



Genetic variability of natural
Saccharomyces cerevisiae strains

Dorit Schuller

cb
centro de biologia
Universidade do Minho

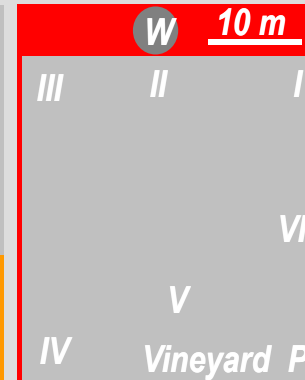
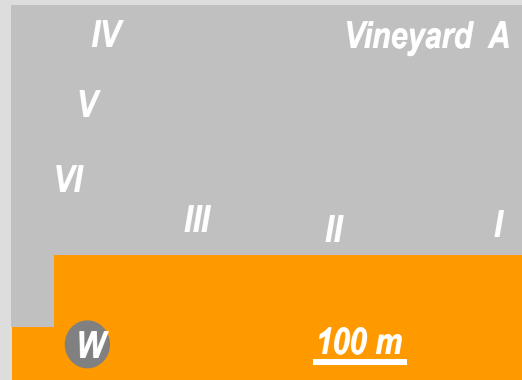
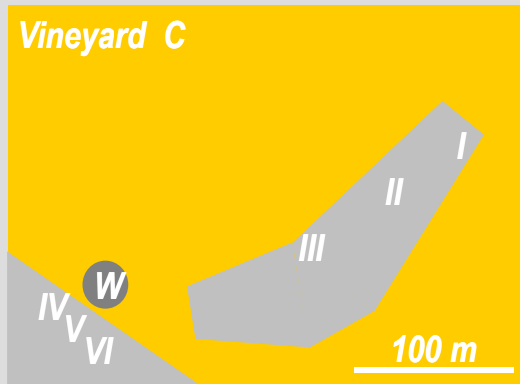
PHENOMICS – Exploratory Workshop
March 2007, Göteborg

The genetic diversity of S. cerevisiae – a biogeographical large-scale survey

Intra-strain genetic variation of the commercial S. cerevisiae strain Zymaflore VL1

Phenotypic characterization of a S. cerevisiae strain collection

The genetic diversity of *S. cerevisiae* – Biogeographical large-scale survey



W Winery

Grapes collection (2 kg per sampling site)

Small-scale spontaneous fermentations

Monitoring fermentation progress: Weight determination



3 vineyards x 6 sampling points x 2 sampling campaigns x 3 years

108 grape samples
54 spontaneous fermentations

1620 *S. cerevisiae* isolates

Methods for the genetic characterization of *S. cerevisiae* strains

Summary of the patterns obtained by all typing methods used.

For each method a different number was assigned to distinct patterns.

