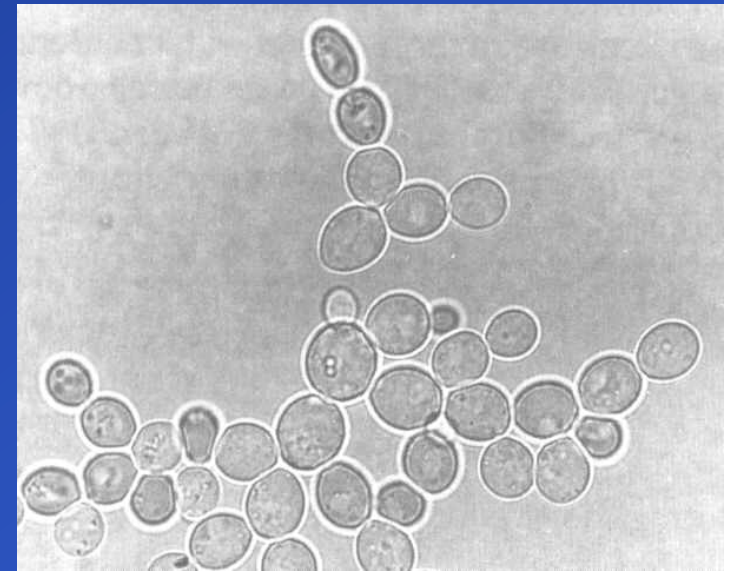


SACCHAROMYCES CEREVISIAE DIVERSITY REFLECTS HUMAN HISTORY

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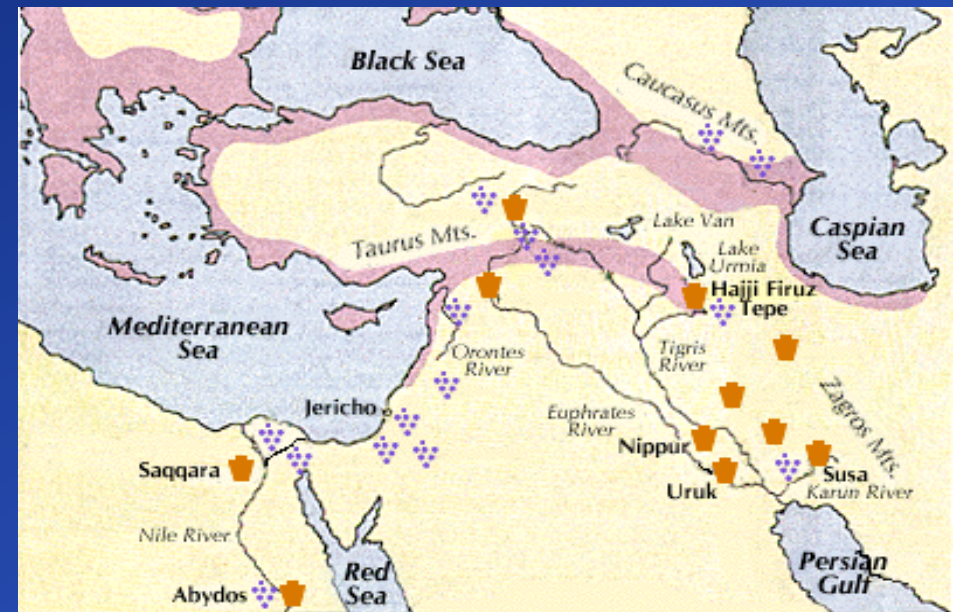


Introduction

- Fermented beverages have a special place in our societies from the economical, cultural and historical points of view.
- On most continents men have been using fermentation to produce local alcoholic beverages (rice wine and Sake, palm wine, wine, beer...).
- Remains of fermented beverages have been found in China, and dated as -7000 BC.

Vine and Wine making Origin

- The origin of vine domestication is located in Caucasus, but the Spanish *Vitis sylvestris* also contributed to actual vine varieties.



