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REVIEW ARTICLE

Diversity, variability and fast adaptive evolution of the wine yeast (*Saccharomyces cerevisiae*) genome—a review

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Abstract Due to the high propensity for genomic alteration of their genomes, wine yeast (*Saccharomyces cerevisiae*) strains are very diverse. Genetic/genomic differences often correlate with different enological and technological properties. Experimental data indicate that the plasticity of the genome makes wine yeast populations capable of adapting to the continuously changing and rather harsh fermentation environment. A model is proposed for this fast adaptive genome evolution (FAGE) that explains the roles of the changing clonal composition of the population during fermentation, genome purification by meiosis at the end of fermentation and subsequent autodiploidisation of the spore clones in the next vintage, and the generation of new genomes through conjugation of non-sister spore clones (heterodiploidisation). Possibilities for genome stabilisation are also considered.

Keywords Genetic instability · Genome stabilisation · Segregation · Adaptation · Selection

Introduction

Winemaking is a complex process, in which many factors, such as the type (variety) of grape, the quality of grape must, technological procedures, alcoholic fermentation by yeasts and malolactic fermentation by bacteria, play important roles. The yeasts are of fundamental importance because they convert the sugar of the grape juice into alcohol and significantly contribute to the taste, flavour, bouquet, and even the colour of the wine (for a review, see Fleet 2003). The principal wine yeast is *Saccharomyces cerevisiae*.

Saccharomyces cerevisiae has both wild and domesticated versions, and the domestication event that resulted in grape wine yeasts took place approximately 2,700 years ago (Fay and Benavides 2005). The long evolutionary process has gradually made the wine yeast physiology and genome capable of coping with the harsh and cyclically changing conditions of fermentation and the long periods that separate successive vintages. Large amounts of experimental data indicate that the success of wine yeasts is largely attributable to their high propensity for genetic/genomic alterations, allowing their properties to change over a short period of time. This review summarises current knowledge on the diversity and flexibility of the wine yeast genome, and proposes a unified model for the fast adaptive genome evolution (FAGE) occurring during grape wine fermentation.

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Genetic diversity of wine strains

Over the last 30 years a large number of observations have demonstrated that the wine strains of *S. cerevisiae* are highly diverse; the population fermenting a grape must is usually polyclonic and the clones can differ significantly in enological performance and genotype. The extent of genetic differences ranges from single-nucleotide substitutions to whole-genome duplication.

Gross genomic diversity—ploidy and chromosome-length polymorphism

Gross genomic diversity (GDD) is illustrated by the fact that wine strains of *S. cerevisiae* are predominantly diploid

(e.g. Thornton 1982; Mortimer et al. 1994; Nadal et al. 1999; Bradbury et al. 2006; Legras et al. 2007; Lopandic et al. 2007), but aneuploids (e.g. Sancho et al. 1986; Bakalinsky and Snow 1990; Martinez et al. 1995; Ibeas and Jimenez 1996; Guijo et al. 1997; Nadal et al. 1999; Infante et al. 2003; Bradbury et al. 2006; Legras et al. 2007; Lopandic et al. 2007), triploids (Cummings and Fogel 1978; Takahashi 1978; Thornton 1986), polyploids (e.g. Takahashi 1978; Bakalinsky and Snow 1990; Guijo et al. 1997; Naumov et al. 2000, 2002) and rarely also haploids (Lopandic et al. 2007) occur in the natural yeast microflora of fermenting wine. Aneuploids are particularly frequent among flor yeasts, where up to 40% of clones have less than 2*n* DNA (Martinez et al. 1995; Infante et al. 2003).

Apart from variability in ploidy, wine strains also show considerable diversity in the number and size of chromosomes (chromosome-length polymorphism). This phenomenon can best be observed by pulse-field gel electrophoresis (PFGE), which separates chromosome-size DNA molecules (Schwartz and Cantor 1984). A marked polymorphism of electrophoretic chromosomal profiles has been observed in naturally fermenting *S. cerevisiae* populations in almost all wine-growing regions of the world for which PFGE analysis has been performed (e.g. Johnston and Mortimer 1986; Bidenne et al. 1992; Frezier and Dubourdieu 1992; Egli et al. 1998; Vezinhet et al. 1990, 1992; Briones et al. 1996; Izquierdo Canas et al. 1997; Povhe et al. 2001; Sipiczki et al. 2004; Schuller et al. 2004; Antunovics et al. 2005). For example, Vezinhet et al. (1990) found 20 different karyotypes in 22 wine yeast strains, and Yamamoto et al. (1991) detected 51 different karyotypes among 77 wine yeasts. Chromosomal length differences are frequently observed between strains isolated from the same fermenting must (e.g. Frezier and Dubourdieu 1992; Vezinhet et al. 1992; Schütz and Gafner 1994; Versavaud et al. 1995; Nadal et al. 1996; Egli et al. 1998; Mesa et al. 1999; Cocolin et al. 2004; Antunovics et al. 2005; Sipiczki et al. 2001, 2004) indicating that clones with different sets of chromosomes propagate simultaneously and in succession during fermentation. These clones may originate from different progenitors or from segregation events occurring during the propagation of the yeast cells in the course of fermentation (see below).