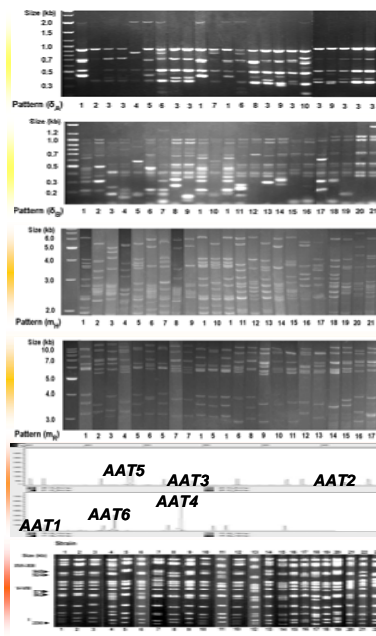
Commercial *S. cerevisiae* strains

Interdelta sequence typing

mtDNA RFLP

Microsatellite typing

Karyotyping



Primer $\delta 1$ - $\delta 2$

Primer $\delta 12$ - $\delta 2$

HinfI

RsaI

Microsatellite loci
ScAAT1-ScAAT6

Karyotype

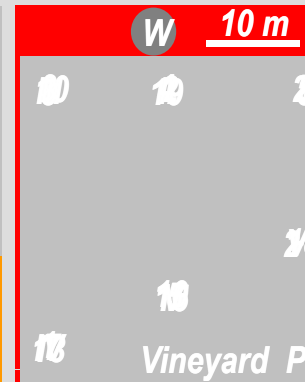
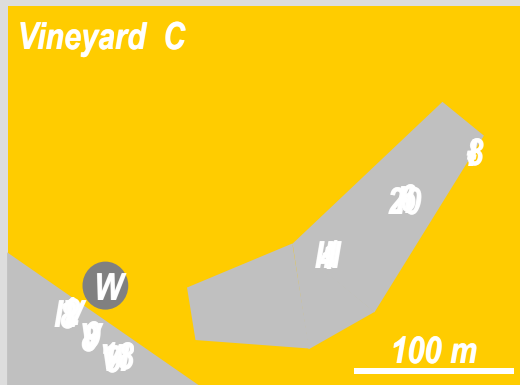
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Primer $\delta 1$ - $\delta 2$	1	2	3	3	4	5	6	3	3	1	7	1	6	8	3	9	3	10	3	9	3	3	3
Primer $\delta 12$ - $\delta 2$	1	2	3	4	5	6	7	8	9	1	10	1	11	12	13	14	15	16	17	18	19	20	21
HinfI	1	2	3	4	5	6	7	8	9	1	10	1	11	12	13	14	15	16	17	18	19	20	21
RsaI	1	2	3	4	5	6	5	7	7	1	5	1	6	8	9	10	11	12	13	14	15	16	17
Microsatellite loci ScAAT1-ScAAT6	1	2	3	4	5	6	7	8	9	1	10	1	11	12	13	14	15	16	17	18	19	20	21
Karyotype	1	2	3	4	5	6	7	8	9	10	11	10	12	13	14	15	16	17	18	19	20	21	22

Schuller et al., 2004

The genetic diversity of *S. cerevisiae* – Biogeographical large-scale survey



W Winery

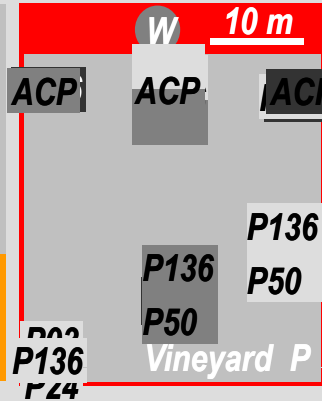
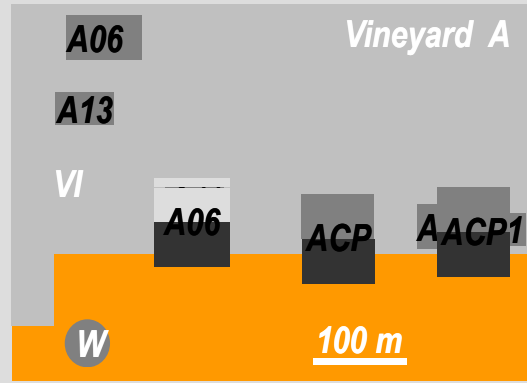
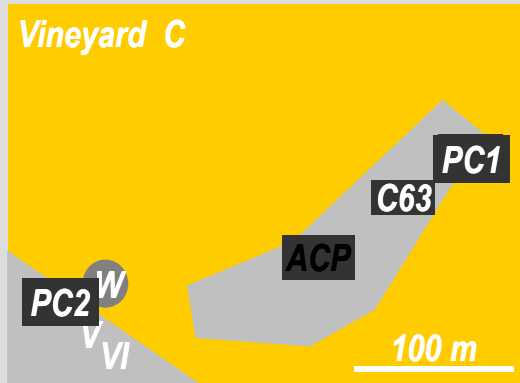


Total distribution of strains

	Vineyard C	Vineyard A	Vineyard P
2001	49	11	84
2002	1	43	23
2003	44	46	66

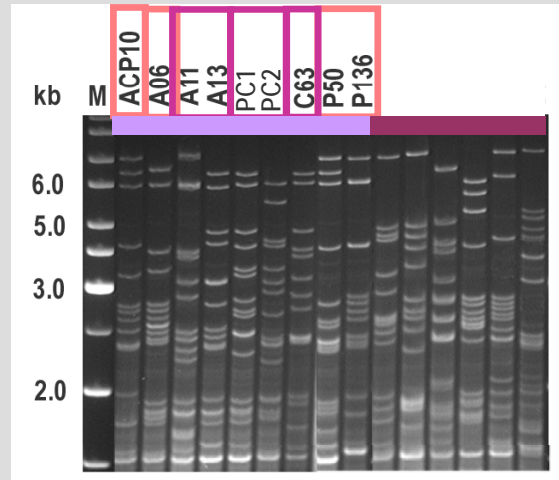
291 unique genetic patterns (mtDNA RFLP)

