



From yeast diversity to yeast domestication

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From yeast diversity to yeast domestication

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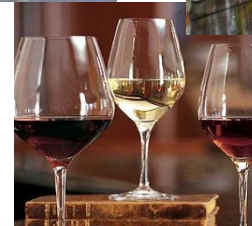


Diversity of *Saccharomyces cerevisiae* habitats

Natural ressources



Environments associated to humans

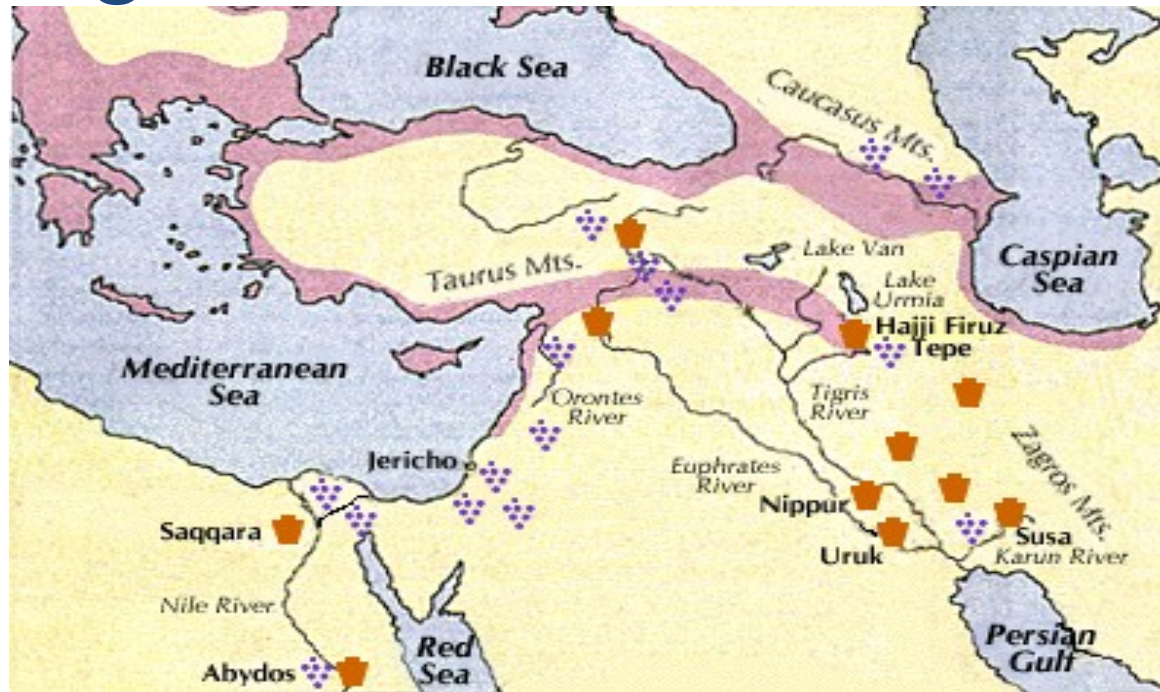




<http://www.penn.museum/research-asian-section/387-the-earliest-alcoholic-beverage-in-the-world.html>

Remains of the most ancient beverage found in China in a Neolithic tomb in Jiahu village (-7000 BC) (Mc Govern PNAS 2004)

Origin of Vine culture and wine making



<http://www.penn.museum/sites/wine/wineneolithic.html>

- potteries dated from 5400 BC in Iran (Hajji Firuz Tepe) (McGovern 1996)
- Identification tartaric acid and resins residuals => wine

Molecular methods for the characterization of yeast diversity

Pulsed

(Schwartz & Cantor, Cell 1984, Blondin et al 1990...)

mDNA RFLP (Aigle et al, J Inst Brew 1984, Querol 1992...)

AFLP (Azumi et Goto-Yamamoto, Yeast 2001)

RAPD (Pafeti et al 1995...)

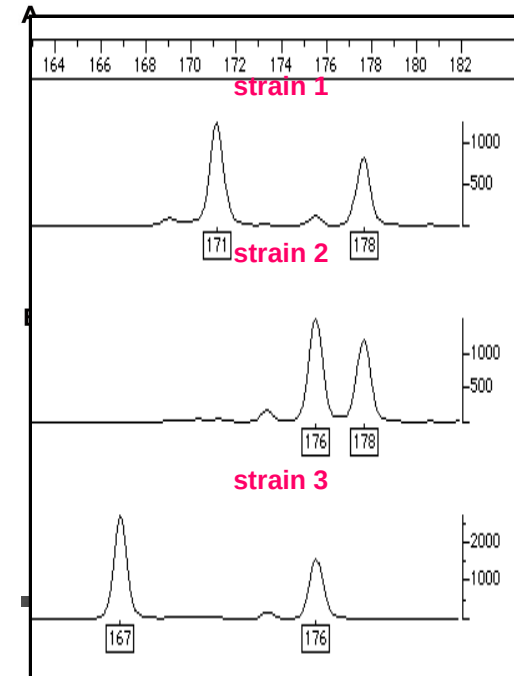
Interdelta typing (Ness et al. 1993, Legras et al. 2003)

Microsatellite Typing (SSR) (Field & Wills PNAS 1998)

Field

Gel

electrophoresis

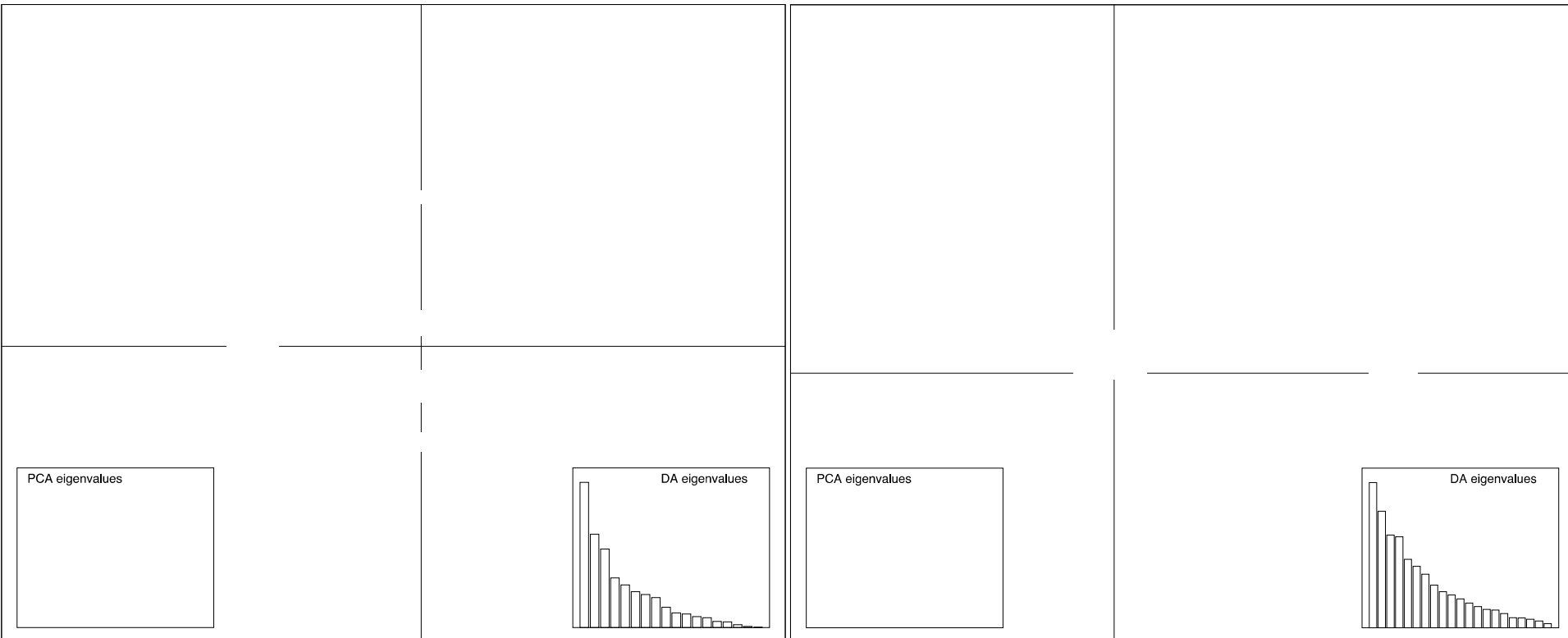


Sequencing

Multi Locus Sequence Typing (Fay & Benavides 2005...)

Population structure obtained with: mDNA RFLP and Microsatellite Typing

- 200 strains from 30 sites in Italian vineyards (DPCA)

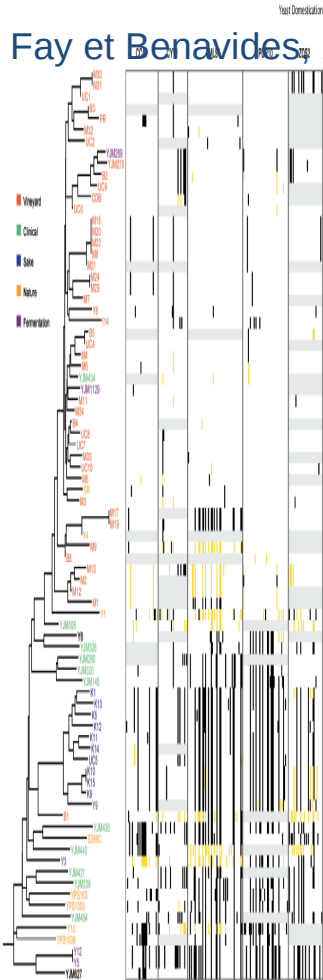




Are strains of various niches different?

