SACCHAROMYCES CERESIAGE 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.

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

(Mc Govern, 2004)

Mc Govern, PE 2003, Ancient wine the search for the origins of viniculture
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?

Fay et Benavides (2005) concluded that 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

<table>
<thead>
<tr>
<th>Origin</th>
<th>tested strains</th>
<th>1 allele max</th>
<th>2 alleles max</th>
<th>3 alleles max</th>
<th>4 alleles</th>
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<td><strong>Vine and Wine</strong></td>
<td></td>
<td></td>
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<td>25</td>
<td>57</td>
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<td><strong>Sake Japan</strong></td>
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<td>5</td>
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</table>
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

Legras et al Mol Ecol 2007
Details of one group of closely related strains

EC1118

CIVC 8130

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

INRA

ulp
Relationships between strains of the “Champagne” group

Meiosis and cross?

6 locus -1al

Meiosis and intratetrad recombination

EC1118

ST2 2.14

ST2 1.13

6093

BV2.5.6

MJ231a

0030

KL21

KL1
Allelic specificity of one group of Bread strains

⇒ 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 seems to have a unique origin
- The position of Lebanon suggests a Mesopotamia origin.
- Association between groups show regional association which correspond to migration pathways.
- We find the connection between bread and beer strains
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

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<tr>
<th>Loc</th>
<th>Austria Klost.</th>
<th>Cognac</th>
<th>Germ Geisen</th>
<th>Italy Firen</th>
<th>Montp.</th>
<th>Nantes</th>
<th>Rhone Valley</th>
<th>Romania</th>
<th>Lebanon</th>
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</table>

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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  – BIV Cognac
  – UHA Colmar
  – ITV Nantes
  – InterRhône Orange
  – INRA UMR SPO Montpellier
  – Clib INA-PG Paris Grignon
  – Several public or industrial collections …