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Oaks, grapevines and the (elusive) ecology of *Saccharomyces*

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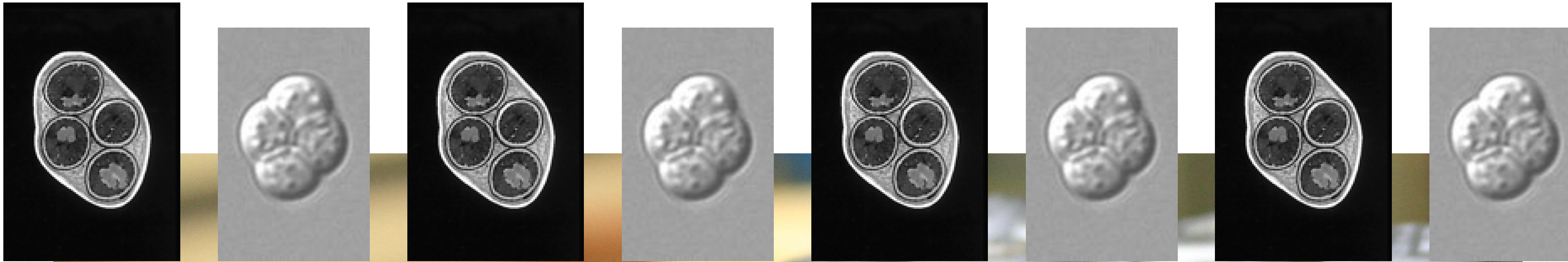
Saccharomyces cerevisiae



Saccharomyces in culture collections

- CBS

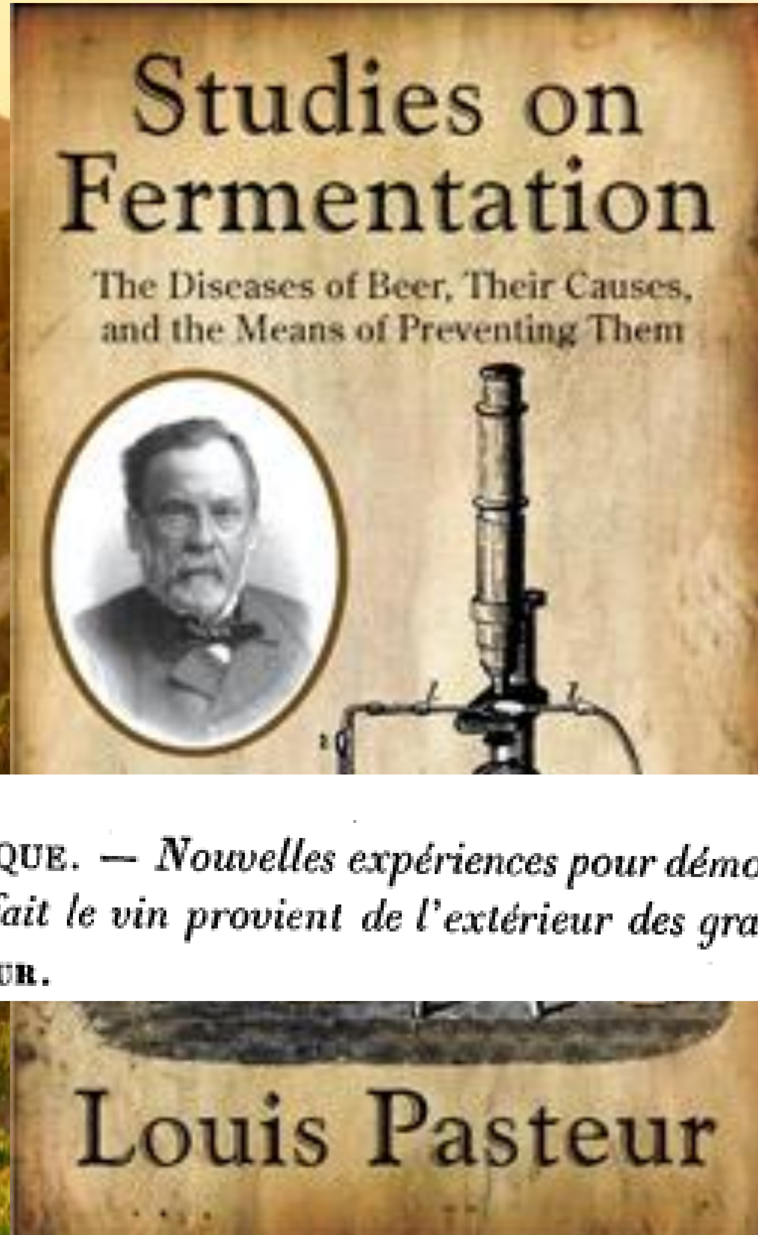
500 *Saccharomyces* strains	5%
10.000 yeast strains	

- PYCC

400 *Saccharomyces* strains	16%
2.500 yeast strains	

outline

- ecology of wine yeasts – an history of changing views

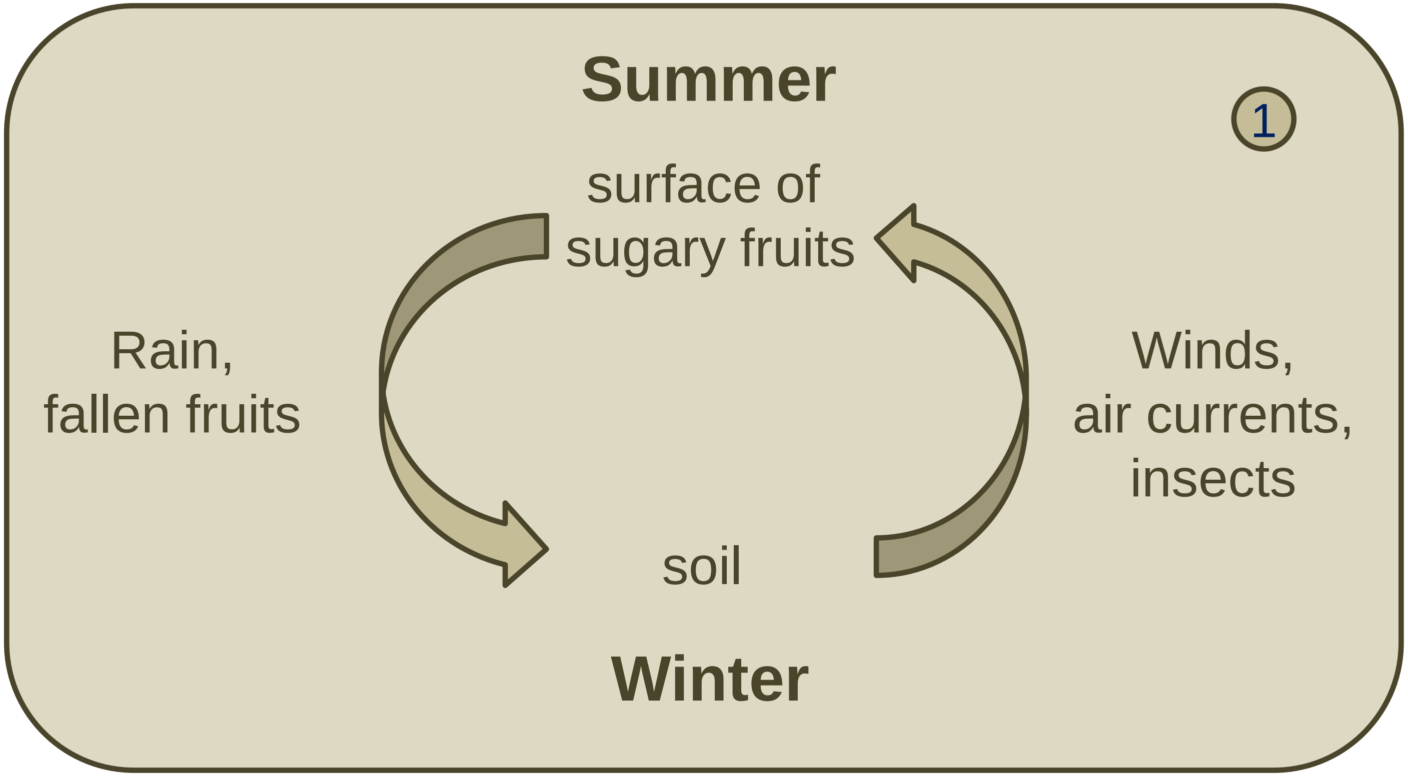


CHIMIE PHYSIOLOGIQUE. — *Nouvelles expériences pour démontrer que le germe de la levûre qui fait le vin provient de l'extérieur des grains de raisin.* Note de **M. L. Pasteur.**

Pasteur 1872

conversion of must into wine is a spontaneous process brought about by the resident yeasts of the grape surface