The genetic diversity of *S. cerevisiae* – Biogeographical large-scale survey

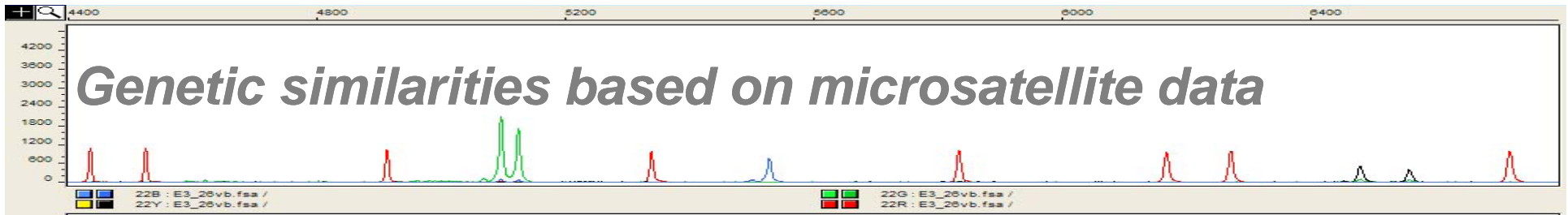


2001
2002
2003

- Indigenous strains
- Commercial starter strains
- Annual strains
- Perennial strains