Population structure and yeast domestication

Fay et Benavides, 2005



Wine

Sake

- lower diversity for wine and sake yeast
=> wine yeast and sake yeast
2 domestication events

divergence

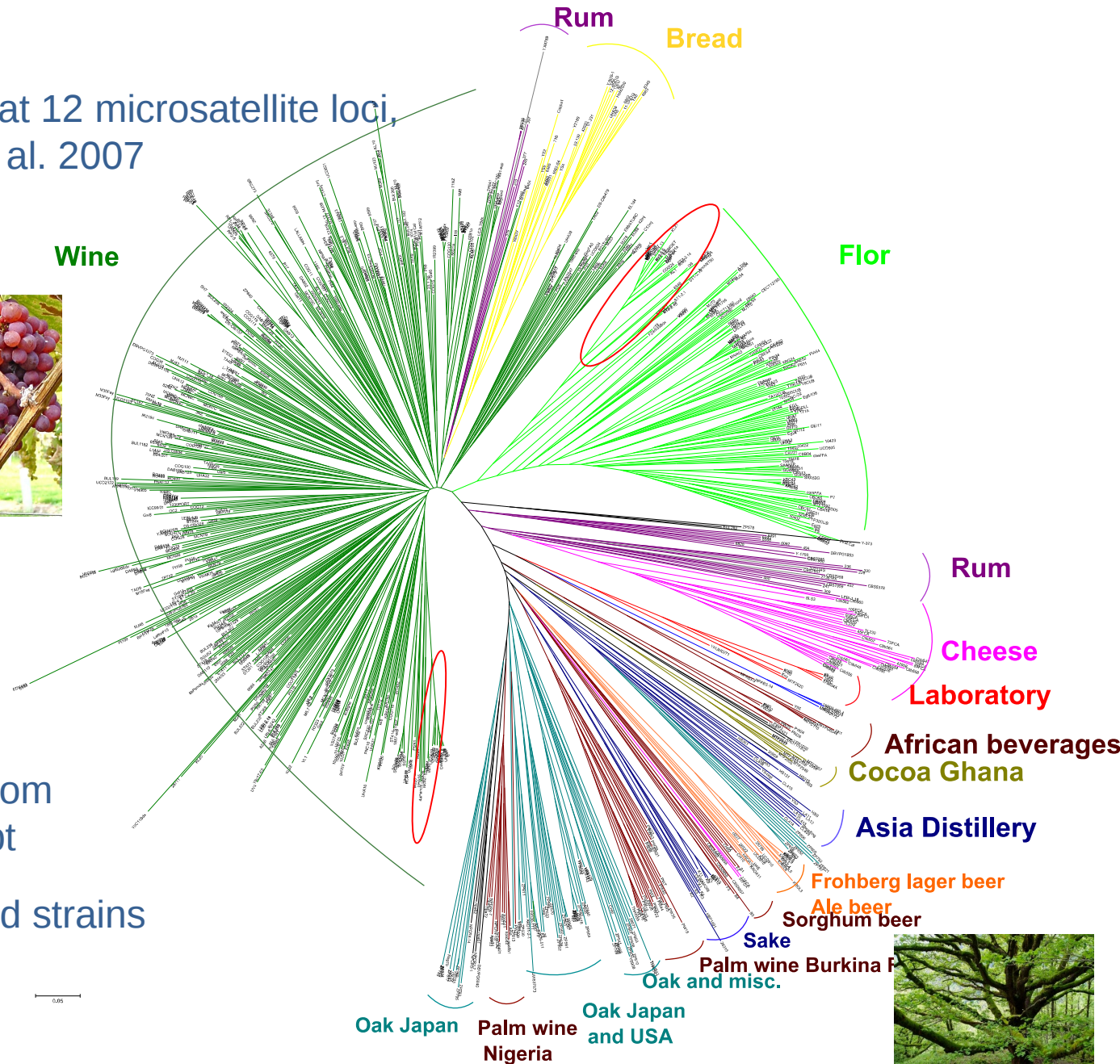
wine strains 2,700 – 27,000 BP

sake strains 3,800 – 38,000 BP

Figure 1. A Neighbor-Joining Tree Showing Divergence among Yeast Strains Isolated from Different Sources

origins

1000 strains genotyped at 12 microsatellite loci,
updated from Legras et al. 2007
Bruvo's distance



Among wine strains:
- Few identical strains from distant vineyards, except starters
- 2 groups of very related strains

Interspecific hybrids are frequent

Several groups of *S. cerevisiae* *
S. kudriavzevii hybrids

isolated in

Alsace (France) (1)

Geisenheim (Allemagne) (1),

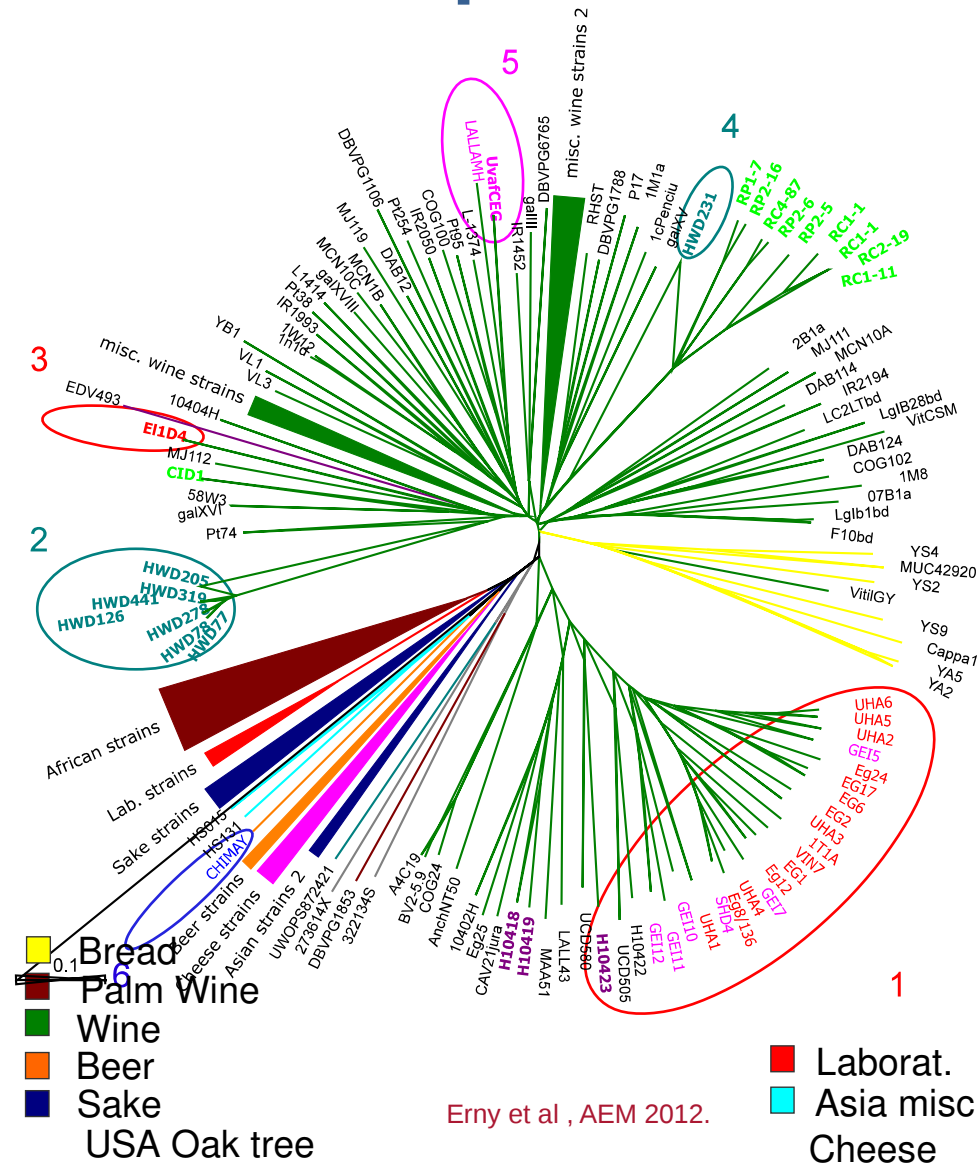
Hungary(1)

OR:

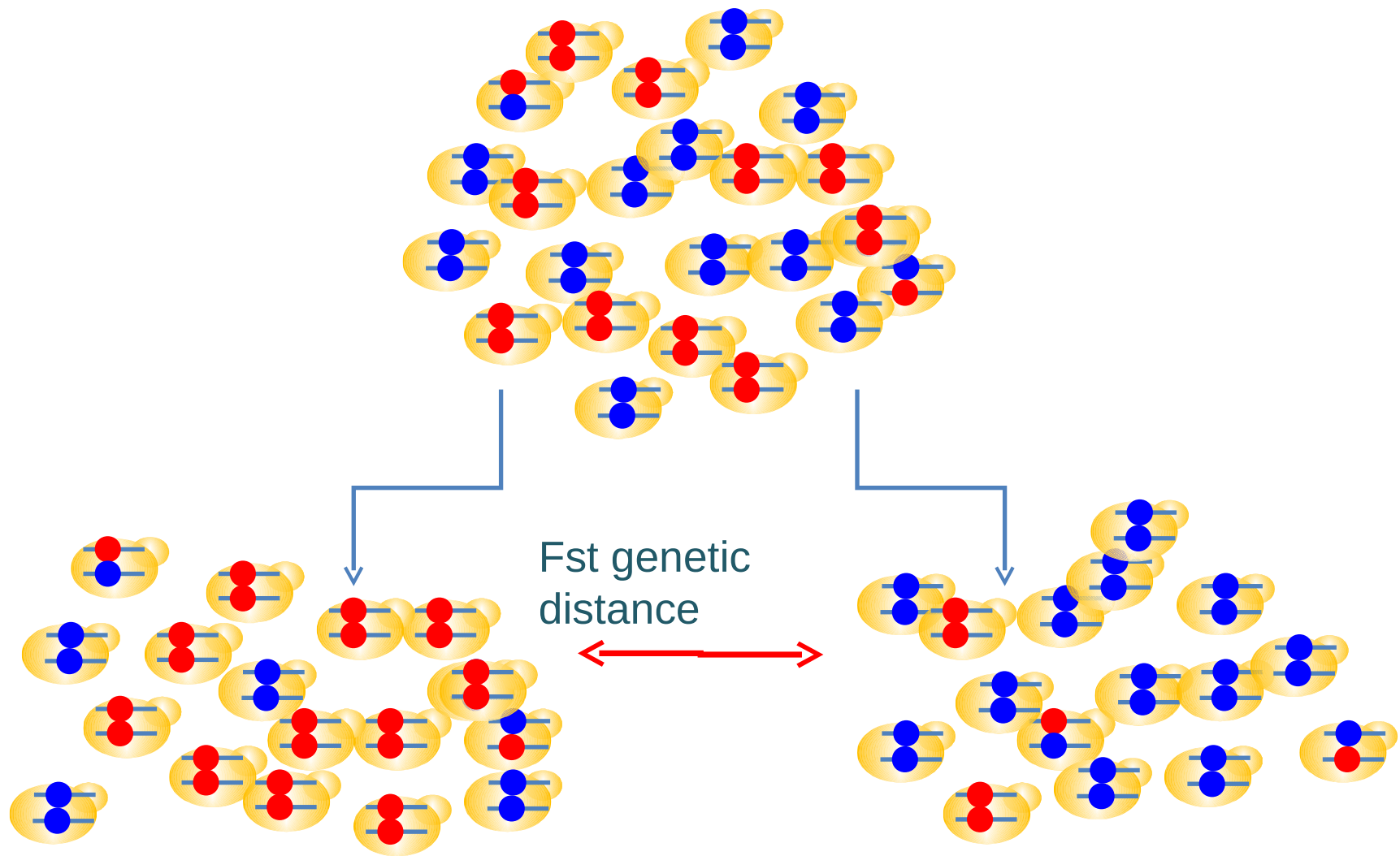
Wadenswill (2 and 4) (Suisse)

Alsace (3) :

Hybrides *S. cerevisiae***S. uvarum*
(Alsace 2004, Diois, ...)