Guilliermond 1912



H. Phaff, A. Martini – 1980's

"strains of *S. cerevisiae* are rarely if ever present on the fruits and berries of wild species of plants"

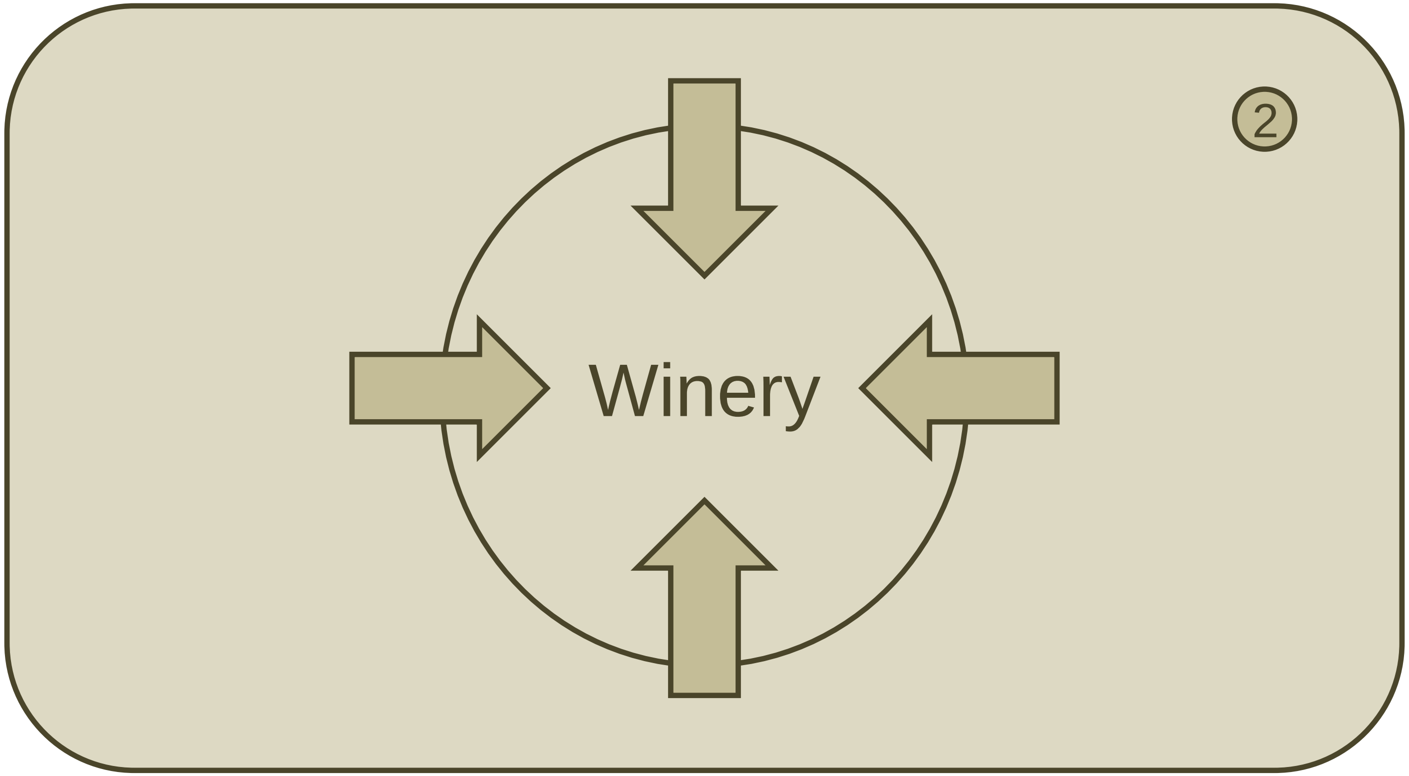
The Life of Yeasts - Phaff et al. 1978

↓

S. cerevisiae must be associated with some other ecological niche …

Peynaud and Domerq.1959. A review on microbiological problems in wine making in France

The primary and exclusive habitats of *S.cerevisiae* are the various surfaces of the winery

