



Genome Characterization & Reconstruction of the Evolutionary History of *S. cerevisiae* x *S. kudriavzevii* Hybrids

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Introduction

Different species have been described in *Saccharomyces* genus: *S. cerevisiae*, *S. paradoxus*, *S. cariocanus*, *S. mikatae*, *S. arboricolus*, *S. kudriavzevii* and *S. bayanus*. Natural hybrids have been found between *S. cerevisiae* and *S. eubayanus*, some of them called *S. pastorianus*, and *S. kudriavzevii*. Natural hybrids between *S. cerevisiae* x *S. kudriavzevii* have been described in wine, beer and cider, and recently in clinical and dietetic supplement. The aim of this work is to explore:

1. The isolation region characteristics. Are these hybrids better adapted to these conditions?
2. Genome composition of natural *S. cerevisiae* x *S. kudriavzevii* hybrids. What is the role of each parental strain in the hybrid genome?
3. What is the most probable mechanism of hybrid formation?
4. Where is the most plausible region of hybrid origin? Hybridization between *S. cerevisiae* and *S. kudriavzevii* is a unique event or has occurred several times? How many parental are involved?
5. Is the hybridization between *S. cerevisiae* and *S. kudriavzevii* an adaptive mechanism?

R1: Geographical Expansion

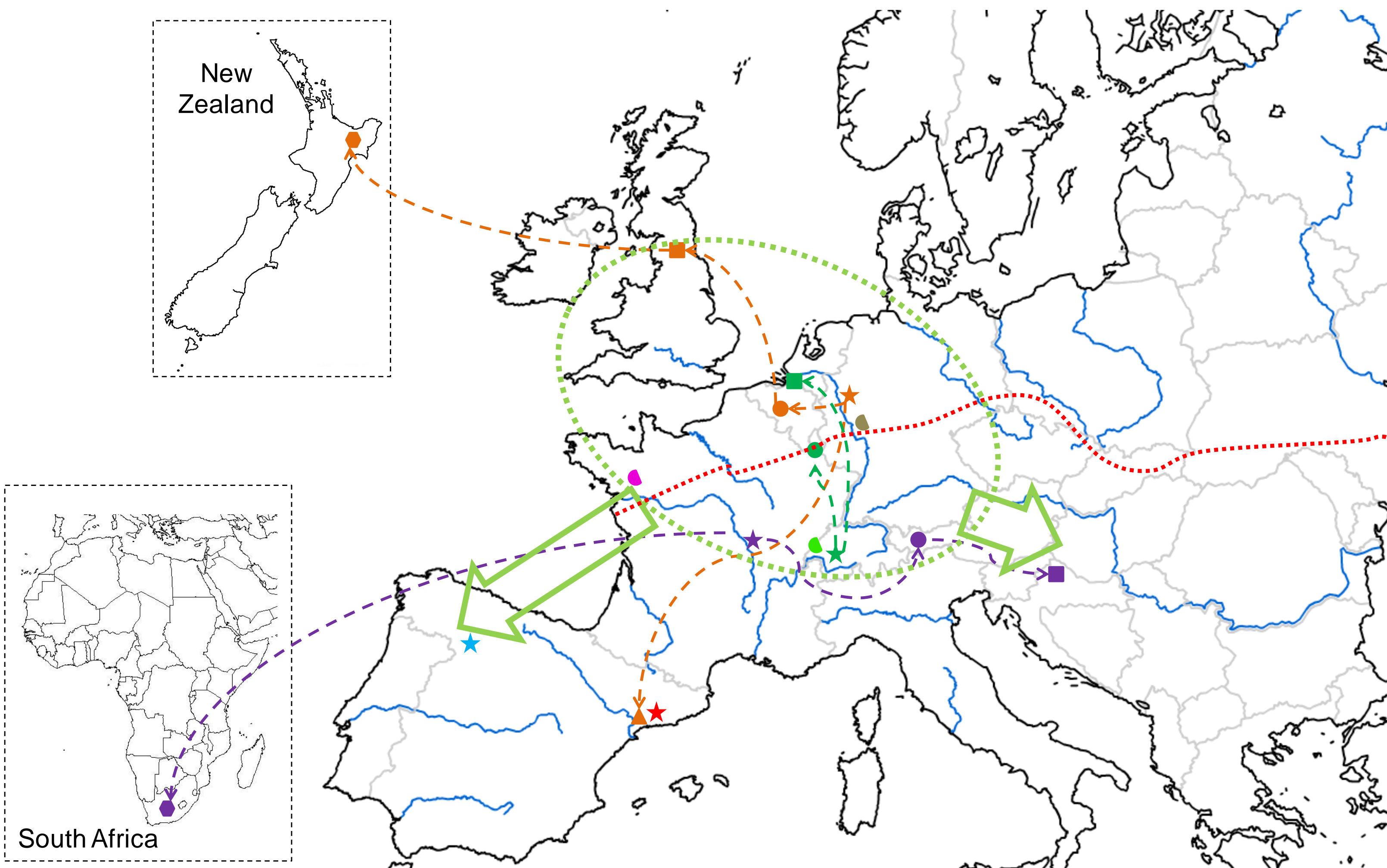
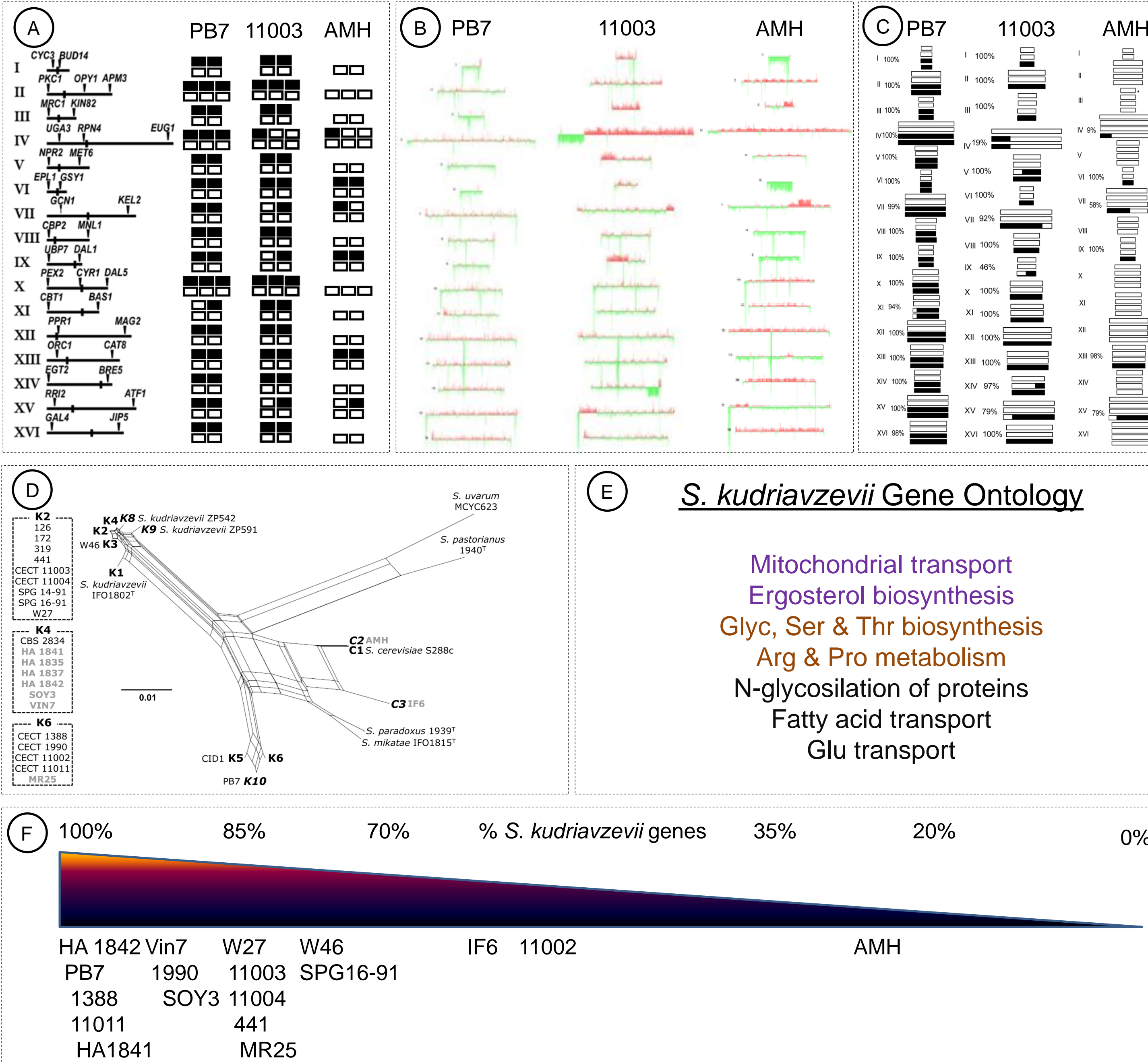