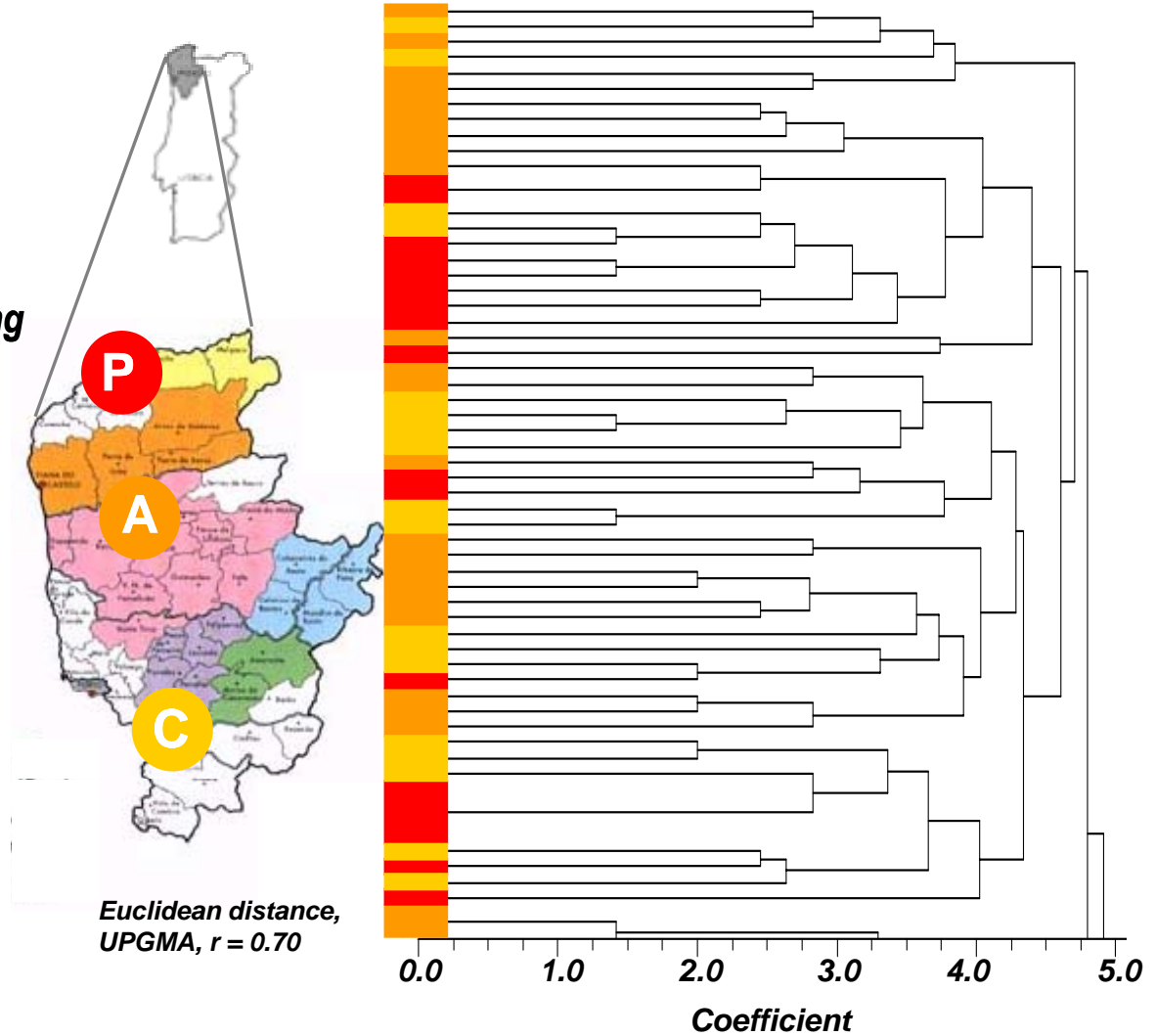


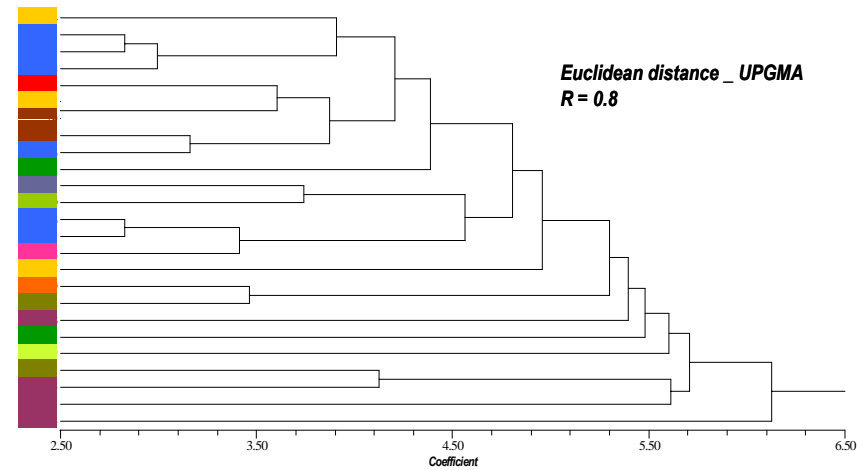
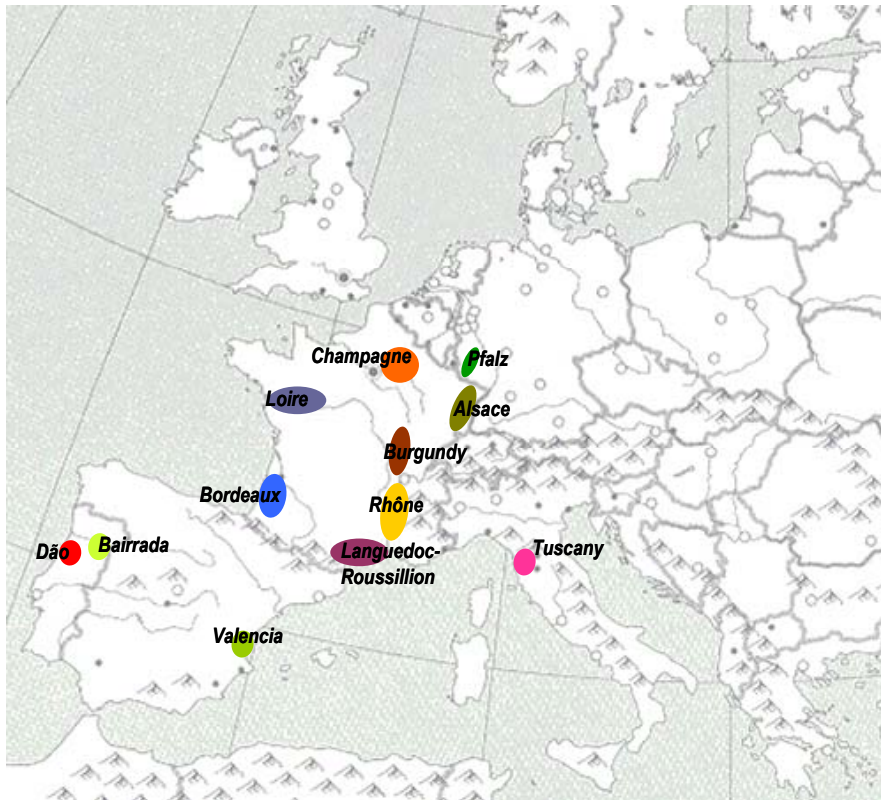
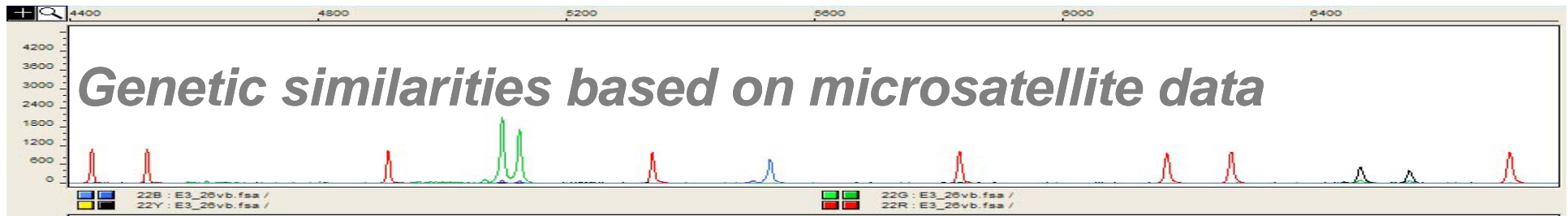
Schuller et al., 2005



