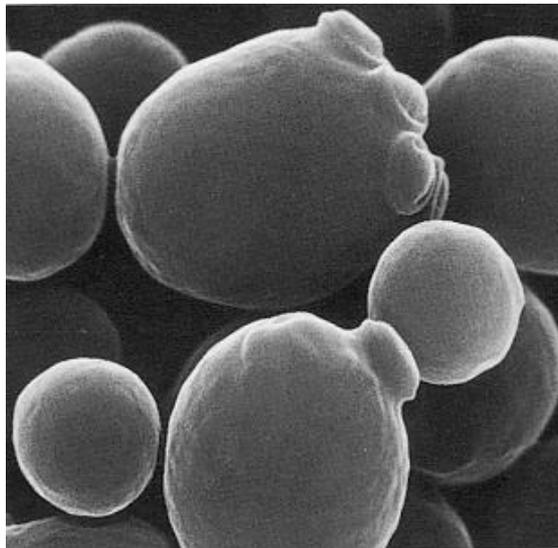
David Scharf



ence Photo Library

# The power of experimental evolution

- Specify selection pressure
- Controls and replicate trials
- Model organisms
  - short generation times
  - ease of genetic manipulation
  - cryopreserve living “fossil record”



# **We can use experimental evolution to study in real time and in vivo:**

1. Adaptation to challenging environments
2. The origin and fate of new genes
3. The stability of novel genomes
4. Connectivity in metabolic and signaling pathways  
– and the resulting evolutionary constraints.

All the above required to understand the progression of cancer, chronic infections, and the evolutionary fate of synthetic organisms

# Adaptation

Insights using experimental evolution

Jared Wenger, Jeff Piotrowski, Sai Nagarajan, Kami Chiotti,  
Dan Kvitek, Karen Schmidt and Gavin Sherlock

*Jack of all trades, master of none,  
though oftentimes better than master of one.*

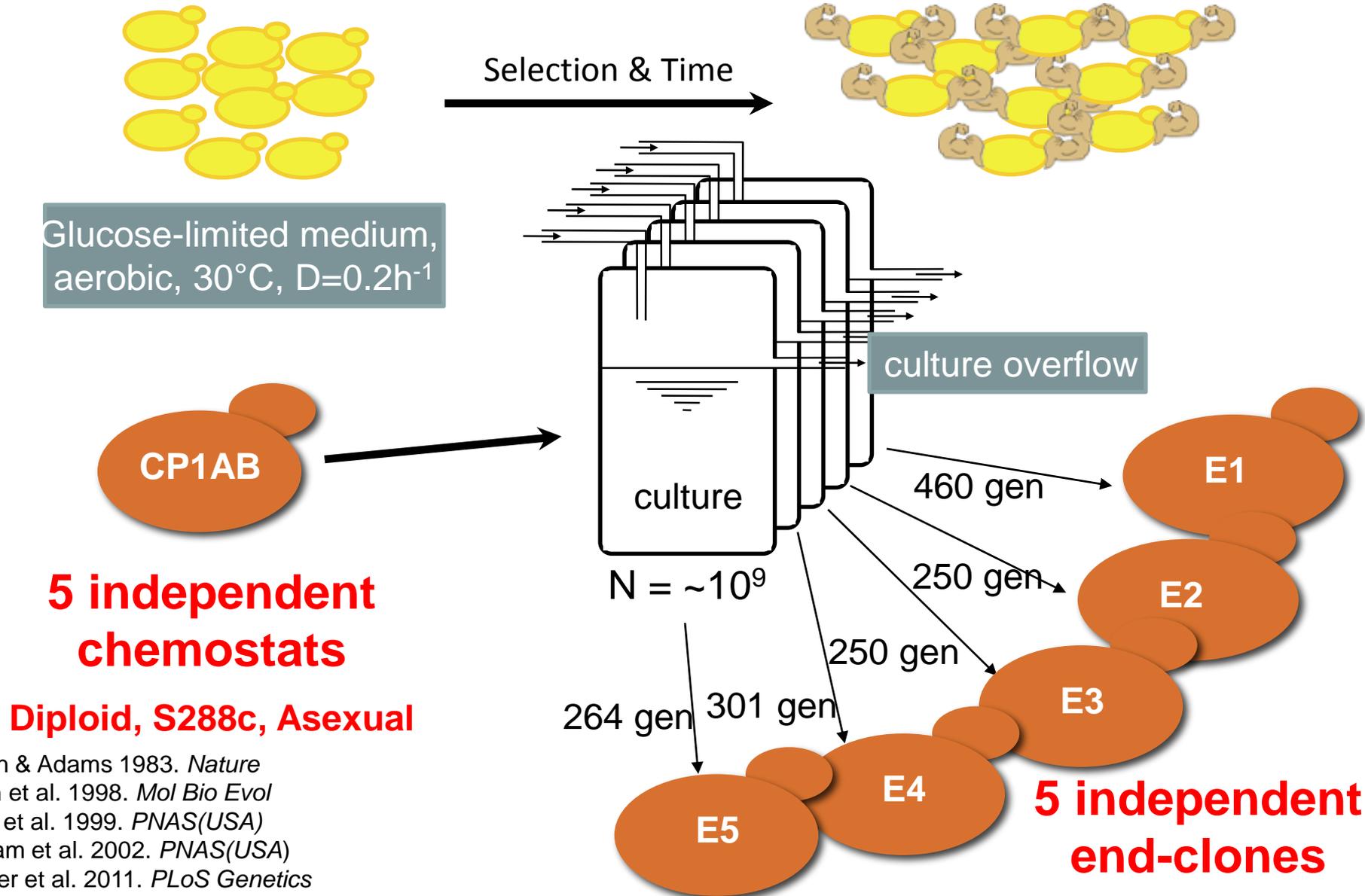
- Generalist— “jack of all trades”
- Broad niche
- Moderately fit in many environments

- Specialist – “master of one”
- Narrow niche
- Highly fit in one environment, but less so others

- What favors evolution of specialists and generalists?
- Must trade-offs occur? what are their genetic bases?

*Mutation accumulation? Antagonistic pleiotropy?*

# Evolving yeast under constant nutrient limitation



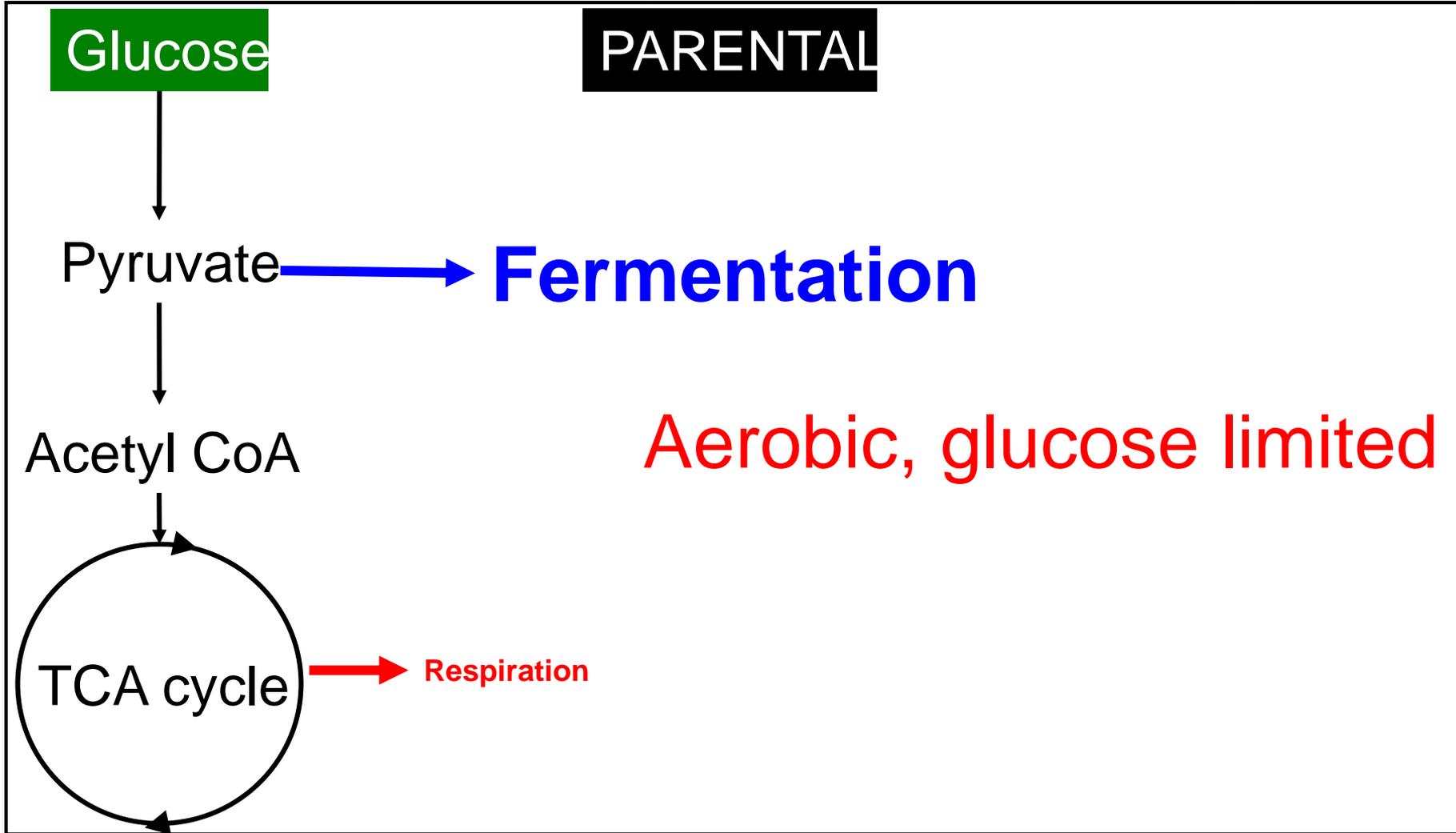
Paquin & Adams 1983. *Nature*  
Brown et al. 1998. *Mol Bio Evol*  
Ferea et al. 1999. *PNAS(USA)*  
Dunham et al. 2002. *PNAS(USA)*  
Wenger et al. 2011. *PLoS Genetics*  
Chiotti et al., 2014. *Genomics*

# Evolved clones share enhanced Pasteur effect”

(Brown, et al. 1998)  
Gene Duplications

(Ferea, et al. 1999)  
Physiology & Gene  
Expression

(Dunham, et al. 2002)  
CNVs & Chr.  
Rearrangements

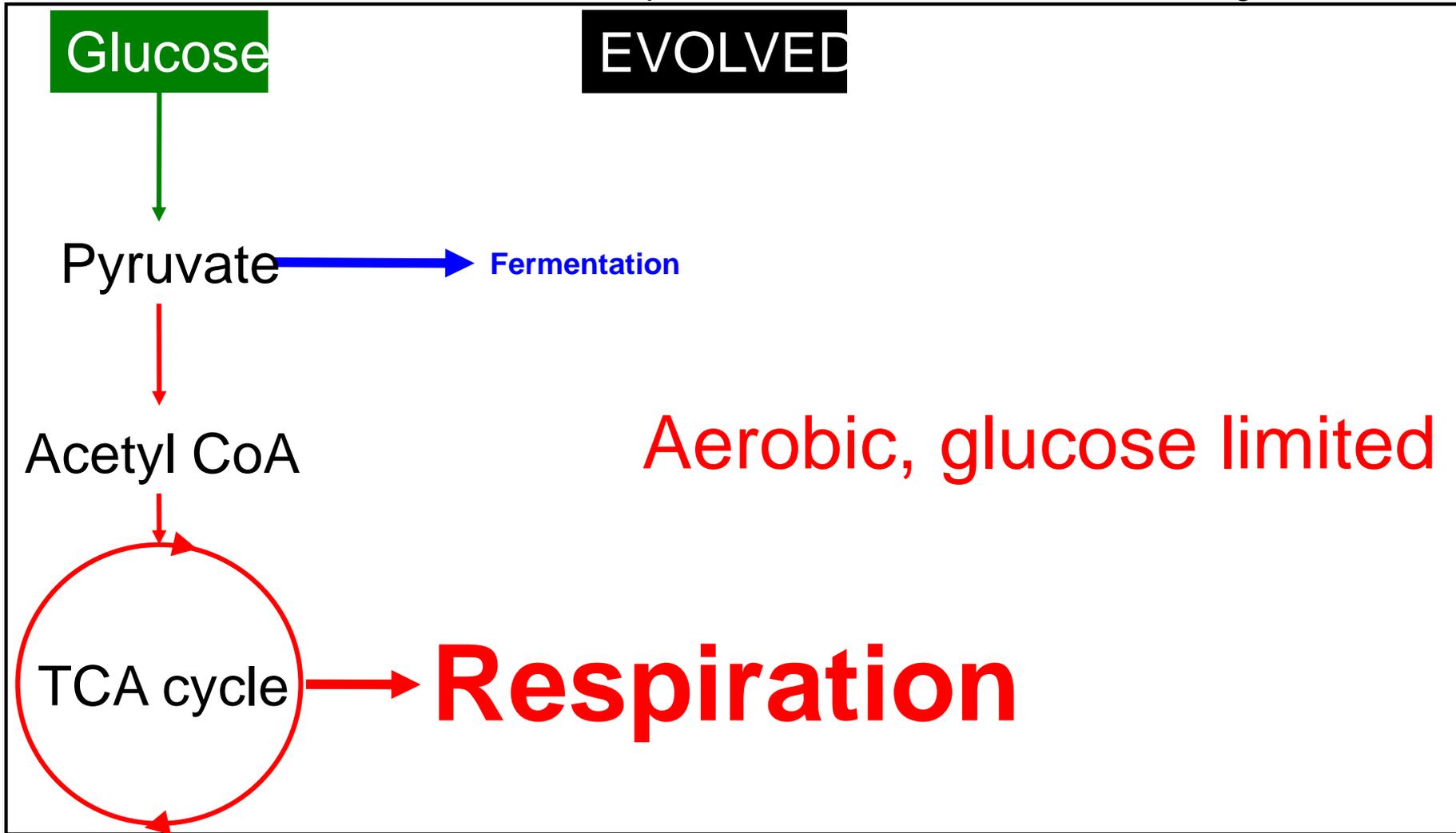


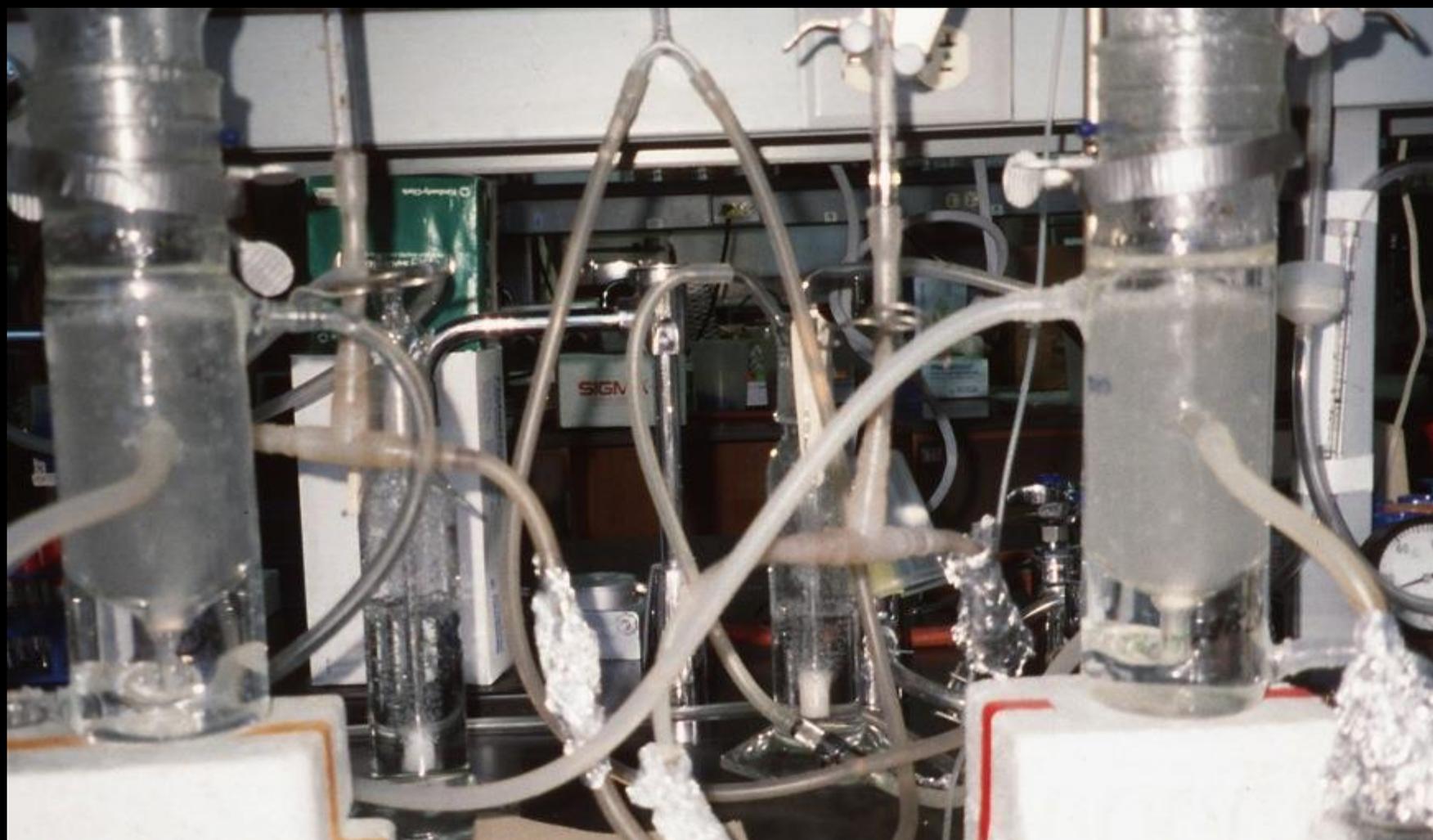
# Evolved clones share enhanced Pasteur effect”

(Brown, et al. 1998)  
Gene Duplications

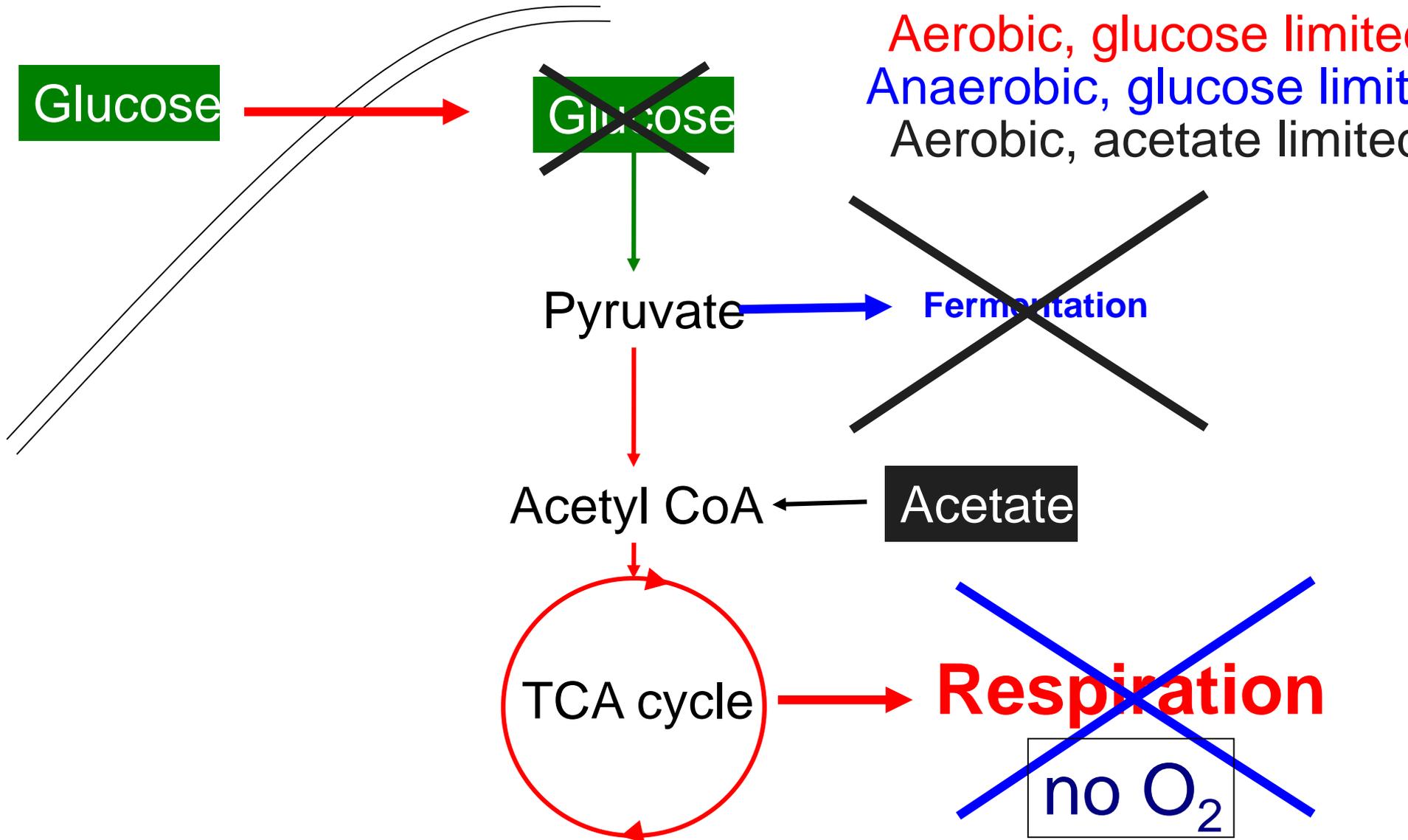
(Ferea, et al. 1999)  
Physiology & Gene  
Expression

(Dunham, et al. 2002)  
CNVs & Chr.  
Rearrangements





# How well do evolutionarily adapted clones perform in different environments?

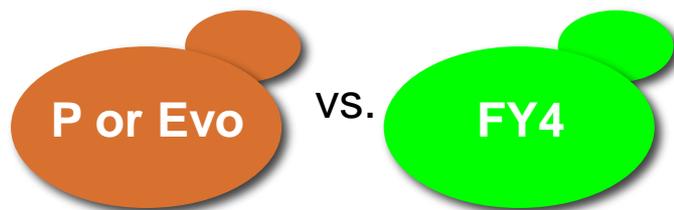


How well do evolutionarily adapted clones perform in different environments?