Population differentiation

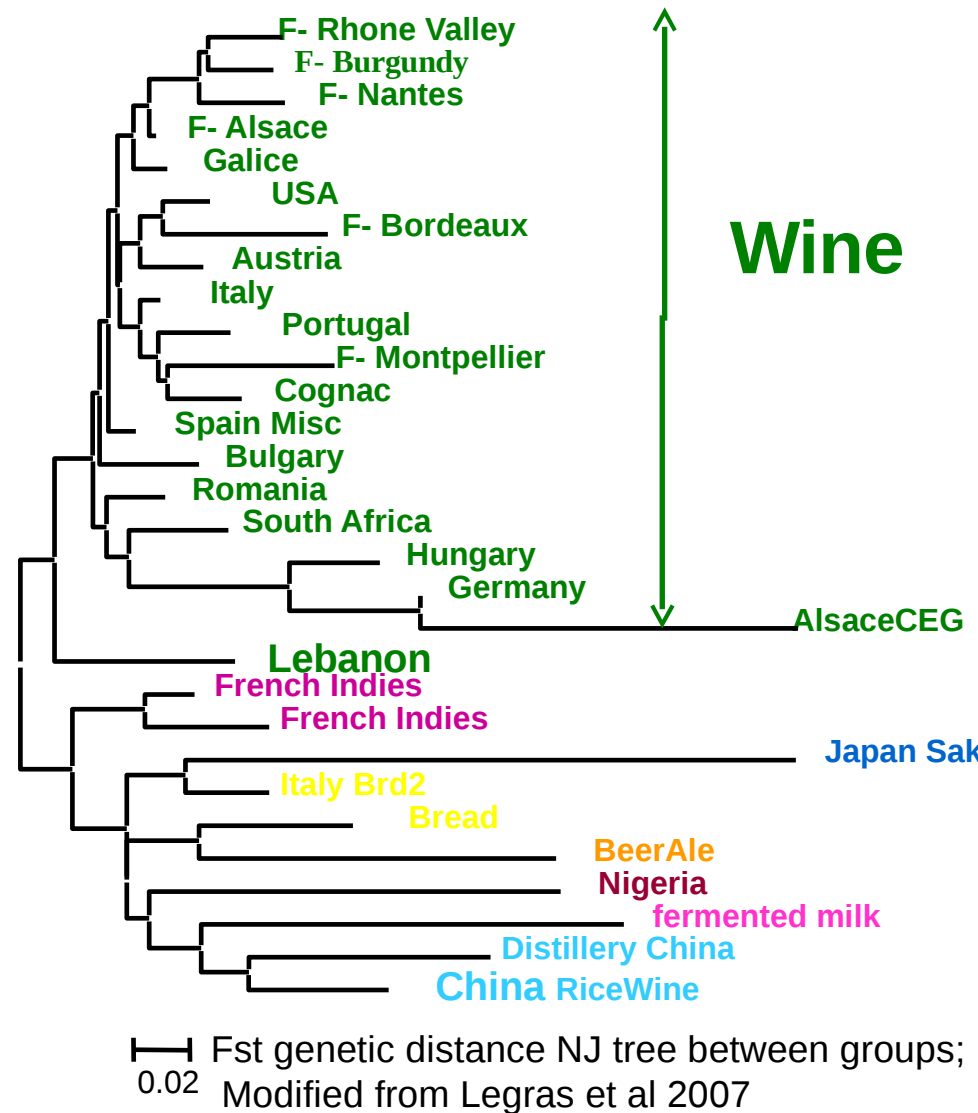


Relationships between populations

Wine yeast separated from other strains
possible Mesopotamia origin .

For other groups

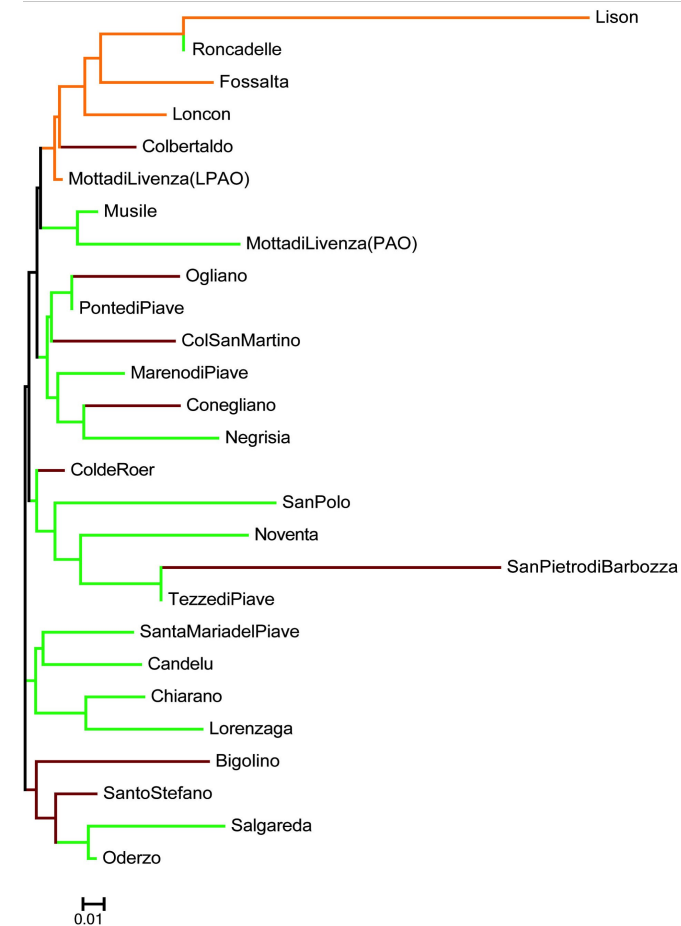
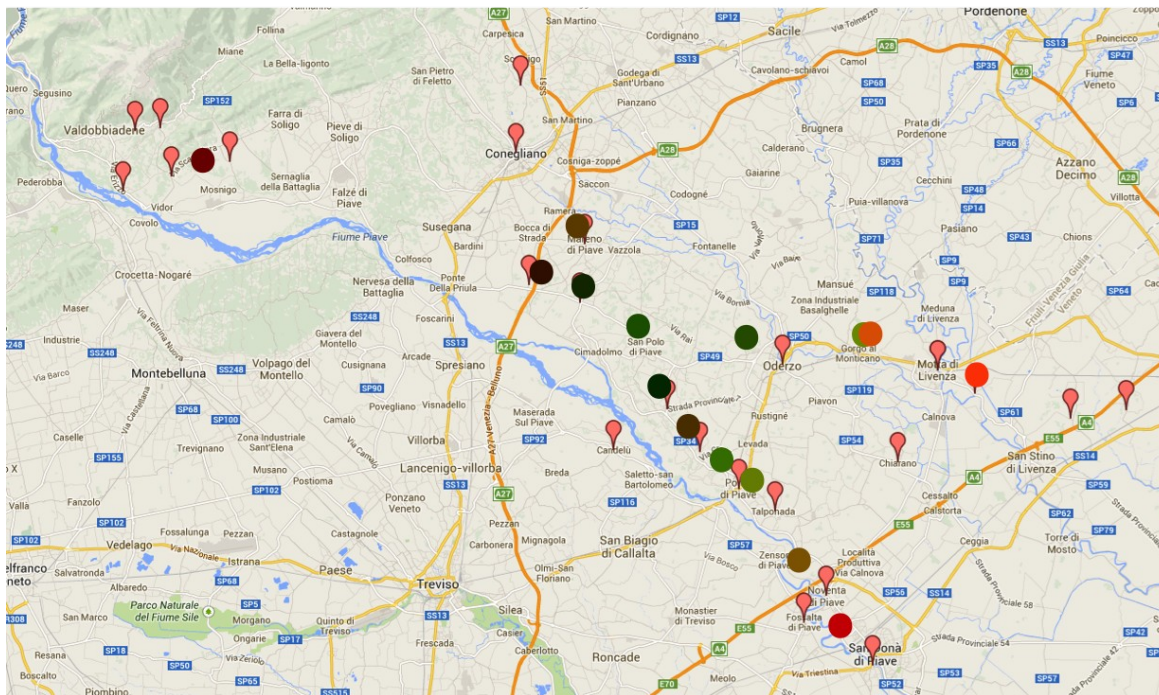
26% of genetic distance between groups explained by geography suggests local domestications