What are the genetic differences among strains from distinct geographical regions?

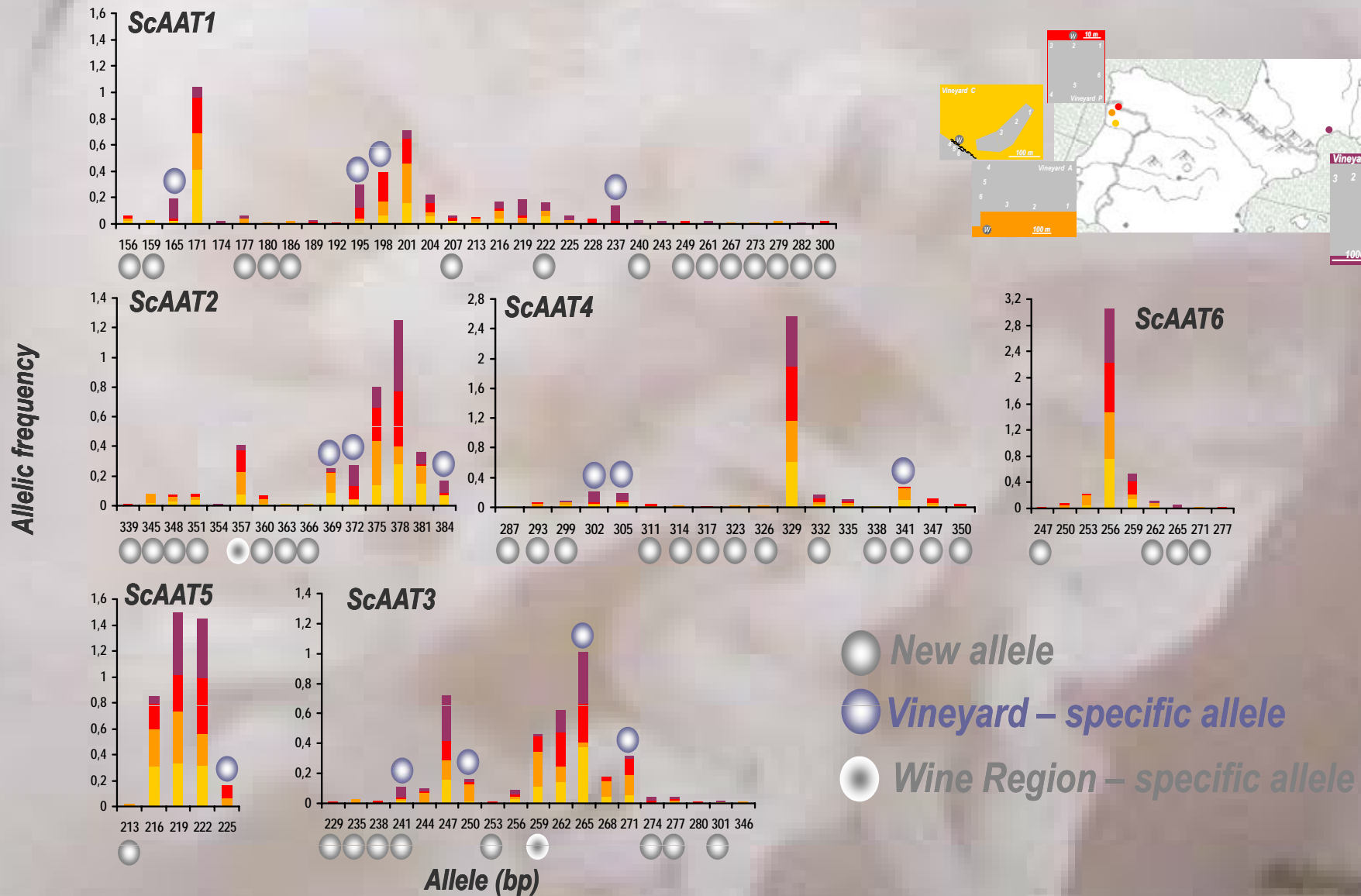
Correlation between geographic distance and genetic similarity?