(Mc Govern, 2004)

- Archeological remains were dated from :
 - 5400 BC in Iran (Hajji Firuz Tepe)
 - 3150 BC in Egypt (Abydos)

Yeast and fermentations

- What are the differences between *S. cerevisiae* strains isolated from these fermentations ?
- How diverse are wine strains? at which scale are the differences noticeable ? (Few vineyards comparison)
- What is the importance of History and natural factors on yeast microflora?

Yeast differences

- Several authors have given some information
 - Azumi and Goto-Yamamoto, 2001 : AFLP
 - Hennequin *et al.*, 2001 : Microsatellites
 - Fay and Benavides, 2005 : MLST

What are the differences between yeast from these fermentations ?

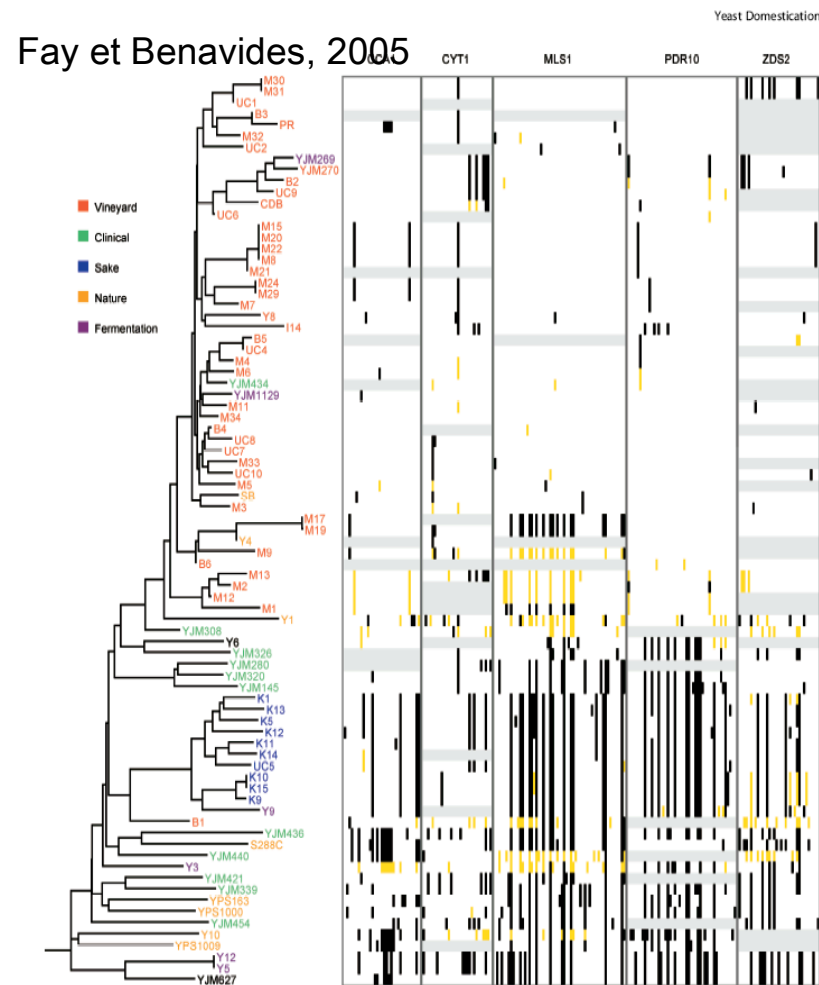


Figure 1. A Neighbor-Joining Tree Shows Differentiation among Yeast Strains Isolated from Different Sources
The tree was constructed from polymorphic sites found at five unlinked loci and was rooted using *S. paradoxus*. Strains are colored according to the substrates from which they were isolated. The right side shows color-coded polymorphism data with minor alleles shown in black, major alleles shown in white, missing data shown in light gray, and heterozygous sites shown in orange.
DOI: 10.1371/journal.pgen.0010005.g001

Fay et Benavides (2005) concluded to wine yeast and sake yeast domestication because of their lower diversity compared to wild yeast

