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Yeast Interspecies Hybrids

Many interspecific chromosomal introgressions are highly prevalent in Holarctic *Saccharomyces uvarum* strains found in human-related fermentations

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Abstract

In the last two decades, the extensive genome sequencing of strains belonging to the *Saccharomyces* genus has revealed the complex reticulated evolution of this group. Among the various evolutionary mechanisms described, the introgression of large chromosomal regions resulting from interspecific hybridization has recently shed light on *Saccharomyces uvarum* species. In this work we provide the *de novo* assembled genomes of four *S. uvarum* strains presenting more than 712 kb of introgressed loci inherited from both *Saccharomyces eubayanus* and *Saccharomyces kudriavzevii* species. In order to study the prevalence of such introgressions in a large population, we designed multiplexed PCR markers able to survey the inheritance of eight chromosomal regions. Our data confirm that introgressions are widely disseminated in Holarctic *S. uvarum* populations and are more frequently found in strains isolated from human-related fermentations. According to the origin of the strains (nature or cider- or wine-related processes), some loci are over-represented, suggesting their positive selection by human activity. Except for one locus located on chromosome 7, the introgressions present a low level of heterozygozity similar to that observed for nine neutral markers (microsatellites). Finally, most of the loci tested showed an expected Mendelian segregation after meiosis and can recombine with their chromosomal counterpart in *S. uvarum*.

Keywords: **wine; cider; introgression; *saccharomyces eubayanus*; *Saccharomyces Uvarum*; MassARRAY**

Introduction

Deciphering genome evolution in yeast has gained momentum in the last two decades with the release of hundreds of genome sequences of several yeast species including *Saccharomyces cerevisiae*, (Gallone et al., 2016; Liti et al., 2009; Goffeau et al., 1996; Borneman et al., 2014; Wang et al., 2009) its sister *Saccharomyces* species (Liti et al., 2009; Hittinger et al., 2010; Scannell et al., 2011; Nakao et al., 2009; Libkind et al., 2011) and other ascomycetes (Souciet et al., 2015; Dujon et al., 2004; Wong et al., 2012; Borneman et al., 2016). Several prominent mechanisms of genomic

evolution have been described, among them interspecific hybridization (Dunn et al., 2012; Leducq et al., 2016), reticulated evolution (Peris et al., 2014) aneuploidization, (Gallone et al., 2016; Bond et al., 2004), recent or ancient polyploidization events (Libkind et al., 2011; Wong et al., 2012), large chromosomal duplication or more limited gene duplication (Steenwyk & Rokas, 2009; Fares et al., 2013), and horizontal transfer (Novo et al., 2009). These mechanisms are usually so closely intertwined that it is difficult to determine which one are causes or consequences, but regardless they have drastically shaped yeast genome along evolution; see for extensive reviews Liti & Louis, (2005), Dujon (2010), and Albertin and Marullo (2012).

Introgression is one such evolutionary mechanism. It has been described so far in various yeast species (Kavanaugh et al., 2006; Mallet et al., 2012), but has been particularly addressed within the *Saccharomyces* genus (Liti et al., 2005; Almeida et al., 2014; Muller & McCusker, 2009; Naumova et al., 2005). Introgression is defined as the transfer of large or more limited genetic information from one species to another, and results in mosaic genomes, whose formal characterization has long been complicated owing to the lack of appropriate molecular tools (Morales & Dujon, 2012). Introgression can be the result of interspecific hybridization followed by the extensive loss of one parental genome, either through repeated backcross with one parental species or through mis-segregation of the hybrid at meiosis. In any case, the preferential loss of one parental genome (except for the introgressed regions) may allow the restoration of meiotic fertility and subsequent successful sexual reproduction. Alternatively, horizontal gene transfer may account for the advent of introgressed regions, as in the case of *S. cerevisiae*, where *Zygosaccharomyces bailii* (Novo et al., 2009) and *Torulaspora microellipsoides* (Marsit et al., 2015) introgressions have been identified in the wine yeasts group. The mechanism of horizontal gene transfer could be mediated by episomal replication (Galeote et al., 2011). Introgression has been largely reported as a mechanism driving rapid adaptive evolution in yeast (Dunn et al., 2013) and other eukaryotes (Ropars et al., 2015; Arnold & Martin, 2009), including human (Huerta-Sanchez et al., 2014), animals (Fitzpatrick et al., 2009), and plants (Martin et al., 2006). It is therefore not surprising that introgression has been frequently associated with domestication in all eukaryotic kingdoms (Ropars et al., 2015; Giuffra et al., 2000; Zhao et al., 2002).

Saccharomyces uvarum is a striking example of a yeast species whose genome is strongly shaped by introgressed regions (Almeida et al., 2014). *S. uvarum* shares partially overlapping ecological niches with *S. cerevisiae*: both are strongly related to human-driven fermentation, but *S. uvarum* is more psychrotrophic and thus is more frequently associated with low-temperature processes: cider-making and winemaking in northern – cooler – French vineyards for example (Masneuf-Pomarede et al., 2016a; Tosi et al., 2009; Demuyter et al., 2004; Naumov et al., 2001). Isolates from natural environments (insect, plant, soil) have also been described (Sampaio & Gonçalves, 2008; Boynton & Greig, 2014). In 2014, Almeida *et al.* performed comparative genomics of 54 *S. uvarum* strains (Almeida et al., 2014). Unexpectedly, 21 of these strains presented introgressions, the number of introgressed regions and their size being highly variable among isolates (up to 900 kb of introgressed regions). These introgressions derived mostly from the sister species *Saccharomyces eubayanus* and possibly resulted from a few inter-specific hybridization events followed by chromosomal rearrangements and the extensive loss of most of the *S. eubayanus* genome, excepting the introgressed regions. These authors pointed out several interesting features: (a) all strains displaying introgressed regions originated from the Northern Hemisphere; (b) within the Holarctic population, *S. eubayanus* introgressions seemed to be more prevalent in strains associated with human activities (and largely absent from wild isolates); and (c) those introgressions were significantly enriched in genes involved in nitrogen and sulphite metabolism. These results feed the hypothesis that selective pressures in anthropic environments have promoted the selection of multiple introgressions in Holarctic domesticated isolates.

In this paper, we developed tools to rapidly assess the presence of introgressed regions in a large population of *S. uvarum* isolates (104 strains). Since introgressed regions were absent from Southern Hemisphere isolates, we decided to focus on Holarctic isolates from natural, cider and wine environments. We confirm that the overall number

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of introgressed regions is significantly higher in cider-associated strains compared with wild strains, and is furthermore higher in wine isolates. However, only a subset of the introgressed regions were found to be over-represented in anthropic activities and their number and quality varied between cider- and wine-making processes. Finally, we investigated the meiotic segregation of those introgressions in F1 hybrid progenies, demonstrating their Mendelian inheritance.