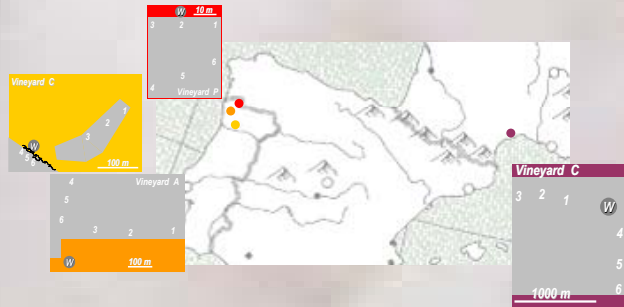


Analysis of individual strains is not the appropriate approach !

The genetic structure of *S. cerevisiae* populations



The genetic structure of *S. cerevisiae* populations



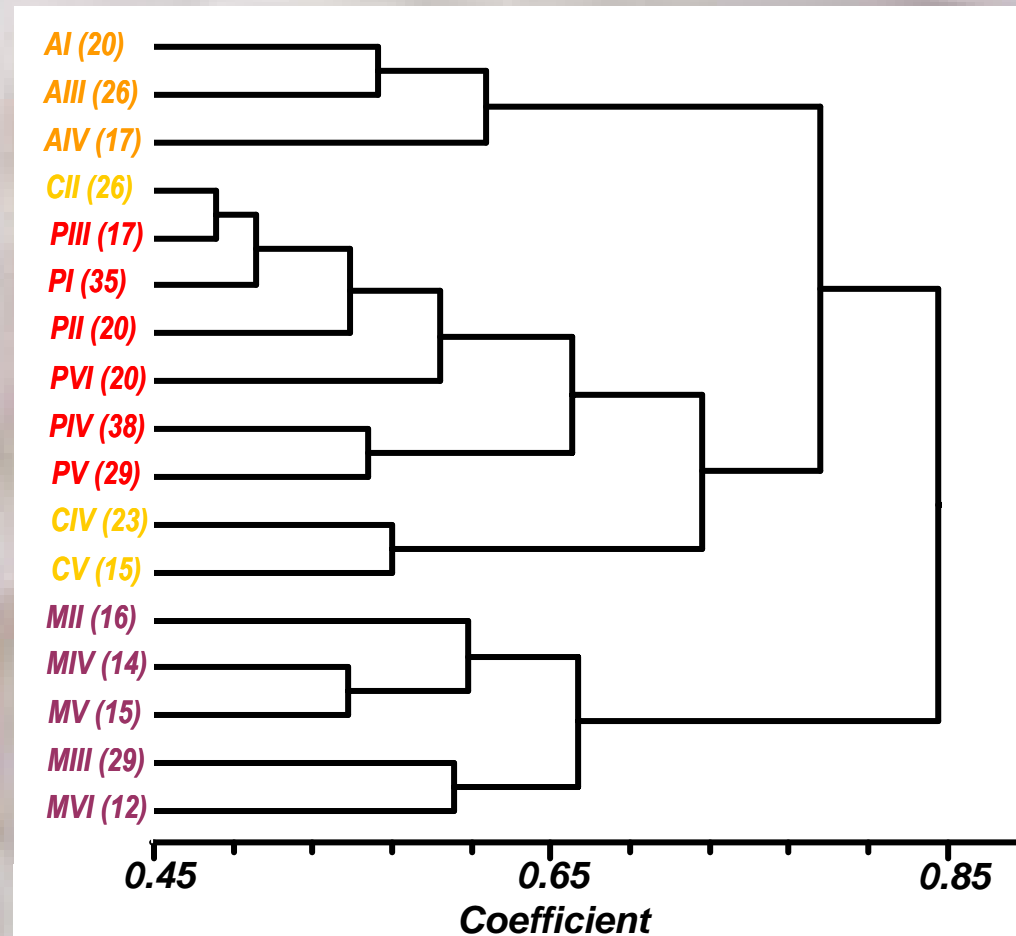
Populational substructure



Genetic differences delimit specific populations - characteristic for each vineyard



Correlation between geographic distance and genetic similarity



() number of strains analyzed

Schuller and Casal, 2007

A grayscale microscopic image showing numerous yeast cells, likely *S. cerevisiae*, with varying morphologies and some appearing to be budding or in different stages of growth. The cells are distributed across the field of view, with some appearing as bright, elongated structures and others as smaller, darker spots.