Evaluation of *S. cerevisiae* diversity with Microsatellite typing

Interest of Microsatellite typing:

used for evaluation of the population structure of other yeast (*C. albicans*)

good markers for comparison of close groups

Tested strains (650)

- Geographic origins :
 - Africa, Asia, Australia, Europe (but mainly wine) and America
- Technological origins :
 - Various fermented beverages, cheese and bread
- Vine and Wine origins :
 - different ancient vineyards countries: Europe and Lebanon
 - or recent vineyards countries: United States, South Africa
 - Most of them were isolated from cellars yet some were from grapes

Microsatellites



Repeated sequences from 2 to 6 bases :

- $(GT)_n$ $(GA)_n$ $(GC)_n$ $(AT)_n$ ou $(CAA)_n$ $(TAA)_n$...
 $(TAAA)_n$...
- variable number of repeats
- located between two conserved zones

we amplified 12 loci and measured the size of the PCR fragment :

- one fragment : homozygosity
- two fragments : heterozygosity...

Maximum number of alleles detected per locus

Origin	tested strains	1 allele max	2 alleles max	3 alleles max	4 alleles
Vine and Wine					
Alsace	102	25	57	10	9
Cognac	27	13	13	1	0
Avignon	20	1	16	2	1
Nantes	21	10	9	2	0
Spain	36	9	22	4	1
Austria	17	4	10	2	1
USA	27	11	10	2	4
Cider (Bretagne)	8	2	6	0	0
Beer	18	0	5	7	6
Bread	29	1	7	9	12
Palm Wine (Nigeria)	20	5	7	4	4
Rhum (Antilles)	19	4	7	5	3
China (Miscellaneous)	13	2	5	6	0
Sake Japan	15	5	8	2	0

Are Yeast different ?

phenetic tree based on Dc
(Cavalli Sforza 1967)

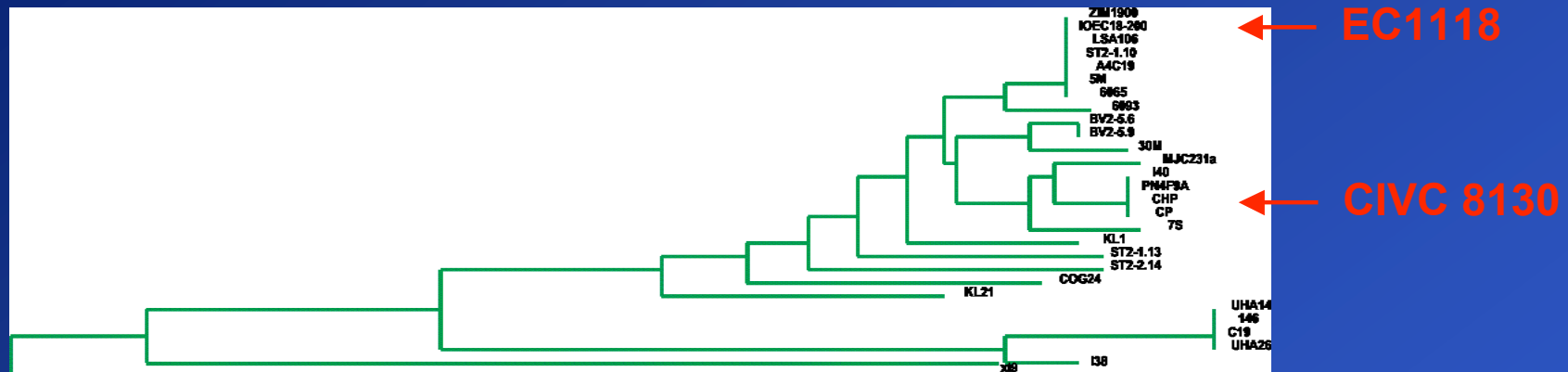
⇒ Strains are clustered
mainly according to the
technological origin