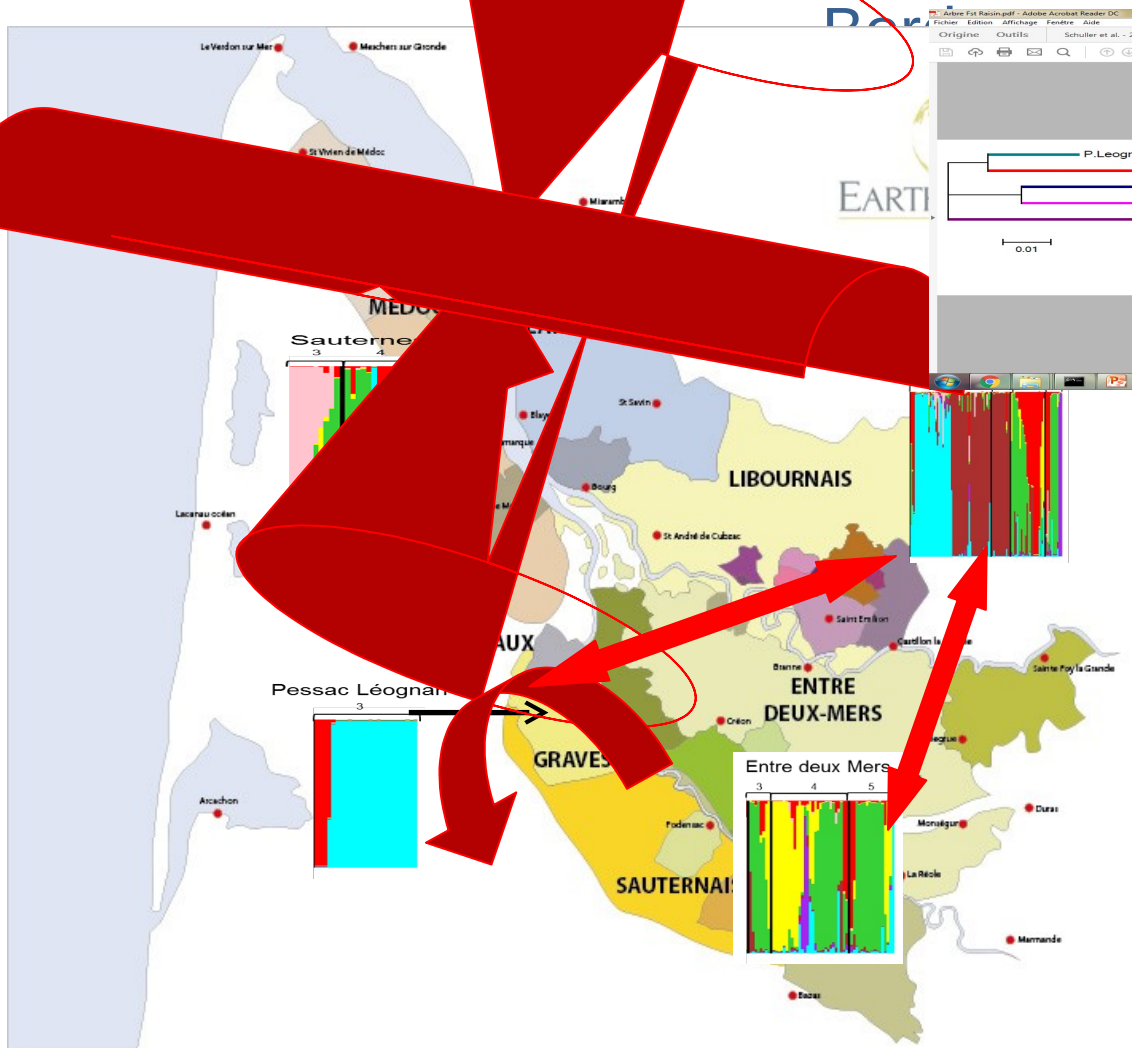
Diversity of *S. cerevisiae* wine populations

Differentiation of yeast populations in 3

Italian vineyards (Treviso) Coll. V Corrich Padova
220 isolates genotyped with microsatellite markers
(Viel et al. 2017 Frontiers Microbiology)



Diversity of yeast populations from vineyards of the Bordeaux region coll. I Masneuf ISVV



Migration pattern
(inferred with Migrate)

Borlin et al. In prep

Vine - Cellar relationship



Wine - cellar relationship

collaboration ISVV Bordeaux

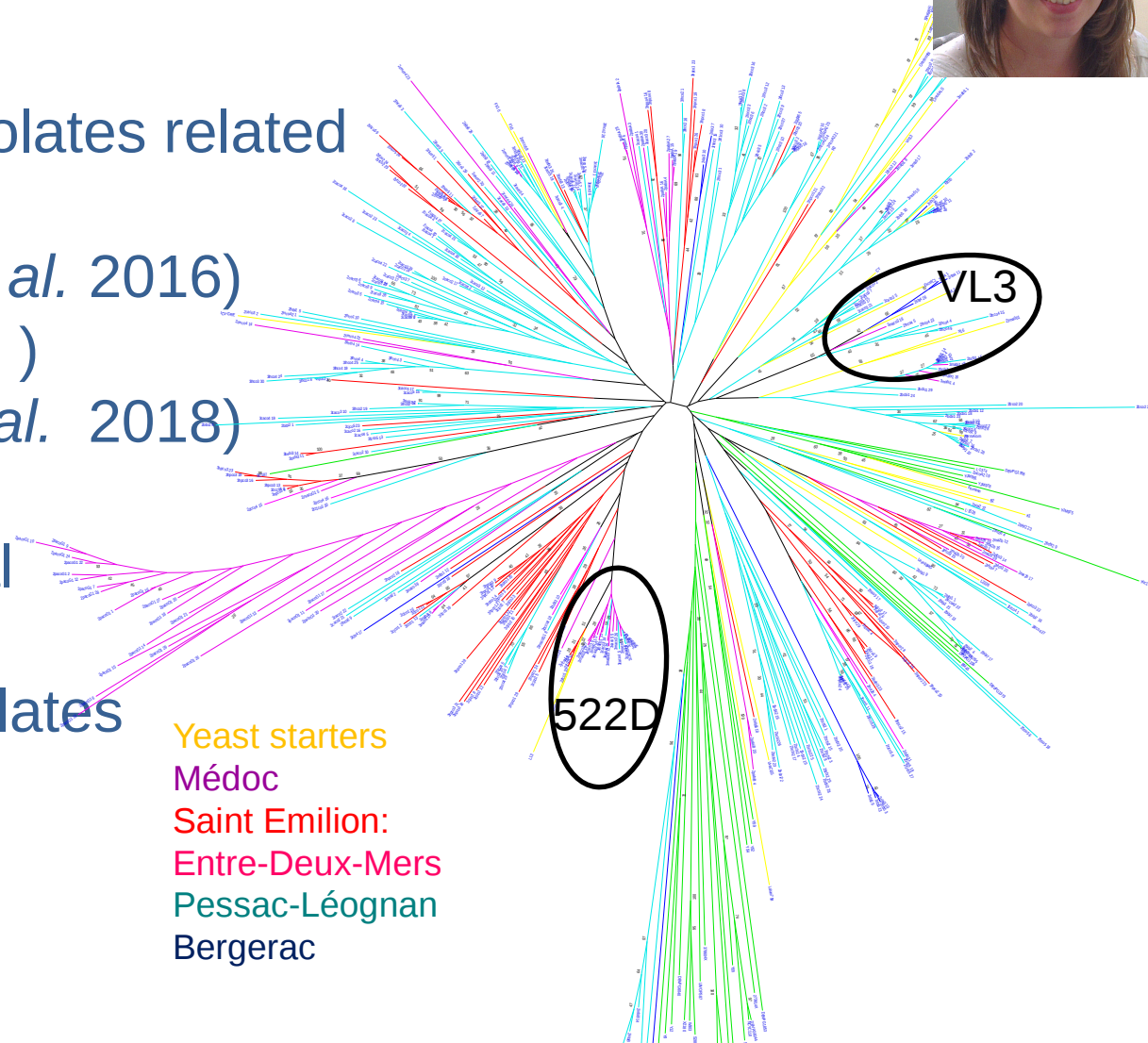


Detection of grapes isolates related to yeast starters in

- Oregon (Martiniuk *et al.* 2016)
- Italy (Viel *et al.* 2017)
- Bordeaux (Borlin *et al.* 2018)

but not 100 % identical

Up to 10 to 25% of isolates





Vine – Cellar connection

collaboration ISVV Bordeaux I. Masneuf

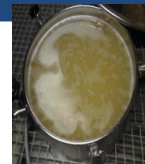
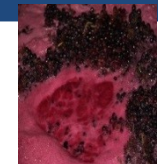


4 cellars et 4 vineyards from 3 areas (2 x 72 souches) (removal of yeast starters)

1 – small differentiation between cellar and vineyard (Nm = 191 : 166-226 = 0.03)

2 – modellisation of gene flow (Bayesian)

**Two compartments in equilibrium
with a higher grapes / vat flow**



(Börlin et al. 2018 in prep)

Nm = 55 : 25-83



Persistence of yeast populations in cellar

collaboration ISVV Bordeaux I. Masneuf



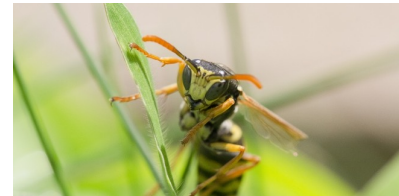
Inter vintage differentiation (Sauternes area, Börlin *et al* 2016 AEM)