Question: Has selection favored evolution of generalists, specialists or both?

Experiments:

**Fitness by competition**



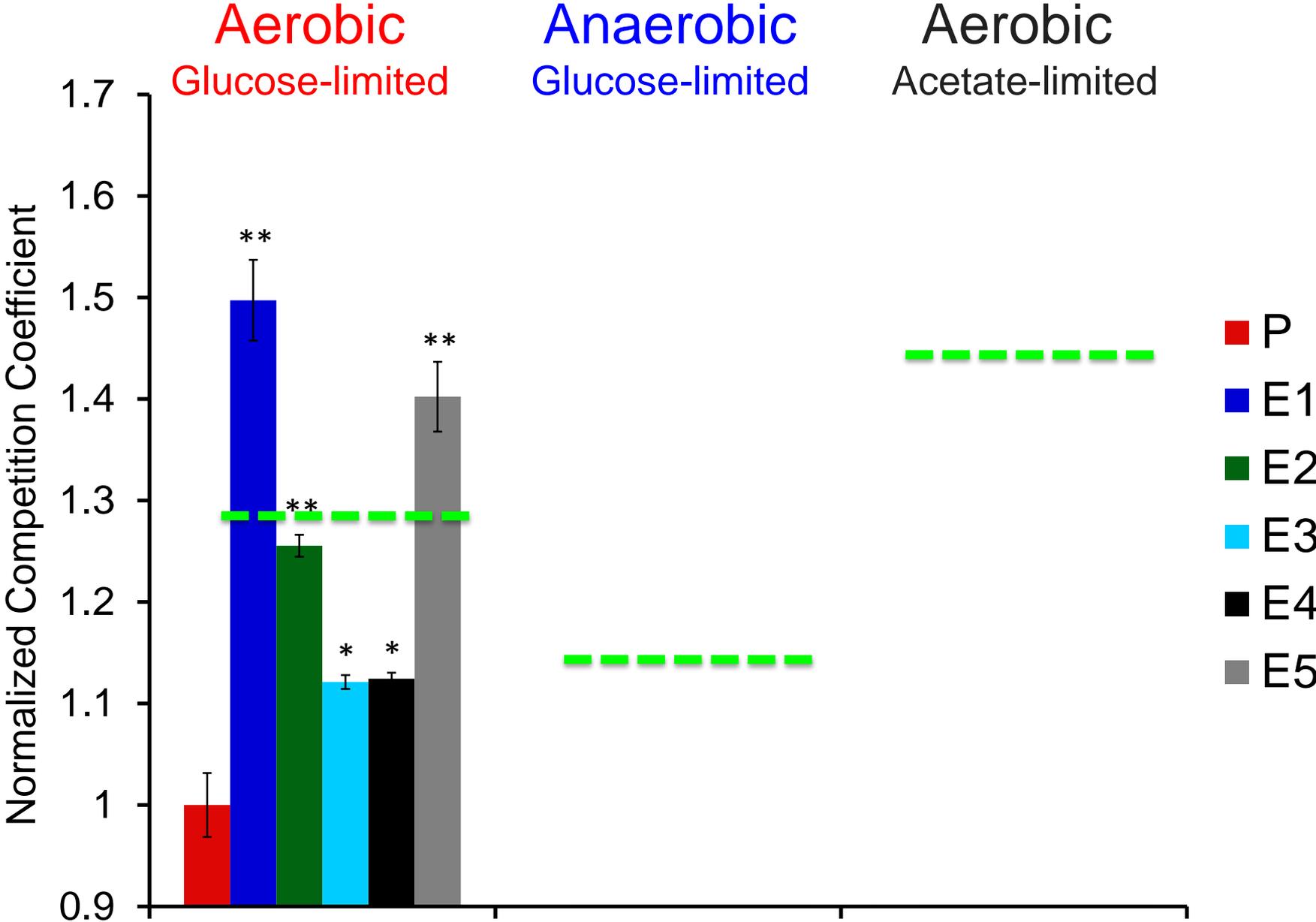
15 generations

**Gene expression profiles**



Steady state  
(monoculture)

# Evolved clones fitter than ancestor in all 3 environ



\*p<0.05, \*\*p<0.01 t-test

Question: **Are evolved strains generalists?**

**Yes**, at least in carbon-limited environments

Follow-up: **What is the genetic basis?**

Experiment: Sequence parent and evolved clones

Call SNPs, Indels, SVs

# SNPs + Indels in evolved relative to ancestor

## E1 (460 generations)

chrII	40354	non-syn	het	I 405 L	BNA4
chrII	336309	non-syn	het	A 170 P	REB1
chrII	649735	non-syn	het	V 619 I	NGR1
chrIV	63484	non-syn	het	Q 512 H	CDC13
chrIV	1E+06	intergenic	het		HXT7
chrIV	1E+06	intergenic	het		HXT7
chrV	246511	intergenic	het		SAP1/CAJ1
chrV	246512	intergenic	het		SAP1/CAJ1
chrVII	96464	syn	het		MIG2
chrVII	674723	non-syn	het	K 845 T	VAS1
chrVIII	71471	non-syn	het	G 400 V	YHLO17W
chrVIII	142567	non-syn	het	E 329 Q	DED81
chrX	514530	non-syn	hom	V 162 F	NUP85
chrXI	247730	syn	het		YKLI02C
chrXI	365542	syn	het		RGT1
chrXII	353830	non-syn	het	S 3304 T	MDN1
chrXII	445331	intergenic	het		YLR152C/ACS2
chrXII	855304	non-syn	het	S 36 L	YLR365W
chrXIII	136753	non-syn	het	S 418 R	POB3
chrXIV	399953	non-syn	het	G 196 V	TOM70
chrXIV	429369	non-syn	het	K 545 Q	MET4
chrXV	172862	non-syn	het	L 598 W	IRA2
chrXV	780680	intergenic	hom		SNR17A/DFR1
chrXVI	113951	non-syn	het	G 1767 D	FAS2
chrXVI	422590	non-syn	het	S 549 *	MUK1
chrXVI	489678	non-syn	het	R 562 L	SVL3
chrXVI	549440	non-syn	het	P 320 S	AEP3
chrXVI	640445	non-syn	het	Q 308 H	ARP7

28

chrXIV	619222	het	+A	early stop (1059->894aa)	SIS1
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## E2 (250 generations)

chrIII	303342	intergenic	het		YCR101/2
chrIV	573014	non-syn	het	V 790 F	MAK21
chrIV	677838	intergenic	het->hom		FOB1/ALT2
chrVII	120136	non-syn	het	T 259 K	MCM6
chrVIII	85160	intergenic	het		YAP3/trNA-Val
chrX	427833	non-syn	het	C 895 F	CYR1
chrXII	898188	5' UTR	het		RPS29A
chrXV	183953	non-syn	het	L 758 I	AVO1
chrXVI	549450	non-syn		E 323 V	AEP3
chrXVI	549451	syn			AEP3

10

## E3 (250 generations)

chrIV	475250	non-syn	het	Y 403 D	RAD61
chrIV	1E+06	non-syn	het	V 98 L	SBE2
chrVI	200805	non-syn	het	T 315 K	PES4
chrVI	215616	non-syn	het	P 773 A	MET10
chrVIII	114170	non-syn	het	L 248 P	GPA1
chrVIII	405595	non-syn	het	C 876 F	RTT107
chrIX	301062	non-syn	het	C 65 Y	YIL029C
chrXIII	46350	intergenic	het		CTK3/BUL2
chrXIV	155355	non-syn	het	L 85 F	ORC5
chrXV	76249	non-syn	het	T 617 K	ALR1
chrXVI	549441	non-syn	hom->het	P 320 Q	AEP3

11

## E4 (301 generations)

chrII	706282	non-syn	het	M 169 I	ALG7
chrVII	147972	intergenic	het		CDC55/RPS26A
chrVII	332626	non-syn	het	K 615 E	PAN2
chrIX	69914	non-syn	hom	N 1180 K	SLN1
chrXIII	729970	non-syn	het	G 385 C	RRP5
chrXV	379626	intergenic	het		HST3/BUB3
chrXV	821294	syn	het		PNT1
chrXV	878197	intergenic			MBF1/BUD7

8

## E5 (264 generations)

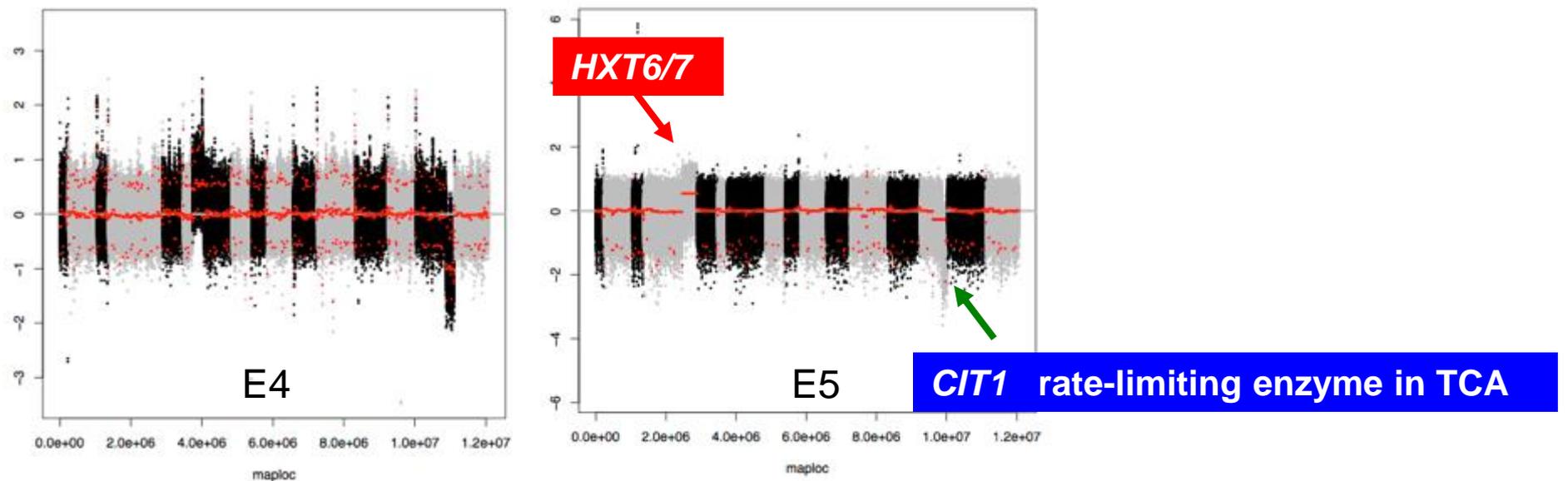
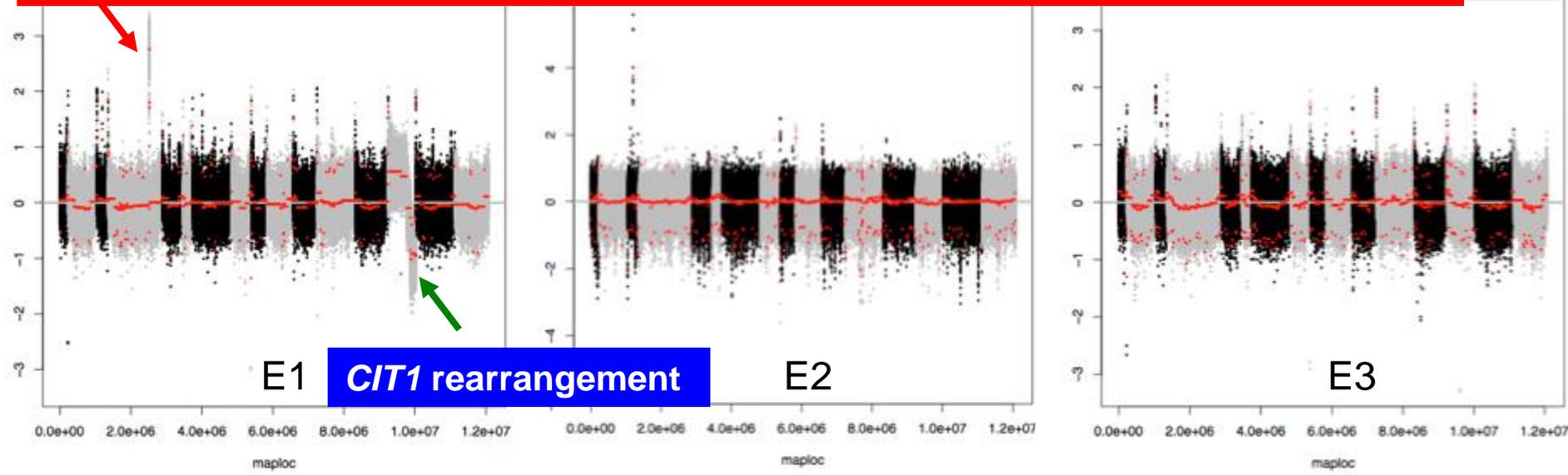
chrVII	34321	syn	het		ZIP2
chrVII	126873	non-syn	het	F 724 S	MDS3
chrVII	155430	non-syn	het	P 197 T	STR3
chrVIII	389339	intergenic	het		YHRCdelta10
chrIX	166514	intron	het		MOB1
chrX	566099	non-syn	het	T 223 I K	TOR1
chrXI	644128	intergenic	het		SIR1/FLO10
chrXII	933373	syn	hom		YLR407W
chrXV	452942	non-syn	het	I 175 F	ALG8

9

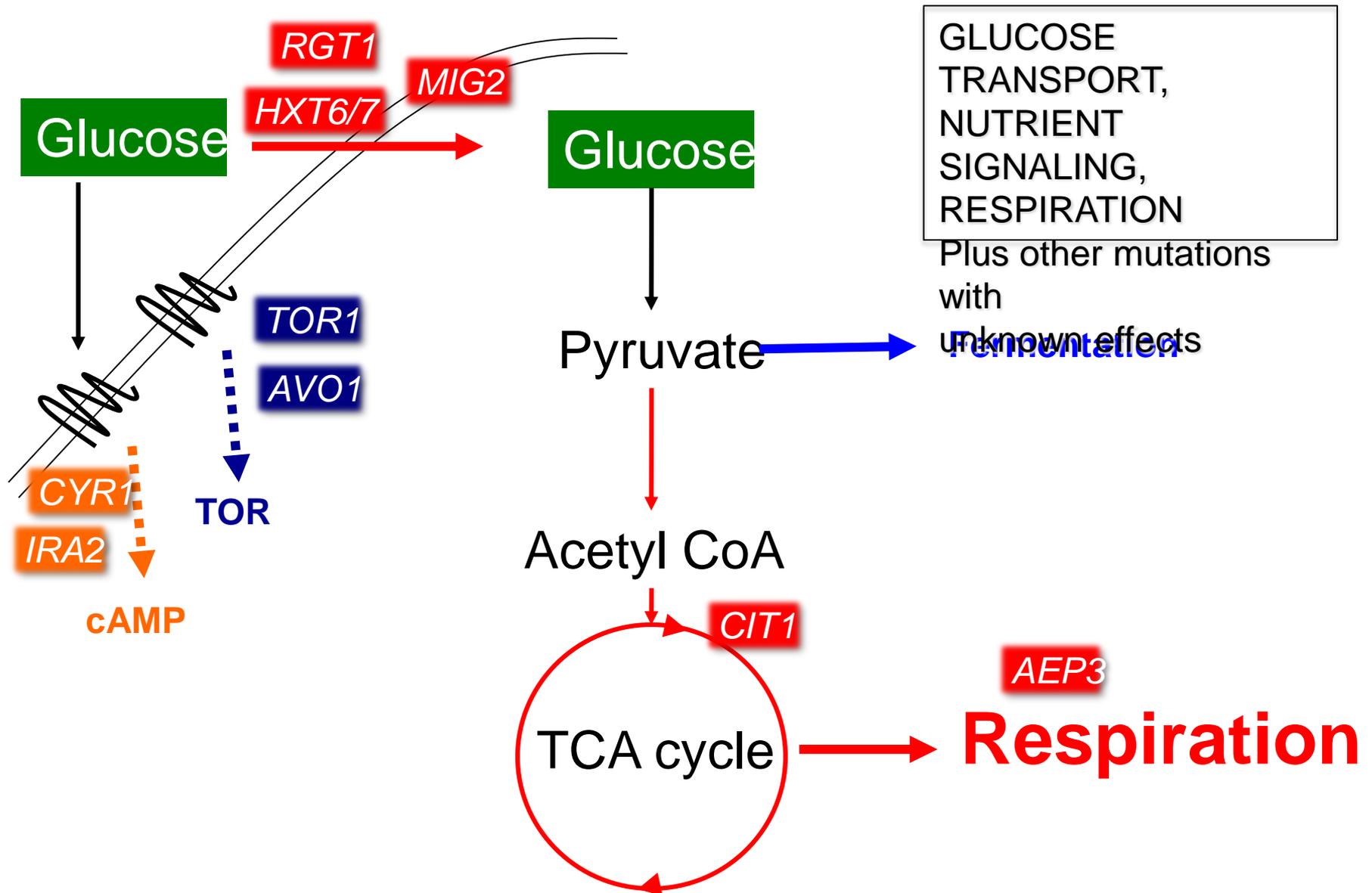
chrVIII	490972	het	-G	early stop (321->119aa)	NVJ1
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# Adaptation via large-scale chromosomal changes (SV)