⇒ Specific groups of
related strains

⇒ few identical strains
in distant vineyards except
industrial strains

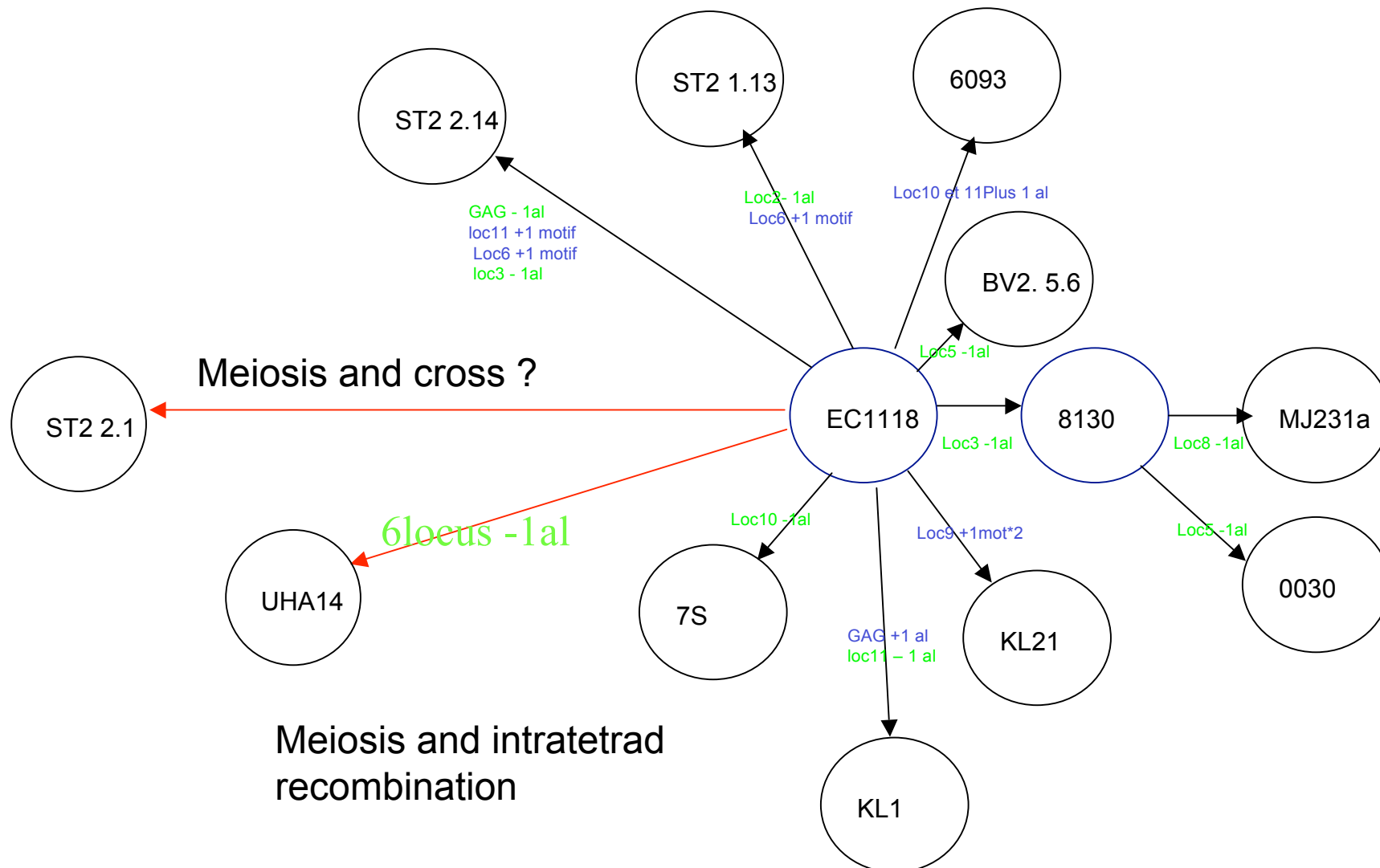


Details of one group of closely related strains



=> occurrence of strains identical (or close) to EC1118 or CIVC8130 in several French or foreign vineyards (South Africa, Austria (grapes), Croatia, Lebanon, Portugal...)