Fine genomic diversity

Besides GDD, the genomes of wine yeasts also show an enormous "small-scale" diversity, or fine genomic diversity (FGD). Genome-wide random amplified polymorphic DNA (RAPD; e.g. Grando et al. 1994; Quesada and Cenis 1995; Martinez et al. 2007), interdelta typing (Ness et al. 1993; Versavaud et al. 1995; Legras and Karst 2003; Le Jeune et al. 2006; Ayoub et al. 2006), microsatellite typing (Gallego et al. 1998; Gonzalez et al. 2001; Howell et al. 2004; Legras et al. 2005; Schuller and Casal 2007), amplified fragment length polymorphism (AFLP; deBarros Lopes et al. 1999), multi locus sequence typing (MLST; Aa et al. 2006; Ayoub et al. 2006), intron splice site analysis (deBarros Lopes et al. 1996) and microarray karyotyping (array-CGH; Winzeler et al. 2003; Infante et al. 2003; Dunn et al. 2005; Carreto et al. 2008) have unveiled extensive chromosomal nucleic acid polymorphism in wine yeasts populations. Since Dubourdieu et al. (1987) introduced the RFLP analysis of the mitochondrial DNA to wine yeast characterisation, its use has revealed high levels of polymorphism also in mitochondrial genomes and mitochondrial genes (e.g. Vezinhet et al. 1990; Querol et al. 1994; Versavaud et al. 1995; Lopez et al. 2003).

Single-nucleotide polymorphism (SNP) genotyping (Ben-Ari et al. 2005) and array-CGH are very powerful tools for analysing FGD. Array-CGH detected single-nucleotide polymorphism variation among laboratory and natural strains (Winzeler et al. 2003), and deletions and amplifications of single genes in the wine yeast genome compared to the genome of a standard laboratory strain (Dunn et al. 2005, Carreto et al. 2008).

More specific analyses identified differences in the sequence (mutations) or the location (translocations) of individual genes or short chromosomal regions. For example, Gogo-Yamamoto et al. (1998) reported on the translocation and copy-number changes of SSU1, Aa et al. (2006) revealed high polymorphism in this and certain other genes, and Divol and van Rensburg (2007) found that in certain endo-polygalacturonase deficient wine strains the PGU1 gene was replaced by a partial Ty mobile element. Erasmus and van Vuuren (2009) found a correlation between osmosensitivity and the deletion of a short region normally located close to the telomere of chromosome XV.

Heterozygosity

Natural wine yeasts are highly heterozygous but their heterozygosity is usually not apparent from the phenotype because the recessive alleles do not have phenotypic effects. Di-, aneu- and polyploid strains can be heterozygous at individual genes (e.g. Romano et al. 1985; Bakalinsky and Snow 1990; Mortimer et al. 1994; Nadal et al 1999; Ramirez et al. 1999; Mortimer 2000; Johnston et al. 2000; Sipiczki et al. 2001, 2004; Ayoub et al. 2006), microsatellite loci (Ayoub et al. 2006) for larger segments, and even for complete chromosomes (Miklos et al. 1996; Nadal et al. 1999; Puig et al. 2000; Castrejon et al. 2004). Heterozygous strains often carry deleterious recessive alleles that would decrease the fitness of the cells by causing slower growth, lower fermentation rate, reduced spore viability, etc., if they were not suppressed by the

dominant wild-type alleles (Ramirez et al. 1999). Genes involved in the production of metabolites or in the determination of resistance to adverse environmental effects can also have more than one allele and cause heterozygosity in natural wine strains (Thornton 1982; Romano et al. 1985; Giudici and Zambonelli 1992; Sipiczki et al. 2001, 2004; Marullo et al. 2004, 2007). Interestingly, commercial wine strains can also be heterozygous (Johnston et al. 2000; Schuller et al. 2004; Bradbury et al. 2006).