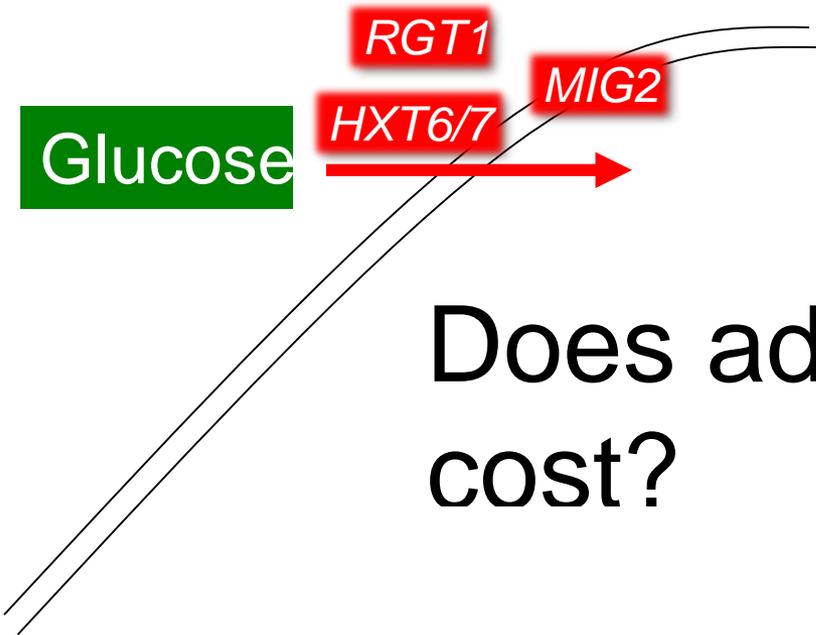
**HXT6/7** a novel chimaeric encoding high affinity glucose transporter



# How do these mutations increase fitness in multiple resource limiting environments?



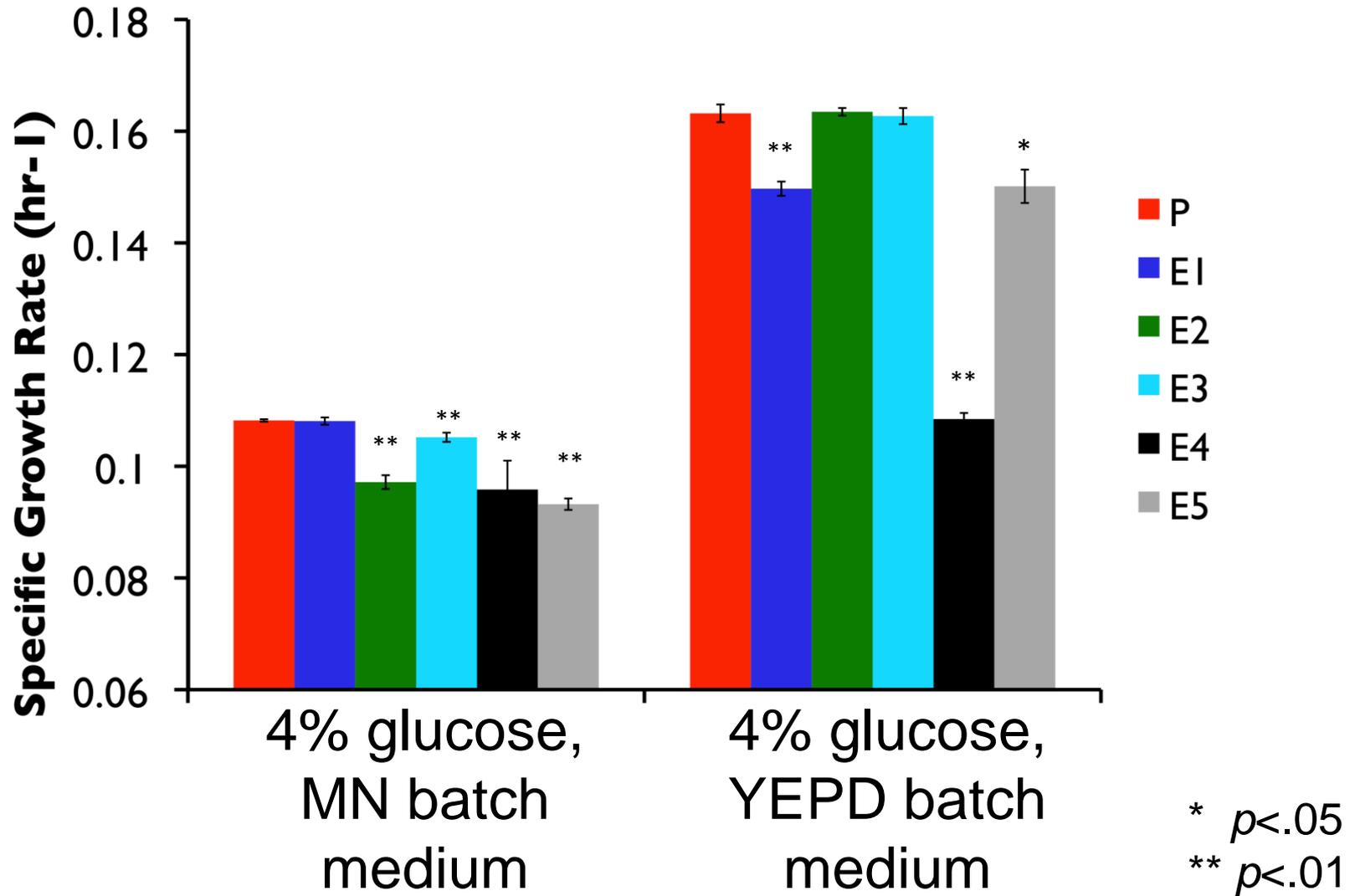
# Do trade-offs result?



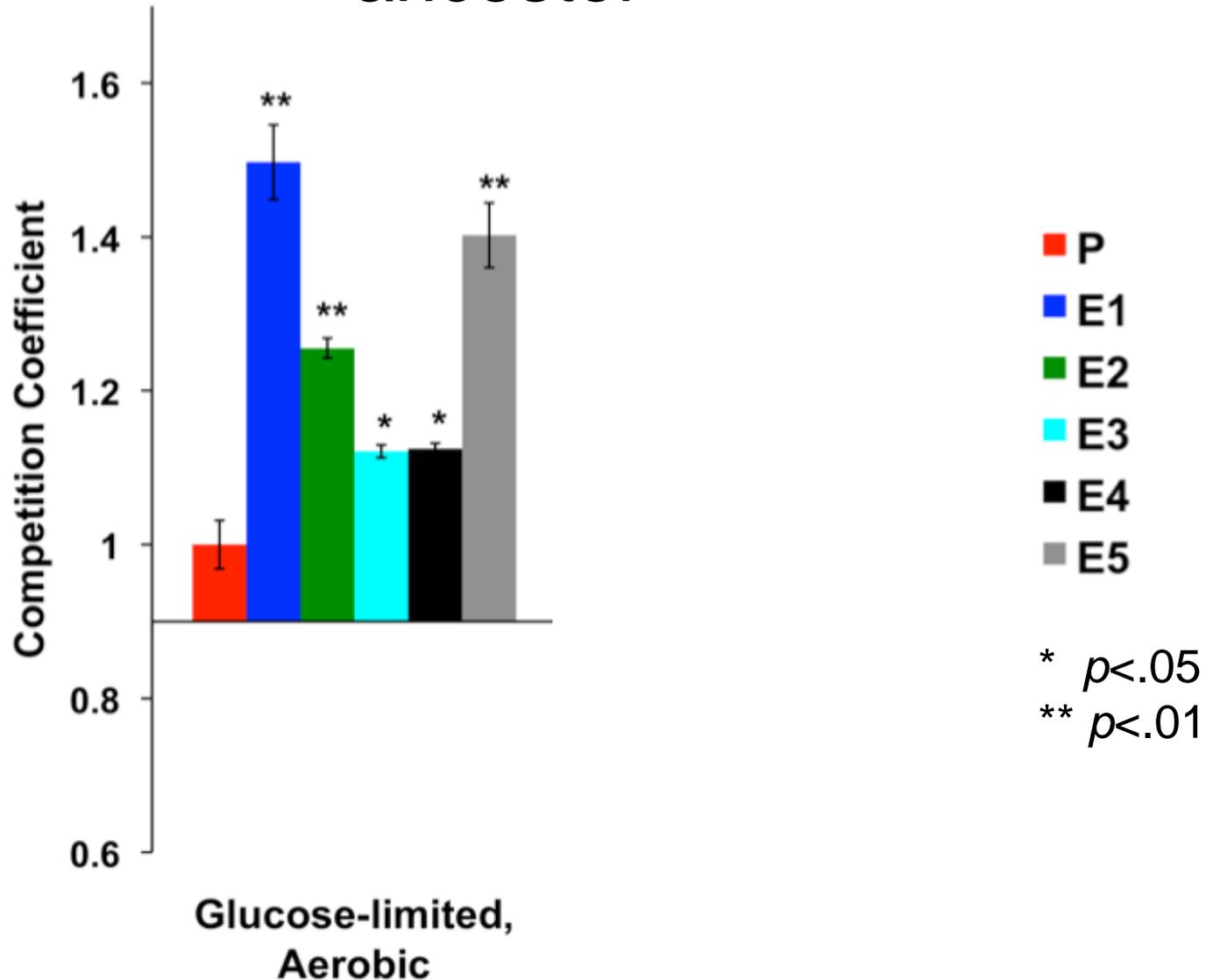
Does adaptation carry a cost?

assimilation be deleterious?

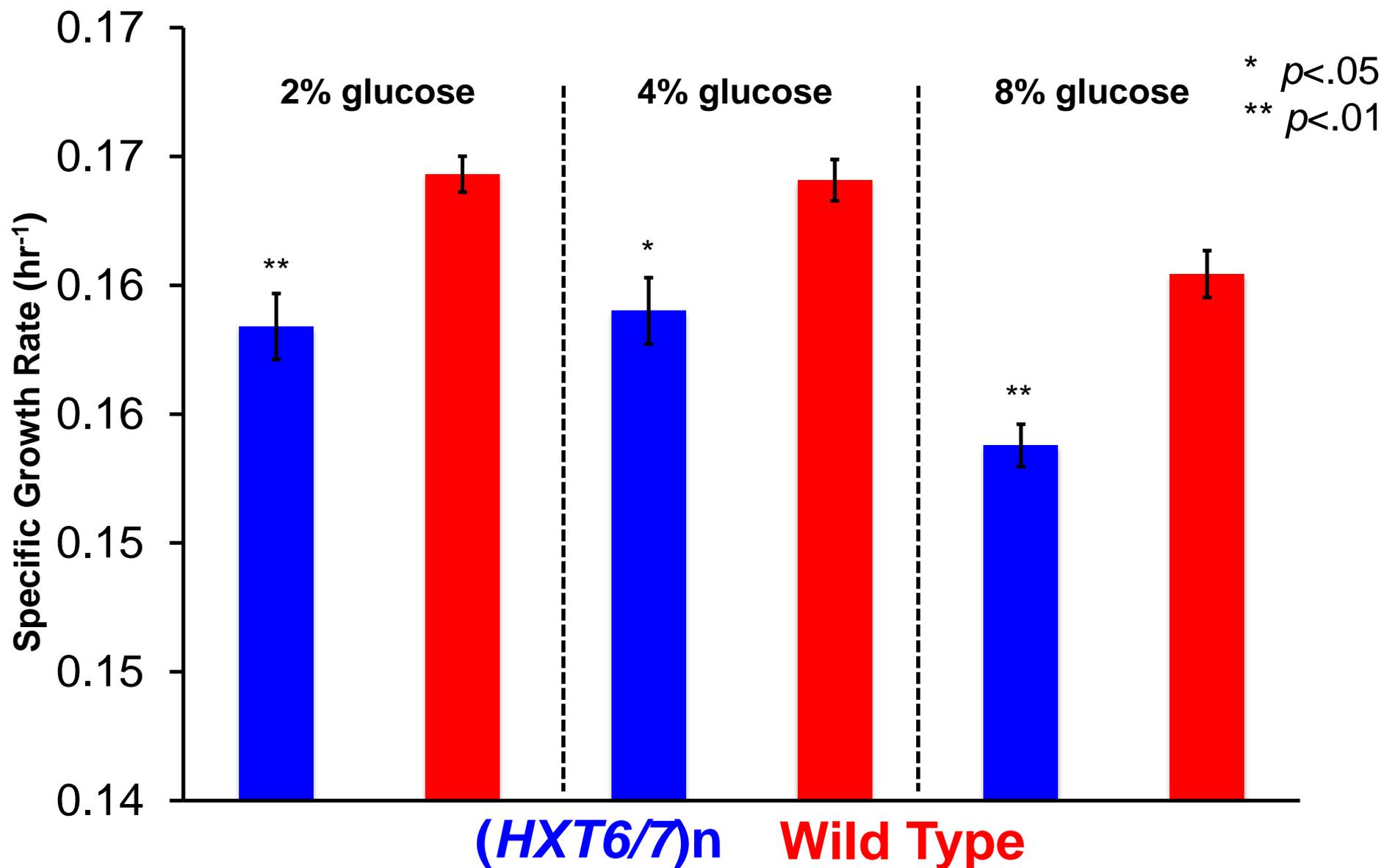
When glucose is non-limiting, evolved clones are no fitter or are less fit than their common ancestor



When glucose is non-limiting, evolved clones are  
no fitter or are less fit than their common  
ancestor



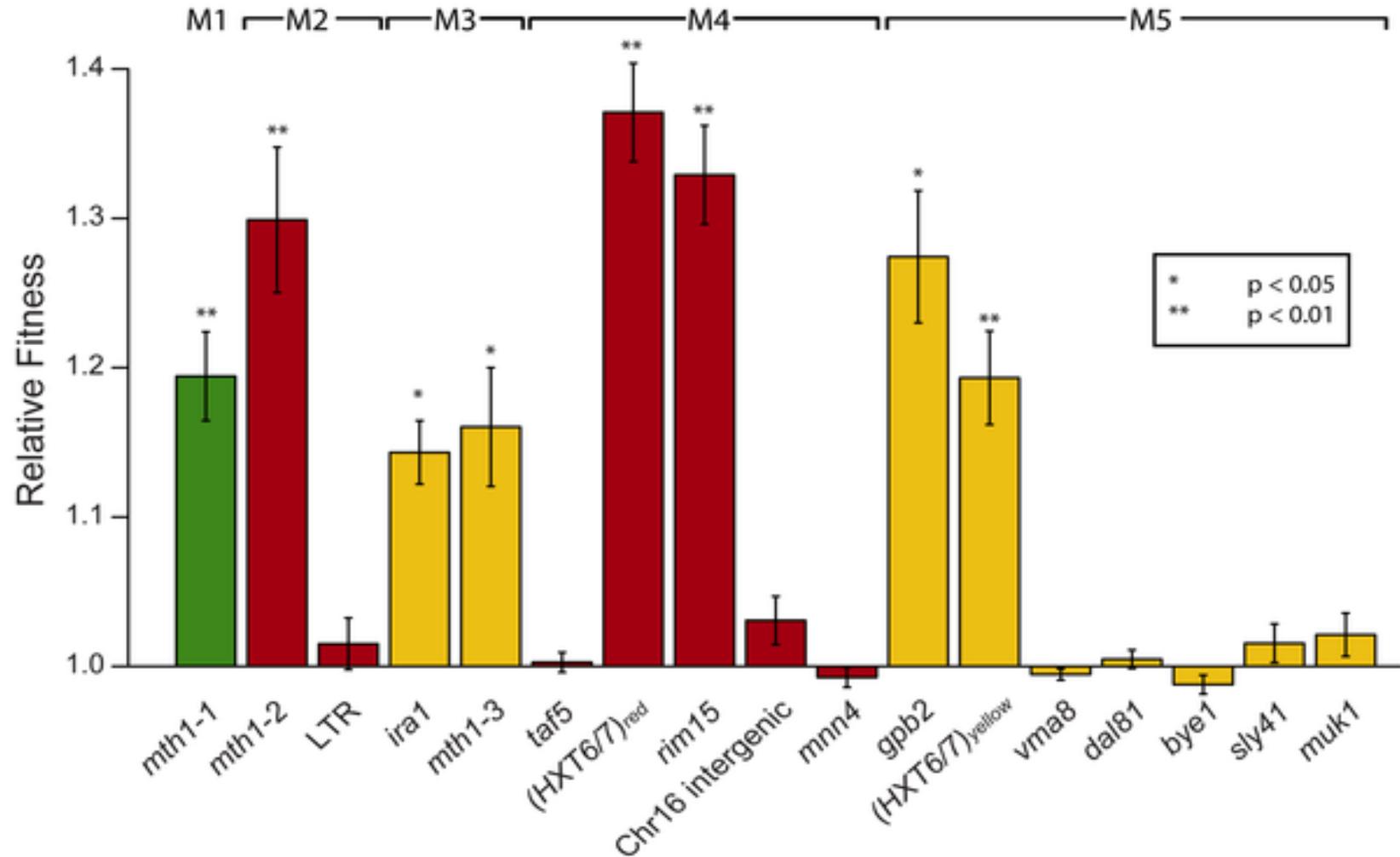
# HXT6/7 amplification results in antagonistic pleiotropy



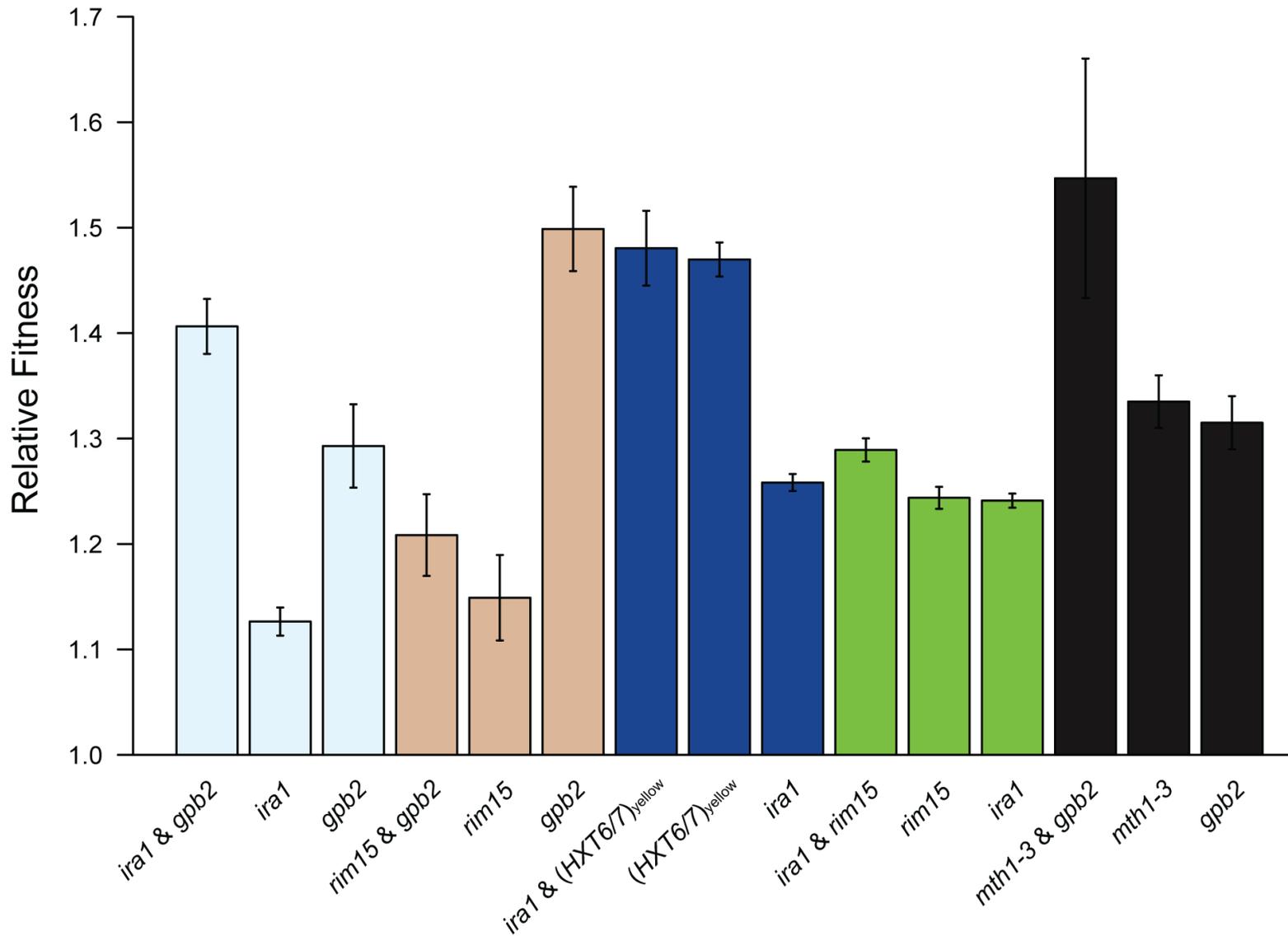
# Insights into adaptation using exp evol