Relationships between strains of the “Champagne” group



Allelic specificity of one group of Bread strains

	LOCUS10														Total
	162	174	180	183	186	189	192	195	198	201	204	207	210	213	
BeerAle (2)	7			5											12
Lebanon		1	5		3	4	7			2	5	7		1	52
Bread (4)	10		6	1				2	9				8	3	39

	locus2								Total
	150	159	162	165	168	171	183	186	
BeerAle (2)	3			9					12
Lebanon		3	12	24	2	4	5	2	52
Bread (4)	8	13		9		8			38

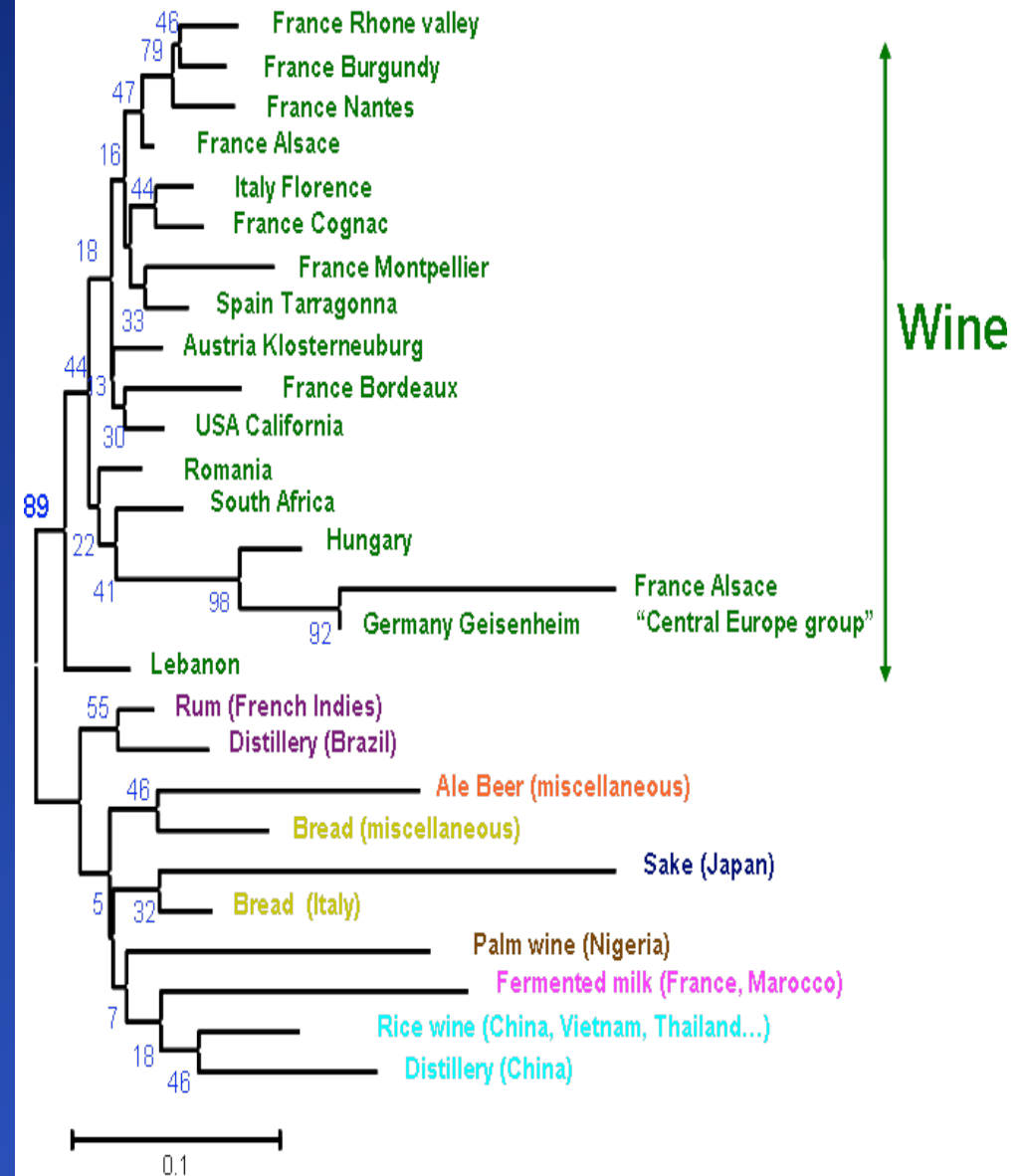
	locus7											Total		
	230	239	251	260	263	266	269	275	281	296	299	302	305	
BeerAle (3)	5	5	1	2	3				4	1				21
Lebanon					1		2	4		9	9	12	15	52
Bread (3)			1			10			8	12	2			33