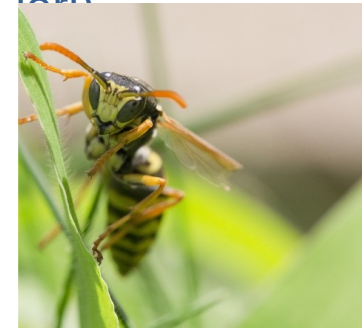
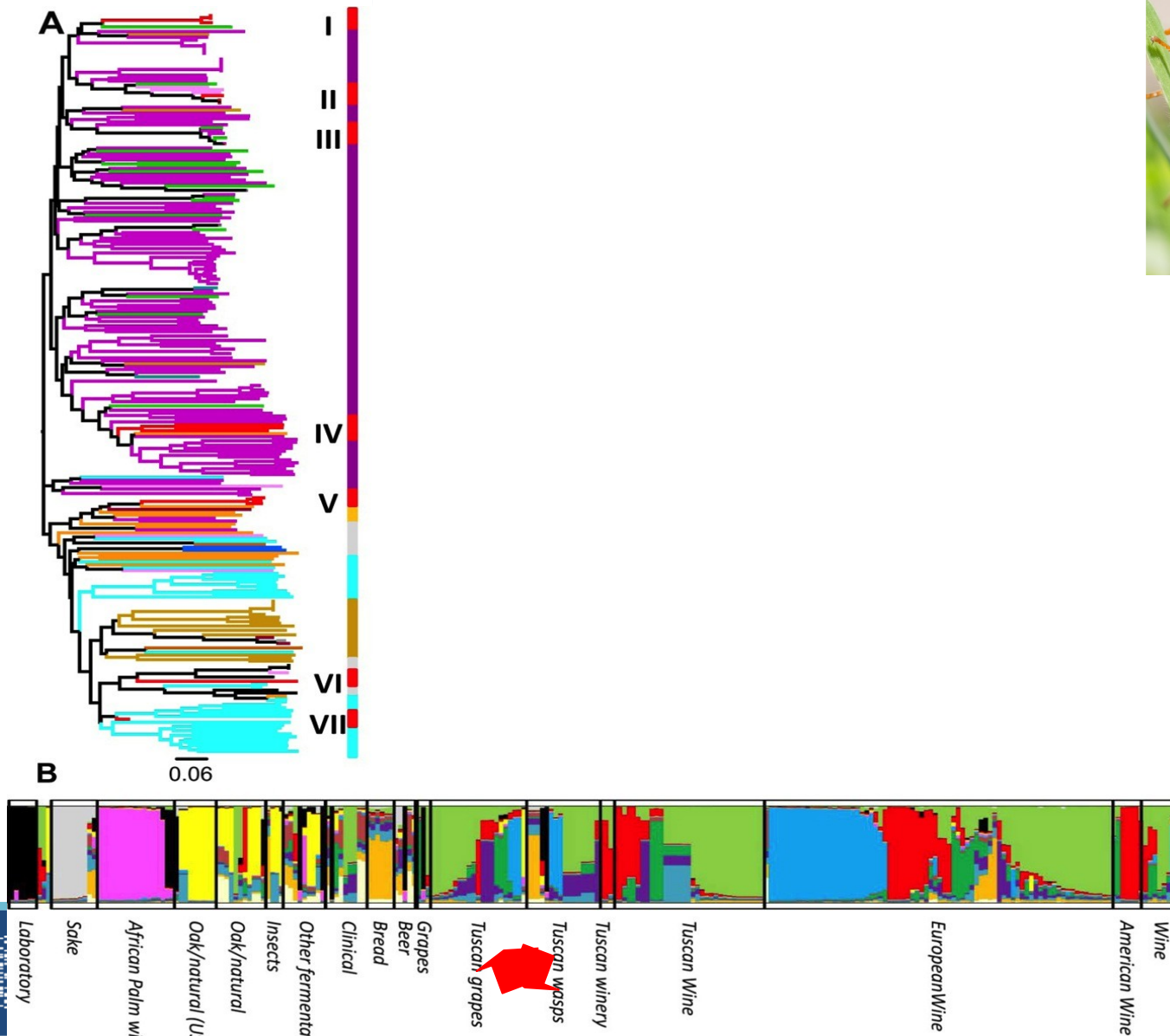
Vintage Comparison	% of genetic variation (APWG)	Fst	P (r<0)
2012 - 2014	5.14	0.113	<0.00000 1
2013-2014	7.17	0.067	<0.00000 1
1992-1993	11.91	0.088	<0.00000 1
1992-2014	15.51	0.294	<0.00000 1

Yeast vectors

- Birds (Francesca et al. 2012)
- Insects :
 - Drosophila
 - Bees (Goddard et al. 2009)
 - Wasp (Stefanini et al. 2012)



Diversity of *S. cerevisiae* isolated from wasps' guts

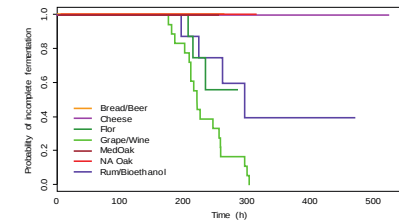




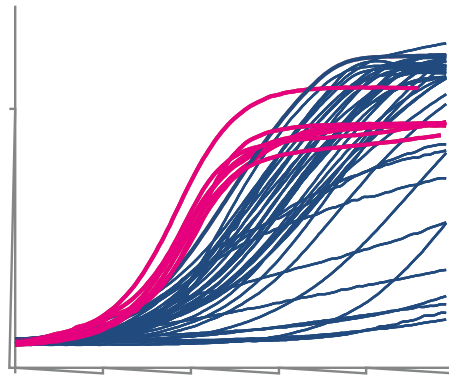
Is there a strain phenotypic diversity
associated to strains of different
origins ?

Two examples of yeast phenotypic diversity

Probability of completing alcoholic fermentation in synthetic grape must (20% glucose)



Growth on a media containing galactose



Obtaining sequencing Data

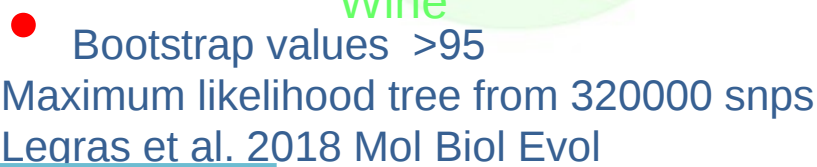
82 strains

- Grapes (8) Wine (23), 6 haploids,
- Flor ageing (10), 3 haploids,
- Bread and Beer (7)
- Cheese(8) , 1 haploid
- Rum and bioethanol fermentations (Brazil) (8) 1 haploid,
- Oak(8)
- African strains (2 palm wine and NCYC110),
- Sake (2)
- Other misc strains ..

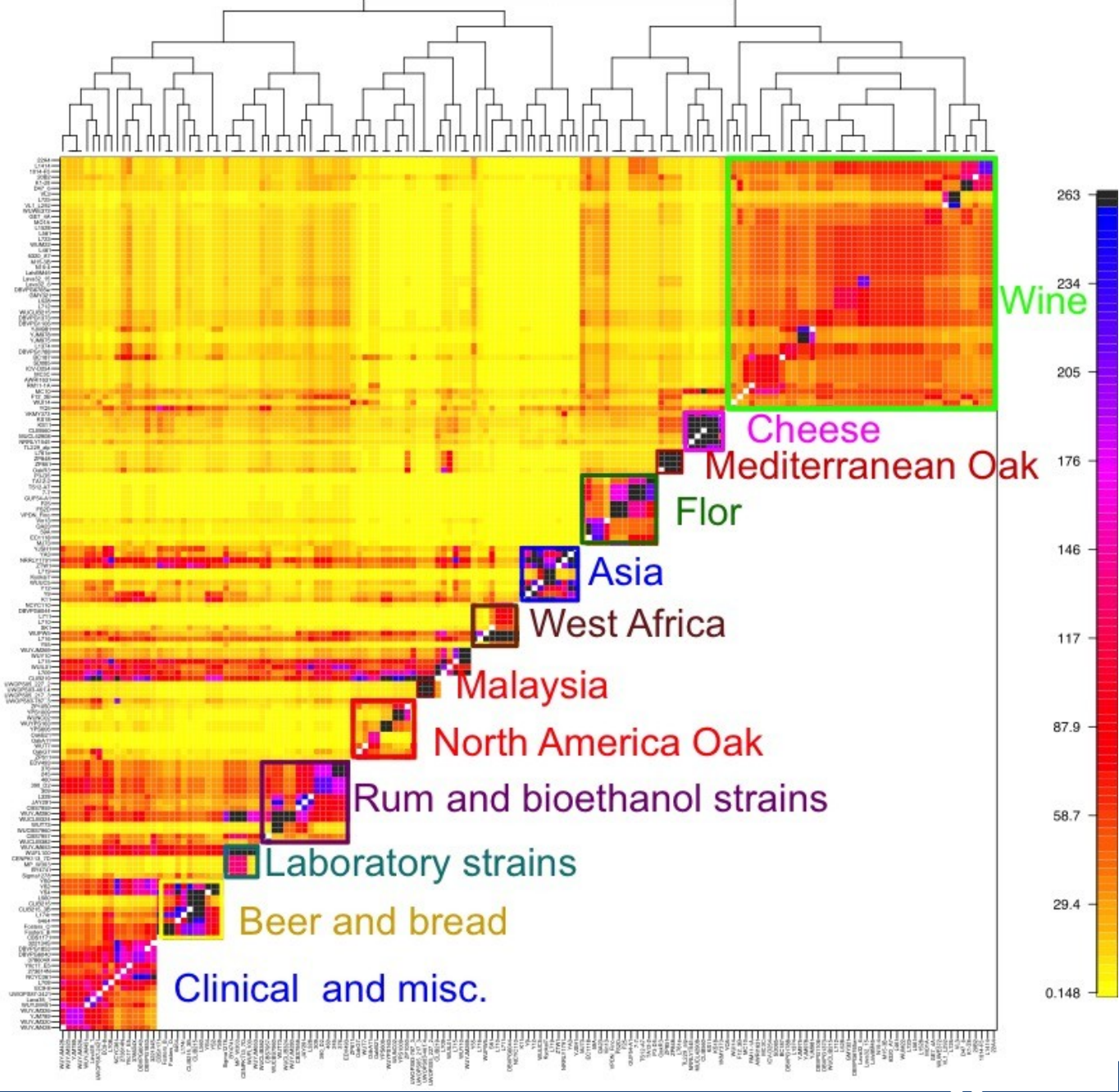
- ❖ Illumina HiSeq 2000 sequencing with (20 to -400X 1)
- ❖ Genotyping with GATK: 367863 biallelic variant positions 20018 indel