- Cells evolving under glucose limitation become carbon limitation generalists, or “Hunger Artists”
- Adaptations arise via *both* gain- and loss-of-function mutations in nutrient transport/nutrient signaling/ respiration
- Trade-offs appear when the resource that was limiting during evolution is presented in excess.
- Trade-offs attributable to antagonistic pleiotropy

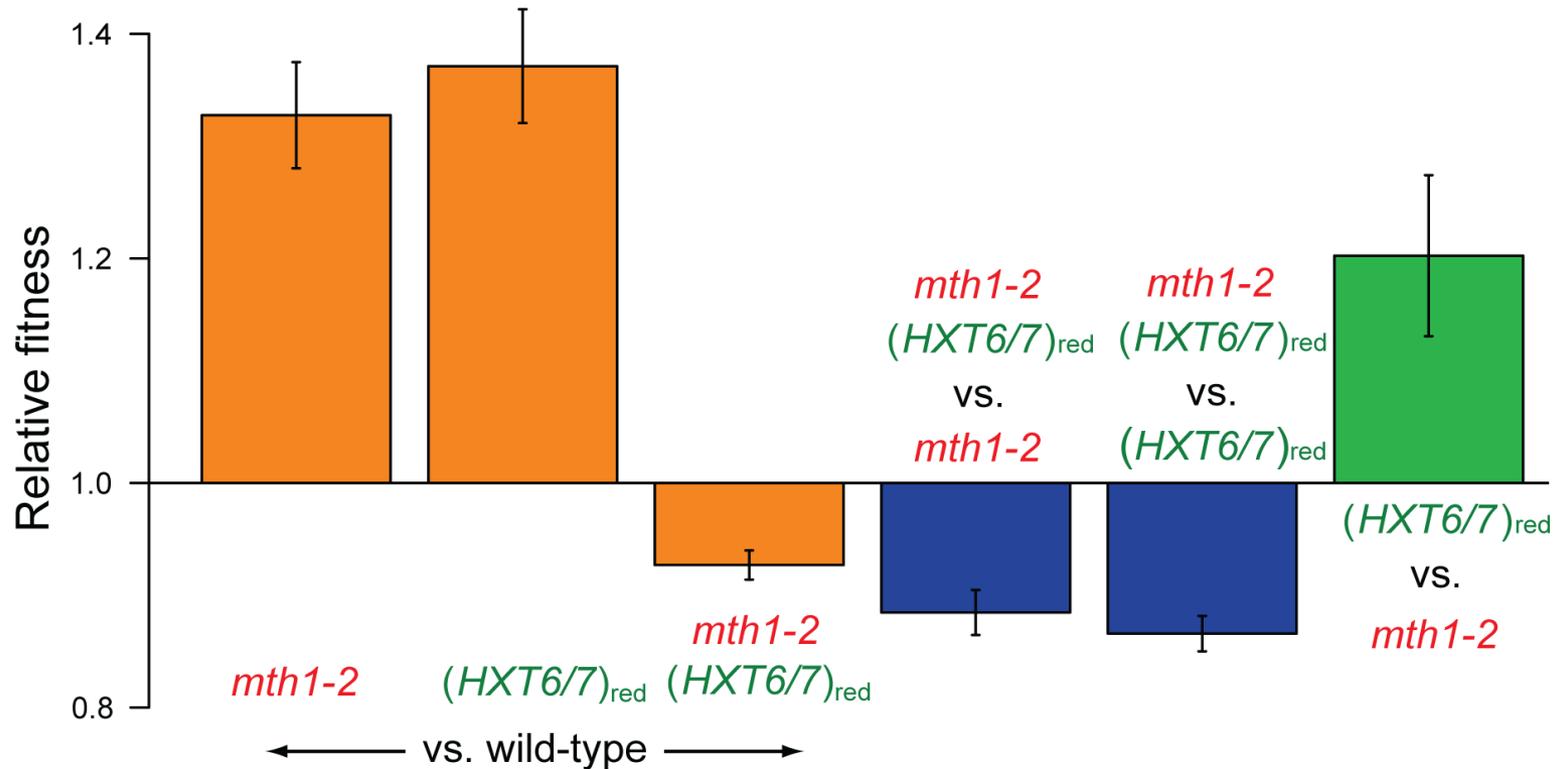
# Pleiotropy notwithstanding, *HXT6/7* amplifications recur when yeast evolve under glucose limitation



# Many beneficial alleles play well together, though their combined fitness effects may not be additive



But, other beneficial alleles play together poorly, e.g., *HXT6/7* amplifications and *MTH1* nonsense mutations



*MTH1* encodes a negative regulator of glucose sensing, whose inactivation increases *HXT* expression

Can the presence of one beneficial allele preclude evolution of another?

Are evolutionary trajectories genetically constrained?

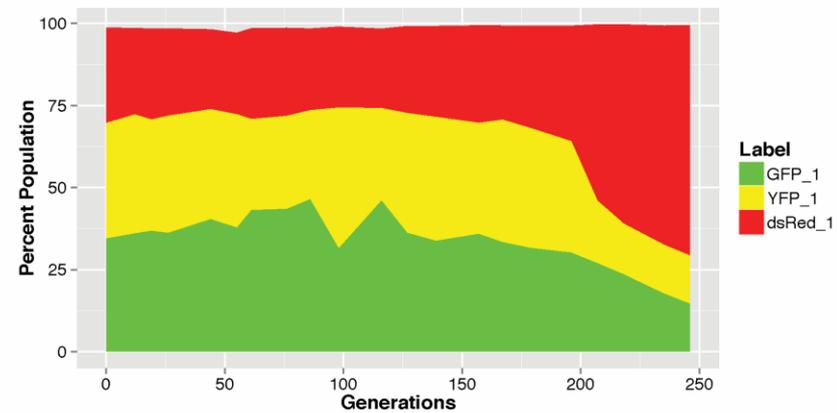
# Can *HXT6/7* evolve in an *mth1-1* background?

1

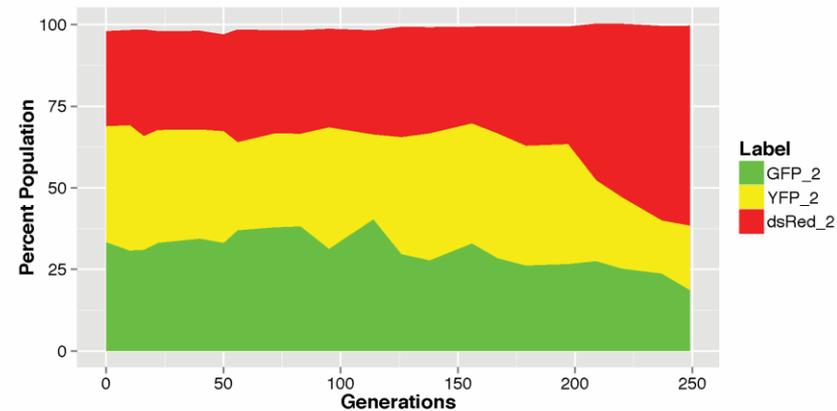
Found replicate populations with 3 isogenic strains, each of which contains *mth1-1*, but also carries a different fluorescent marker:

**GFP**, **DsRed**, **YFP**

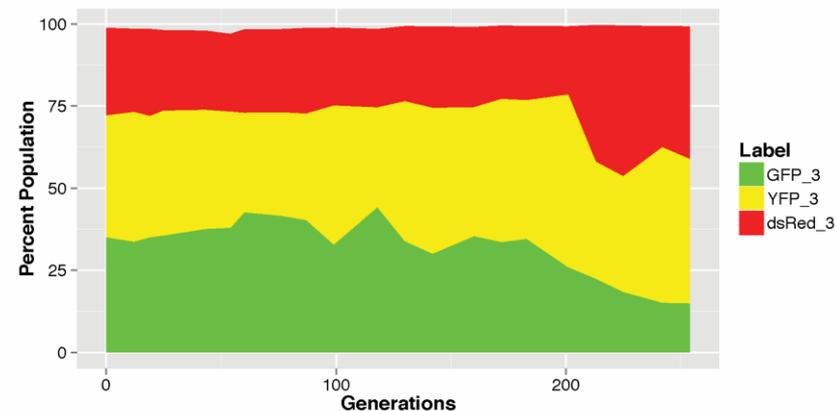
*Mth1-1* Gln → Stop at aa 338 that removes terminal 96 amino acids.



2



3



- PCR screen every 50-gens
- WGS individual clones

# Can *HXT6/7* evolve in an *mth1-1* background?

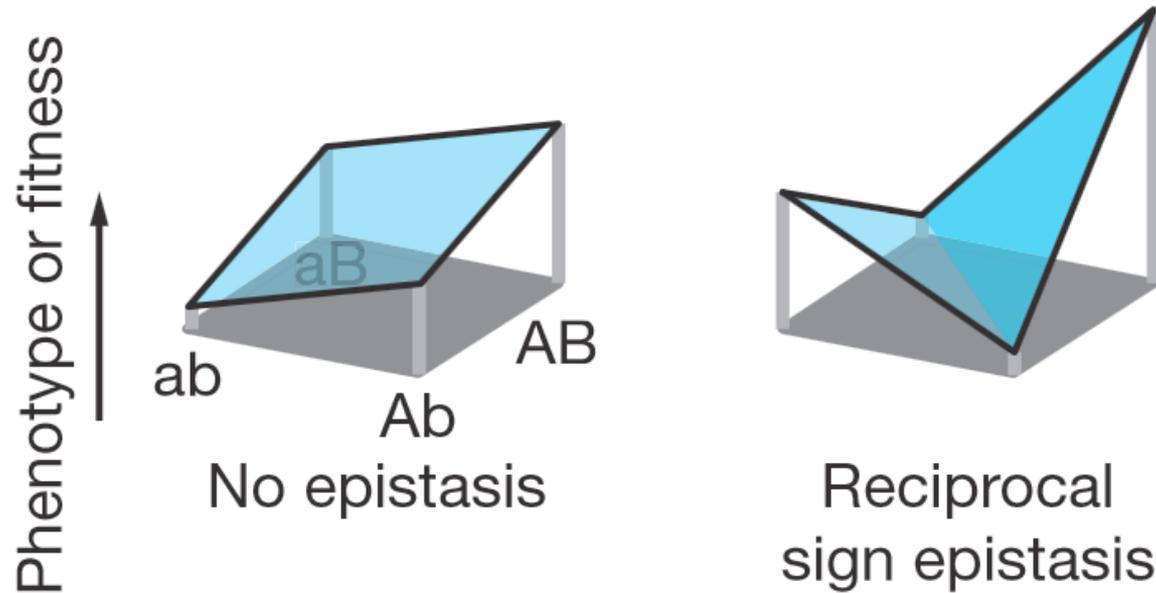
**No**, at least not over 250 gen, *HXT6/7* copy # incr. **never** detected

Other likely beneficial mutations do arise in *mth1-1*

#CHROM	POS	GENE(S)	Effect	Codon	AA	REF	ALT	GSY2750	GSY2754	GSY2756	GSY2751	GSY2755	GSY2757	GSY2753
chrII	371865	<i>TIP1</i>	DOWNSTREAM			T	A							
chrI	128481	<i>SYN8</i>	FRAMESHIFT			S/	Frameshift							
chrV	364256	<i>SSA4</i>	UPSTREAM			A	G							
chrVIII	519892	<i>CRG1</i>	SYNONYMOUS	ccc/ccT	P/P	C	T							
chrXV	178574	<b><i>IRA2</i></b>	NON_SYNONYMOUS	aCa/aAa	T/K	C	A							
chrMito	80513	<i>COX3</i>	DOWNSTREAM			C	G							
chrIV	1289911	<i>SIZ1</i>	NON_SYNONYMOUS	tCt/tAt	S/Y	C	A							
chrX	666710	<i>STR2</i>	NON_SYNONYMOUS	tCt/tTt	S/F	G	A							
chrXII	147914	<i>DNM1</i>	NON_SYNONYMOUS	Cct/Act	P/T	C	A							
chrXV	606620	<i>MDM32</i>	NON_SYNONYMOUS	cGt/cCt	R/P	G	C							
chrIV	67810	<i>TIM22, YDL218W</i>	DOWNSTREAM			C	A							
chrIV	183707	<i>RPC53</i>	NON_SYNONYMOUS_CODING	tCt/tTt	S/F	C	T							
chrVIII	167568	<i>YHR028W-A</i>	NON_SYNONYMOUS	YHR028W-A:c.217T>G,218C>G,219C>A		TCC	GGA							
chrX	304208	<i>JEM1</i>	NON_SYNONYMOUS	tCg/tGg	S/W	C	G							
chrXI	158736	<i>RPS27A</i>	INTRON			A	G							
chrXI	647912	<i>FLO10</i>	SYNONYMOUS	tcT/tcA	S	T	A							
chrXVI	416459	<i>YTA6</i>	NON_SYNONYMOUS	Caa/Aaa	Q/K	C	A							
chrII	452501	<i>PHO88</i>	UPSTREAM			G	T							
chrXV	557840	<i>UBP2</i>	NON_SYNONYMOUS	aGa/aCa	R/T	C	G							
chrIII	283793	<i>CDC39</i>	NON_SYNONYMOUS	tAt/tGt	Y/C	A	G							
chrVII	321148		INTERGENIC			T	C							
chrVIII	524447		INTERGENIC			C	G							
chrIV	675571	<i>YDR109C</i>	NON_SYNONYMOUS	Caa/Aaa	Q/K	G	T							
chrIV	785856	<i>TRM82</i>	NON_SYNONYMOUS	aCa/aTa	T/I	C	T							
chrVIII	418602	<i>TDA11</i>	STOP_GAINED	Gag/Tag	E/*	G	T							
chrIX	89756	<i>TMA108, TPM2</i>	INTERGENIC			G	C							
chrXII	838026	<i>ILV5</i>	DOWNSTREAM			C	A							
chrXV	179275	<b><i>IRA2</i></b>	STOP_GAINED	Gag/Tag	E/*	G	T							
chrXVI	95218	<i>CIN2, IQG1</i>	INTERGENIC			G	A							
chrXVI	421772	<b><i>MUK1</i></b>	STOP_GAINED	taC/taA	Y/*	C	A							
chrV	143507	<b><i>MIT1</i></b>	NON_SYNONYMOUS	gAa/gCa	E/A	A	C							
chrXII	921722	<i>BDF1, YLR400W</i>	UPSTREAM			C	G							
chrXV	213387	<i>PRS5</i>	NON_SYNONYMOUS	Acc/Gcc	T/A	A	G							
chrIX	69868	<b><i>SLN1</i></b>	NON_SYNONYMOUS	Cca/Gca	P/A	G	C							
chrXIV	119311	<i>BOR1</i>	NON_SYNONYMOUS	aCa/aGa	T/R	C	G							
chrXVI	898617	<i>SEC23</i>	NON_SYNONYMOUS	Gat/Cat	D/H	C	G							
chrMito	10368		INTERGENIC			T	C							
chrMito	84816	<i>RPM1, tM(CAU)Q2</i>	UPSTREAM			A	T							

**Table 3** Summary of SNPs detected in sequenced clones from population 3, where yellow indicates the presence of the SNP. Gene names in **red**, bold fonts are genes which were either mutated recurrently in our experiments, or in which mutations have been observed previously in other glucose limited chemostat evolutions.

# The Valley-of-Death: Reciprocal sign epistasis constrains adaptive trajectories



Chiotti et al. *Genomics*. The Valley-of-Death: Reciprocal sign epistasis constrains adaptive trajectories in a constant, nutrient limiting environment.

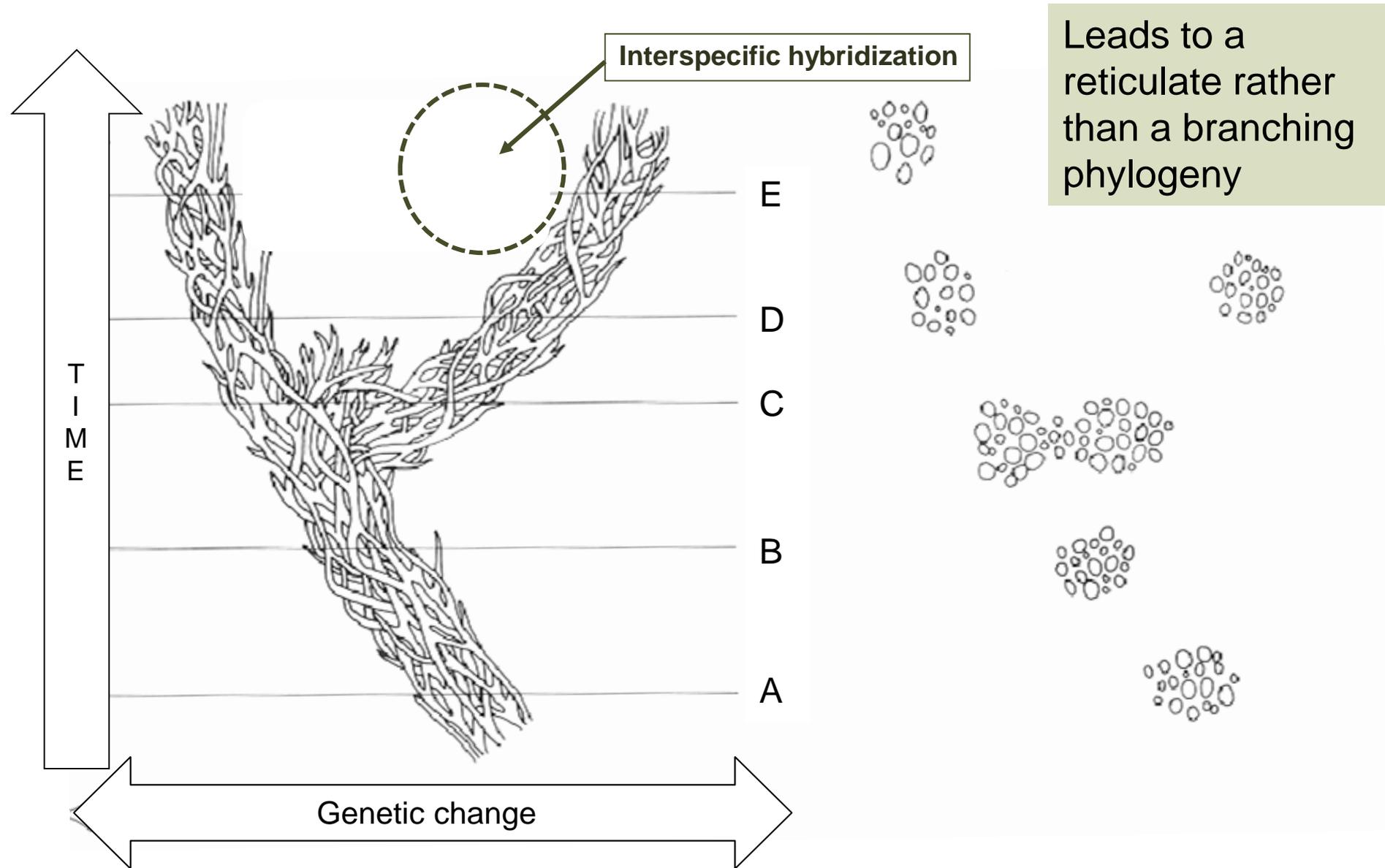
Figure adapted from [http://tansgroup.amolf.nl/research\\_evolutionary\\_pathways.html](http://tansgroup.amolf.nl/research_evolutionary_pathways.html)