Intra-strain genetic variation of the commercial *S. cerevisiae* strain Zymaflore VL1

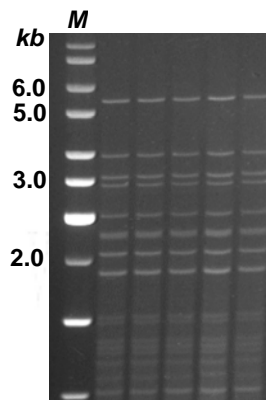
Intra-strain genetic variation of the commercial *S. cerevisiae* strain Zymaflore VL1

1620 yeast isolates

100 isolates recovered from natural environments with mtDNA RFLP of the commercial strain Zymaflore VL1



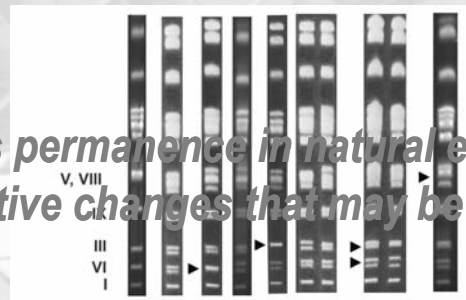
Comparison with 30 isolates of the parental commercial strain Zymaflore VL1



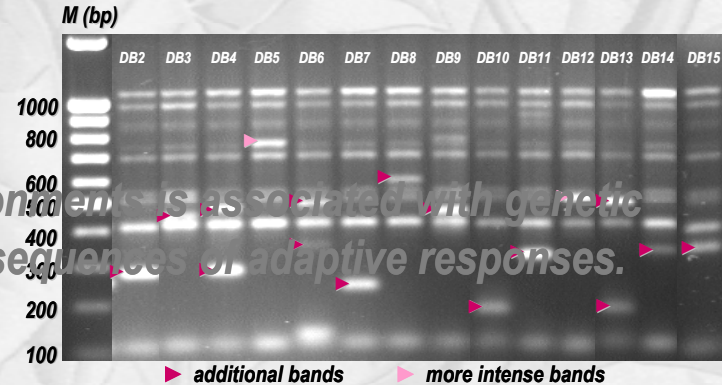
Loci	Alleles (bp) of distinct microsatellite patterns						
	M1	M2	M4	M5	M6	M7	M8
ScAAT1	204/219	219	204	204/219	204/219	204/219	204/219
ScAAT2	372/381	372	384	381	372	372/381	372/381
ScAAT3	265	265	265	265	265	265	265
ScAAT4	329	329	329	329	329	329	329
ScAAT5	219/222	222	219	219/222	219/222	222	219/222
ScAAT6	256/259	256	256	256/259	256/259	256/259	259
N° of natural VL1 isolates	89	1	1	1	1	2	5
N° of parental VL1 isolates	30	0	0	0	0	0	0

Intra-strain genetic variation of the commercial *S. cerevisiae* strain Zymaflore VL1

Chromosomal profile (PFGE)



Interdelta sequence amplification



The strain's permanence in natural environments is associated with genetic microevolutive changes that may be consequences of adaptive responses.

Summary

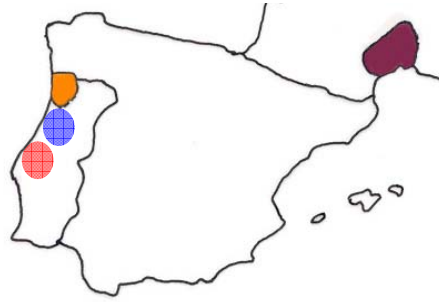
	N° of Isolates	Percentage of genetic changes		
		Microsatellite	Chromosomal profile	Interdelta patterns
<i>Recovered VL1 isolates</i>	100 (100%)	11	67	15
<i>Parental VL1 isolates</i>	30 (100%)	0	7	3

Schuller et al., submitted

A grayscale microscopic image showing a large number of yeast cells, likely *S. cerevisiae*, arranged in a somewhat circular pattern. The cells are bright against a dark background, and some show distinct internal structures. The text is overlaid on the left side of the image.