	locus9								Total
	90	93	96	99	102	105	108	117	
BeerAle (2)					3	7	3		13
Lebanon	6	35	10					1	52
Bread (3)		8	7	11	1	9			36

⇒ This suggests that bread yeast are originating from beer strains and from a second origin that could be a wine yeast.
 ⇒ Only parasexuality could explain the two alleles from both origins

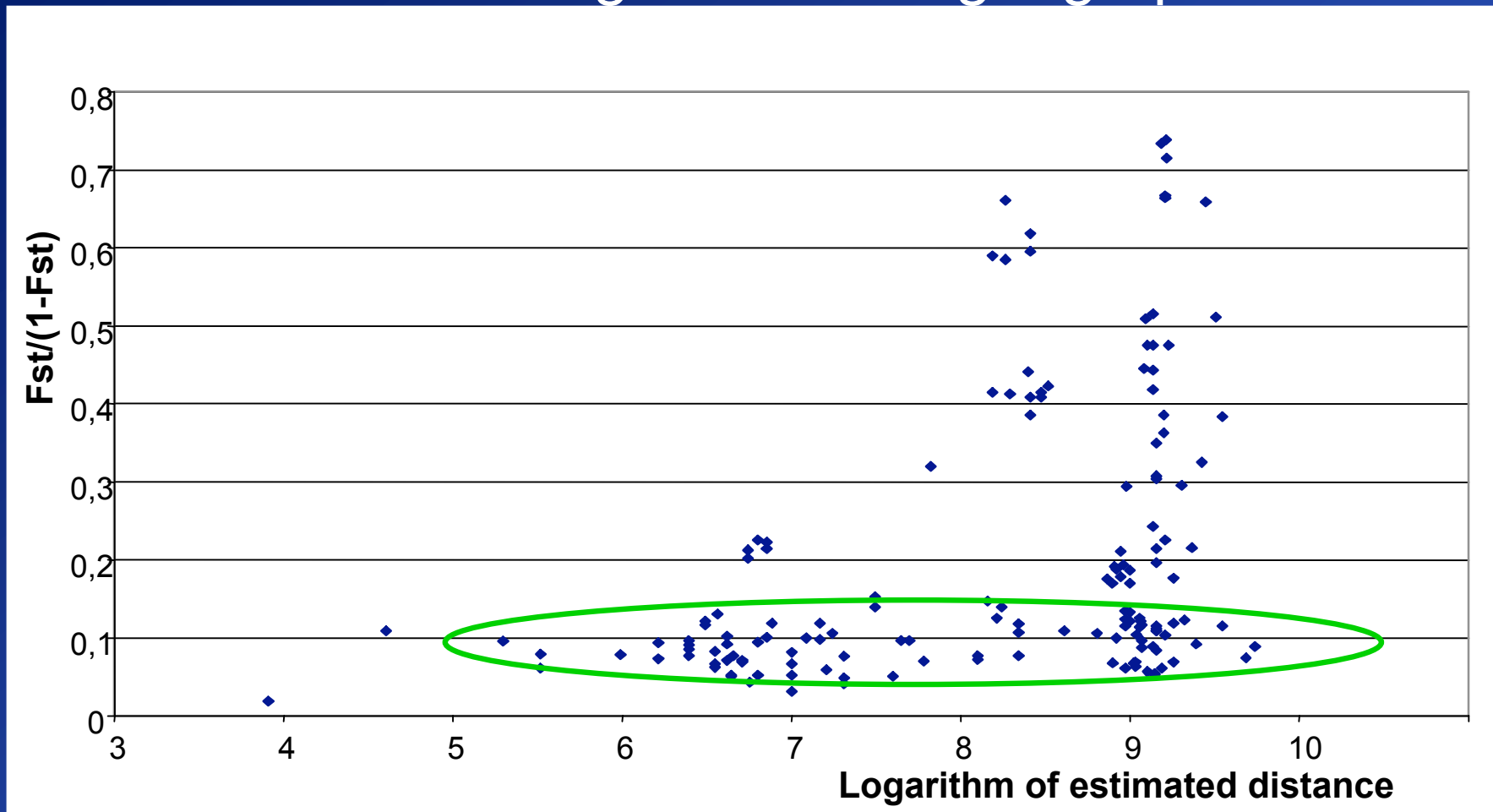
Relationships between groups of strains

- A group of wine yeast separated from other strains
- Wine yeast seem to have a unique origin
- The position of Lebanon suggest a Mesopotamia origin .
- Association between groups show regional association which correspond to migration pathways.
- We find the connection between bread and beer strains



Fst genetic distance NJ tree between groups

Correlation between genetic and geographic distances



⇒ link between genetic and geographic distance ($r^2 = 0.28$)

⇒ gene flow

Allelic richness of the different wine origins