# Speciation

Insights using experimental evolution

Barbara Dunn, Eugene Kroll, Terry Paulish, Alison Stanbery, Jeff Piotrowski, Gregory Koniges, Ed Louis, Gianni Liti and Gavin Sherlock

# Often think of speciation as branching and divergence



Many hybrids are inviable,  
or cannot reproduce



Yet hybrid speciation is  
widespread



$2n - 4n$



$2n-8n$   
(allopolyploid)



$2n-10n$



Allo- and  
autopolyploids

Polyploid hybrids  
Chromosome number in  
new species > ancestors

Wood et al 2009



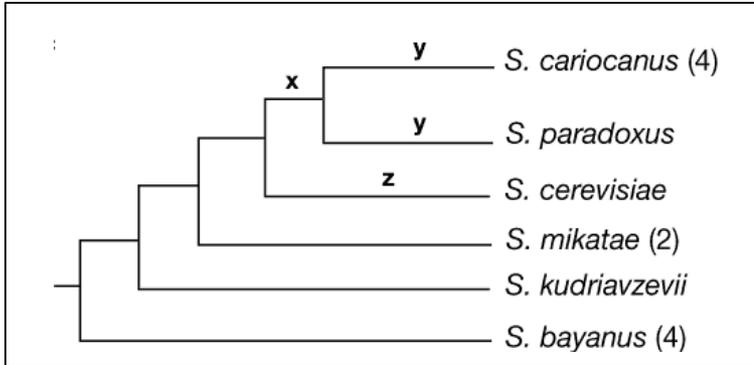
Homoploid hybrids  
Chromosome number in  
new species = ancestors

Gompert et al 2006

Inferred retrospectively

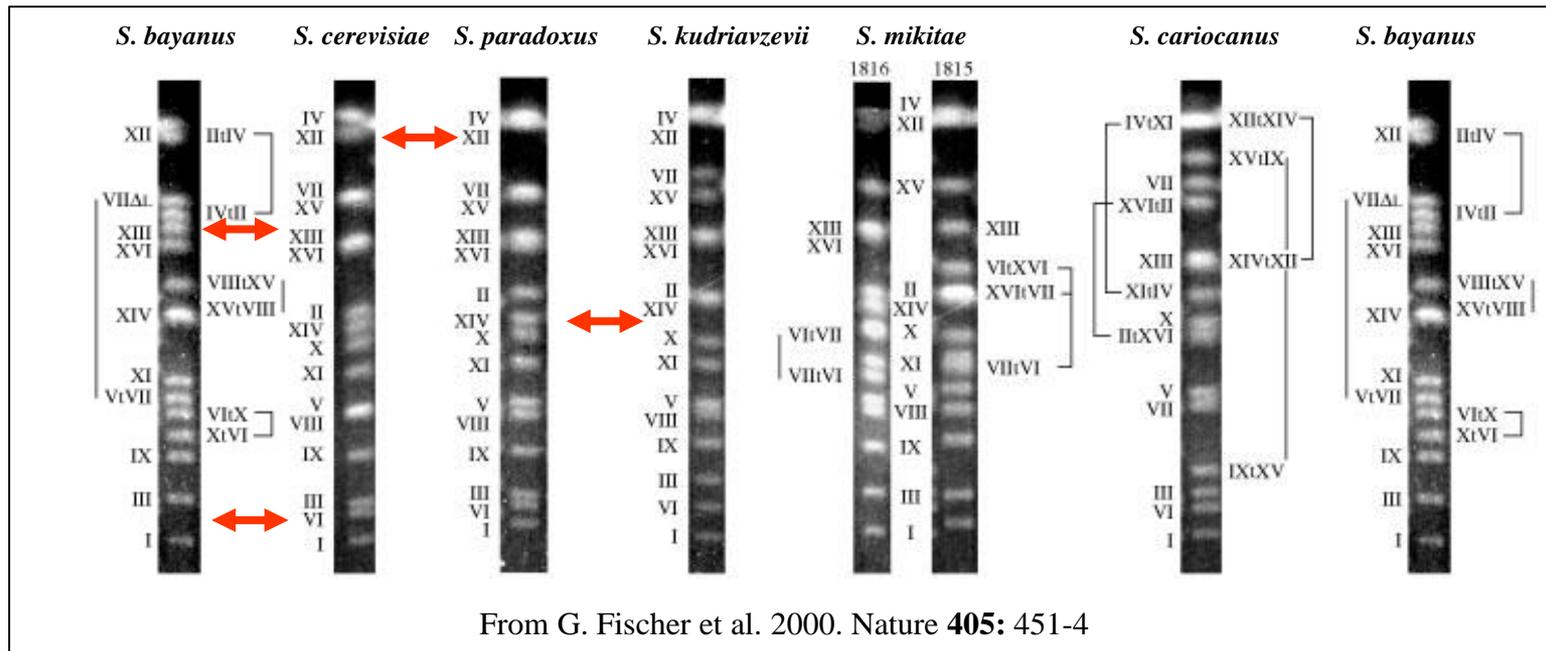
# We can study real-time hybrid speciation in yeast

## The *Saccharomyces* 'sensu stricto' complex

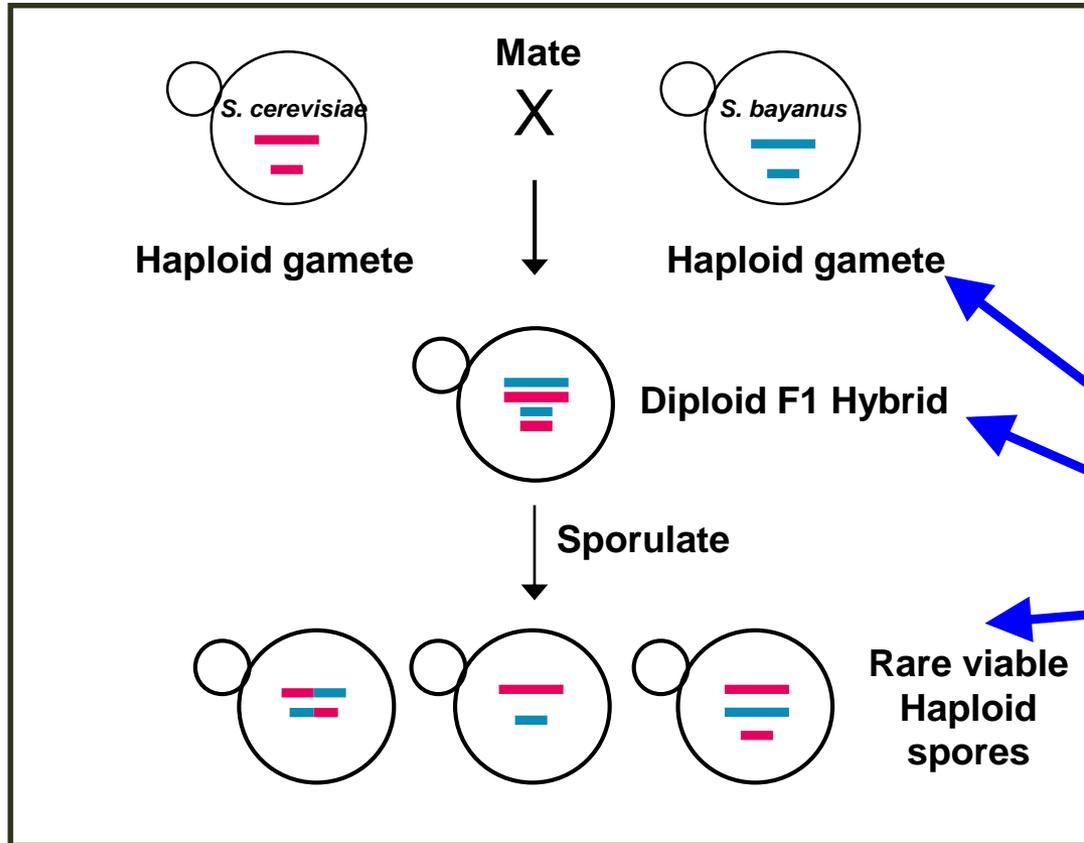


Distinguishable by molecular techniques, e.g., 18S rDNA phylogeny, CHEF electrokaryotype of whole chromosomes

Reciprocal translocations & chromosomal rearrangements may play a role in hybrid spore inviability



# First, we create new yeast species in the lab



Put different selectable markers into *S. cerevisiae* and *S. bayanus* haploids → mate → recover rare F1s by selecting for both markers → sporulate to form F2 hybrids

Each reproductively isolated from the other, thus new spp.

Then, we experimentally evolve new species & ask

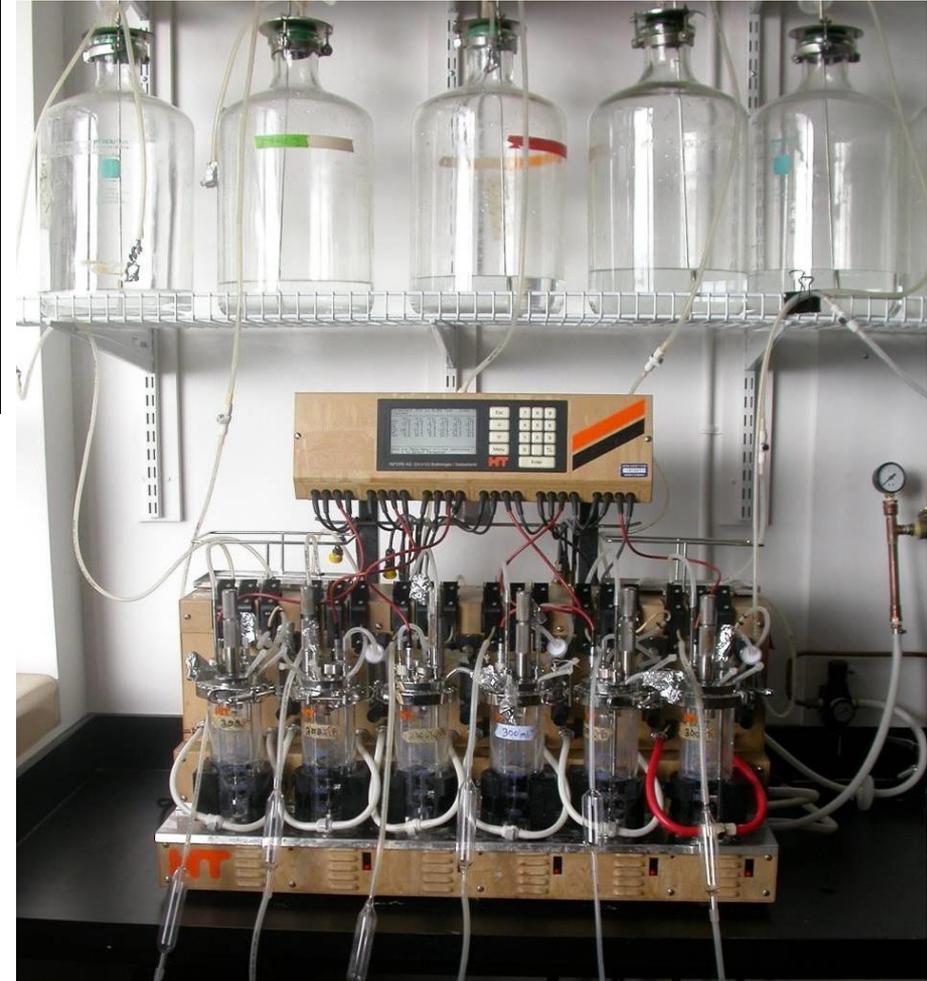
Do ancestral species and newly-formed F1 and F2 homoploid hybrids differ in genome stability?

Do certain genome rearrangements recur, and if so, are they adaptive?

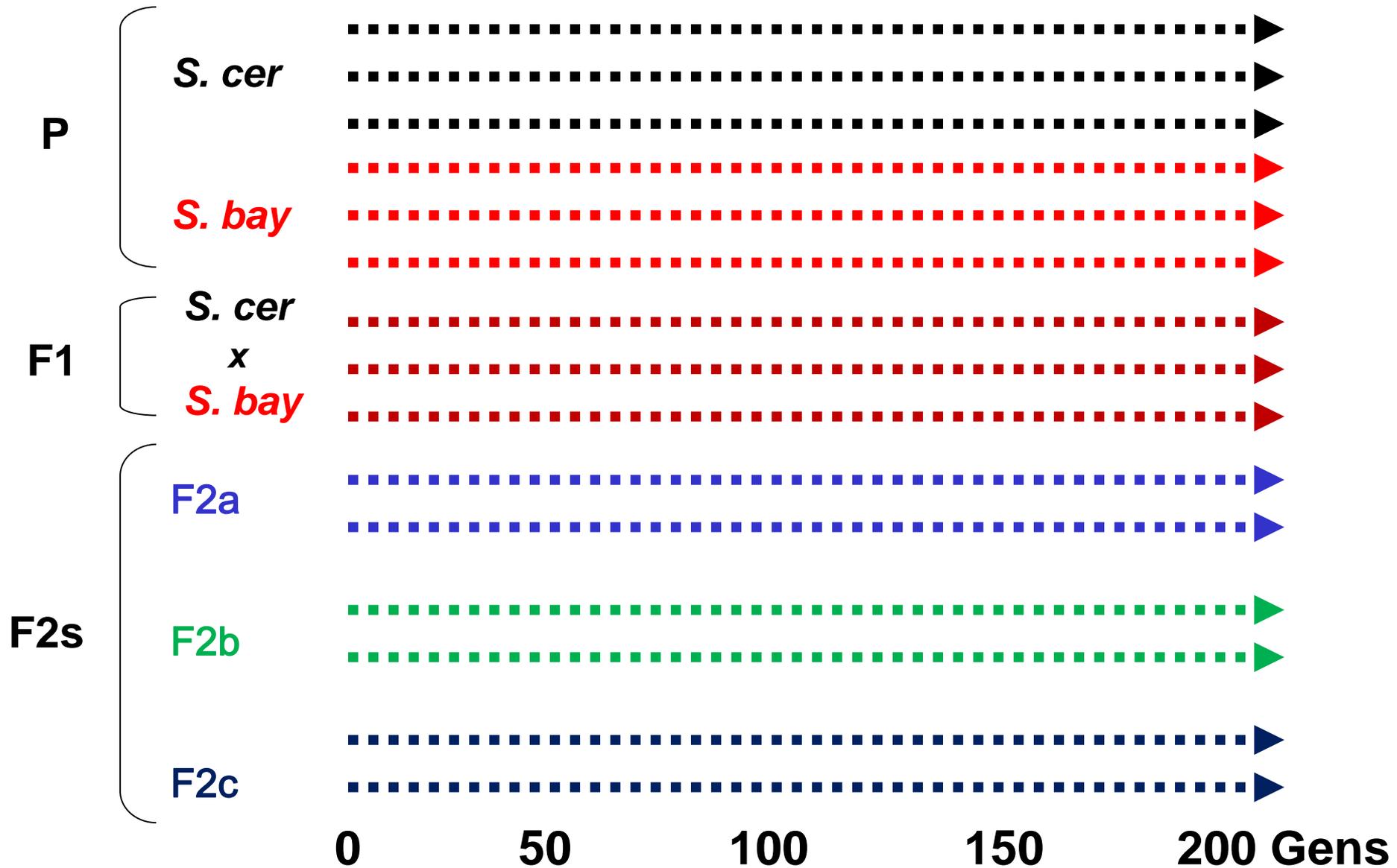
# The ecological theater for our evolutionary play

## Continuous resource limitation in chemostats

- Nitrogen ( $\text{NH}_4$ )-limited
- 0.9% glucose-sufficient
- $D \approx 0.16 \text{ h}^{-1}$