Materials and methods

Yeast strains used and culture media

All of the strains used in this study are described in Table 1. The genomes of four strains of *S. uvarum* (U1, U2, U3 and U4) have been sequenced in this work. The strains so named were obtained by tetrad microdissection (da Silva et al., 2015) and are monosporic clones of the strains PM12, PJP3, BR6-2 and RC4-15, respectively. Their genomic sequences (short reads) have been previously released (Almeida et al., 2014). A collection of 104 strains of *S. uvarum* sampled from various isolation substrates (grape/wine, nature, cider and others fermented beverages) was also genotyped. All of these strains were isolated in the Northern Hemisphere and could be considered to belong to the Holarctic group of *S. uvarum* (Almeida et al., 2014). Furthermore, a few interspecific hybrids (CBS 3008, CBS 425, CBS 1480, CID1) were genotyped. Finally, two sets of meiotic progeny clones of *S. uvarum* F1 hybrids carrying different introgressions were also obtained by tetrad microdissection. The F1 hybrids used, UU23 and UU34, were previously obtained from haploid derivatives of U2, U3 and U4 (da Silva et al., 2015). In order to set up the genotyping method of introgressions in *S. eubayanus*, the strains belonging to *S. kudriavzevii* (ZP542), *S. cerevisiae* (VL3) and *S. eubayanus* (CBS 12357) were used. All strains were usually grown at 24°C in YPD medium containing 1% yeast extract (Difco Laboratories, Detroit, MI, USA), 1% Bacto peptone (Difco) and 6% glucose, supplemented or not with 2% agar. Sporulation was induced in ACK medium (1%, potassium acetate, 2% agar) for 3 days at 24°C after an overnight preculture on YPD medium.

Genome assembly of four *S. uvarum* strains

The genomic sequences of strains U1, U2, U3 and U4 were obtained by combining both Illumina Paired End and Mate Pair datasets. Briefly, genomic DNA was extracted from a saturated culture of 100 mL under anaerobic conditions (YPD) using the genomic tip-100 kit (Qiagen, Courtaboeuf, France). Paired-end and 2.5 kb mate pair Illumina libraries were prepared according to manufacturer protocols (Genomic DNA Sample Preparation) from sonicated genomic DNA. Sequencing was performed on Illumina Genome Analyzer IIx (Illumina, CA, USA) with a read length of 54 pb by the Genomic and Transcriptomic facility of Bordeaux, France. A mapping dataset was obtained by mapping reads on the reference genome *S. uvarum* CBS 7001 (Scannell et al., 2011) using the Stampy program. Variant calling was performed by mapping short reads to the reference genome using Stampy (Lunter & Goodson, 2011) followed by Samtools. (Li et al., 2009; Danecek et al., 2011). Single Nucleotide Polymorphisms (SNPs) were called using Samtools mpileup with mapping quality ≥30, base quality ≥20, and varFilter depth ≥10. Single amino-acid polymorphisms were identified using snpEff (Cingolani et al. 2012), requiring quality QUAL ≥30 and genotype GEN[*].GQ ≥ 20. A *de novo* assembly was then carried out from an initial set of 80× single reads combined with 180× paired-end sequences from 2500 ± 250 bp inserts. Intitial contigs from GAIIx reads were assembled using Mira 3.2.1 (Chevreux et al., 1999) with eight passes. They were oriented and joined into scaffolds with paired-end sequences as follows. To anchor initial contigs into the paired-end assembly, they were fragmented into 45 × 160 bp libraries using simLibrary 1.3 then into overlapping reads by simNGS 1.6 (Massingham & Goldman, 2012) to simulate the AllPaths-LG sequencing protocol. These fragment reads were combined with the paired-end reads and reassembled using AllPaths-LG (Gnerre et al., 2011). The supercontigs for the four strains were deposited on a GenBank database with the following BioProject ID: PRJNA388544. The genomes of the strains U1–U4 are registered with the accession number SAMN07178572 to SAMN07178575; the genomes were not annotated. Whole-genome synteny was computed using Sibelia (Minkin et al., 2013) using the 'loose' parameter, pairwise between CBS7001 and strains

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Table 1. Yeast strains used.

Strain name	Isolated/obtained from	Origin	Area	Species	References
Sequenced strains	—	—	—	—	—
U1	Monosporic clone of PM12	Laboratory	—	*Saccharomyces uvarum*	da Silva *et al.*, 2015
U2	Monosporic clone of PJP3	Laboratory	—	*S. uvarum*	da Silva *et al.*, 2015
U3	Monosporic clone of BR6–2	Laboratory	—	*S. uvarum*	da Silva *et al.*, 2015
U4	Monosporic clone of RC4–15	Laboratory	—	*S. uvarum*	da Silva *et al.*, 2015
F1 hybrids and related progeny	—	—	—	—	—
UU23	F1 hybrid U2 × U3	Laboratory	—	*S. uvarum*	da Silva *et al.*, 2015
UU34	F1 hybrid U3 × U4	Laboratory	—	*S. uvarum*	da Silva *et al.*, 2015
UU23 msp clones	Monosporic clones (n = 73)	Laboratory	—	*S. uvarum*	This work
UU34 msp clones	Monosporic clones (n = 48)	Laboratory	—	*S. uvarum*	This work
other species	—	—	—	—	—
CBS 12357	Bark/tree	Nature	Patagonia	*Saccharomyces eubayanus*	Libkind *et al.,* 2011
CBS 3008	Beer	Beer-cereal	Unknown	*S. eubayanus* × *S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
CBS 425	Cider/apple juice	Cider-fruit	Switzerland	*S. eubayanus* × *S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
ZP542	Unknown	Unknown	Portugal	*Saccharomyces kudriavzevii*	Sampaio and Gonçalves, 2008
CBS 1480	Sorghum brandy	Beer-cereal	Unknown	*Saccharomyces pastorianus*	Masneuf-Pomarede *et al.*, 2016b
VL3	Industrial wine starter	Grape-wine	SW of France	*S. cerevisae*	Masneuf-Pomarede *et al.*, 2016b
CID1	Cider/apple juice	Cider-fruit	Brittany/Normandy	Triple hybrid	Masneuf-Pomarede *et al.*, 2016b
S. uvarum isolates	—	—	—	—	—
BR46–1	Cider/apple juice	Cider-fruit	Brittany/Normandy	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
BR23–1	Cider/apple juice	Cider-fruit	Brittany/Normandy	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
CBS 1608	Fruit/fruit juice	Cider-fruit	Unknown	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
CBS 1605	Fruit/fruit juice	Cider-fruit	Unknown	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
BR9–2	Cider/apple juice	Cider-fruit	Brittany/Normandy	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
BR46–2	Cider/apple juice	Cider-fruit	Brittany/Normandy	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
BR7–2	Cider/apple juice	Cider-fruit	Brittany/Normandy	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
BR11–1	Cider/apple juice	Cider-fruit	Brittany/Normandy	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
BR9–1	Cider/apple juice	Cider-fruit	Brittany/Normandy	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
LC11a	Cider/apple juice	Cider-fruit	Brittany/Normandy	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
BR5–2	Cider/apple juice	Cider-fruit	Brittany/Normandy	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
BR1–1	Cider/apple juice	Cider-fruit	Brittany/Normandy	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
BR43–1	Cider/apple juice	Cider-fruit	Brittany/Normandy	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
BR18–1	Cider/apple juice	Cider-fruit	Brittany/Normandy	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
BR45–1	Cider/apple juice	Cider-fruit	Brittany/Normandy	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
DJ7T10A	Cider/apple juice	Cider-fruit	Brittany/Normandy	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
BR23–2	Cider/apple juice	Cider-fruit	Brittany/Normandy	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
LJ8TM2	Cider/apple juice	Cider-fruit	Brittany/Normandy	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
BR7–3	Cider/apple juice	Cider-fruit	Brittany/Normandy	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
BR7–1	Cider/apple juice	Cider-fruit	Brittany/Normandy	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
Cat19	Cider/apple juice	Cider-fruit	Brittany/Normandy	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
BR20–1	Cider/apple juice	Cider-fruit	Brittany/Normandy	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b