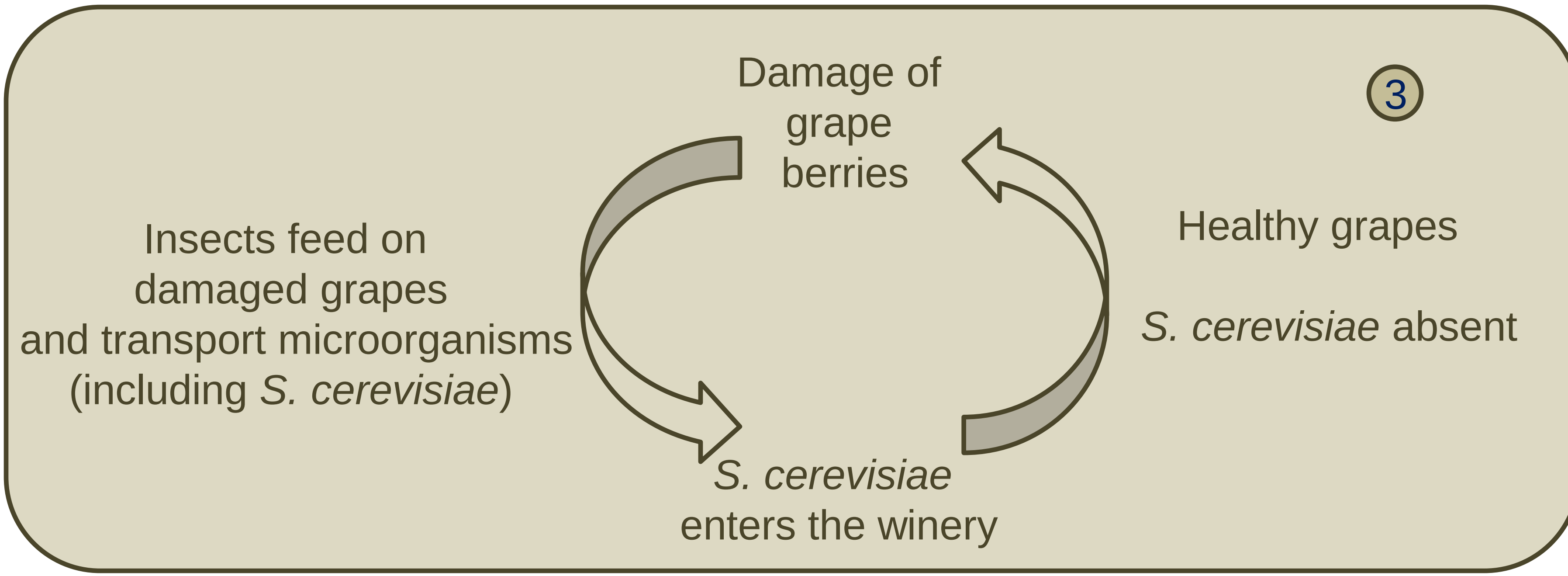


S. cerevisiae does not exist in natural environments – it is therefore a domesticated organism

Mortimer and Polsinelli 1990's

1. Most grape berries do not have *S. cerevisiae* (1/1000)

2. Damaged berries have much higher frequencies of *S. cerevisiae* (1/4)

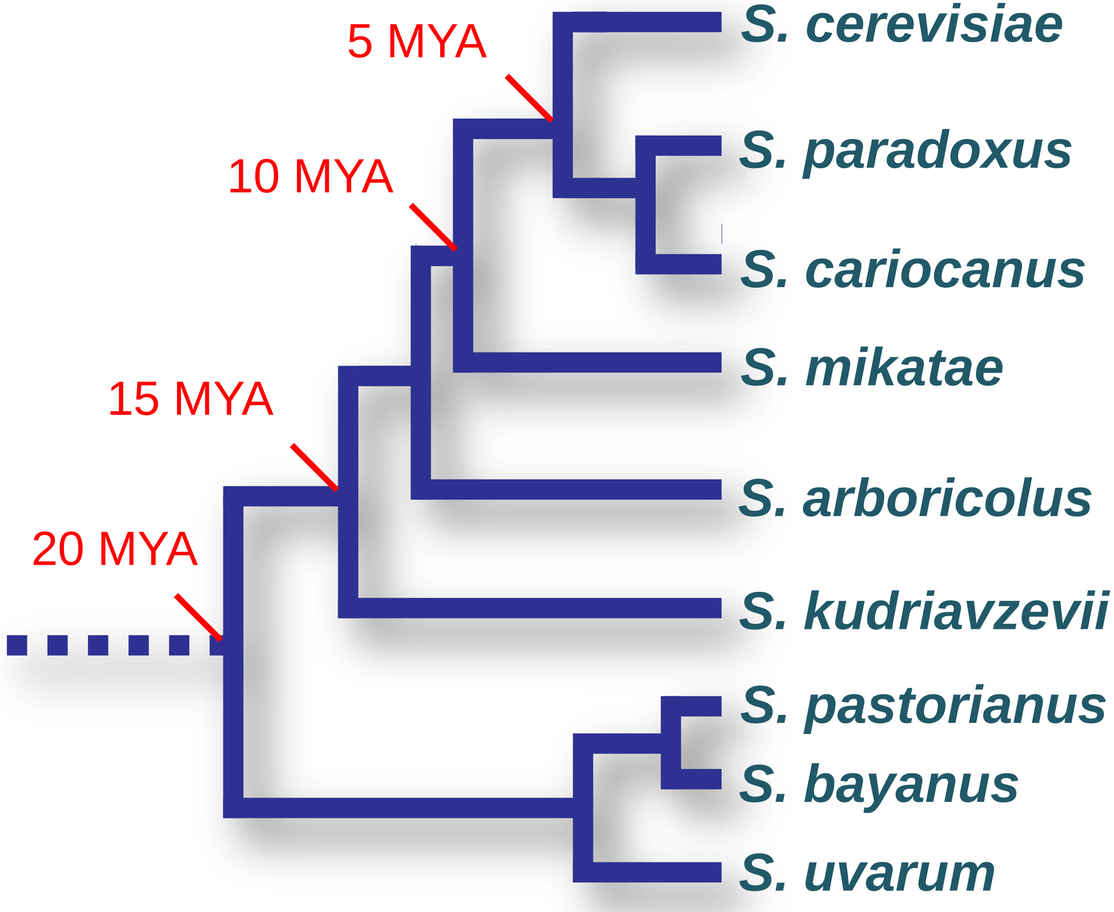


Origin of the yeasts vectored by insects is unknown

outline

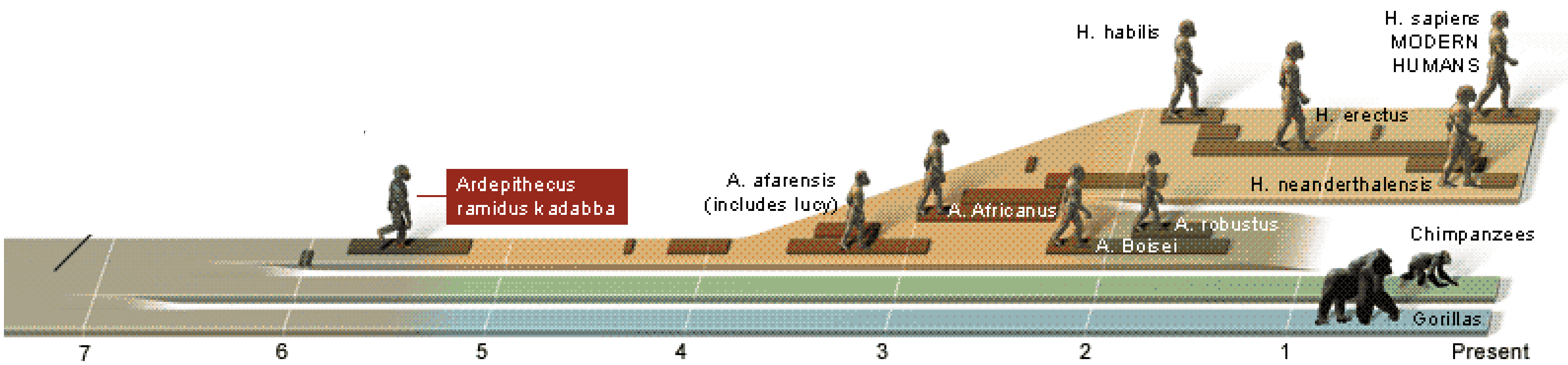
- ecology of wine yeasts – an history of changing views
- Finding the natural habitats of *Saccharomyces*

Genus *Saccharomyces*



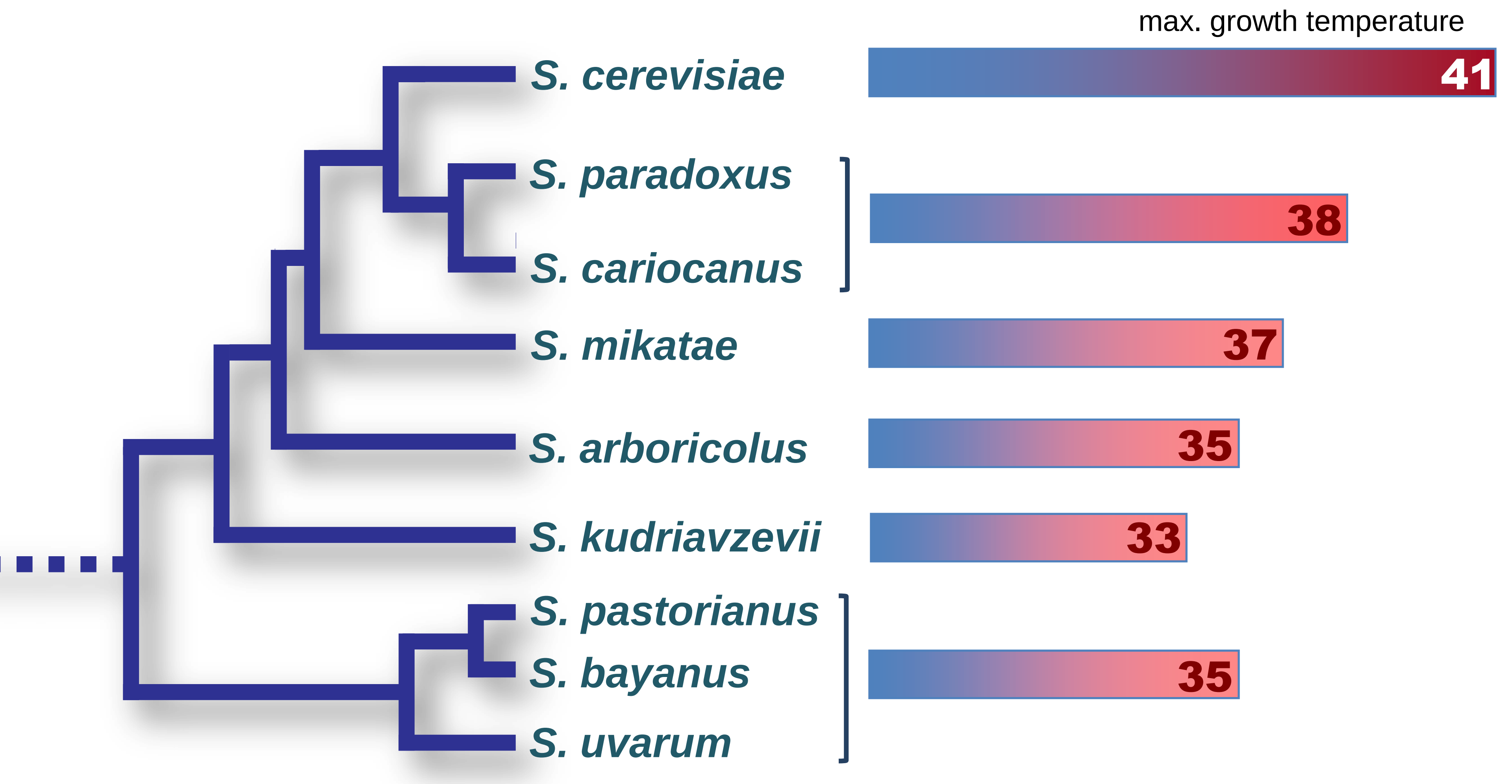
sensu stricto group, van der Walt, 1970

Nucleotide identity in coding regions


H. habilis
H. sapiens
MODERN
HUMANS
H. erectus
Ardepithecus
ramidus kadabba
A. afarensis
(includes lucy)
A. Africanus
H. neanderthalensis
A. robustus
A. Boisei
Chimpanzees
Gorillas
7
6
5
4
3
2
1
Present


S. mikatae
S. paradoxus
S. cerevisiae

Phenotypes



max. growth temperature
S. cerevisiae
41
S. paradoxus
S. cariocanus
38
S. mikatae
37
S. arboricolus
35
S. kudriavzevii
33
S. pastorianus
S. bayanus
S. uvarum
35

[artificial environments created by man cannot be a *Saccharomyces* natural habitat because they are to young (less than 10 000 years)]

Question 1:

What are the natural habitats of *Saccharomyces* spp?

(do the different *Saccharomyces* species have distinct habitats?)