C



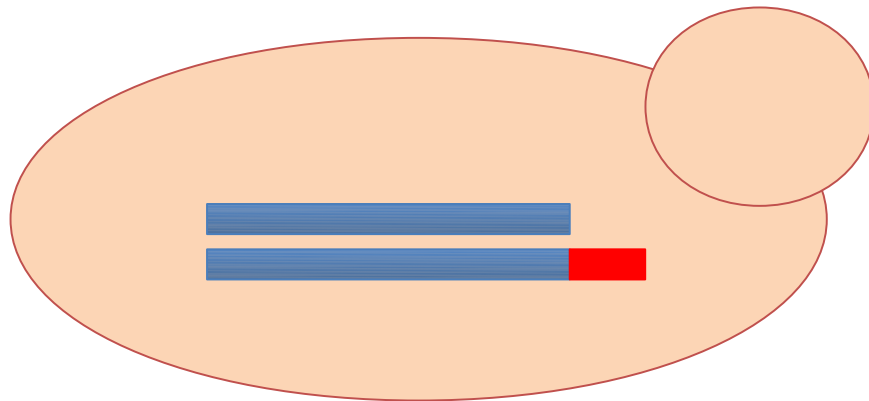
Analysis with FineStruc (phased data) (Lawson PloS Genetics 2012)



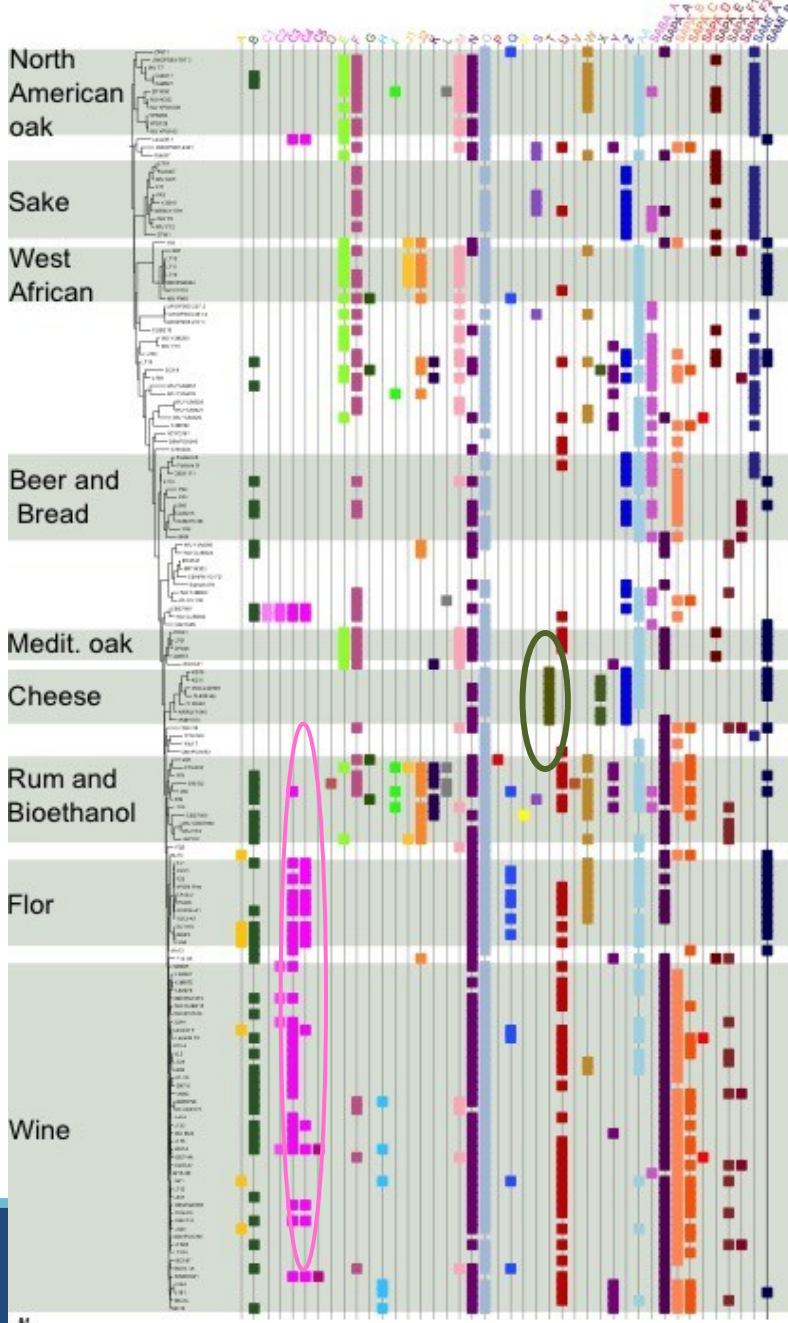
Legras et al. 2018 Mol Biol Evol



Can phenotypic diversity be explained by specific genomic content?



Distribution of new genomic regions among *S. cerevisiae*



36 transfer events from 1.5 to 42 kb,
28 are prevalent in several populations,
and 6 are specific to one or two niches

Region C3 and C4 65kb (*T. microellipsoides*, Marsit et al. 2015):

truncated in wine yeast (**FOT1-2**)

full length in flor yeast
PSY1: High affinity fructose symporter

Region T 6.8 kb



GAL1: Galactokinase

GAL7: Galactose-1-phosphate uridyl transferase

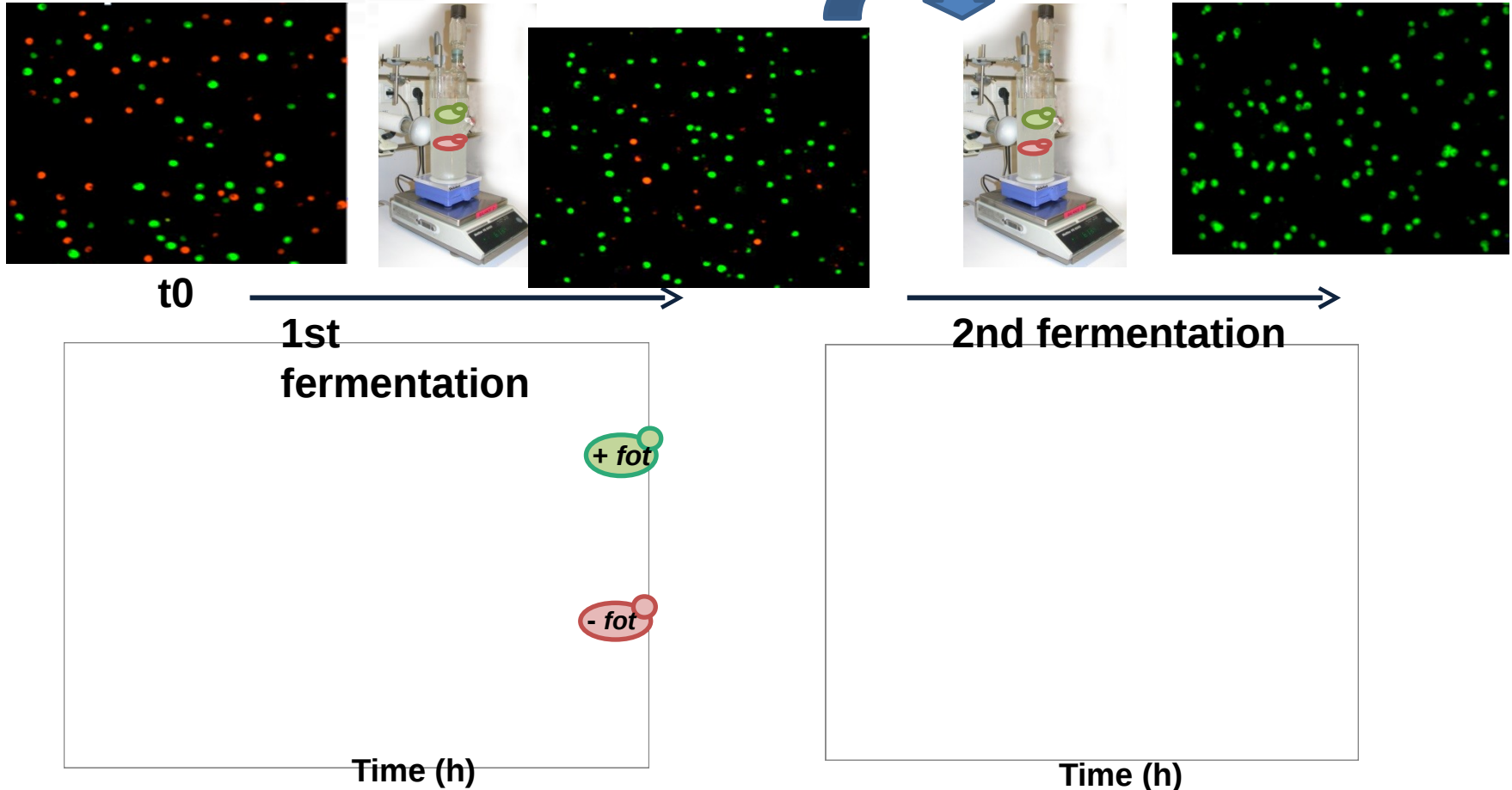
GAL10: UDP-glucose-4-epimerase

Legras et al. 2018 Mol Biol Evol

Ecological advantage offered by one HGT : region C

Evolution of frequencies of strains carrying or not  / 

FOT genes



Marsit et al. (2015)

Ecological advantage offered by Region T in cheese strains

Figure S6: Total nucleotide divergence between cheese strain (NRRLY1545) across *Saccharomyces* clade and one species of each of the *Naumovozyma*, *Kazachstania*, *Torulaspora*, *Vanderwaltozyma*, *Zygosaccharomyces* and *Kluyveromyces* clades