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Table 1. (Continued)

Strain name	Isolated/obtained from	Origin	Area	Species	References
J32T10c	Cider/apple juice	Cider-fruit	Brittany/Normandy	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
ZP646	Cider	Cider-fruit	Germany	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
CBS 1606	Fruit/fruit juice	Cider-fruit	Unknown	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
CBS 377	Fruit/fruit juice	Cider-fruit	Germany	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
CBS 1547	Fruit/fruit juice	Cider-fruit	Brittany/Normandy	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
BR6–2	Cider/apple juice	Cider-fruit	Brittany/Normandy	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
CBS 1604	Fruit/fruit juice	Cider-fruit	Unknown	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
PJS28	Wine/fermenting grape	Grape-wine	Sancerre	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
PJS25	Wine/fermenting grape	Grape-wine	Sancerre	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
PJS26	Wine/fermenting grape	Grape-wine	Sancerre	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
PJS17	Wine/fermenting grape	Grape-wine	Sancerre	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
PJS18	Wine/fermenting grape	Grape-wine	Sancerre	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
PJS19	Wine/fermenting grape	Grape-wine	Sancerre	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
LC5	Wine/fermenting grape	Grape-wine	Sancerre	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
PJP14	Wine/fermenting grape	Grape-wine	Sancerre	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
PJP15	Wine/fermenting grape	Grape-wine	Sancerre	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
PJS30	Wine/fermenting grape	Grape-wine	Sancerre	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
PJS16	Wine/fermenting grape	Grape-wine	Sancerre	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
LC6	Wine/fermenting grape	Grape-wine	Sancerre	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
LC8	Wine/fermenting grape	Grape-wine	Sancerre	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
PJS24	Wine/fermenting grape	Grape-wine	Sancerre	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
NCAIM Y.00677	Fermented drink	Grape-wine	Hungary	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
NCAIM Y.00676	Fermented drink	Grape-wine	Hungary	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
Sapis 21	Wine/fermenting grape	Grape-wine	SW of France	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
RC4–15	Wine/fermenting grape	Grape-wine	Alsace	*S. uvarum*	Muller and McCusker, 2009
RC4–5	Wine/fermenting grape	Grape-wine	Alsace	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
D11	Wine/fermenting grape	Grape-wine	Clairette de Die	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
D3	Wine/fermenting grape	Grape-wine	Clairette de Die	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
RP1–21	Wine/fermenting grape	Grape-wine	Alsace	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
RP1–16	Wine/fermenting grape	Grape-wine	Alsace	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
RP2–32	Wine/fermenting grape	Grape-wine	Alsace	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
RC2–20	Wine/fermenting grape	Grape-wine	Alsace	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
RR1–3	Wine/fermenting grape	Grape-wine	Alsace	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
LC3	Wine/fermenting grape	Grape-wine	Sancerre	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
LC2	Wine/fermenting grape	Grape-wine	Sancerre	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
SU3	Wine/fermenting grape	Grape-wine	Tokai	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
PJP3	Wine/fermenting grape	Grape-wine	Sancerre	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
D6	Wine/fermenting grape	Grape-wine	Clairette de Die	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
SU7	Wine/fermenting grape	Grape-wine	Tokai	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
D15	Wine/fermenting grape	Grape-wine	Clairette de Die	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
D8	Wine/fermenting grape	Grape-wine	Clairette de Die	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b

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Table 1. (Continued)

Strain name	Isolated/obtained from	Origin	Area	Species	References
D45	Wine/fermenting grape	Grape-wine	Clairette de Die	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
PJS2	Wine/fermenting grape	Grape-wine	Sancerre	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
TB95VIC3	Wine/fermenting grape	Grape-wine	SW of France	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
DDI4	Wine/fermenting grape	Grape-wine	Sauternes	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
PM12	Wine/fermenting grape	Grape-wine	SW of France	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
GM14	Wine/fermenting grape	Grape-wine	SW of France	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
D50	Wine/fermenting grape	Grape-wine	Clairette de Die	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
D2	Wine/fermenting grape	Grape-wine	Clairette de Die	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
TB3IVC28	Wine/fermenting grape	Grape-wine	SW of France	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
RC3 U1	Wine/fermenting grape	Grape-wine	Alsace	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
TB95VIC28	Wine/fermenting grape	Grape-wine	SW of France	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
PJS5	Wine/fermenting grape	Grape-wine	Sancerre	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
PJS21	Wine/fermenting grape	Grape-wine	Sancerre	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
D4	Wine/fermenting grape	Grape-wine	Clairette de Die	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
D19	Wine/fermenting grape	Grape-wine	Clairette de Die	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
D17	Wine/fermenting grape	Grape-wine	Clairette de Die	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
PJS3	Wine/fermenting grape	Grape-wine	Sancerre	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
PJS10	Wine/fermenting grape	Grape-wine	Sancerre	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
PJS4	Wine/fermenting grape	Grape-wine	Sancerre	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
PJS6	Wine/fermenting grape	Grape-wine	Sancerre	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
PJS13	Wine/fermenting grape	Grape-wine	Sancerre	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
PJS14	Wine/fermenting grape	Grape-wine	Sancerre	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
PJS15	Wine/fermenting grape	Grape-wine	Sancerre	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
PJS7	Wine/fermenting grape	Grape-wine	Sancerre	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
PJS8	Wine/fermenting grape	Grape-wine	Sancerre	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
PJS27	Wine/fermenting grape	Grape-wine	Sancerre	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
CBS 7001	Insect	Nature	Spain	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
ZP1021	Soil under cherry tree	Nature	Portugal	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
ZP664	*Quercus robur*	Nature	Germany	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
ZP663	*Quercus robur*	Nature	Germany	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
ZP556	*Quercus garryana*, Homby Island	Nature	Canada	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
ZP554	*Quercus garryana*, Homby Island	Nature	Canada	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
ZP558	*Quercus garryana*, Homby Island	Nature	Canada	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
ZP830	*Quercus glauca*, Takamatsu	Nature	Japan	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
8.4 ACF	Bark/tree, *Quercus*	Nature	Alsace	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
8.5 ACF	Bark/tree, *Quercus*	Nature	Alsace	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
CBS 2954	Insect	Nature	USA	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
CBS 426	Honey	Nature	Unknown	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
CECT 10192	Insect	Nature	Spain	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
ECO K4	Unknown	Unknown	Unknown	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b
D24 Cu	Unknown	Unknown	Unknown	*S. uvarum*	Masneuf-Pomarede *et al.*, 2016b

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U1–U4. A total of 1484 blocks longer than 5 kbp were found, with a median length of 45 kbp. Selected syntenies are shown in Fig. S1 (Supporting Information) for U1 (CBS7001 chromosomes 1, 10, 11), U2 (CBS7001 chromosomes 1, 7, 13, 16) and U4 (CBS7001 chromosomes 1, 8, 13), illustrating both coverage and collinearity.