# The actors



# Separation of whole chromosomes via Pulsed Field Gel Electrophoresis.

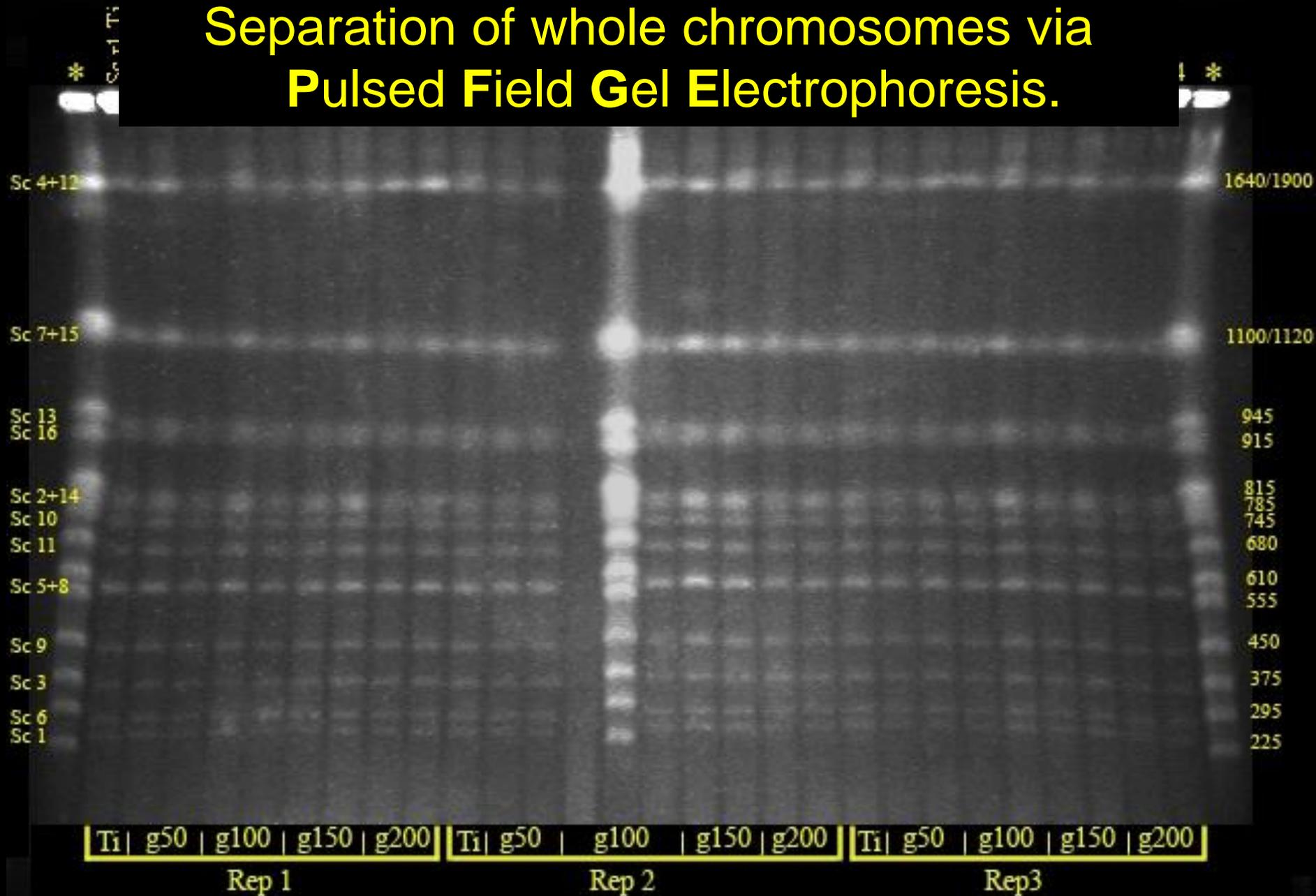


Fig. 4A

*Saccharomyces cerevisiae*

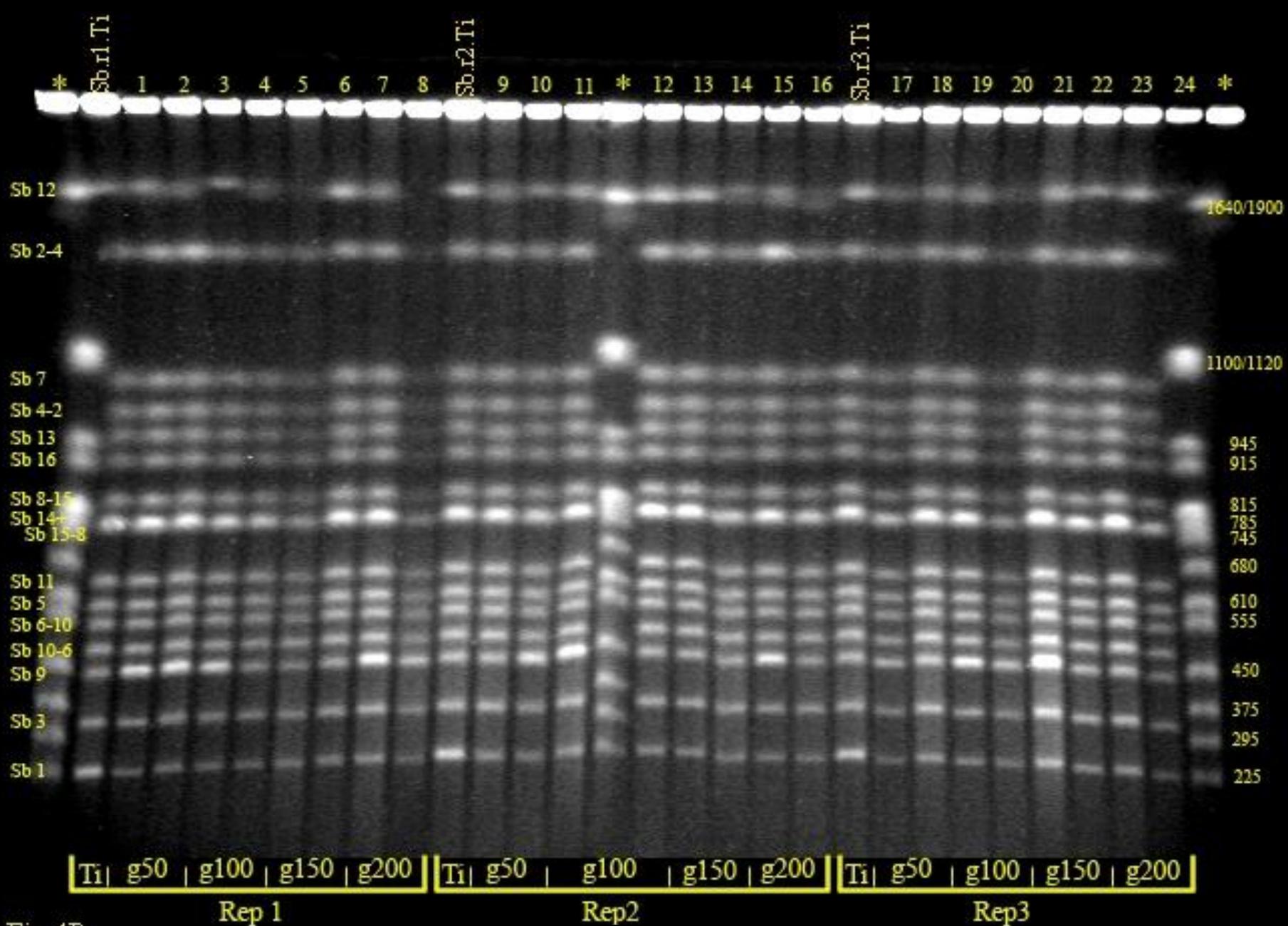
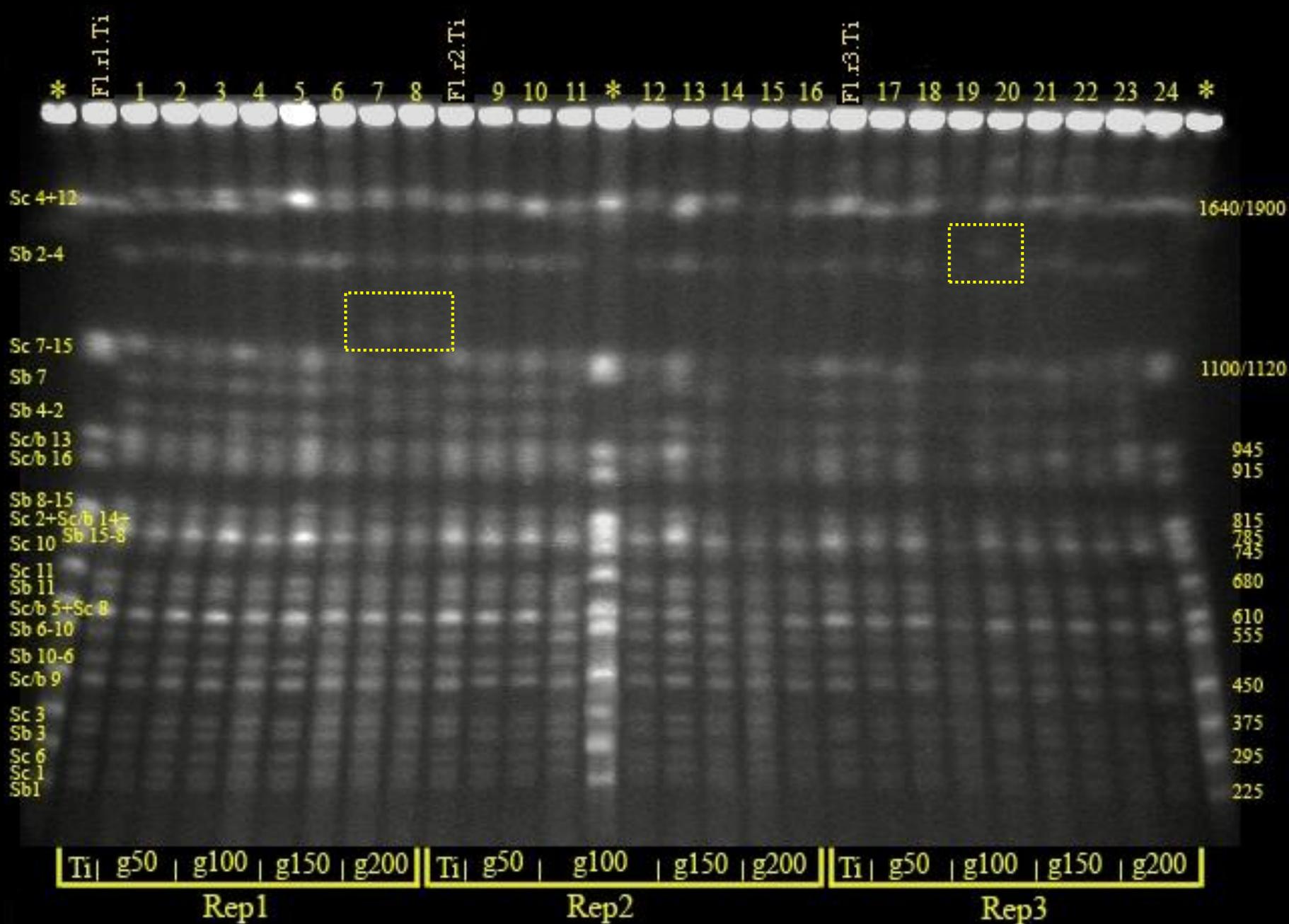


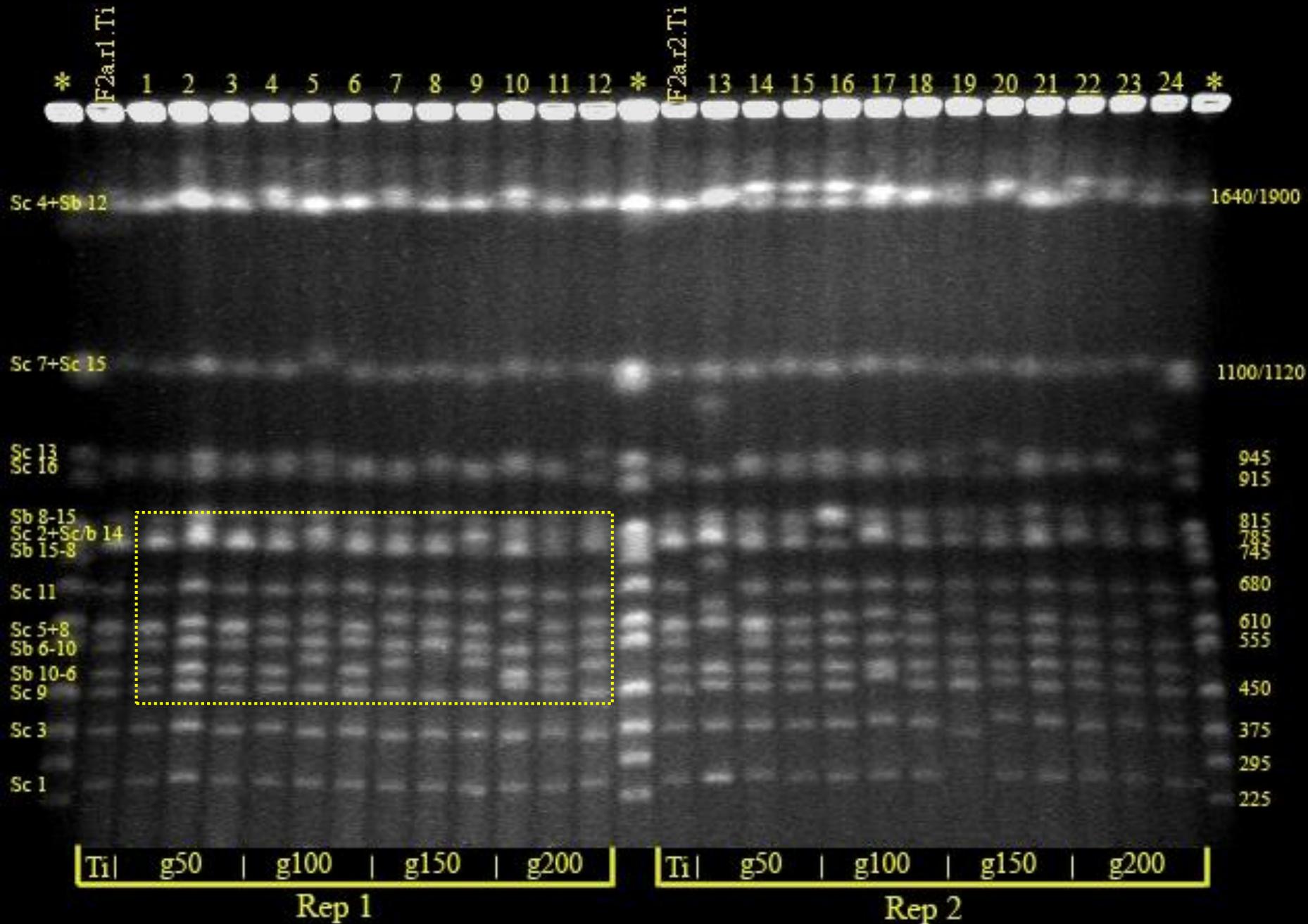
Fig. 4B

*Saccharomyces bayanus*



F1 (*S. cerevisiae* x *S. bayanus* hybrid)

Fig.4C



F2a (*S. cerevisiae* x *S. bayanus* hybrid)

F2b & F2c similar

# Speciation

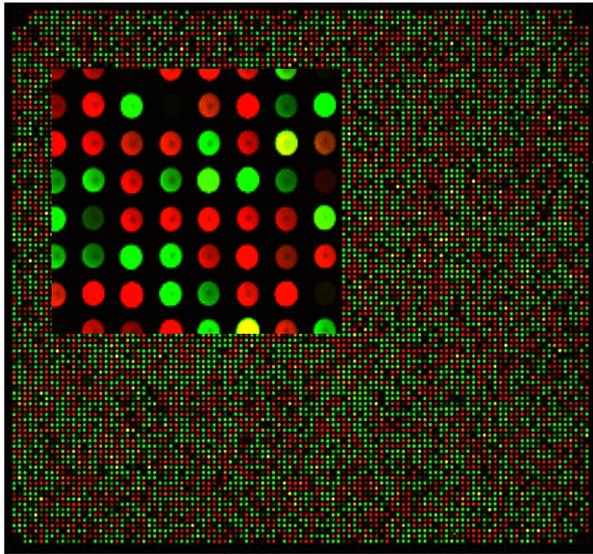
Genome stability differs among ancestral species and de novo hybrids as they evolve under resource limitation

Ancestors > F1s >> F2s

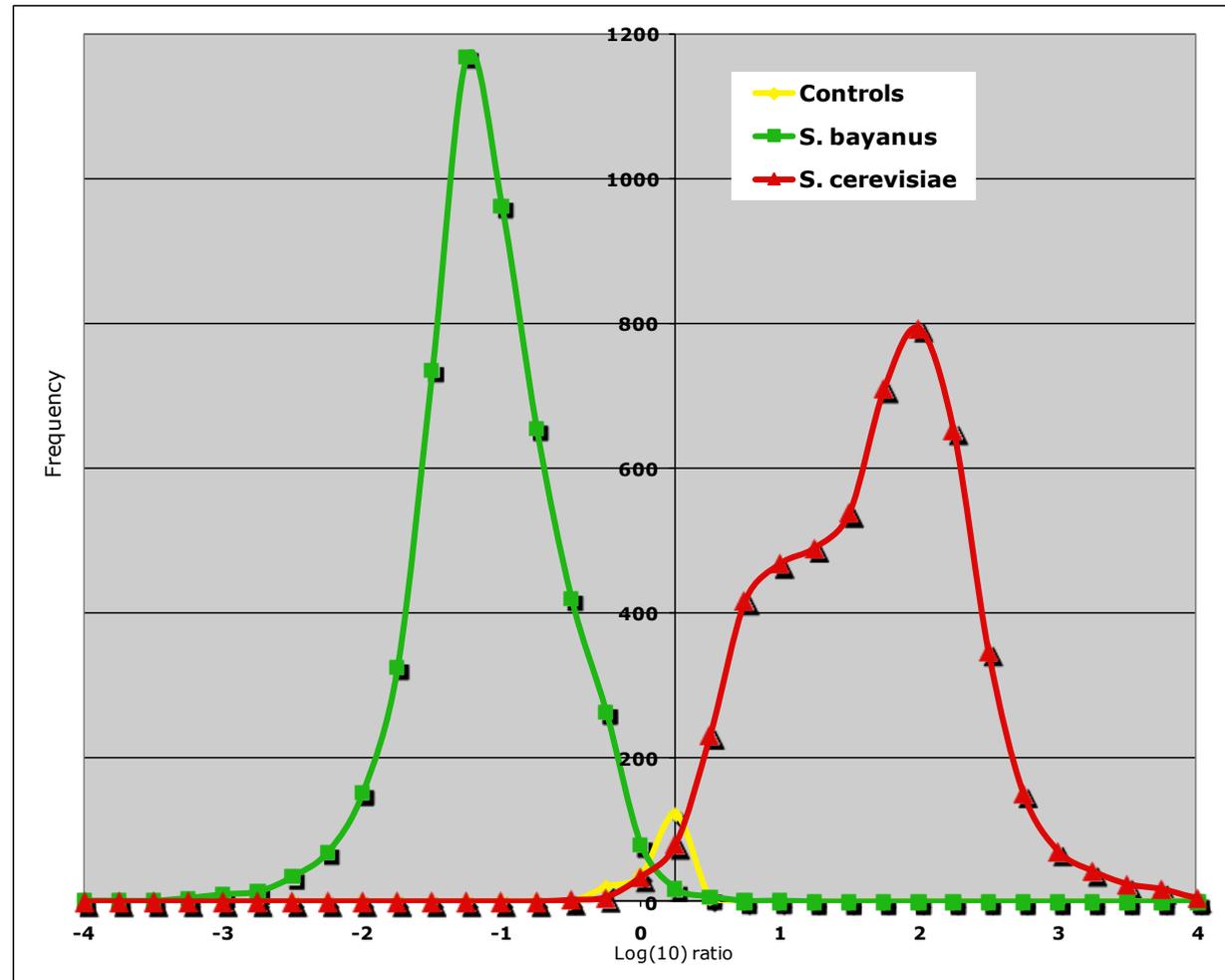
Do certain genome rearrangements recur?  
If so, are they adaptive?

# A 2-species microarray for comp genome hyb

- Label *S. bayanus* (= *uvarum*) gDNA with green fluor (Cy3)
- Label *S. cerevisiae* gDNA with red fluor (Cy5)



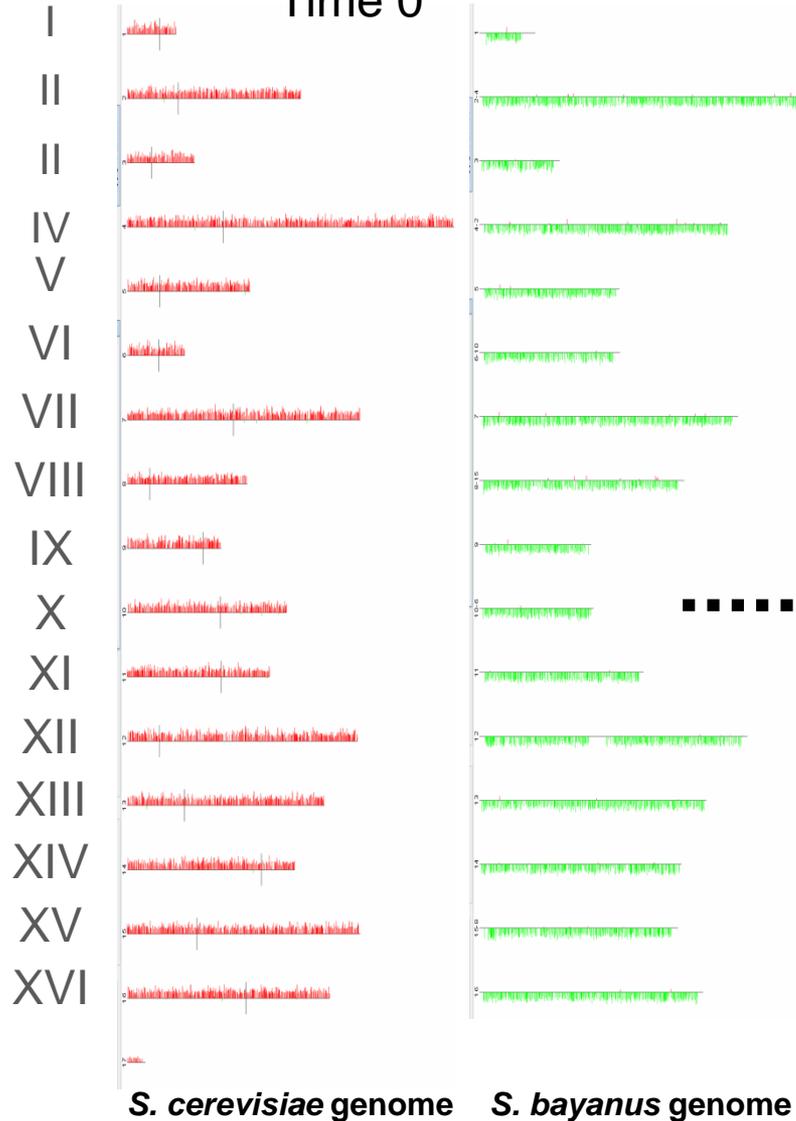
(A) 2-species Agilent array of *S. cerevisiae* (red) vs. *S. bayanus* (green);  
(B) Histogram of log<sub>10</sub> ratios of *S. cerevisiae* (red) vs. *S. bayanus* (green) spots. For most probes there is 10 to 100-fold discrimination between species and therefore, little cross-hybridization.