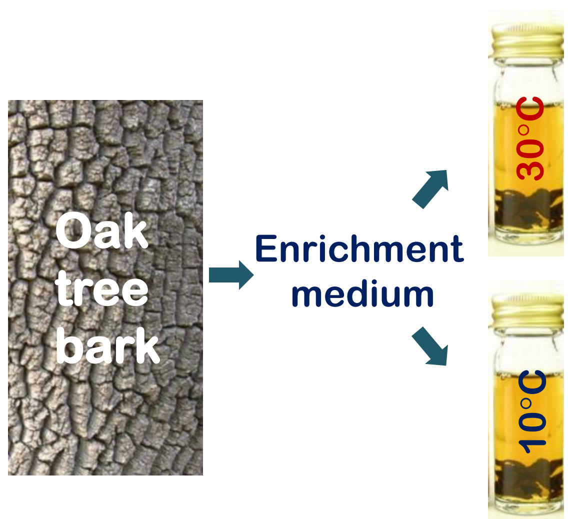
Naumov
1980's – 1990's

S. paradoxus found in tree exudates

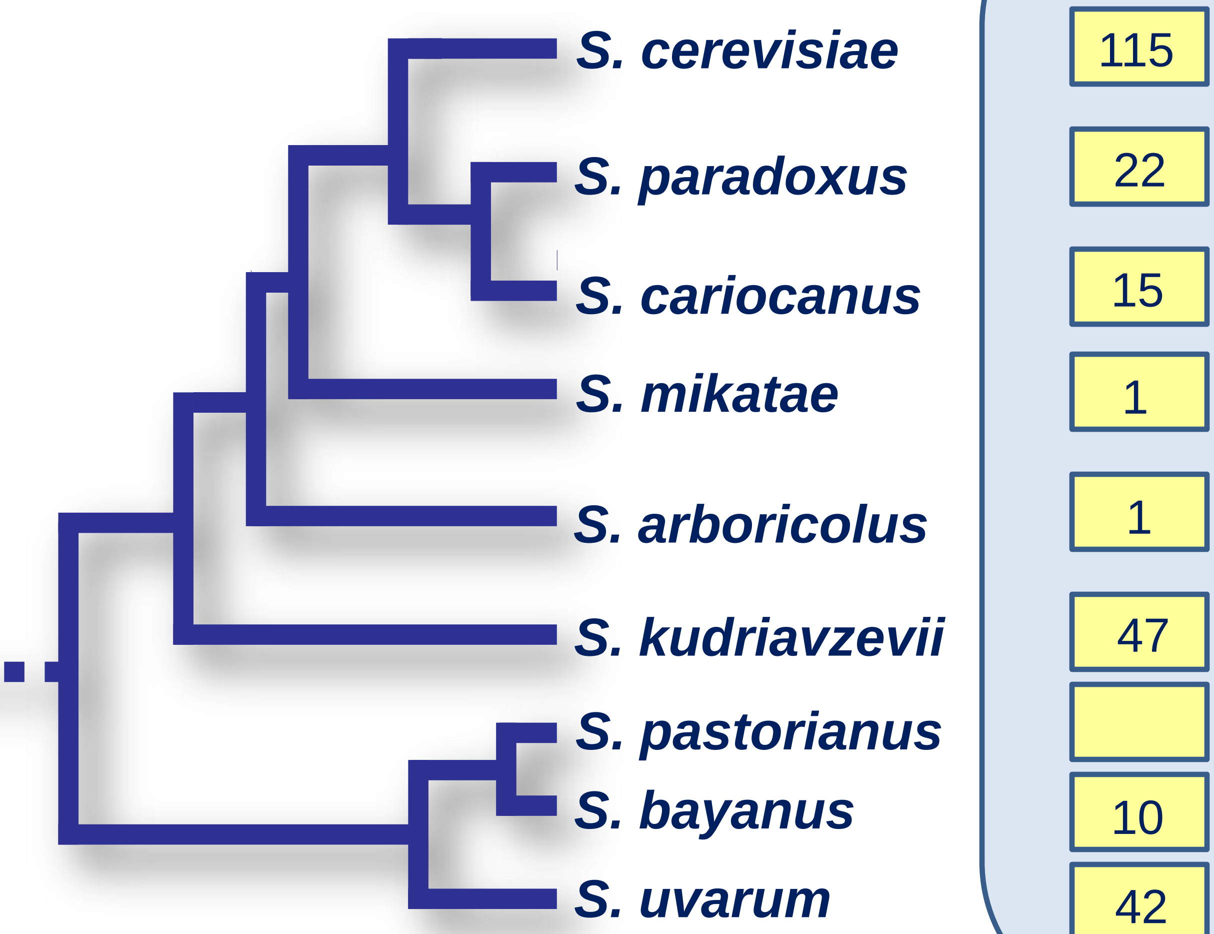
Sniegowsky 2002
FEMS Yeast Research 1: 299-306

- North America (Pennsylvania)
- oak trees
- **selective enrichment**
- **consistent** isolation of *S. paradoxus* and *S. cerevisiae*

Isolation strategy



The tree bark system harbors multiple (all?) *Saccharomyces* species



Localities	Samples
Europe (Portugal, Germany)	164
North America (Canada)	96
South America (Argentina)	52
Oceania (Tasmania, New Zeal.)	64
Asia (Japan)	155
Total	531

Quercus pyrenaica 73%
Quercus faginea 71%

Sampaio and Gonçalves. 2008. Appl Env Microbiol 74: 2144-2152
and unpublished work

The emerging *Saccharomyces* ecology

The oak tree system is a natural habitat for *Saccharomyces*

outline

- ecology of wine yeasts – an history of changing views
- Finding the natural habitats of Saccharomyces
- A model of ecological speciation

Question 2:

If oak bark is an habitat for various *Saccharomyces* species how can competitive exclusion be avoided?

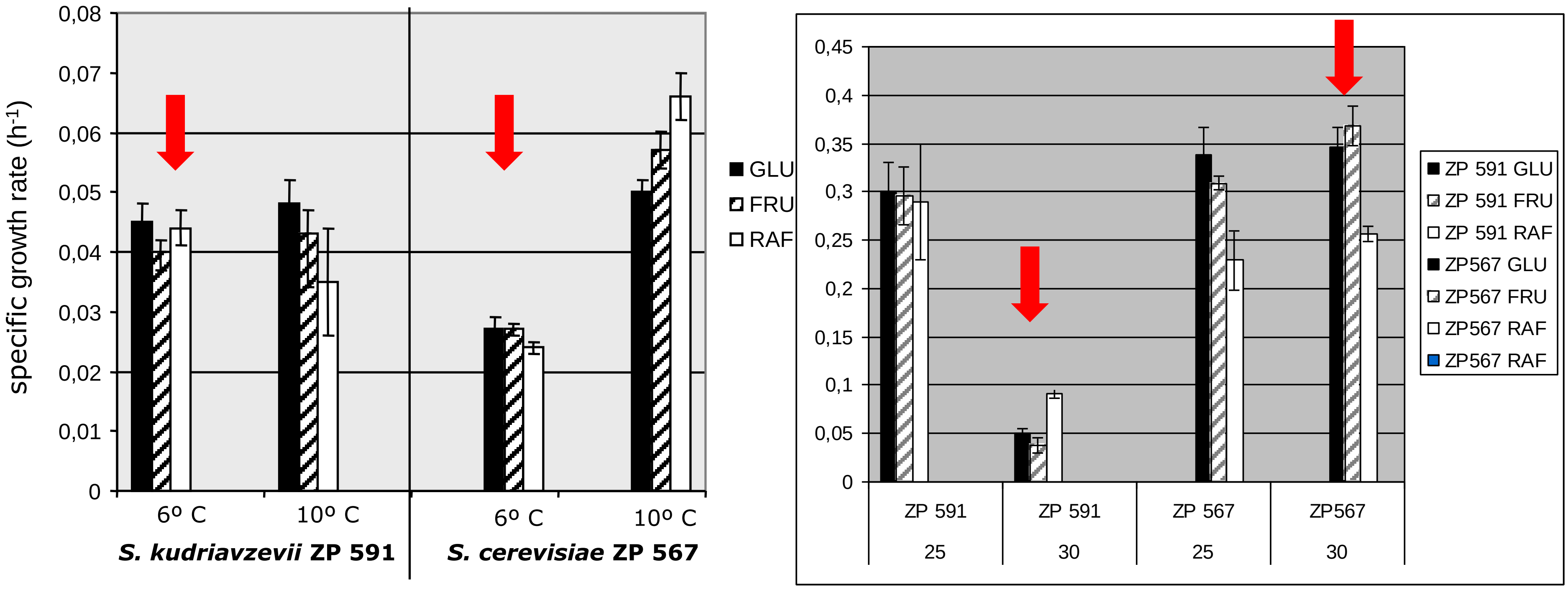
Locality		Country	CER	PAR	UVA	KUD
1	Adagoi	P	■			■
2	Alvão, Olo	P		■		
3	Aldeia das Dez, S. Estrela	P	■	■		■
4	Marão, Campeã	P	■			
5	Lisbon	P				■
6	Lagoa de Albufeira	P		■		■
7	Arrábida	P				■
8	Sines	P		■		
9	Castelo de Vide	P	■			■
10	Paul Boquilobo	P	■			
11	Tübingen	G		■		
12	Murrhardt	G		■		
13	Vancouver	C	■	■		
14	Hornby Island	C		■	■	