Introgression genotyping

Rapid DNA extraction

The genomic DNA of *S. uvarum* isolates and monosporic clones was quickly extracted in 96-well microplate format using a customized LiAc-SDS protocol. Basically, 5×10^6 cells were pelleted on a PCR microplate and incubated with 50 µL of 200mM LiAc/1% SDS at 70°C for 5 min. Genomic DNA was then extracted by mixing cell lysates with 150µL of pure ethanol and vortexed for 15 s. After a brief centrifugation (5 min, 4400 rpm) the supernatant was removed and the pellet washed with 70% ethanol. Genomic DNA was then solubilized in 200 µL of milliQ water at 60°C for 5 min. After a brief centrifugation, cell debris were pelleted and 150 µL of supernatant containing genomic DNA was recovered in a new microplate. The genomic DNA was then analysed by MassARRAY genotyping.

MassARRAY genotyping

Initially, 20 sequences located in the eight introgressed regions were screened, corresponding to 74 polymorphic sites, including SNPs and INDELs. Candidate markers were submitted for assay design using the MassARRAY Assay Design version 4.0.0.2 (Agena Biosciences, Hamburg, Germany). To circumvent the high polymorphism in each sequence (two to eight polymorphisms within 103–151 bp), we decreased the allowed PCR primer length to 16 bases, reduced the minimum peak separation to 10 Da and extended the mass array window to between 3000 and 10,000 Da. One multiplex of 30 polymorphisms was selected (Table S2), covering 16 out of the 20 sequences tested. We used 15 ng of DNA for genotyping with the MassARRAY iPLEX platform (Agena Bioscience) following the manufacturer's instructions. Raw data analyses were performed using Typer Viewer v 4.0.26.75 (Agena Bioscience). We filtered out monomorphic SNPs and loci with weak or ambiguous signals (loci displaying more than three genotypic clusters or unclear cluster separation). The markers showed mean amplification rates of 95.5% (84.9–100%).

Genetic and statistical analyses

The genotypes of a subpanel of 72 *S. uvarum* strains were obtained from a previous genetic analysis using nine microsatellite markers (Masneuf-Pomarede et al., 2016b). Expected and observed heterozygosity were calculated from Hardy–Weinberg equilibrium using the *ade4* package (R). To assess whether the proportion of heterozygous individuals was higher for introgressed markers compared with microsatellite ones, χ^2 tests were performed (statistical test of the presence/absence of the introgressed markers). The χ^2 test was applicable as all groups displayed >10 individuals as recommended by Cochran (Cochran, 2012). A non-parametric statistical test (Kruskal–Wallis) was used to determine whether the strains of the different groups presented significantly different numbers of introgressed markers using R package *agricolae*.

The subpanel of 72 *S. uvarum* strains was then used to draw dendrogram trees using either microsatellite data or introgressed markers. A microsatellite tree was built using Bruvo's distance and NJ clustering (*poppr* package, R). An introgression tree was built using Euclidean distance and Ward's clustering. The genetic distance was estimated using the Haldane relation $d = -1/2\ln(1 - 2r)$, were r is the recombination rate.

Results and discussion

Genome sequences of four monosporic clones of *S. uvarum* strains

The four monosporic clones U1–U4 were obtained by tetrad microdissection respectively from strains PM12, PJP3, BR6 and RC4-15 and were previously sequenced by a Paired end strategy (Almeida et al., 2014). In order to improve their genome quality, an additional sequence dataset was obtained with a 2.5 kb mate pair approach (see methods). Using both datasets, the *de novo* assembly delivered nearly 50 scaffolds for the strains U2, U3 and U4 (Table S1). For some chromosomes,

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the assembled scaffolds correspond to an entire chromosome (Fig. S1). The assembly of U1 is more fragmented than those of U2–U4, probably owing to the poor quality of the mate pair library. Although not completely finished, the scaffolds released will contribute to the genomic databases.

In the present study, we focused our attention on some genomic regions showing a strong SNP polymorphism density (>5% of divergence) with respect to the reference genome (CBS 7001). This high polymorphic rate contrasts with the relative low SNP polymorphism found for the remaining part of the genome that varies between 1.92 (U2) and 2.24 (U1) SNP/kb according to the strain (Fig. 1A). The high polymorphic regions encompass 712 kb and are located in eight *S. uvarum* chromosomes (chromosomes 2, 4, 6, 7, 9, 13, 14, 16) corresponding to the interspecific introgressions from *S. eubayanus* and *S. kudriavzevii* described by Almeida (2014). Except for two large regions in chromosomes 12 and 15 (detected for the strains DBVPG 7787 and 148.01 respectively), the four clones sequenced showed almost all of the interspecific introgressions described until now for this species. For each genome, a blast analysis confirmed that all of the the introgressed loci belong to distinct scaffolds confirming that these regions were not physically linked.

Design of multiplexed PCR experiment for tracking *S. eubayanus* and *S. kudriavzevii* introgressions in a large set of *S. uvarum* strains

To confirm the inheritance of these introgressions, 20 species-specific PCR markers covering the