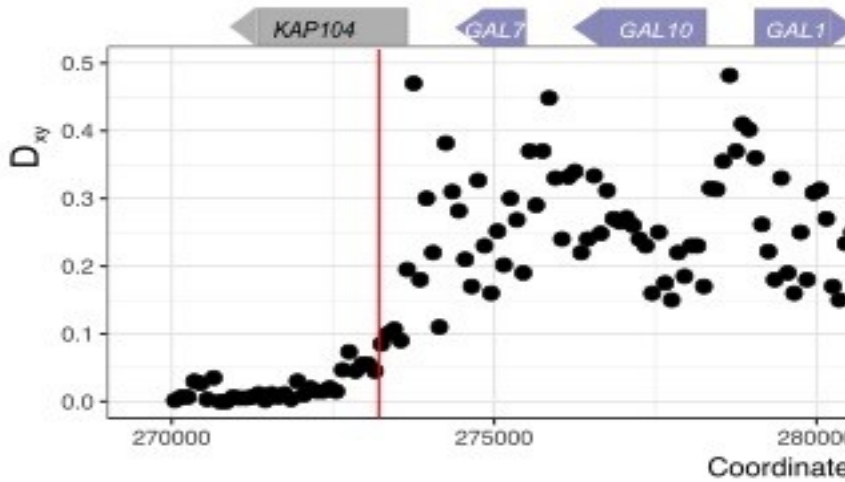
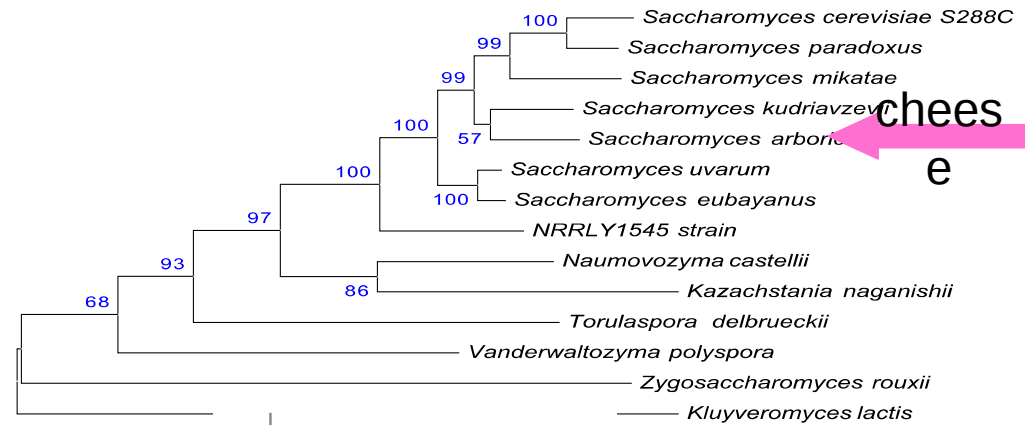
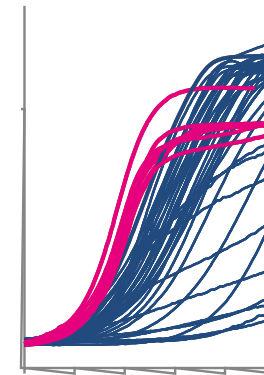


Figure S5: Phylogeny based on the concatenated amino acid sequences of the *GAL1*, *GAL10* and *GAL7* genes in a *S. cerevisiae* cheese strain (NRRLY1545) across *Saccharomyces* clade and one species of each of the *Naumovozyma*, *Kazachstania*, *Torulaspora*, *Vanderwaltozyma*, *Zygosaccharomyces* and *Kluyveromyces* clades



The same region of *S. uvarum* when tra improves growth on galactose improves growth on glucose + galactose (Roop et al 2016 Nature)



S. cerevisiae



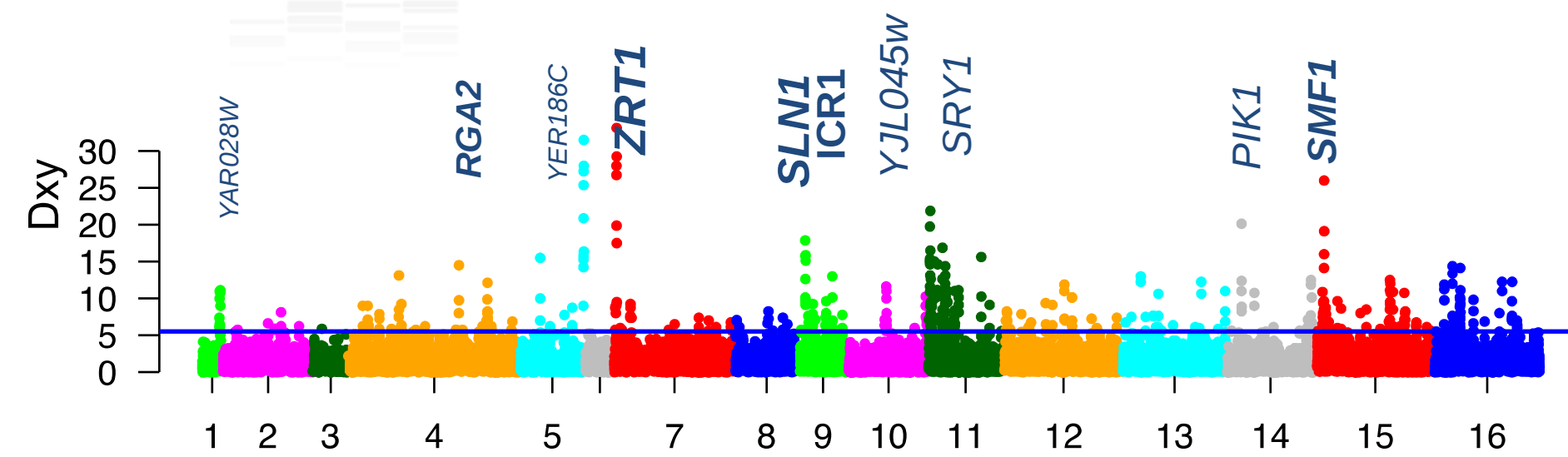
May highly differentiated genomic regions explain phenotypic diversity and adaptation?

May highly differentiated genomic regions explain phenotypic diversity and adaptation?

Flor strain in comparison to wine : Dxy , PCA
(Coi et al. 2017 Mol Ecol)



Chromosomal regions differentiating wine and flor yeasts

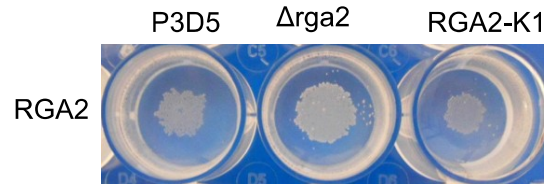
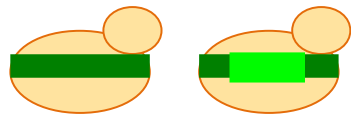


Genes involved in key functions:

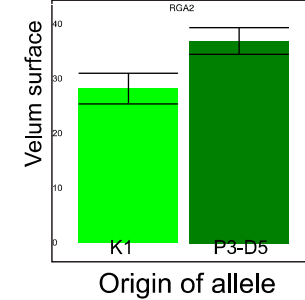
- ✓ Metal ions homeostasis (*ZRT1 SMF1 COT1 ALR1 SKY1 ...*)
- ✓ *HXT3, HXT4 HXT6*
- ✓ ...
- ✓ Extra cellular cell wall(*FLO5 MUC1 DAN4 PIR3 HPF1...*)
- ✓ *ICR1, PWR1, FLO11 + IRA1*

Impact of allelic variation on flor phenotypes

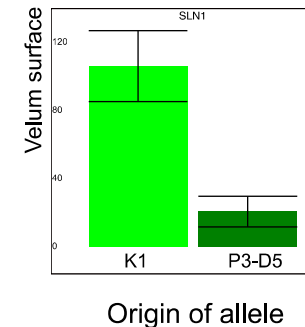
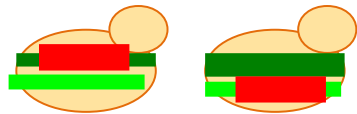
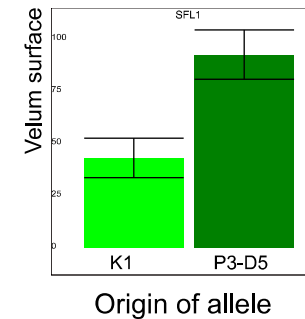
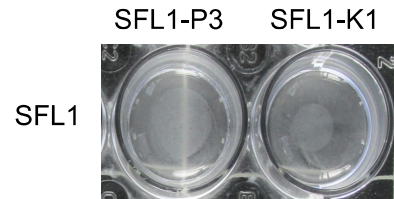
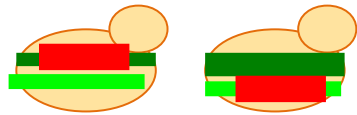
P3-D5 background