Question 2:

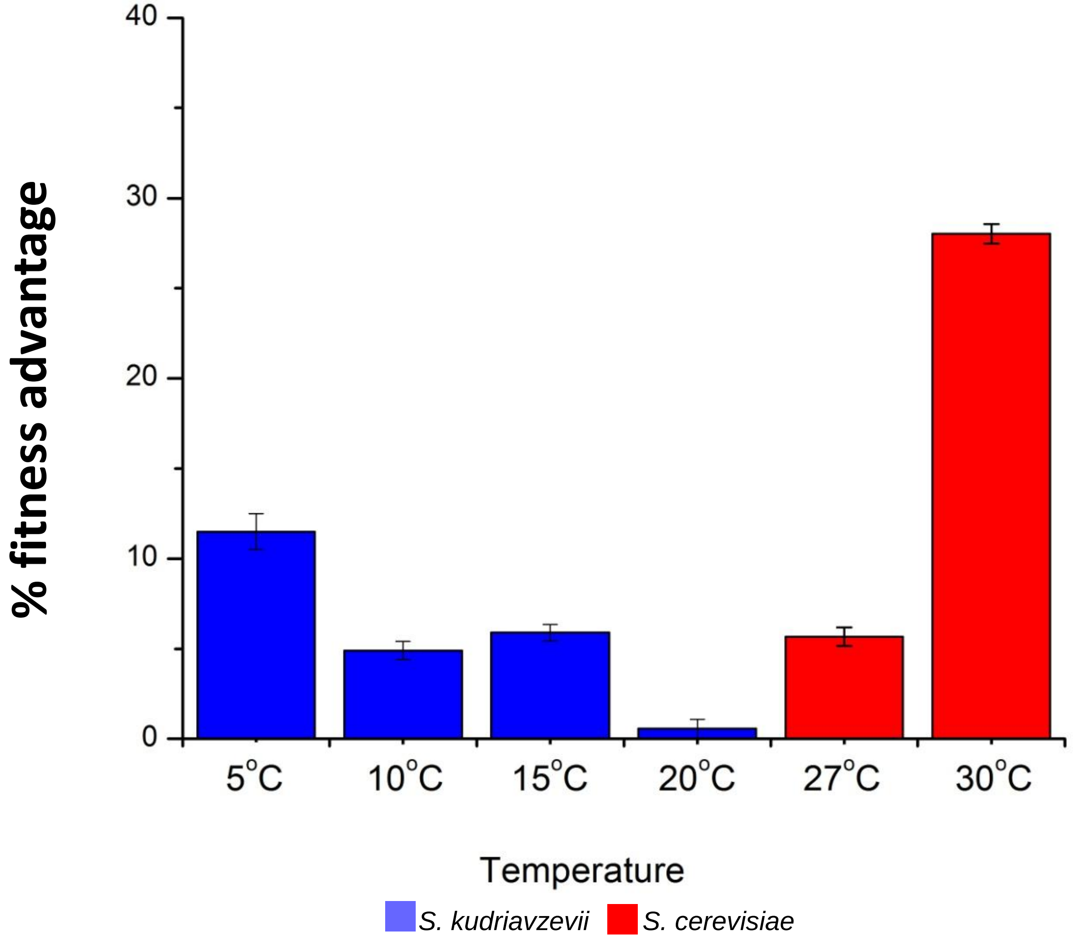
If oak bark is an habitat for various *Saccharomyces* species how can competitive exclusion be avoided?

Locality		Country	CER	PAR	UVA	KUD
1	Adagoi	P	30			10
2	Alvão, Olo	P		30		
3	Aldeia das Dez, S. Estrela	P	30	30		10
4	Marão, Campeã	P	30			
5	Lisbon	P				10
6	Lagoa de Albufeira	P		30		10
7	Arrábida	P				10
8	Sines	P		30 / 10		
9	Castelo de Vide	P	30			10
10	Paul Boquilobo	P	30			
11	Tübingen	G		10		
12	Murrhardt	G		10		
13	Vancouver	C	30	10		
14	Hornby Island	C		30 / 10	10	

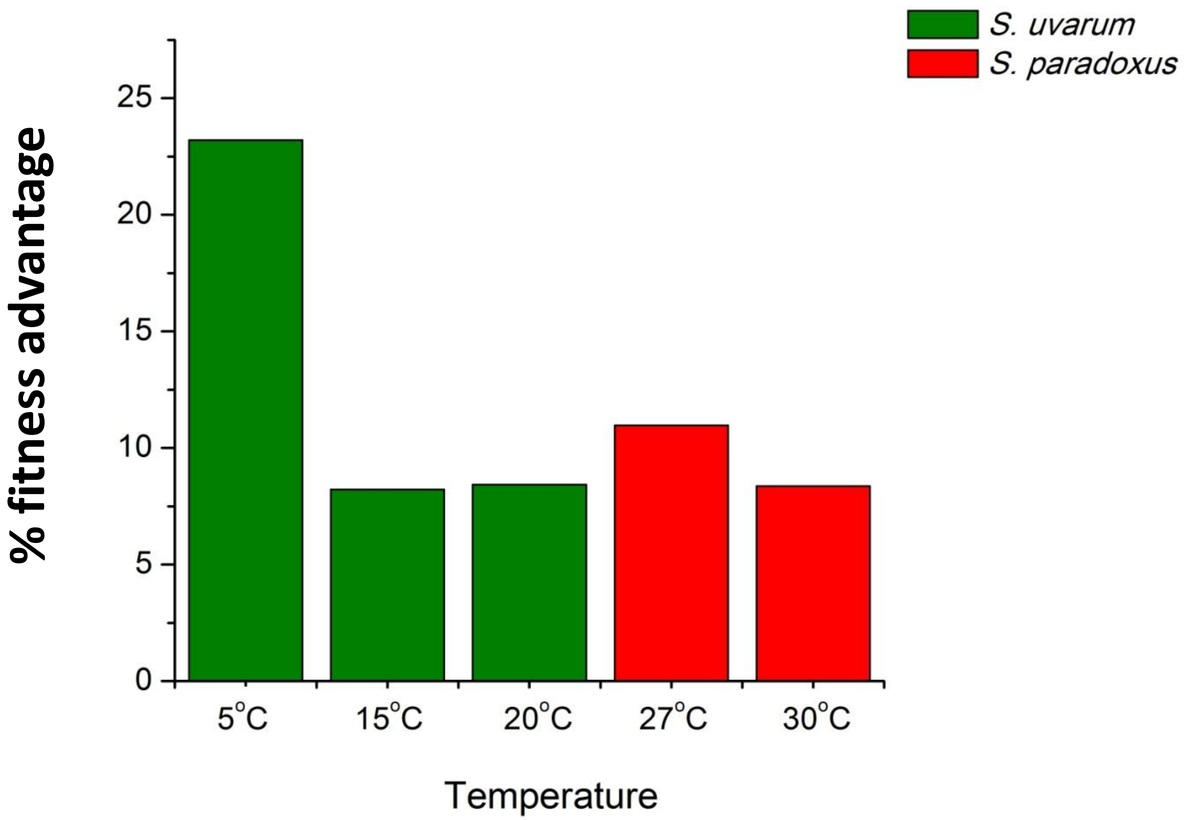
Growth rates of sympatric partners



Competition experiments



Competition experiments



Question 2:

If oak bark is an habitat for various *Saccharomyces* species how can competitive exclusion be avoided?

Sympatric species have evolved different temperature adaptations and circadian or seasonal temperature oscillations seem to facilitate their coexistence

1. *Saccharomyces* species are found in sympatry

2. sympatric partners have different temperature preferences

Question 3:

Did adaptation to distinct temperature ranges play a crucial role in the evolution of (sympatric) *Saccharomyces* species?

[model of ecological speciation]

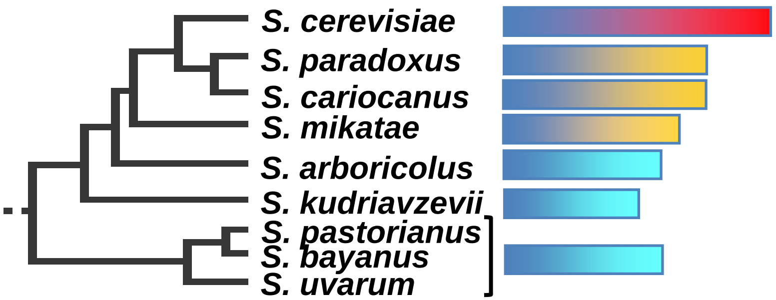
1. *Saccharomyces* species are found in sympatry

2. sympatric partners have different temperature preferences

Question 3 (reformulated)**:**

Is it possible to identify the genes involved in temperature adaptation?