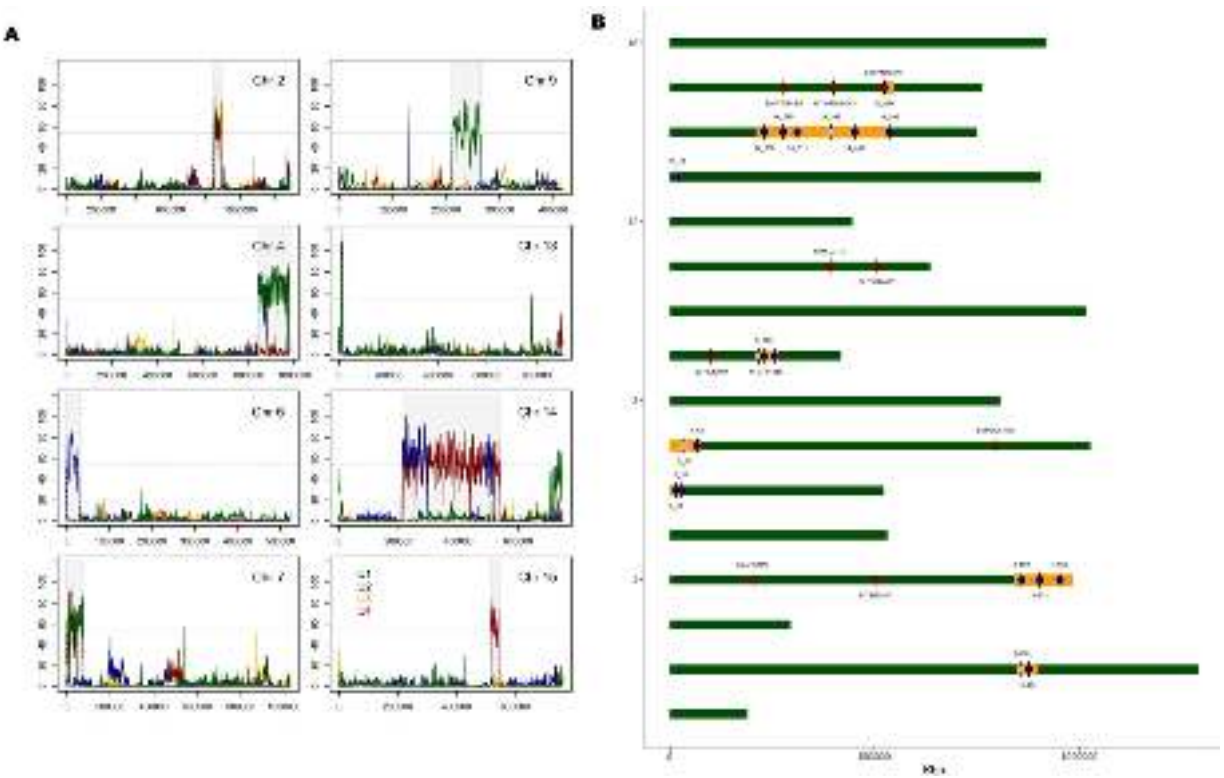

Figure 1. SNP scanning of four *Saccharomyces uvarum* genomes defined eight interspecific introgressions tracked by MassARRAY genotyping. (A) The number of SNP per kb relative to the *S. uvarum* reference (CBS 7001) genome was given for the sequenced strains U1–U4 colour-coded according to the key. The eight genomic regions, having an SNP divergence >5% and longer than 9 kb are shaded in grey and are located on the chromosomes 2, 4, 6, 7, 9, 13, 14 and 15. (B) The names and positions of 20 markers designed are shown on the genetic map of the CBS 7001 reference genome. Dark blue and grey dots represented the markers multiplexed or not by MassARRAY. Finally, the names and positions of the nine microsatellites markers (Masneuf-Pomarede *et al.,* 2016b) used for calculating heterozygosity are shown by dark red dots [Colour figure can be viewed at wileyonlinelibrary.com]

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eight introgressions were designed. Each locus was covered by at least one marker and, for the larger ones, by few markers spaced every ~30 kb. The specificity of each marker was confirmed by using as templates the DNA of the strains CBS7001 (*S. uvarum*), ZP542 (*S. kudriavzevii (European)*), CBS 12357 (*S. eubayanus*) and VL3 (*S. cerevisae*). As expected, the locus located on chromosome 13 (13_17) was amplified with the DNA of the strain ZP542 (*S. kudriavzevii*). All of the other loci were positively amplified using the strain CBS 12357 (*S. eubayanus*) but were not amplified by other reference strains of *S. uvarum*, *S. kudriavzevii* and *S. cerevisiae* (data not shown). For all the markers, the strains U1–U4 showed the allele inheritance predicted by the genomic sequence. The names, positions and relative inheritance of these 20 PCR-markers are given in Table 2.

In order to readily track these interspecific introgressions within a large set of *S. uvarum* strains, a high-throughput PCR screening was then developed. We used the MassARRAY technology, which allows genotyping up to 48 SNP in a single multiplexed reaction (Gabriel et al., 2009). Owing to the very divergent sequence between the *S. uvarum* genome and the introgressed regions, only 16 loci of the 20 designed were positively multiplexed; each of the eight chromosomes was covered by at least one marker. Figure 1(B) shows the relative position of the MassARRAY markers on the *S. uvarum* CBS 7001 map.

Prevalence of introgression in strains associated to alcoholic fermentation

The prevalence of the 16 MassARRAY markers was evaluated in a population of 104 holarctic *S. uvarum* strains isolated from different substrates: 13 isolates from nature, 60 strains from grape or wine, 29 from cider or fruits (except grapes) and two isolates of unknown origin. In addition, four interspecific hybrids, CBS 3008, CBS 425, CBS 1480 and CID1, the *S eubayanus* type strain (CBS 12357) as well as the fully homozygous strains U1–U4 were genotyped. The whole dataset is represented in Fig. 2. Only five *S. uvarum* strains (CBS 7001, CECT 10192, ZP1021, ZP554 and ZP556) displayed no introgressed markers, confirming the high prevalence (95%) of introgressed regions in Holarctic *S. uvarum* population. All of them belong to the 'nature' group

Table 2. *S. eubayanus* and *S. kudriavzevii* introgressions detected by genome sequencing and confirmed by PCR

Locus name[a]	Chromosome[b]	Position[b]	U1 (PM12-msp)[c]	U2 (PJP3-msp)[c]	U3 (BR6-msp)[c]	U4 (RC4–15 msp)[c]	Maximal range of introgressed locus
2_858*	2	858 180	U	U	E	E	853 000–890 000
2_877	2	877 220	U	U	E	E	
4_859	4	859 484	E	E	U	U	841 000–983 000
4_903	4	903 570	E	U	U	U	
4_952	4	952 806	E	U	U	U	
6_15	6	15 260	U	E	U	U	0–30 000
6_26	6	26 601	U	E	U	U	
7_35*	7	35 910	E	U	U	U	—
7_65	7	65 845	E	U	U	U	0–76 000
9_217*	9	217 324	E	U	U	U	211 000–255 000
9_229	9	229 110	E	U	U	U	
9_255	9	255 205	E	U	U	U	
13_17	13	17 267	K	K	K	K	9000–16 000
14_230	14	230 688	U	E	U	E	214 000–517 000
14_276	14	276 520	U	E	U	E	
14_311	14	311 301	U	U	U	E	
14_392	14	392 621	U	U	U	E	
14_451	14	451 083	U	U	U	E	
14_535	14	535 571	U	U	U	E	
15_524	15	524 107	U	U	U	E	517 000–545 000

[a]The markers labeled with an asterisk failed to be multiplexed by MassARRAY.
[b]The positions were given according to the reference genome *S. uvarum* from Scannell *et al.* (2011)
[c]The letters U, E and K stand for *S. uvarum*, *S. eubayanus* and *S. kudriavzevii* alleles, respectively.