Phenotypic characterization of a S. cerevisiae strain collection

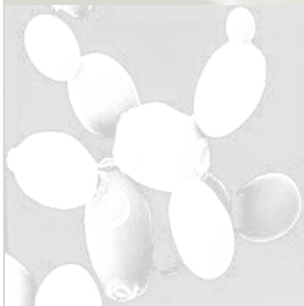
Saccharomyces cerevisiae Strain collection

	Minho 2001 – 2003 2006	Bairrada 2006	Torres Vedras 2006	Languedoc 2001-2003	Total
Number of isolates	2520	620	1080	2160	6380
<i>S. cerevisiae</i> strains (unique genetic profiles)	340	*	*	100	440

* ongoing

Phenotypic characterization of a *S. cerevisiae* strain collection

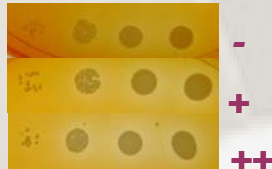


Ethanol tolerance



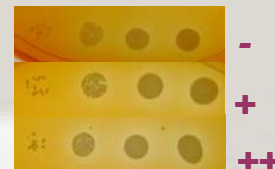
YNB + glucose (2 %, w/v),
acetic acid (0,25 %, v/v)
ethanol (10%, v/v) pH 4,0

Malic acid utilization



YP + 0,5 % (w/v)
malic acid

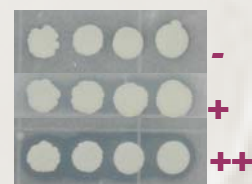
Acetic acid utilization



YP + 0,25 % (v/v)
acetic acid

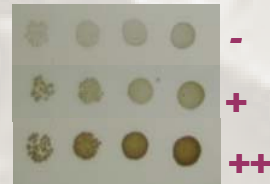


Acetic acid production



CaCO₃ Agar

H₂S production



BIGGY Agar



Ethanol resistance	Malic acid utilization	Acetic acid utilization	H ₂ S production	Number of isolates
-	-	-	+	4
-	-	-	++	2
-	-	+	-	2
-	-	+	+	20
-	-	+	++	10
-	-	++	+	12
-	-	++	++	6
-	+	+	+	6
-	+	+	++	4
-	+	++	+	5
-	++	+	+	1
-	++	++	+	1
+	-	-	+	6
+	-	+	-	5
+	-	+	+	48
+	-	+	++	6
+	-	++	-	2
+	-	++	+	20
+	-	++	++	3
+	+	-	+	1
+	+	+	-	1
+	+	+	+	10
+	+	+	++	5
+	+	++	+	6
++	-	-	+	1
++	-	+	+	7
++	-	+	++	3
++	-	++	-	1
++	-	++	+	6

Phenotypic characterization of a *S. cerevisiae* strain collection

Colony morphology in GMA medium
(Kuthan et al., 2003)

225 strains
65 strains

smooth colonies
fluffy colonies

Microsatellite patterns of strains with characteristic phenotypes

	119	384	346	192	237	235	177	136	242	VL1	S1	S2	S4	A14	ACP10
ScAAT1	171	171	171 198	171	201	201	171	204 222	201	171	204 219	201	201	195	201
ScAAT2	381	357 378	378	375	375	357	357	351	375	372 381	378	378	378	369	381
ScAAT3	262	259 265	265	247	265	247 262	271	250	250	265	247	241 277	265	247	256
ScAAT4	329	341	341	329	329	329	329	329	329	329	329	305 329	329	329	329
ScAAT5	219	219 222	216	222	216	219	219	222	222	219 222	222	222	222	216	219
ScAAT6	256	253 259	253	256	256	256	256	259	256	256 259	256	256	256	256	259

Acknowledgements

Universidade do Minho
Biology Department

Margarida Casal

Hugo Alves
Barbara Dellinger
Sofia Machado
Ricardo Duarte

INRA Montpellier
Sciences pour
Oenologie

Sylvie Dequin
Brigitte Cambon

IMIDRA, Madrid

Eva Valero

Ciência.Inovação
2010

Programa Operacional Ciência e Inovação 2010
MINISTÉRIO DA CIÊNCIA, TECNOLOGIA E ENSINO SUPERIOR

FCT Fundação para a Ciência e a Tecnologia
MINISTÉRIO DA CIÊNCIA, TECNOLOGIA E ENSINO SUPERIOR



UNIÃO EUROPEIA
FUNDOS ESTRUTURAIS



GRICES
MINISTÉRIO DE RELAÇÕES INTERNACIONAIS DA CIÊNCIA E DO ENSINO SUPERIOR
Ministério da Ciência, Tecnologia e Ensino Superior



GOVERNO DA REPÚBLICA PORTUGUESA

PHENOMICS – Exploratory Workshop
March 2007, Göteborg

EUROPEAN
SCIENCE
FOUNDATION

ch
centro de biologia
Universidade do Minho