Protein evolution

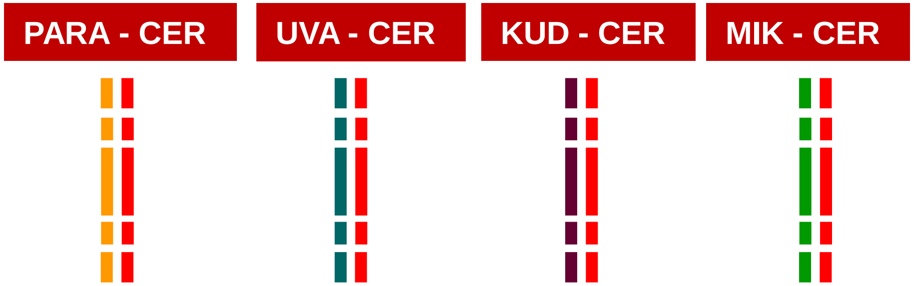


- similar between *S. cerevisiae* / *S. paradoxus*

- more dissimilar than expected between *S. cerevisiae* / *S. uvarum*

Approach

- Generate a set of databases of orthologs (using bidirectional Blast)



Genome wide assessment of gene divergence from *S. cerevisiae* by measuring evolution rates

Evolution rate – Ka/Ks

Ka- nonsynonymous substitutions

e.g. CGC (ARG) → CAC (HIS)

Ks- synonymous substitution

e.g. CGC (ARG) → CGA (ARG)

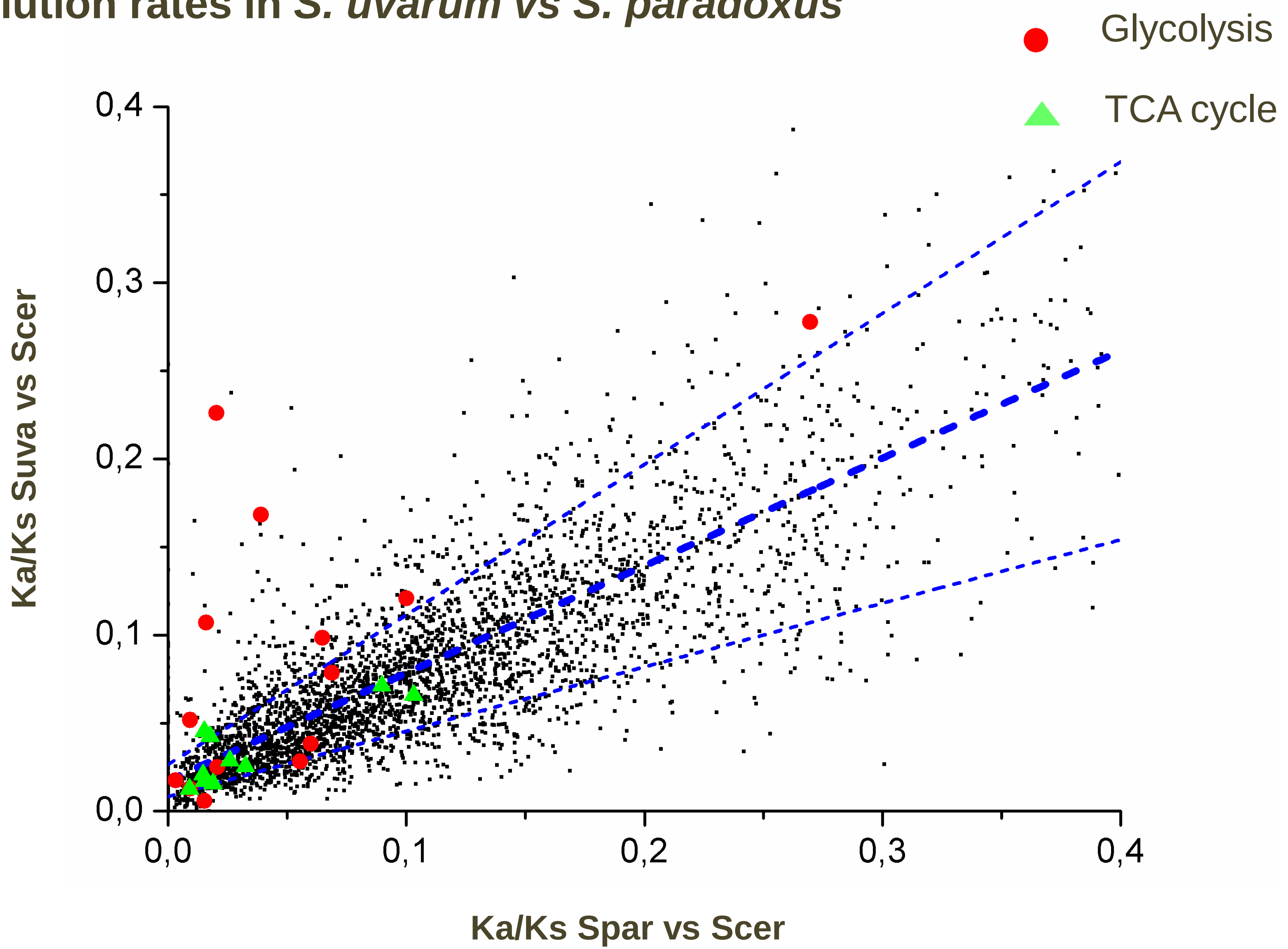
Ka/Ks <1 purifying selection

Ka/Ks =1 neutral

Ka/Ks >1 adaptive evolution

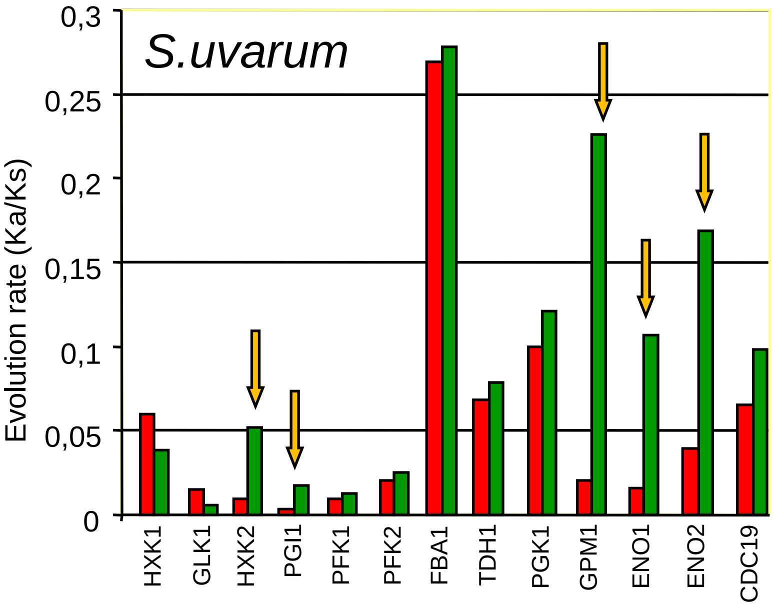
- Aim: to find, among genes with a wide range of evolution rates those for which *Ka*/*K*s UVA-CER >> *Ka*/*K*s PAR-CER

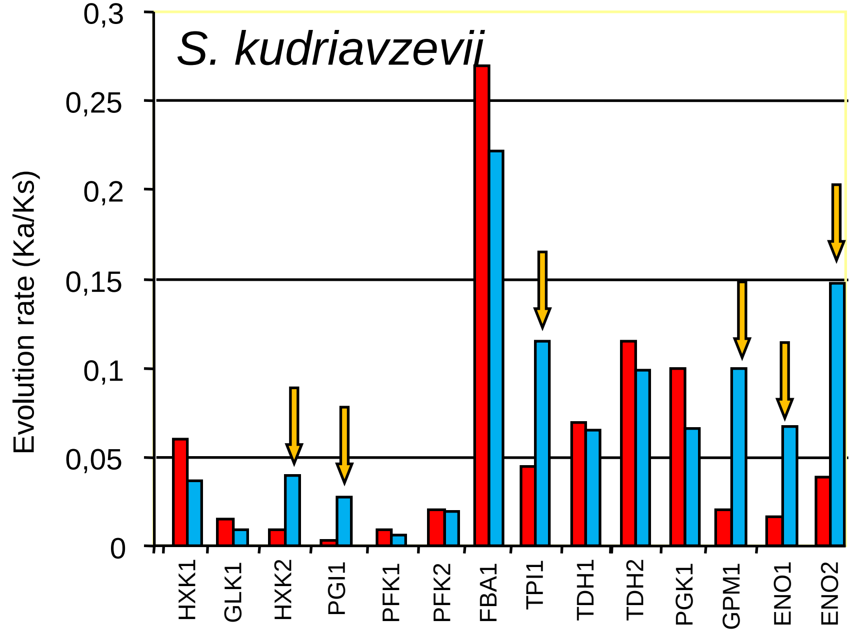
Evolution rates in *S. uvarum vs S. paradoxus*