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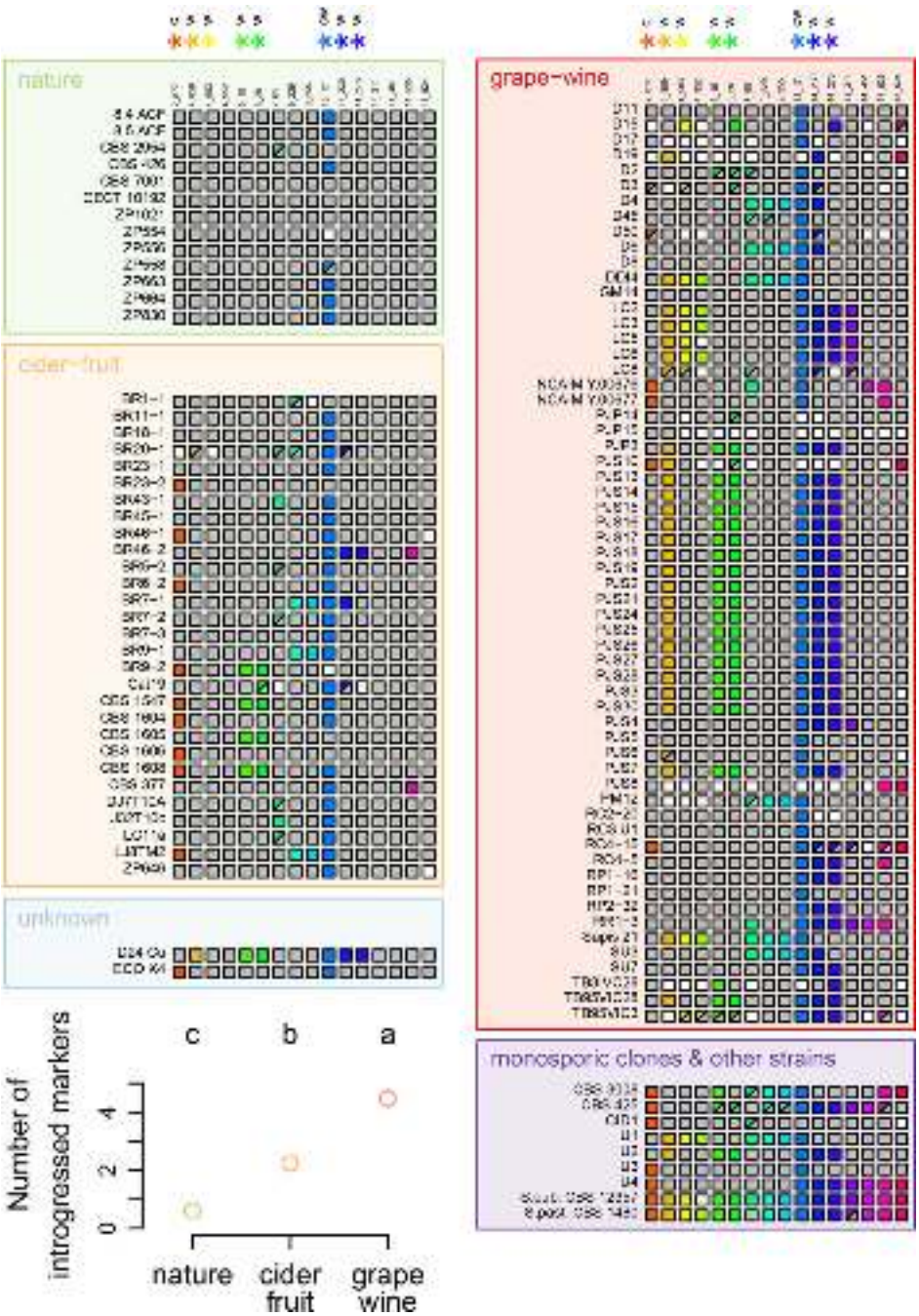

Figure 2. Detection of introgressed 16 markers in 113 strains of *S. uvarum* and related species. For each strain and each marker, a grey square indicates the presence of the *S. uvarum*-like allele, while a coloured square indicates introgressions. Heterozygosity is represented by grey/coloured triangles, and missing data by white squares. For *S. uvarum* only, χ^2 tests were performed to assess whether the introgressions were over- or under-represented depending on the substrate origin [nature (13 strains), cider-fruit (29 strains), grape-wine (60 strains)]. Coloured stars indicate significant distribution differences (α = 0.05, Benjamini–Hochberg correction for multiple testing), and 'c' or 'w' indicates whether the introgressed markers are over-represented for 'cider-fruit' and/or 'grape-wine' groups compared with the nature one. The number of introgressed markers harboured by the *S. uvarum* of different substrate origins was calculated (bottom-left graph), and was found to be significantly different (Kruskal–Wallis, α = 0.05, different letters indicates different means) [Colour figure can be viewed at wileyonlinelibrary.com]

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and have a very limited number of introgressed loci (only the markers 13_17 and 7_65). Although the number of strains from the 'nature' group is limited in this study, this result confirms that most of the introgressions described are rare for such strains. The highest number of introgressed markers was eight for strain TB95VIC3 (grape-wine group) and many strains have more than five introgressed markers. To determine whether the number of introgressions was significantly different depending on the substrate origin, we computed the average number of introgressed markers per group (Fig. 2). Overall, strains from 'nature' displayed a mean of 0.61 introgressed markers, while strains from cider-fruit and grape-wine possessed 2.24 and 4.48 introgressed markers, respectively. A Kruskal–Wallis test indicated that the over-representation of introgressed markers in both anthropic groups was significant compared with wild strains, and furthermore that grape-wine strains had a higher number of introgressed markers.

All of the introgressed regions derived from *S. eubayanus* species (chromosomes 2, 4, 6, 7, 9, 14, 16) were not or were poorly detected within the 'nature' population. Thus, we tested whether each marker was over-represented in cider-fruit and/or grape-wine groups compared with nature one (χ^2 test $\alpha = 0.05$, Fig. 2). Grape-wine strains displayed a significant over-representation of six markers distributed over three chromosomes (4, 6, 14). In contrast, cider-fruit isolates displayed only one over-represented marker, located on chromosome 2. For the cider group the allele frequency was 3.7-fold higher than for the wine group (0.31 vs. 0.083). One possible explanation of this enrichment could be the presence of the *ASP1* gene encoding the cytosolic L-asparaginase (type I) and required for asparagine anabolism (Dunlop et al., 1978). Asparagine is the most abundant amino acid in most apple juices (10–30 mg/100 mL apple juice) (Burroughs, 1957; Dizy et al., 1992), while grape juices usually display 100-fold lower asparagine concentration (Dizy et al., 1992). Interestingly, when L-asparagine is a major nitrogen source, the activity of L-asparaginase strongly impacts yeast growth as well as acetic acid production (Marullo et al., 2007), which are important traits in both cider and wine industry.

The introgression located on chromosome 13 and derived from *S. kudriavzevii* showed an atypical inheritance and was the unique introgression harbouring a relatively high frequency in the 'nature' group (allele frequency 0.29). Nonetheless, the marker 13_17 was still significantly over-represented in both cider- and wine-related populations and represented by far the most frequent allelic form. The relative high frequency of this *S. kudriavzevii* region in *S. uvarum* natural isolates might be explained by the fact that European *S. kudriavzevii* and *S. uvarum* shared the same biotope (bark tree) and temperature optima (cold regions) (Sampaio & Gonçalves, 2008). This environmental proximity might have promoted hybridization and/or horizontal transfer events.