	Austria Klost.	Cognac	Germ Geisen	Italy Firen	Montp.	Nantes	Rhone Valley	Romania	Lebanon	Spain Penedes
Nb	11	26	10	16	19	17	17	8	20	15
GAG	5.9	5.3	4.9	5.7	5.4	6.4	4.2	6.7	6.1	6.3
loc10	5.4	5.1	5.5	5.0	6.1	6.5	6.3	6.2	7.1	8.2
loc11	6.3	5.9	6.2	5.1	5.3	6.1	5.1	7.6	6.5	6.5
loc2	4.1	2.9	2.3	2.8	2.0	3.5	3.1	5.1	4.3	2.9
loc3	3.4	3.7	3.2	4.7	2.1	2.9	2.8	3.6	3.3	3.2
loc4	3.8	3.8	6.4	5.0	4.2	5.4	5.6	5.2	6.8	4.4
loc5	5.8	6.3	6.8	6.6	3.7	7.1	7.7	7.0	6.4	6.8
loc6	3.9	3.3	4.9	3.1	3.6	3.2	2.6	5.5	5.0	3.5
loc7	5.0	4.6	4.5	4.5	3.7	4.1	5.1	5.0	4.9	5.0
loc8	4.8	4.0	4.3	4.4	4.3	4.7	4.5	5.1	4.5	5.2
loc9	1.9	2.1	2.8	1.8	2.7	1.6	2.0	3.6	2.7	3.5
Mean	4.6	4.3	4.7	4.4	3.9	4.7	4.4	5.5	5.3	5.1

⇒ Allelic richness slightly higher in Romania , Lebanon and Spain

Time Divergence estimation between groups

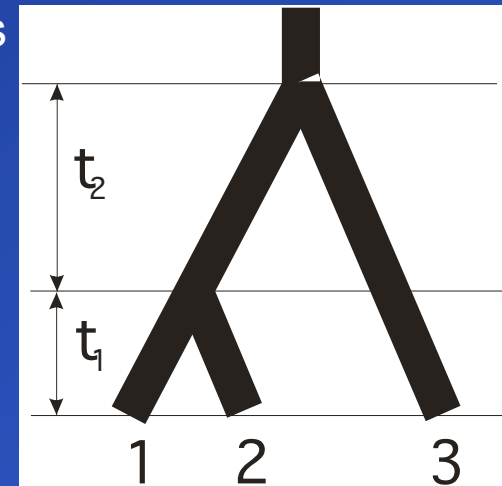
Bayesian Model ABC (JM CORNUET)

Advantages no mutation rate or number of generations estimation based on historical hypothesis.