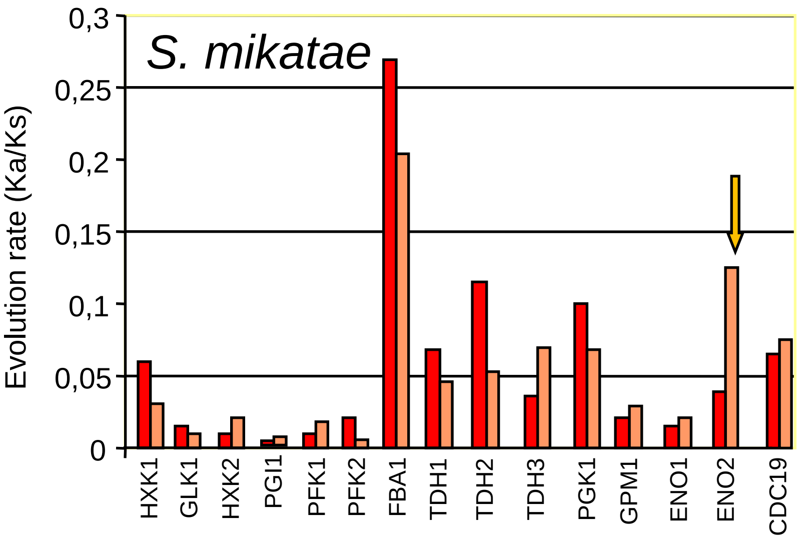


Ka/Ks computation: Yang, Z. 1997. PAML: a program package for phylogenetic analysis by maximum likelihood

Evolution rates of glycolytic genes







S. paradoxus vs S. cerevisiae

A significant number of glycolytic genes presents higher evolution rates in *S. uvarum* and *S. kudriavzevii*, but not in *S. mikatae*

Question 3:

Is it possible to identify the genes involved in temperature adaptation?

It is likely (although unsuspected!) that some glycolytic genes are involved in temperature adaptation

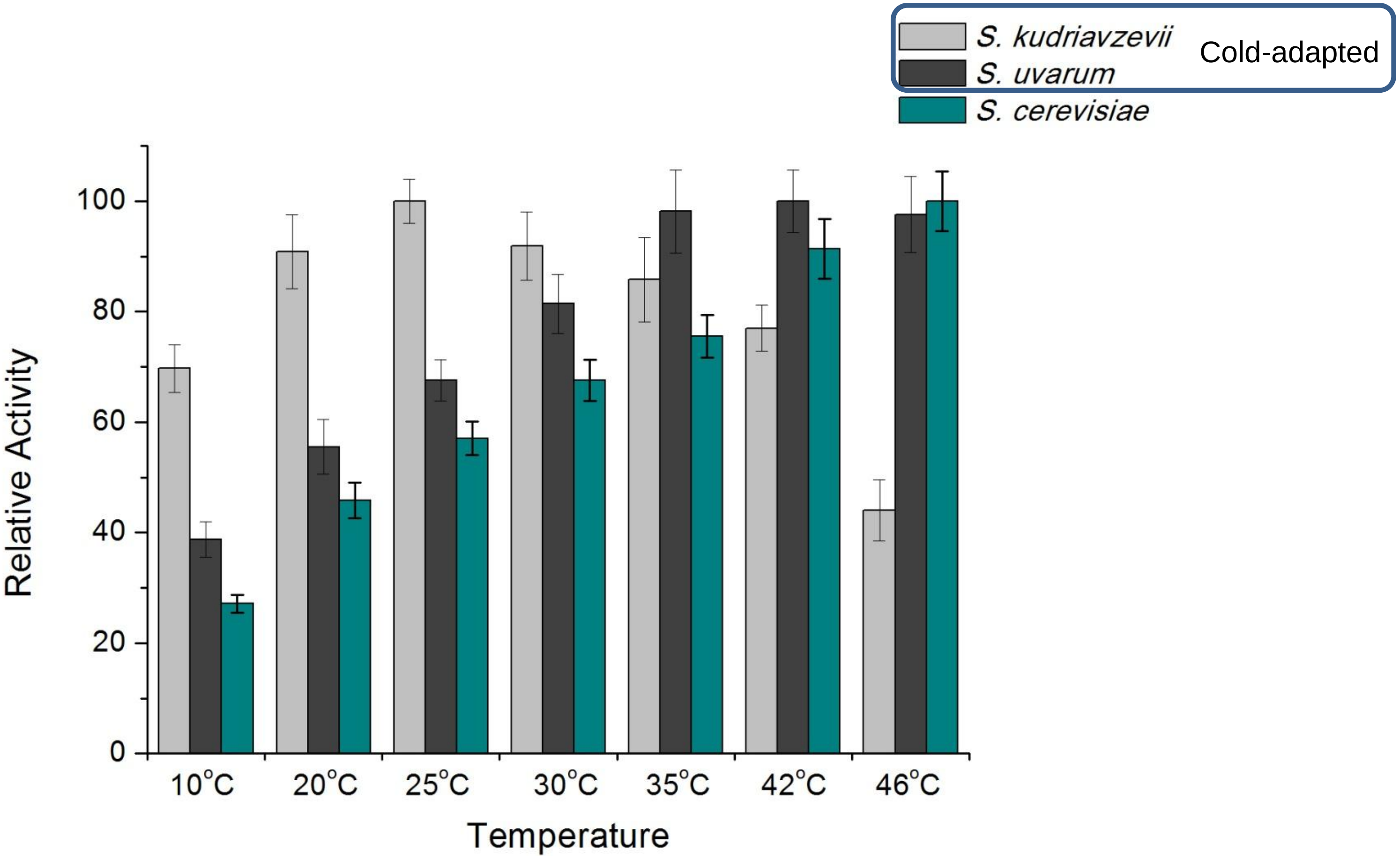
Question 4:

Did adaptation to distinct temperature ranges involved changes in the kinetic properties of glycolytic enzymes in *Saccharomyces* species?

EXPERIMENTS**:**

- Temperature profiles of individual enzymes
- Measure the performance of the entire glycolytic pathway at different temperatures

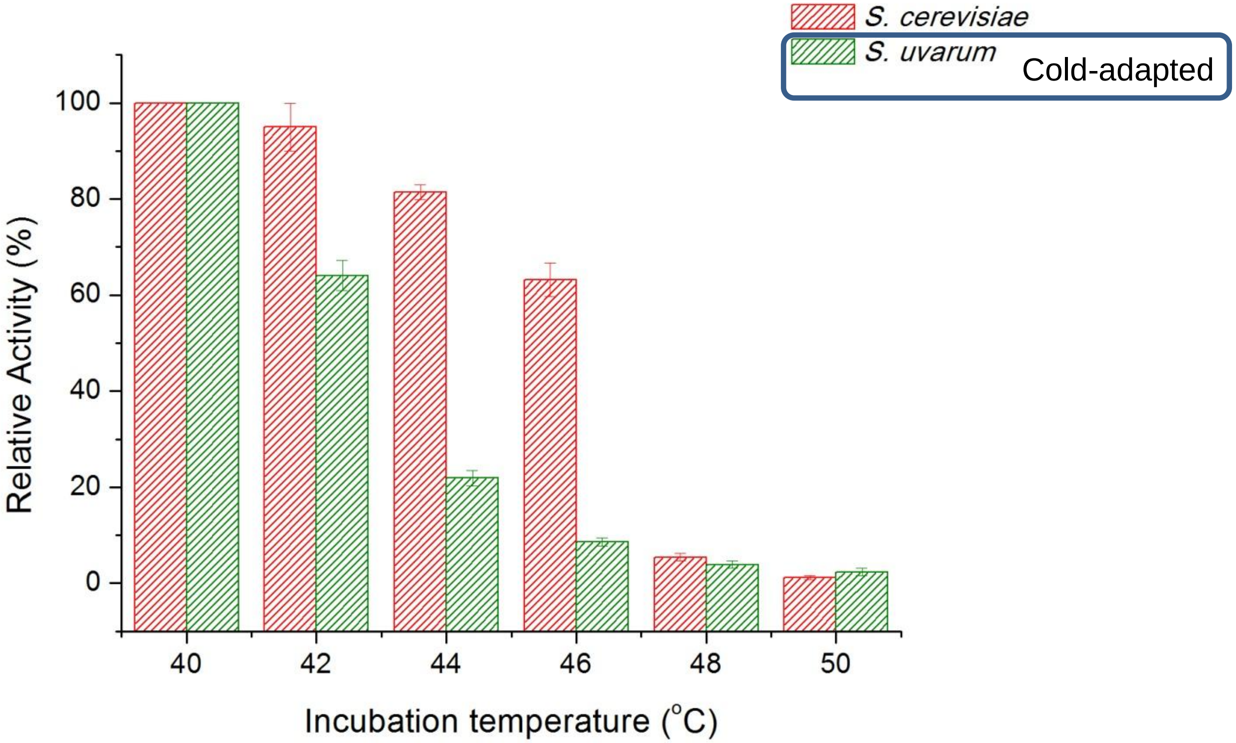
Temperature profiles of hexokinase activity