Two additional introgression regions (not screened in this work) have been identified in only two 'nature' isolates: DBVPG 7787 (chromosome 12) and 148.01 (chromosome 15) (Almeida et al., 2014). Our method could be applied to large nature isolates to test if these regions are more frequently found in natural populations and might confer any adaptation to wild habitat conditions. However their low frequency (each found only twice in 54 genomes) seems to be in contraction with any positive selection.

Finally, we tested whether the introgression patterns could be used as a proxy for genetic distance between *S. uvarum* strains. Among the 104 strains genotyped, a subpanel of 72 has been previously genotyped using nine microsatellite markers (Table S3) (Masneuf-Pomarede et al., 2016b). Two dendrograms were built using either microsatellite or introgression data (Fig. 3). The trees were not completely congruent, except for the most distant group (called 'A' in the microsatellite tree) that globally is well conserved in the introgression tree. However, most of these strains have unique geographical and source origins (fermented grape juice, Sancerre, France) and might be strongly similar clonal variants. Therefore, further experiments are needed to increase the *S. uvarum* collection tested in order to have a more precise idea of the relationship between genetic diversity, geographical origin and possible domestication events.

'Genetic behaviour' of introgressed loci

Previous analysis reported very low levels of heterozygosity in *S. uvarum* using microsatellite genotyping, probably as a consequence of a high selfing rate (>95%) (Masneuf-Pomarede et al., 2016b). In order to test whether introgression and

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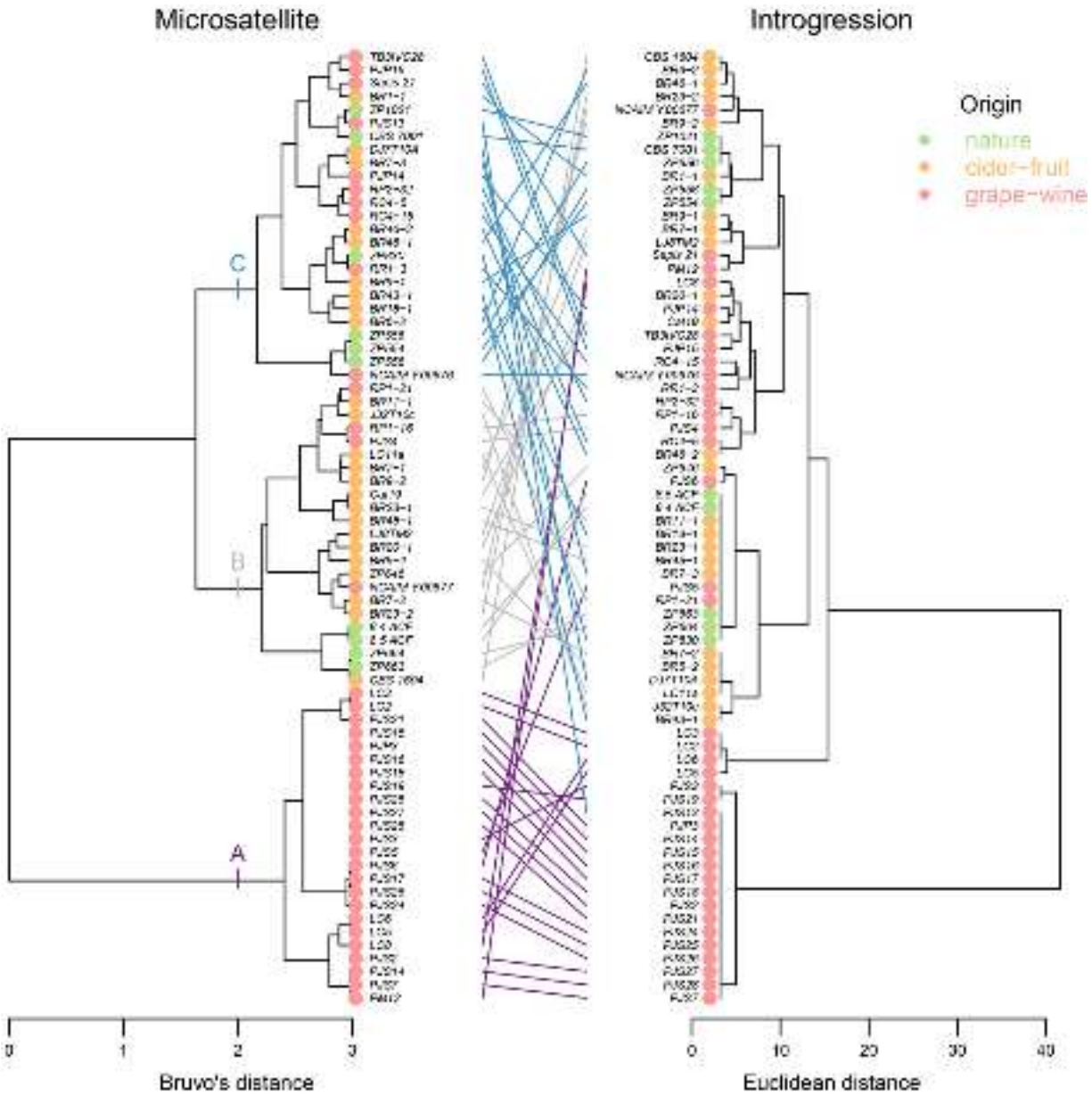

Figure 3. Dendrogram trees from microsatellite and introgressed markers. Seventy-two *S. uvarum* strains were genotyped for both sets of markers and were used. The microsatellite tree was built using Bruvo's distance and NJ clustering. The three main groups (A, B, C) were then reported on the introgression tree, built using Euclidean distance and Ward's clustering [Colour figure can be viewed at wileyonlinelibrary.com]

microsatellites displayed similar patterns regarding heterozygosity levels, we computed the observed and expected heterozygosity (from Hardy–Weinberg equilibrium) for both sets of markers (Fig. 4). For all markers, observed heterozygosity is around 10-fold lower than expected, in agreement with a high selfing rate. Expected heterozygosity is higher for microsatellite markers compared with introgression markers, probably as a consequence of the increased number of alleles for microsatellites. Observed heterozygosity ranged from 0 to 10%, the mostly heterozygous locus being 7_65. Interestingly, Almeida et al. (2014) discussed the possible selective advantage of chromosome 7 introgression, as it contains the *FZF1* gene involved in sulphite resistance