Figure shows previous natural hybrids isolation region (dotted green circle) in Europe and the new isolation regions (green arrows). These regions are characterized by a Continental or Oceanic climates with low temperatures. Red dotted line indicates the vineyard expansion limit. Symbol colors indicate hybrid strains (as in Figure R3C) and dashed lines indicate plausible expansion paths mediated by monks in the Middle Age. AMH (●), CBS 2834 (●) and CID1 (●) hybrids are also indicated. Blue lines indicate European rivers.

R2: Genome Characterization



R3: Parental Strains & Origins

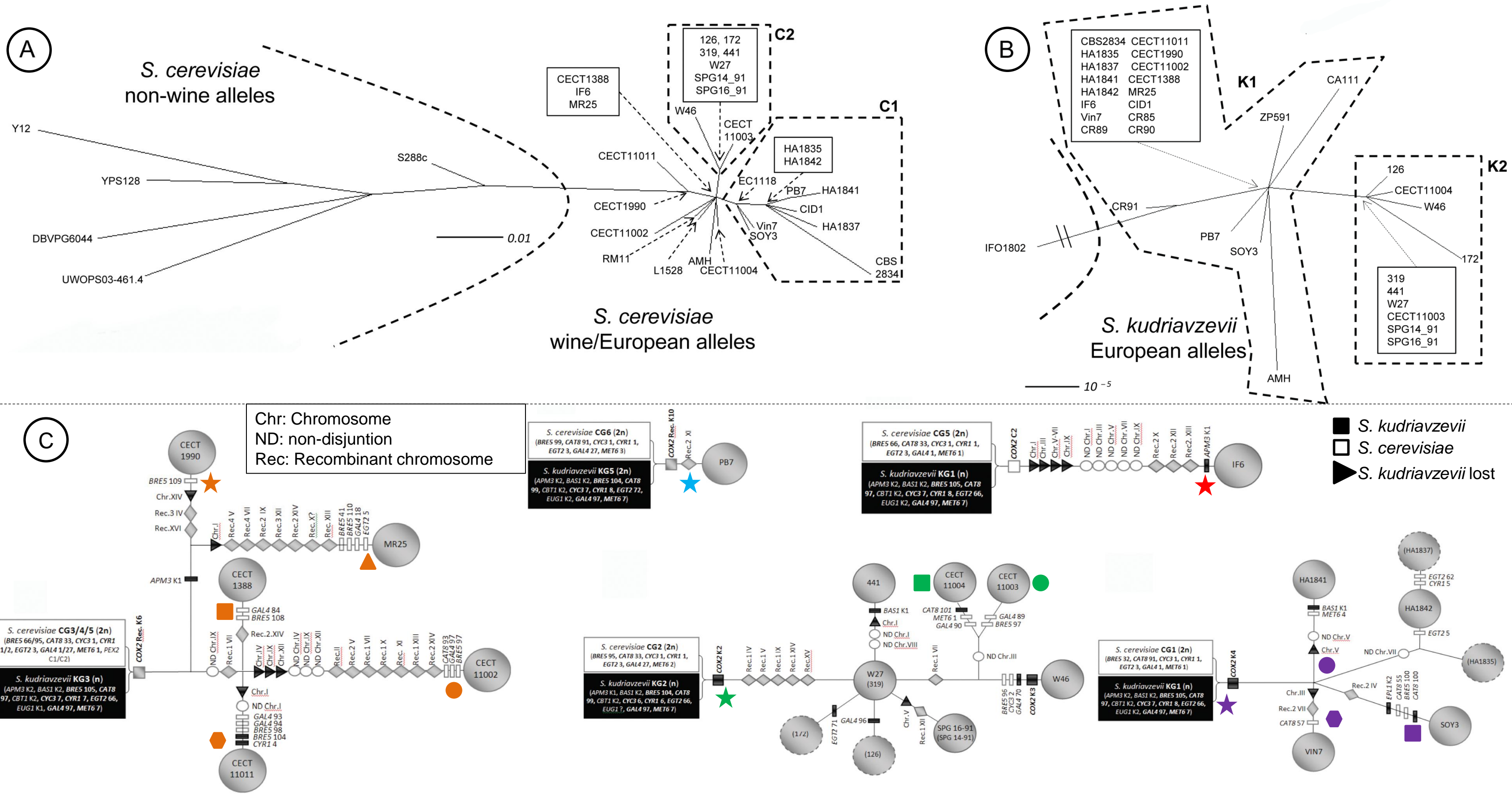


Figure A shows the *S. cerevisiae* Supernetwork computed using an input 7 Neighbor-Joining trees reconstructed from 7 *S. cerevisiae* nuclear genes. 4 of 7 NJ and Maximum Parsimony trees (data not shown) supported C1 and C2 groups. Hybrids are close to wine/European *S. cerevisiae* strains, also confirmed by a pool of *S. cerevisiae* gene deletions inferred from microarray data (data not shown). Figure B shows the *S. kudriavzevii* Supernetwork computed as in Figure A but using *S. kudriavzevii* gene sequences. 4 of 7 NJ and MP trees supported groups K1 and K2. Hybrids were closer to European *S. kudriavzevii* strains, also supported by RFLP data. Figure C indicates the possible multiple origins for hybrids (AMH, CBS 2834 and CID1 independent origins are not shown) based on Supernetworks (Fig. A), Polymorphic sites (data not shown), Parsimony and NJ trees (data not shown), PCR-RFLP (Fig. R2A), COX2 sequence data (Fig. R2D) and MP analysis of chromosome rearrangements (using data from Fig. R2C). It's a summary of genome rearrangements. Symbol colors are used in Fig. R1.

Material & Methods

24 Double hybrids
S. cerevisiae x *S. kudriavzevii*

Switzerland: W27, W46, SPG 14_91, SPG16_91, SPG 126, SPG 172, SPG 319, SPG 441
Germany: AMH
South Africa: Vin7
Croatia: SOY3
Spain: PB7
Austria: HA 1835, HA 1837, HA 1841, HA 1842