# Evolution of *S. cerevisiae* parent

Chr.

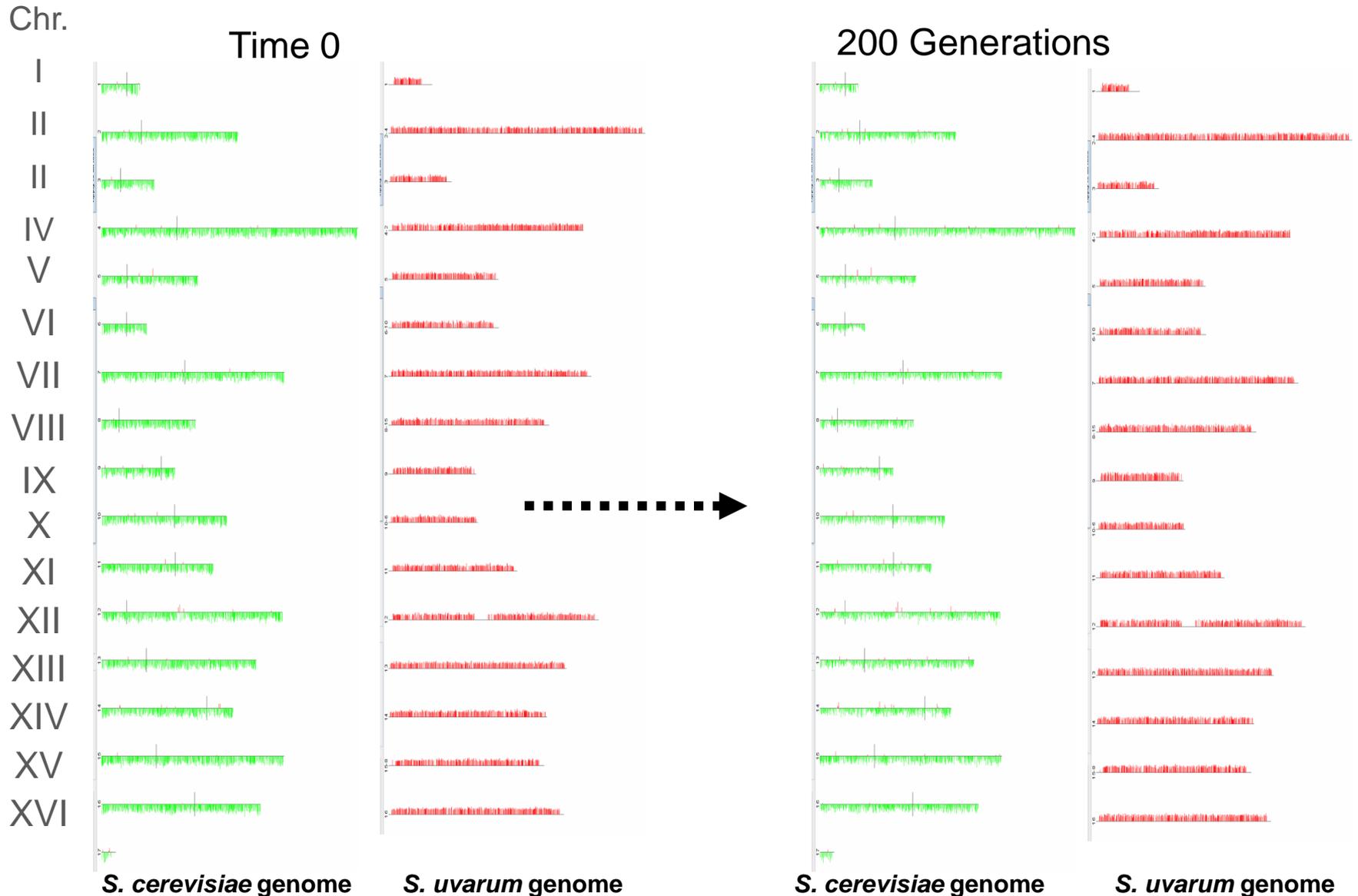
Time 0



Red = Present

Green = Absent

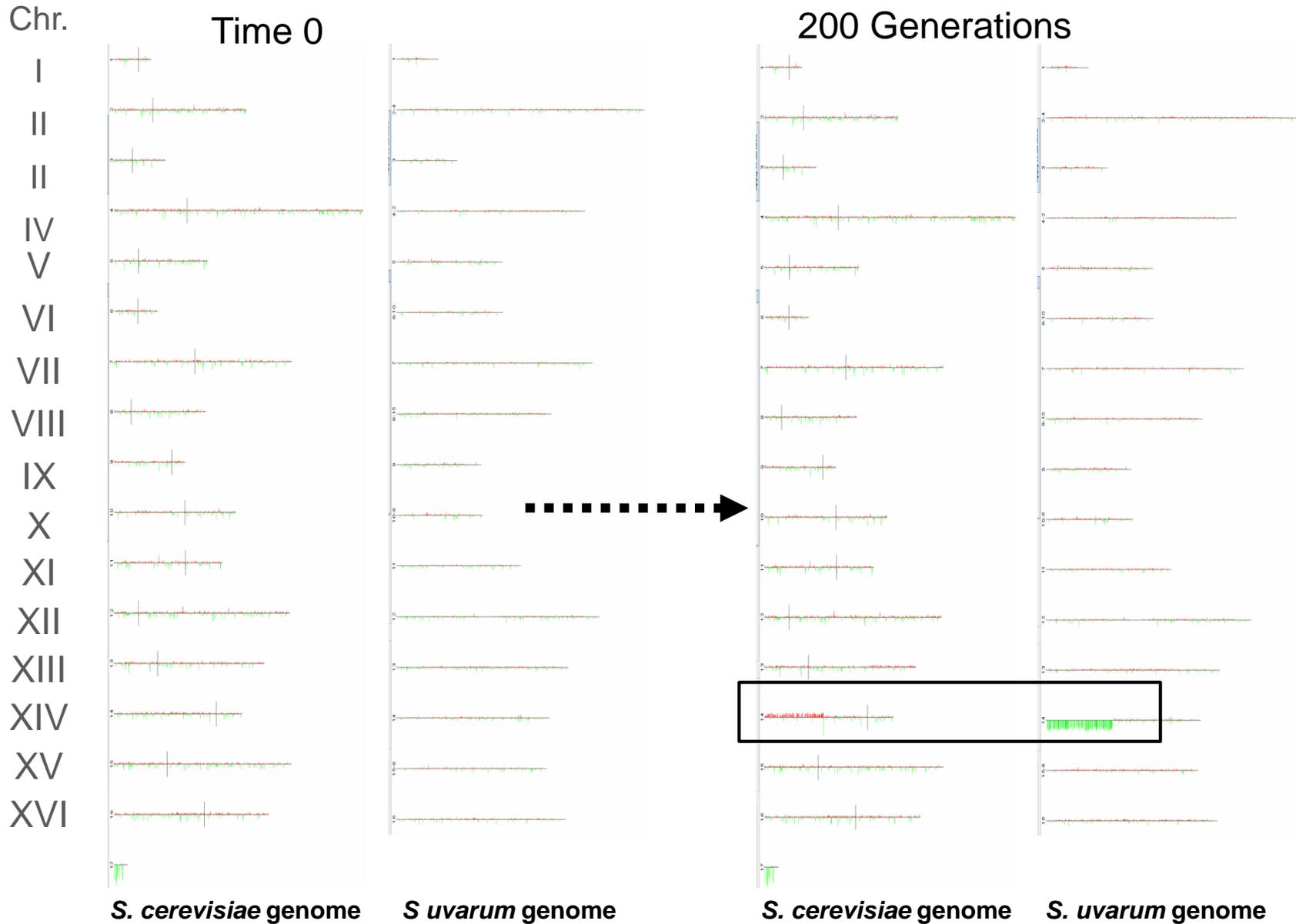
# Evolution of *S. uvarum* parent



Red = Present

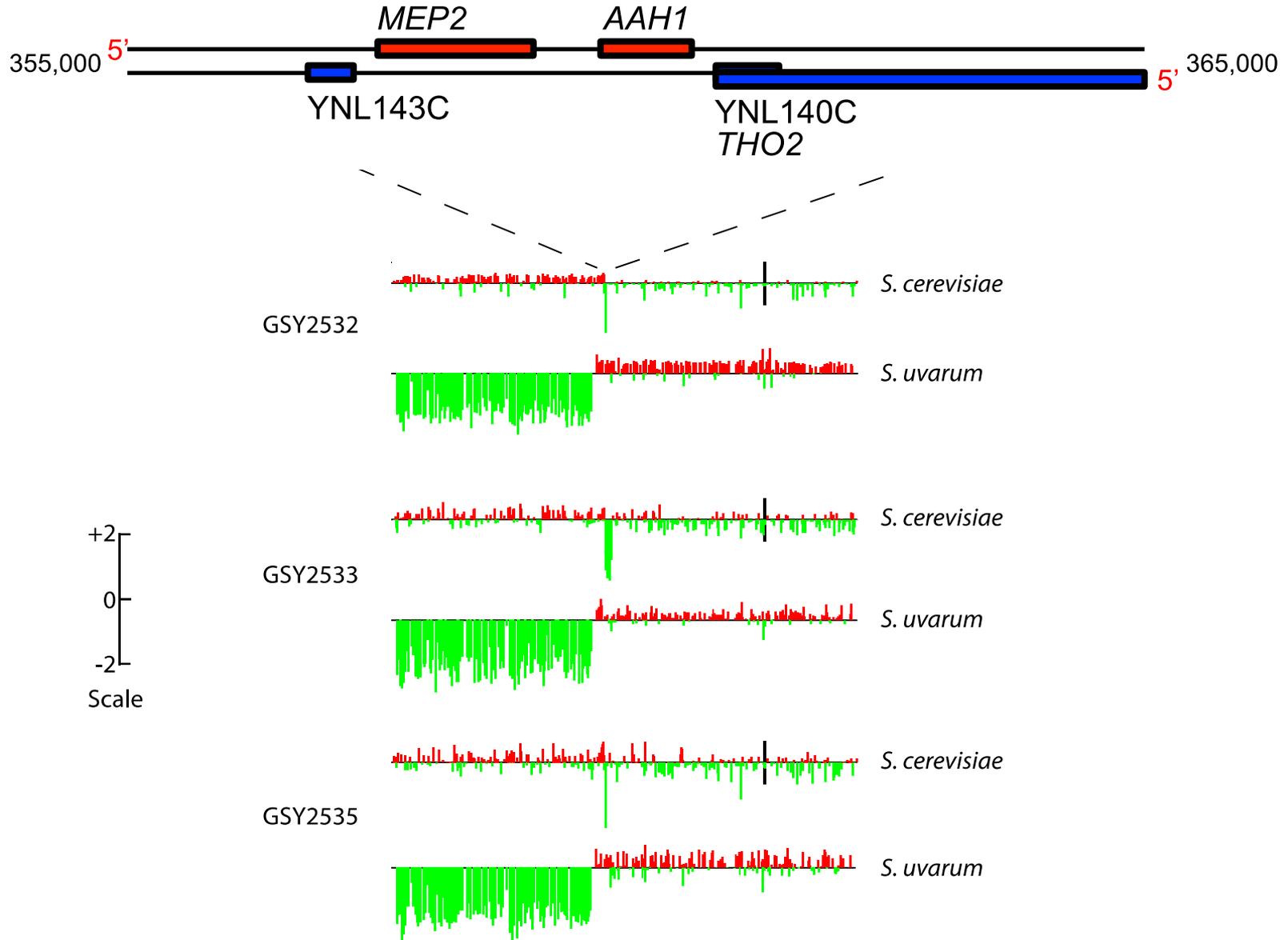
Green = Absent

# Evolution of F1 *S. uvarum* x *S. cerevisiae*



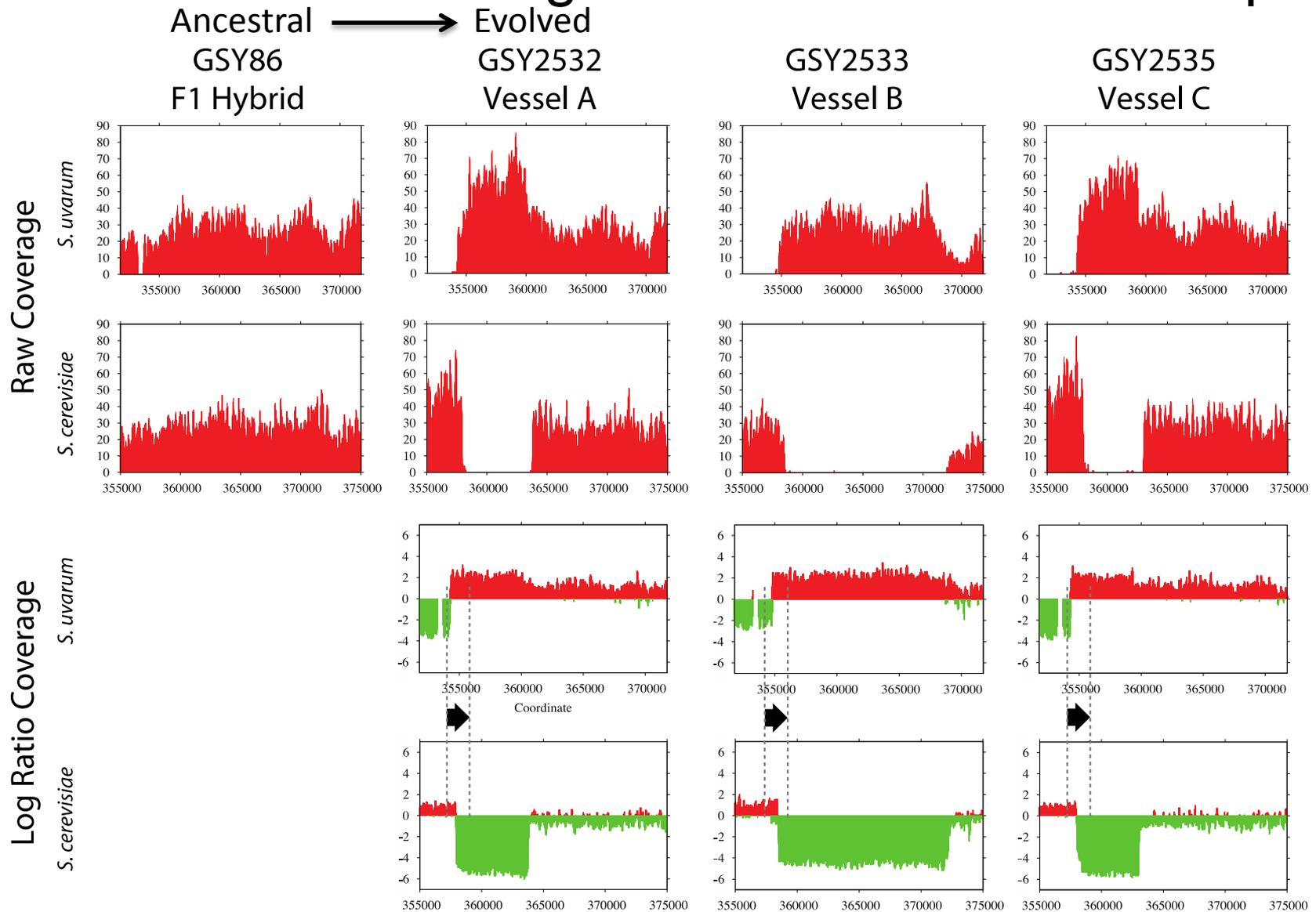
Straight line = R & G Balanced  
Red = amplified, Green = Lost

# aCGH shows recurrent Chr XIV rearrangement in F1s



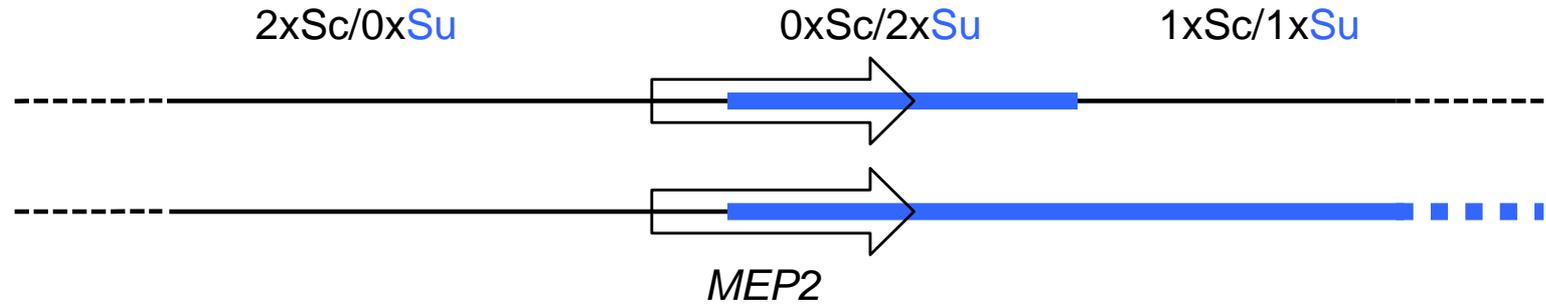
*Mep2* encodes for a high-affinity  $\text{NH}_4^+$  transporter

# WGS confirms rearrangement and locates breakpoints



Chimaeric *Mep2* genes repeatedly arise as F1s evolve under  $\text{NH}_4^+$  limitation

# Genome configuration in the *MEP2* fusion region for *S. cerevisiae* and *S. uvarum* chromosome 14s



Thin black line = *S. cerevisiae*, **thick light blue line** = *S. uvarum*, Arrowed box = *MEP2* coding region

Diverse *MEP2* gene fusions found by sequencing clones from independently evolved populations.

■ = vessel A    ■ = vessel B    ■ = vessel C



# Speciation

Chimaeric genes, an evolutionary innovation and a path to rapid introgression, recur in de novo hybrids under  $\text{NH}_4^+$ -limitation.

As they arise in every experiment, go to high frequency, encode the protein for  $\text{NH}_4^+$  uptake, they're likely adaptive.