S. kudriavzevii - higher HXK activity in at low temperatures but lower stability above 30ºC

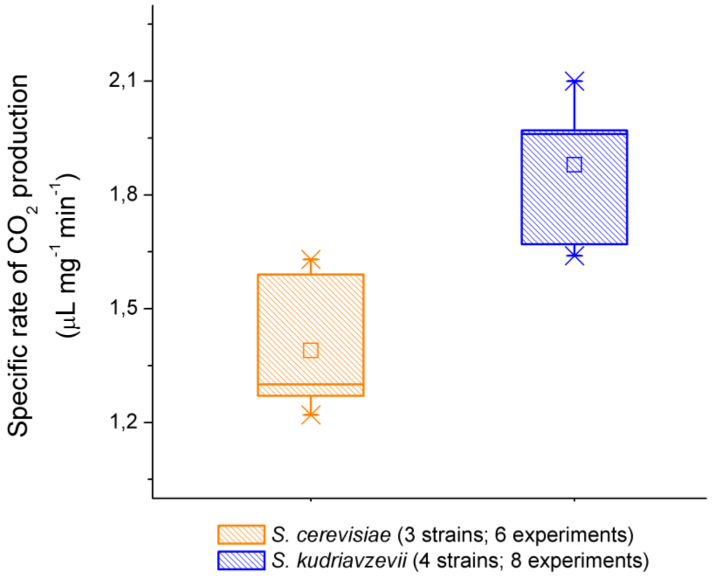
S. cerevisiae and *S. uvarum* have similar profiles

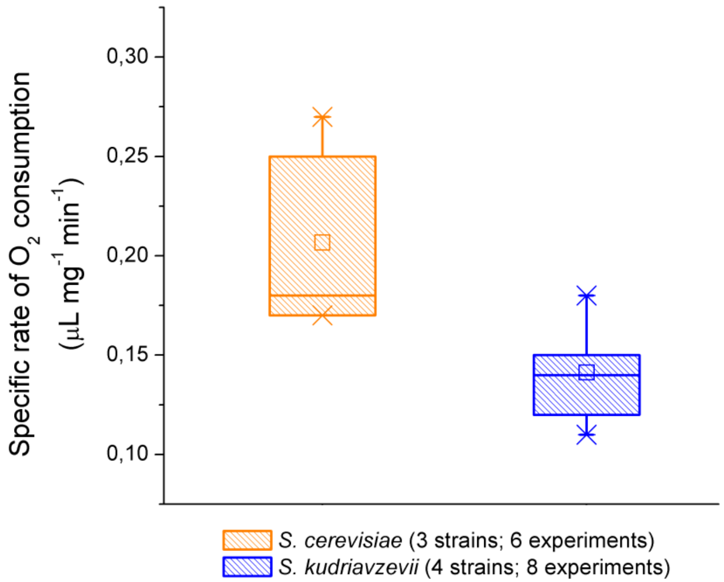
Hexokinase stability



Lower stability of *S. uvarum* HXK above 42ºC, when compared with *S. cerevisiae*

Measurement of the glycolytic flux (CO_2 production) – Warburg apparatus





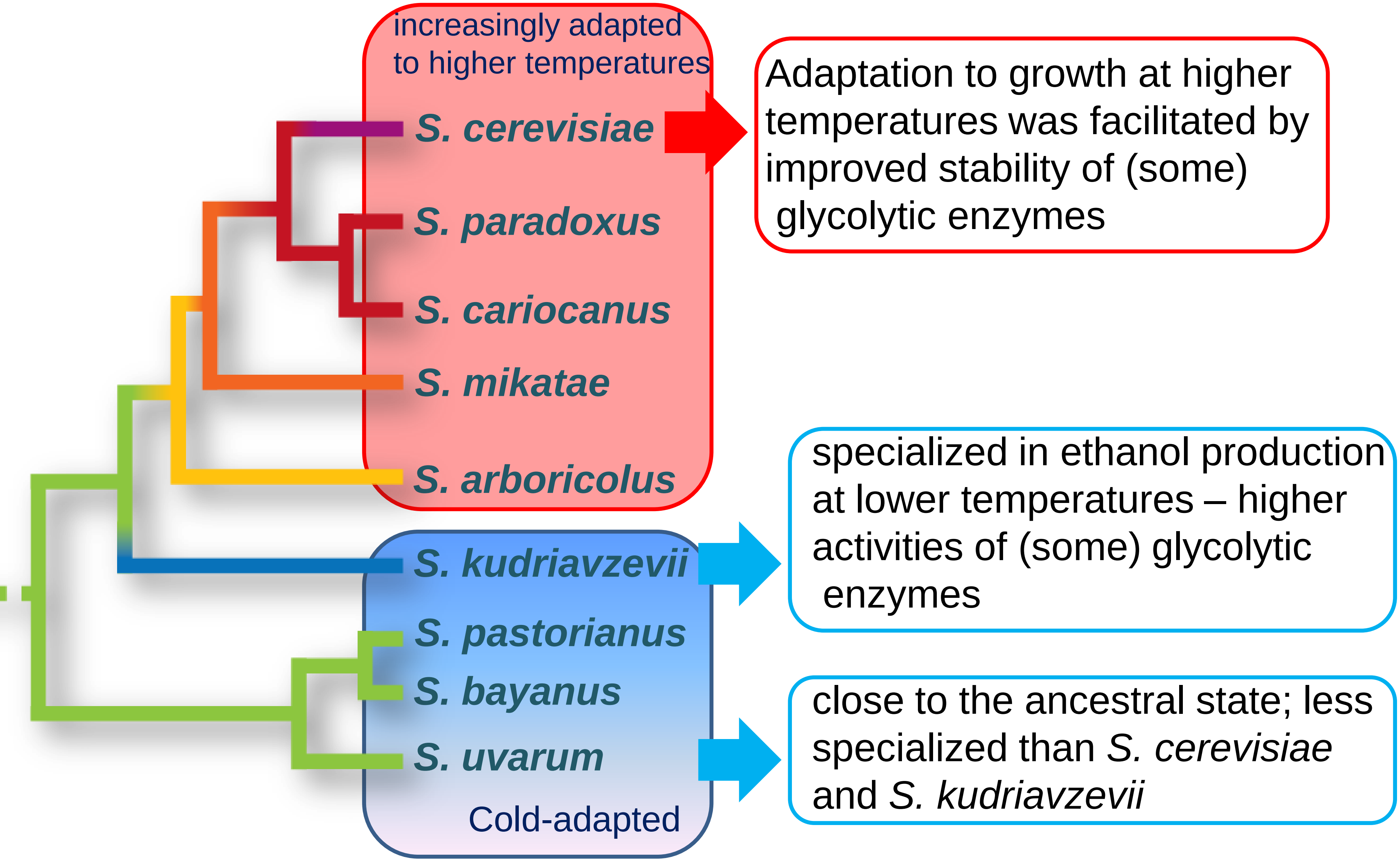
- Higher glycolytic flux for *S. kudriavzevii* at low temperatures (10°C)

Question 4:

Did adaptation to distinct temperature ranges involved changes in the kinetic properties of glycolytic enzymes in *Saccharomyces* species?

Yes (also for glycolytic flux)

An ecological model for the evolution of the genus *Saccharomyces*



The emerging *Saccharomyces* ecology

- **The most consistent natural habitat of *Saccharomyces* is the oak tree system**
- **In nature *Saccharomyces* species are sympatric**
- ***Saccharomyces* species that share the same habitat minimize competition because they have different temperature adaptations**
- **Speciation in *Saccharomyces* might have an ecological basis related with temperature adaptation**
- **Divergent adaptation of glycolytic enzymes (and of glycolysis) to perform optimally under distinct temperatures**

Acknowledgments

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Evolutionary ecology of wild populations of *Saccharomyces* from the bark of Fagaceae