England: CECT 1388
Germany: CECT 1990
Belgium: CECT 11002, CECT 11003, CECT 11004
New Zealand: CECT 11011

Spain: IF6
Spain (Respiratory tract): MR25

2 Triple hybrids
S. cerevisiae x *S. kudriavzevii* x *S. uvarum*

Switzerland: CBS 2834
France: CID1

PCR Amplification:
35 Nuclear Genes
1 COX2 (mt Gene)

Gene Sequencing
7 Nuclear Genes
1 COX2 (mt Gene)

Hybrid Identification
by RFLPS
S. cerevisiae
&
S. kudriavzevii

Genome Characterization
by Microarrays
S. cerevisiae subgenome

Flow Cytometry
DNA content

Bioinformatics
Phylogenetic Trees &
Networks reconstructions
Microarrays analysis

Conclusions

1. *S. cerevisiae* x *S. kudriavzevii* hybrids have been found in regions of Continental or Oceanic climates, characterized by lower temperatures.
2. Hybrids are characterized by the lost of *S. kudriavzevii* genes and the maintainance of at least one copy of each *S. cerevisiae* chromosome. The plausible role of each parental subgenome is the ethanol tolerance and fermentation power (acquired from *S. cerevisiae* moiety) and low temperature adaptation (acquired from *S. kudriavzevii*).
3. Due to the presence of a DNA content near to 3.00C and in some cases 4.00C (PB7 and AMH), and heterozygotes genes (in the cases of brewing hybrids), the most plausible mechanism of hybrids formation, in the nature, is the rare-mating.
4. Hybrids share haplotypes with wine/European *S. cerevisiae* strains and European *S. kudriavzevii*. Moreover, reconstructed parental genotypes are found in *S. cerevisiae* strains isolated in Europe (data not shown). These results indicate Europe as the most probable region of hybrid formation.
5. Hybridization between *S. cerevisiae* and *S. kudriavzevii* is not a unique event and has occurred several times due to the presence of different combination of reconstructed parental genotypes.
6. Hybridization is a powerful adaptive mechanism conferring to hybrids the ability to grow in conditions where parentals can not.