- The most distant groups are the « Central Europe » and «Rhône Valley » or Montpellier groups.

Starting Hypothesis :

- Strains are originating from Lebanon or close
 - The France/Lebanon divergence dated from 2500 years ago
-
- => when took place the divergence between « Lebanon » and « Central Europe » ?



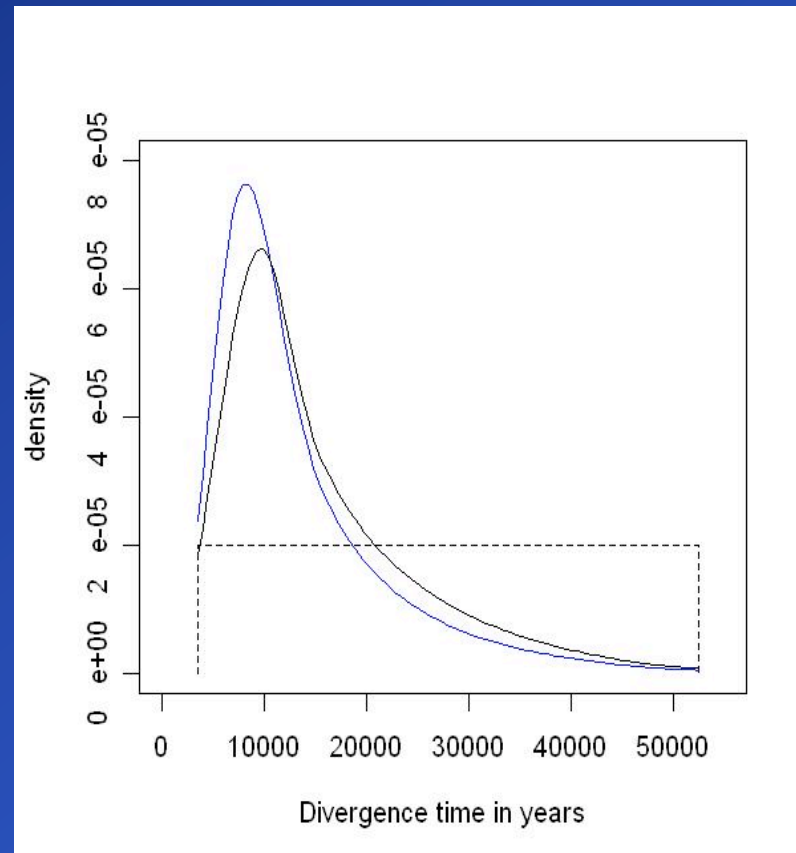
Time Divergence estimation between groups

- Estimated divergence:

Rhone Valley - Liban :
vs Liban/ « Central Europe »
- 10500 (-4500, -32000)

Montpellier - Liban
vs Liban /«Central Europe »
- 11750 (-4750, -36000)

=> Most probable period posterior
to the last glaciations era



Conclusions

- Yeast strains are specific to the technological origins
- Genetic distances are partially correlated to geographic distances which suggests several domestications from local flora (Beer, rice-wine/sake, grape wine, palm wine...)
- Number detected alleles per locus show different levels of ploidy according to the origin of the strains (bread, beer, wine...)
- A special cluster for wine strains which suggests a unique origin and extension that parallels the extension of wine making through history and can be noticed in the most recent vineyards (USA, South Africa, ...)
- The migration of wine yeast started very likely during the Neolithic era (with wine making?)

Conclusions

- *A part of human history also lies in yeast diversity !*

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 - University of Chicago
 - BIV Cognac
 - UHA Colmar
 - ITV Nantes
 - InterRhône Orange
 - INRA UMR SPO Montpellier
 - Clib INA-PG Paris Grignon
 - Several public or industrial collections ..