Velum surface

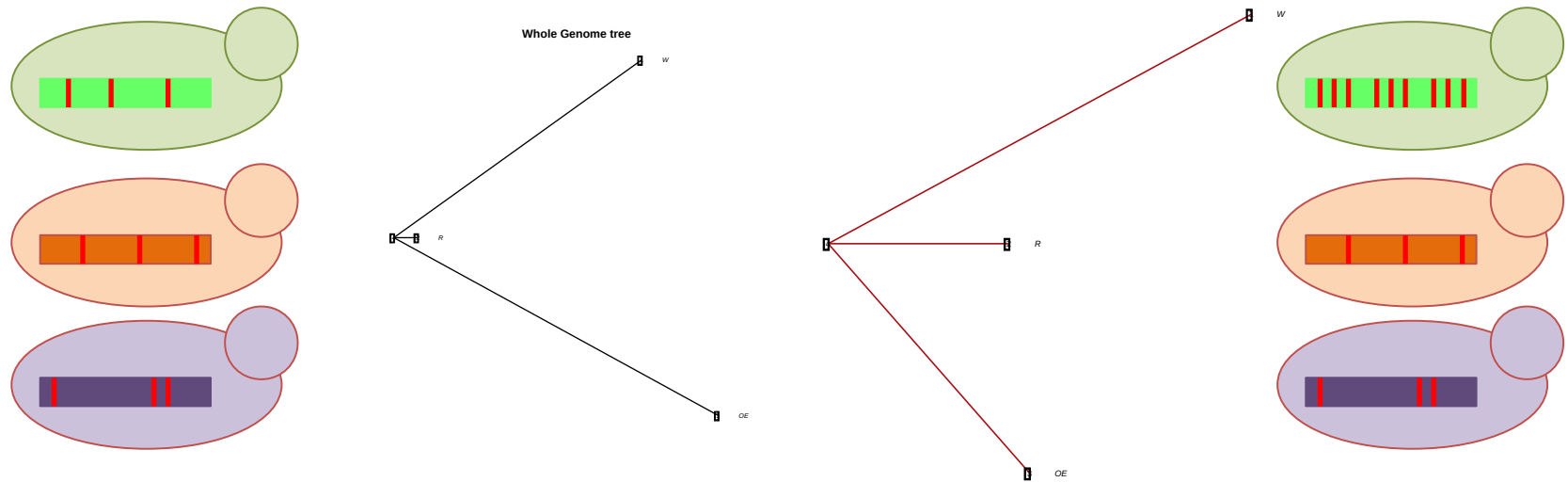


P3-D5 x K1 background



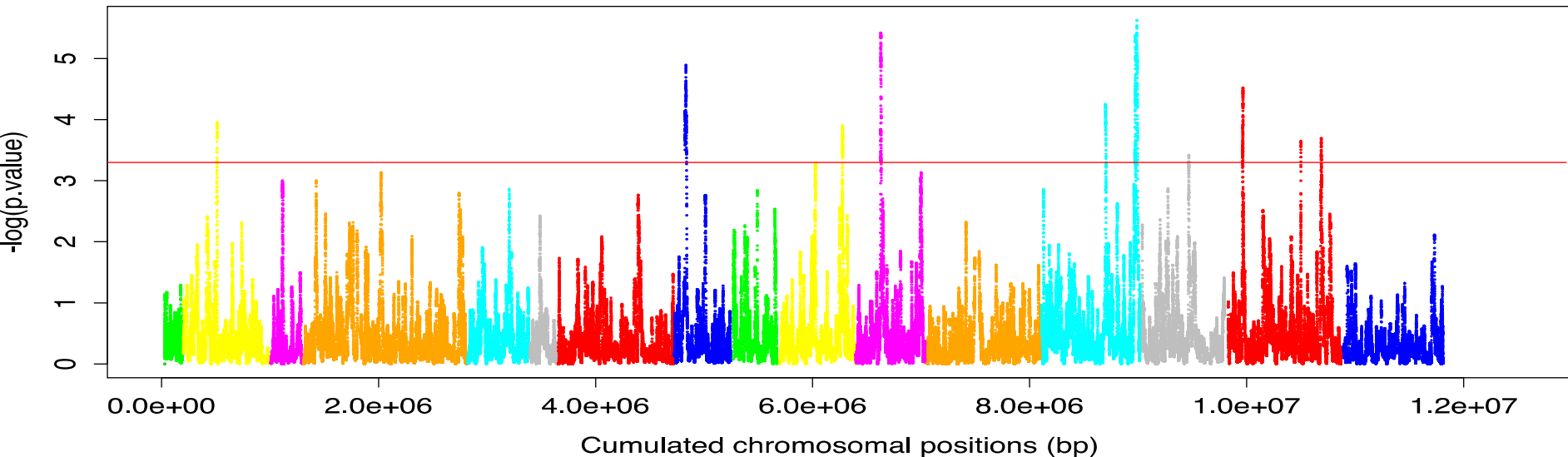
Searching for highly differentiated regions that may explain phenotypic diversity and adaptation

haplotypes (Pardo 2013 Genetics): for multiple populations wine, Rum, Mediterranean Oak



Search for selection from population differentiation (hapFlk, Fariello, 2013 Genetics)

4 populations : Wine Rum, Mediterranean oak, USA oak as a root

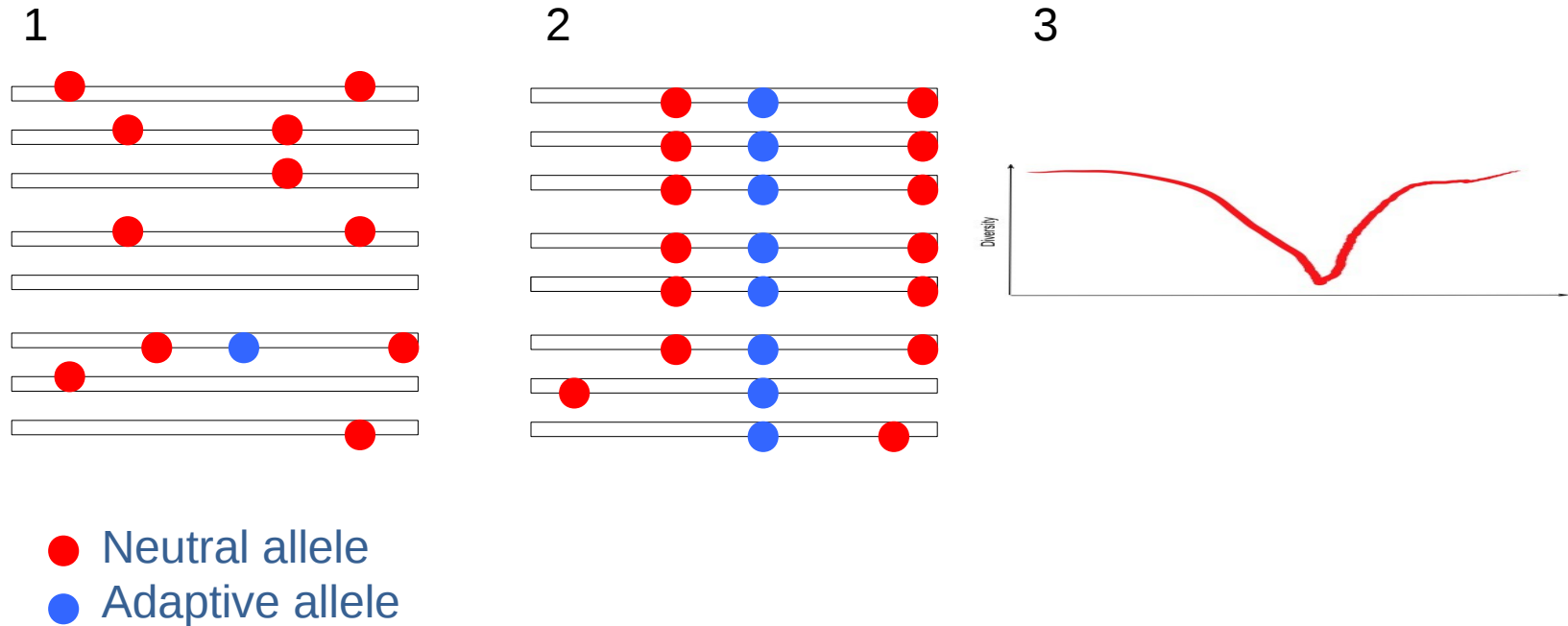


...

- $q\text{-value} < 0.05$:

- **AUS1, ATG34 –ATG19:** Wine ,
- **YME2- ADH2- FKS3 :** Oak and rum
- **THI72, IME1, HAP4 :** Oak Med Oak and rum

Searching adaptation to environment from the signatures of selective sweeps inside a population

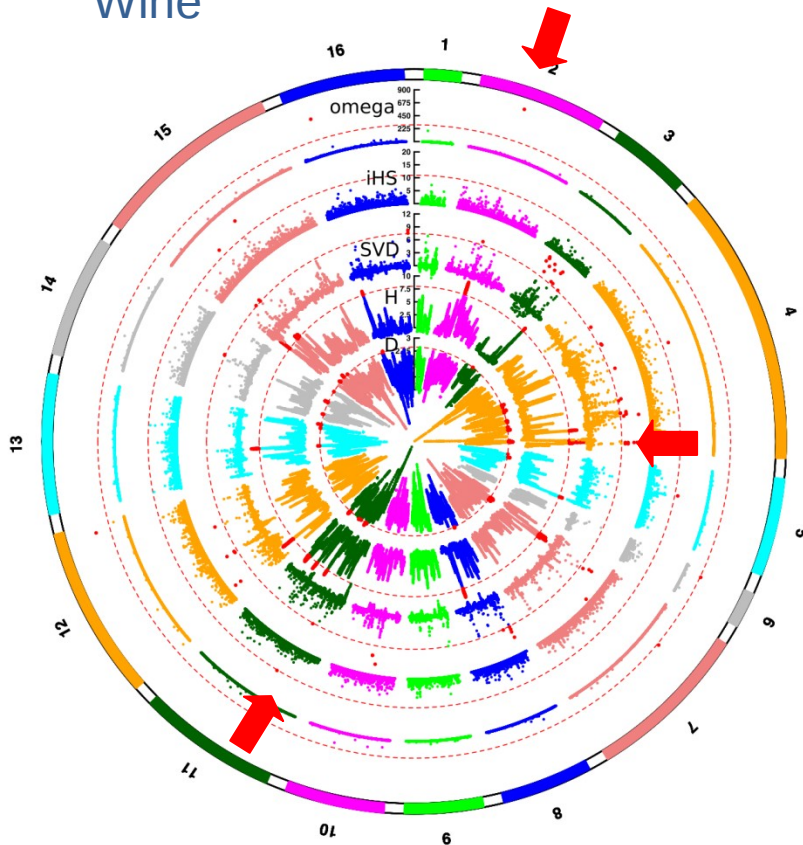


The fixation of an advantageous allele is accompanied by a reduction of diversity

searching adaptation to environment
from the signatures of selective sweeps inside a
population

A -

Wine



- Wine : Enrichment for amino acid transport genes (*BAP2*, *TAT1*, *AVT3*, *GNP1*)
- Cheese sweep for genes involved in galactose metabolism : Region T



- **Conclusions**

- The use of new molecular methods has changed our vision of *S. cerevisiae* diversity :
 - revealed the existence of specific clusters per human associated environments
 - revealed the domestication of some groups
 - offered the possibility to estimate gene flow between regions , cellar and vineyards ...
 - enabled the evaluation of demographic/historical scenarii

=> necessity to adopt these molecular methods especially for ecological studies



• Conclusions

- *S. cerevisiae* populations from anthropic environments present several clues for adaptation :
 - for wines : amino acid transport and sterol uptake are the two major known constraints : and we found targets for these two constraints
 - for cheeses : adaptation to galactose assimilation (*GAL1 GAL7 GAL10* cluster + *GAL2* + *GAL80*) => *rewiring of galactose assimilation network*
- mechanisms permitting adaptation : CNV, **HGT**, mutation accumulation
- *S. cerevisiae*: a species complex of specialized populations with customized genomes

Acknowledgments



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Delphine Sicard



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University Lisboa : JP Sampaio

CEA – Genoscope: Julie GUY



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AL Coi

University Braga : D Schuller
R Franco-Duarde

CRG Universitat PF Barcelona
T Gabaldon



THANK YOU FOR YOUR ATTENTION !