# Conclusions

Experimental evolution can illuminate:

- The stability of novel genomes
- The origin and fate of new genes
- Adaptive escape from stress
- Connectivity in metabolic and signaling pathways
- Clarity on these issues is key to understanding the progression of cancer, chronic microbial infections, and the evolutionary fate of synthetic organisms

# Another perspective on yeast adaptation and speciation

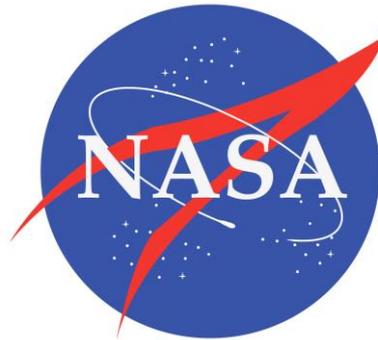
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Barbara Dunn  
Katja Schwartz

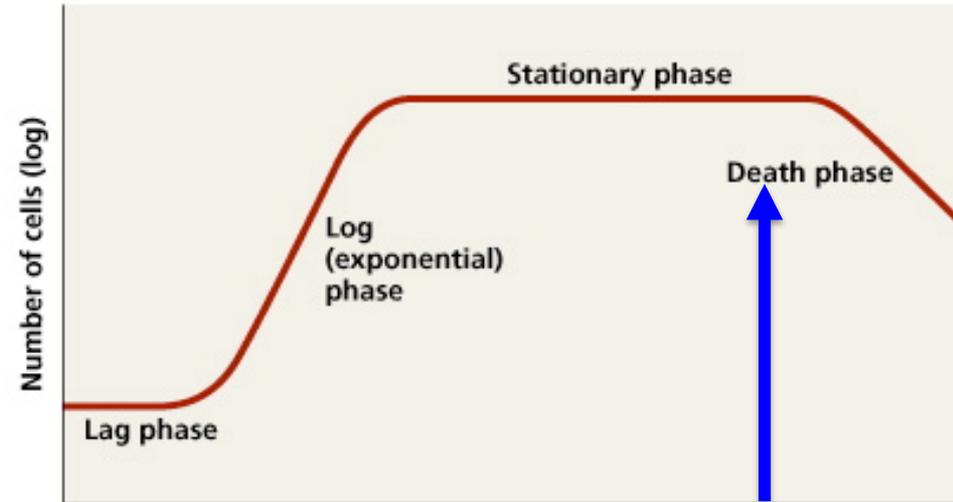
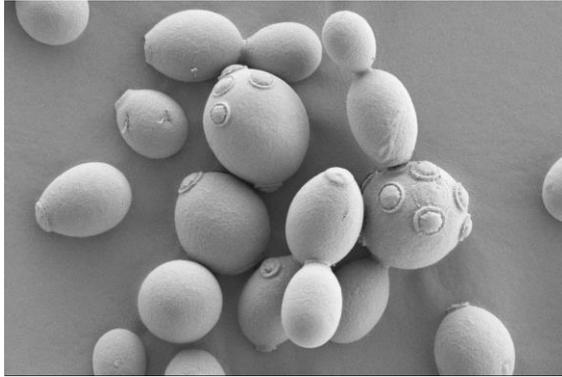
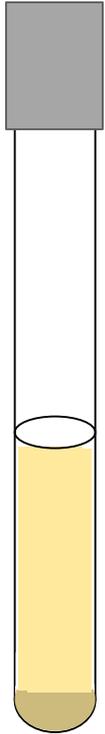


**VIBE**

Virtual Institute for Bio-inspired Exploration



# A different kind of ecological theater . . .

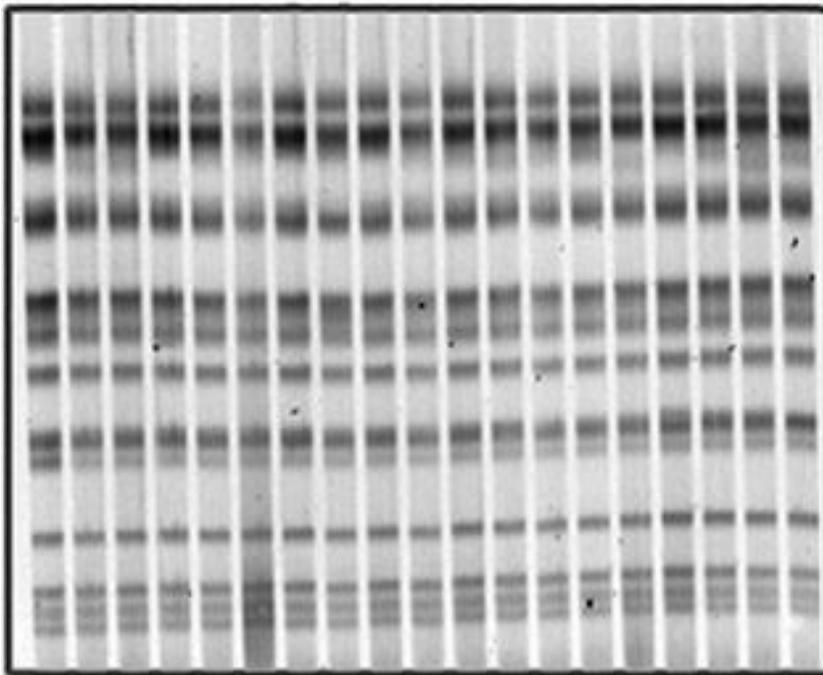


How do cells respond to prolonged starvation?

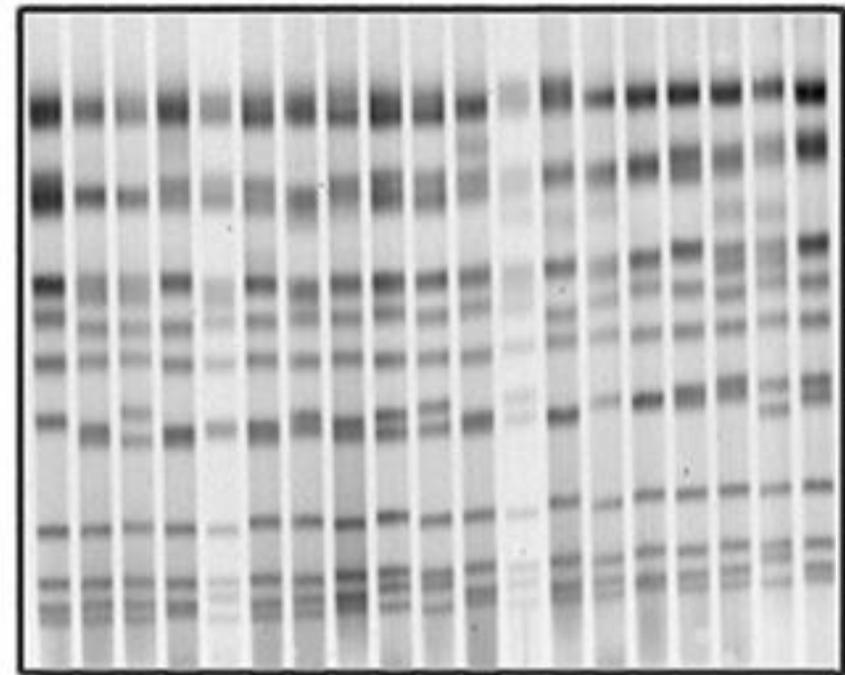
# Starvation induces genomic rearrangements

Nonstarved population

Starved population



-VIIIb  
unid-3  
unid-2  
unid-1  
-V  
-VIIIa  
-IX  
-III  
-VIb  
-VIa  
-Ia/Ib



-VIIIb  
unid-3  
unid-2  
unid-1  
-V  
-VIIIa  
-IX  
-III  
-VIb  
-VIa  
-Ia/Ib

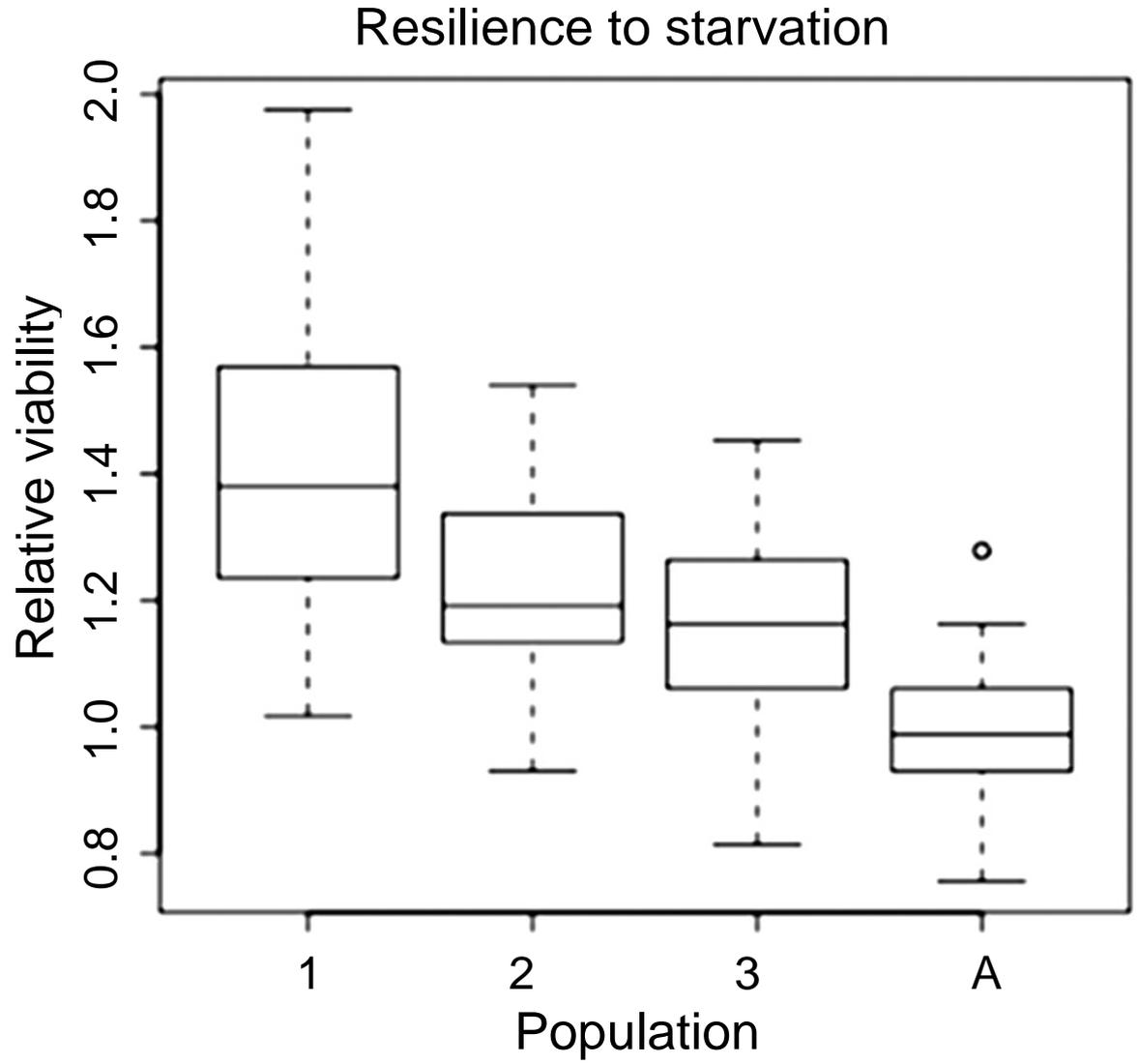
1-1  
1-2  
1-3  
1-4  
1-5  
1-6  
1-7  
1-8  
1-9  
1-10  
1-11  
1-12  
1-13  
1-14  
1-15  
1-16  
1-17  
1-18  
1-19

**Starvation-Associated Genome Rearrangements (SAGR)** are common in stressed populations but not in control.

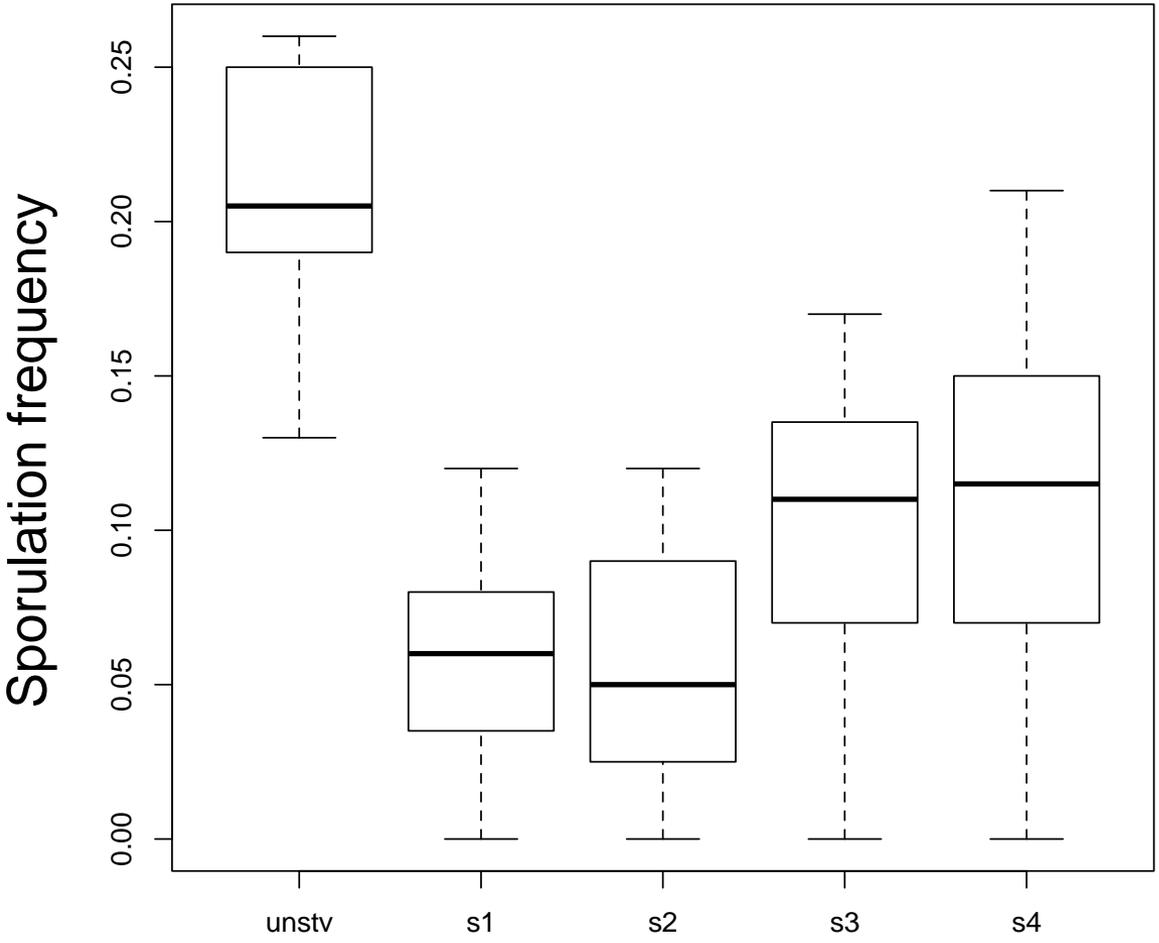
Incidence of SNPs no different ( $5$  vs  $8 \times 10^8$ )

Incidence of rearrangement orders of magnitude higher

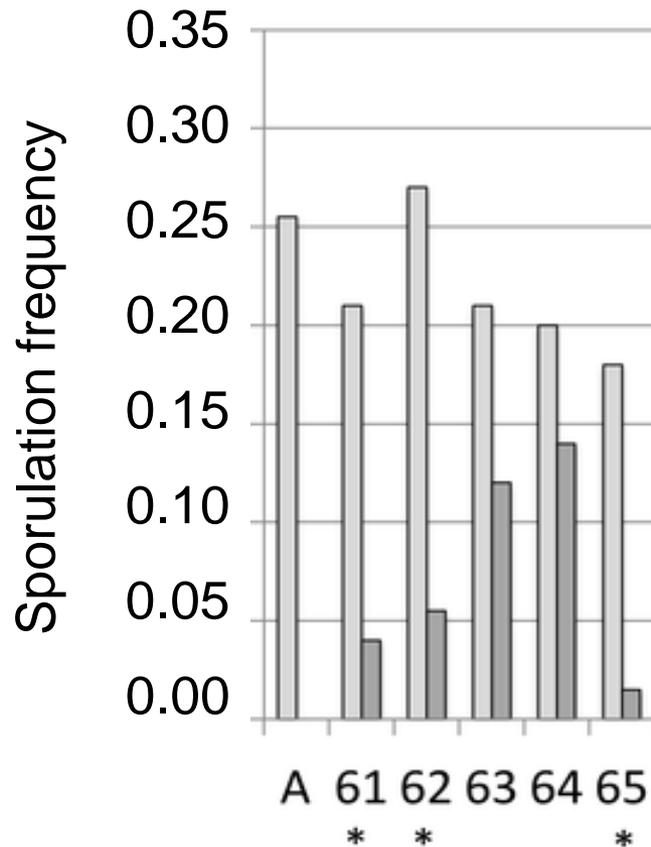
# Cells that survive starvation are more fit than their ancestor when restarted



Yeast make gametes by undergoing sporulation; cells that survive starvation often sporulate poorly



# Certain starved isolates can self-cross but cannot easily backcross to their ancestor



Starved isolates 61, 62, 65 are reproductively isolated from their Ancestor.

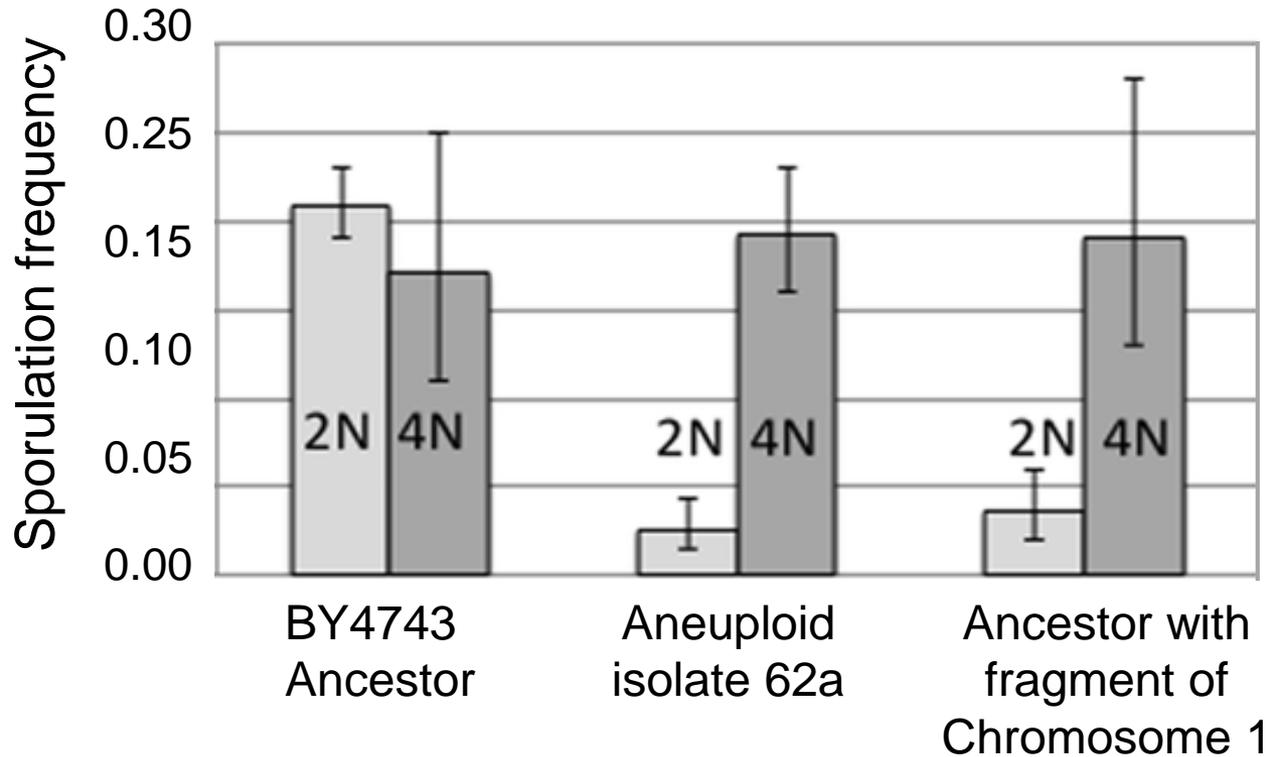
Can starvation promote speciation?

**Figure 2. Sporulation frequencies of backcrosses and self-crosses.** Crosses were made using haploid derivatives of starved isolates from four starved cultures. A – unstarved diploid control. Light grey bars are self crosses, dark grey bars are backcrosses. “\*” denote significant differences between the corresponding self-cross and backcross sporulation frequencies



# Chr. I aneuploidy causes a sporulation defect, which can be cured by restoring euploidy

4N=Tetraploidized





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