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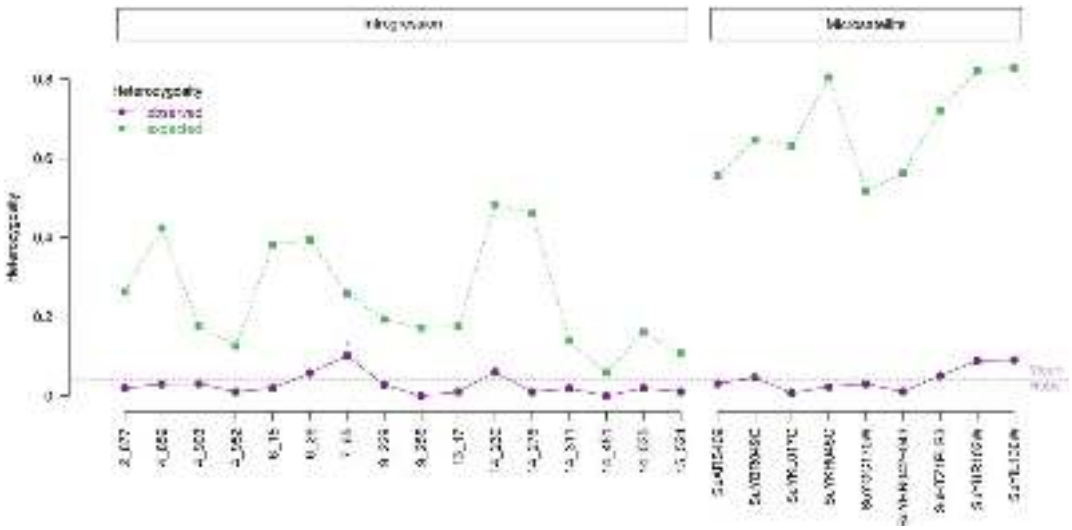

Figure 4. Observed and expected heterozygosity for introgressed and microsatellite markers. Expected heterozygosity was calculated from Hardy–Weinberg equilibrium using the *ade4* package (R), using only *S. uvarum* strains (nature, cider-fruit, grape-wine and unknown groups). Microsatellite data were extracted from Masneuf-Pomarede I *et al.* 2016b. 'Mean Hobs' stands for mean observed heterozygosity calculated from microsatellite markers only. χ^2 tests were performed to assess whether the proportion of heterozygous individuals was higher for introgressed markers compared with those for microsatellites; only 7_65 marker was significant (α = 0.05) [Colour figure can be viewed at wileyonlinelibrary.com]

(Avram et al., 1999; Avram et al., 1999; Park et al., 2000) and the *ZRT1* gene that presents traces of balancing selection (Coi et al., 2017). In this work, we show that chromosome 7 introgression is not significantly over-represented in cider- and wine-making processes compared with natural ones, in apparent contradiction to any selective advantage. This result underlines the difficulty of drawing a correlation between functional genetics and the presence/absence of particular alleles in limited populations. Interestingly, it has to be noted that chromosomes 7's introgression displays a higher level of heterozygosity (>10%), which is significantly higher than the proportion of observed heterozygosity within microsatellites (χ^2 test, α = 0.05). Such observation raises the hypothesis of a possible heterozygous advantage.

Finally, we investigated the segregation of five introgressed regions (chromosomes 2, 4, 6, 14 and 15) by analysing their inheritance in the meiotic progeny of two *S. uvarum* F1 hybrids. The hybrids UU23 and UU34 were obtained by crossing haploid derivatives of the strain U3 with U2 and U4, respectively (da Silva et al., 2015). The germination rate of each hybrid is close to 50% and few complete tetrads were obtained in both cases (Table S4). All of the spore clones (UU23 = 73 and UU34 = 48) were genotyped by MassARRAY for the 10 markers covering the five introgressions (Table 3). As expected, most of the markers showed a Mendelian segregation and the five complete tetrads dissected displayed a 2:2 segregation (data not shown). For the introgression of chromosome 4, a slight but significant enrichment for the *eubayanus* allele was found (χ^2, α = 0.05). This result may indicate a trend toward positive selection of the *S. eubayanus* allele, which is also suggested by the strong frequency (52%) of this introgressed region in the wine group. For two loci, few recombination events were observed (chromosome 6: 1/73 within 9 kb; chromosome 14 6/48 within 305 kb) between *S. uvarum* and *S. eubayanus* alleles. The maximal ratio between genetic and physical distance for the two loci ranged between 0.05 and 0.17 cM/kb. Although lower than the average ratio observed in *S. cerevisiae* (0.33 cM/kb), the rare crossing overs observed demonstrate that these interspecific regions have been successfully incorporated in the meiotic machinery of *S. uvarum* despite their high genetic divergence with the *S. uvarum* genome. The selection of appropriate spore clones of UU23 and UU34 containing *S. eubayanus* markers and their successive mating

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Table 3. Segregation analysis and recombination frequency of *S. eubayanus* introgressed loci

Segregating background	Locus	UV inheritance	EUB inheritance	Khi2	cM/kb observed
UU23 (73 progenies)	2_877	32	40	n.s.	n.r.
	4_859	28	45	<0.05	n.r.
	6_15	37	34	n.s.	0.15
	6_26	38	33	n.s.	
UU34 (48 progenies)	14_230	27	21	n.s.	0.04–0.17
	14_276	27	21	n.s.	
	14_311	27	21	n.s.	
	14_451	26	22	n.s.	
	14_535	22	26	n.s.	
	15_524	24	24	n.s.	n.r.

n.r. and n.s. stand for not significative and not relevant, respectively.

would result in the construction of strains presenting all the introgressed regions for chromosomes 2, 4, 6, 14 and 15. By crossing such strains with selected U1 spore clones, most of the *S. eubayanus* introgressions should be grouped in the same hybrid in two crosses, offering new perspectives for studying whether those introgressions may confer a selective advantage and/or a phenotype of interest. Indeed, breaking the linkage disequilibrium existing within *S. eubayanus* alleles would be efficient for addressing the effect of introgression on phenotypes. The development of MassARRAY markers allowing the genotyping of numerous spore clones in a short time paves the way for quantitative genetics programmes that are very efficient in yeast (Liti & Louis, 2012).

Conclusion

In this work, we show that 95% of Holarctic isolates of *S. uvarum* harbour introgressions where the number and the size of the introgressed regions depend on the strains. We confirm that anthropic isolates possess significantly more introgressions than wild strains. In addition, we show that only one introgressed region is over-represented for cider-making environment, and up to three regions for wine-related process. Interestingly, Almeida et al. (2014) reported that strains from the Northern Hemisphere showed remarkably low diversity across their genomes compared with Southern Hemisphere isolates, while previous microsatellite analysis failed to detect a significant clustering based on substrate origin (Masneuf-Pomarede et al., 2016b). This quite low genetic diversity contrasts with the relative high phenotypic variability found for technological traits (Masneuf-Pomarède et al., 2010). This contradiction suggests that interspecific introgressions found among Holarctic *S. uvarum* strains could be the most important source of genetic, and by extension, phenotypic variability. The high-throughput genotyping method developed here paves the way for studying the impact of these regions on the phenotypic variability of *S. uvarum* strains.

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Conflict of interest

P.M. is an unpaid member of BIOLAFFORT group developing yeast strains starters for winemaking.

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Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article.

Table S1. de novo assembly of 4 *S. uvarum* genomes
Table S2. Primers and SNP detected with MassARRAY technology
Table S3. Genomic location and name of microsattelite loci used
Table S4. Tetrad analysis of F1-hybrids UU34 and UU23
Figure S1. Selected syntenies of de novo assemby

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