Interspecies hybrid and mosaic chimerical genomes

Wine yeasts with genomes consisting of elements from two or more species have been identified in numerous wine-growing regions (for a review, see Sipiczki 2008). These yeasts were either alloploids (true interspecies hybrids) or only had chimerical genomes consisting of a (nearly) complete S. *cerevisiae* genome and small genomic fragments from related species (e.g. *Saccharomyces uvarum* and *Saccharomyces kudriavzevii*) or from *Zygosaccharomyces bailii* (Novo et al. 2009). The yeasts that have chimerical genomes are also frequently referred to as interspecies hybrids, which is misleading. True hybrids with complete genomes of both partner species were described between *S. cerevisiae* and *S. uvarum* (*S. bayanus* var. *uvarum*). They were either allodiploids, producing mostly nonviable spores, or allotetraploids that produced viable spores. Allodiploid and allopolyploid hybrids can also be constructed under laboratory conditions. Laboratory-bred *S. cerevisiae* x *S. uvarum* hybrids are unstable and usually undergo a complex genome reduction process resulting in stabile segregants with an (almost) complete *S. cerevisiae* genome containing genes or chromosomal segments from *S. uvarum*. Presumably, a similar process accounts for the formation of natural interspecies chimeras. It is reasonable to suppose that such segregants can later backcross with *S. cerevisiae* strains, which stabilizes the acquired "foreign" sequences as introgressions.

Genetic instability and segregation in wine strains

Many studies have shown that the natural wine yeasts have a large capacity for genome reorganisation and thus the wine yeast genome is in a sort of continuous evolution due to frequent structural rearrangements and mutations occurring during vegetative propagation and at meiosis–sporulation.

Vegetative instability and mitotic segregation

Wine yeast populations can undergo various multiple genetic changes when cultured for longer periods of time under conditions (e.g. in a medium rich in nutrients) that keep the cells in vegetative phase (propagation by mitosis). These events can affect individual genes, groups of genes or larger segments of the genome, and can take place independently in different cells of the growing population, resulting in diverse subpopulations (e.g. Longo and Vezinhet 1993; Schütz and Gafner 1993; Nadal et al. 1996). The presence of multiple subpopulations may account for the increased number of bands and the heterogeneous brightness of bands in karyotypes of the cultures (Miklos et al. 1997). The brighter bands represent chromosomes present in all (or in the larger) subpopulations whereas the fainter bands correspond to chromosomes that are present only in minor subpopulations. These subpopulations can be identified and separated by plating samples of the culture onto an agar medium and selecting individual colonies that show different karyotypes, usually containing fewer but uniformly bright bands (Longo and Vezinhet 1993; Miklos et al. 1996, 1997). The rate of chromosome rearrangement events is highly variable in various strains. Carro et al. (2003b) reported rearrangement rates of up to 1% chromosome changes per generation. Nadal et al. (1999) detected one to five changes per 100 doublings in sparkling-wine strains. The phenomenon referred to as loss of heterozygosity (LOH) is another type of vegetative instability (Ramirez et al. 2004). Although the exact mechanism is unknown, LOH is probably the consequence of gene conversion or mitotic (somatic) crossing over, which produces two different sister cells, one being homozygous for the dominant allele and the other being homozygous for the recessive allele.

Meiotic instability: meiotic segregation

Although meiosis and sporulation occur only rarely during fermentation, they can take place at the end of the vintage when low-nutrient conditions switch on the meiotic developmental programme. The haploid spores produced are very resistant to adverse environmental conditions (long periods of nutrient depletion, extreme temperatures, radiation, dryness, etc) and can survive until the next vintage.

Meiosis represents a much more severe threat to genetic stability than mitosis because it halves the genome by separating the homologous chromosomes. When the diploid genome is heterozygous, which is mostly the case when natural strains are considered, the spores will have genotypes different from that of the parental strain and of those of the other spores. Moreover, meiotic recombination can cause rearrangements in the structure of chromosomes. Thus, meiotic segregation of karyotypes is quite common in natural wine yeasts (e.g. Miklos et al. 1996, 1997; Budroni et al. 2000; Puig et al. 2000; Carro and Pina 2001; Sipiczki et al. 2004; Marullo et al. 2004, 2007). Low sporulation

efficiency and low spore viability (also referred to as low fertility) hampers meiotic segregation and renders the genome more stable. This is usually the case in aneuploids (Martinez et al. 1995; Ibeas and Jimenez 1996) and allodiploids (reviewed in Sipiczki 2008), which either sporulate poorly or their spores are incapable of germination. In contrast to allodiploids, allotetraploids can produce viable spores that have allodiploid or recombinant chimerical genomes.

Several laboratories have found that single-spore derivatives can lose characteristic traits of the mother strains, among them also technologically important properties (Romano et al. 1985, 2003; Johnston et al. 2000; Gimeno-Alcaniz and Matallana 2001; Giudici and Zambonelli 1992; Sipiczki et al. 2001, 2004; Marullo et al. 2004, 2007). This happens if the strain is heterozygous for different alleles of the gene that determines the trait. In this case, each ascus (tetrad of spores) will have two types of spores in a 2:2 ratio (e.g. Romano et al. 1985; Giudici and Zambonelli 1992; Sipiczki et al. 2001, 2004). However, the segregation pattern is frequently more complex, and variable proportions of 0:4, 1:3, 2:2 and 1:1:1:1 tetrads occur in the asci of the strain. Numerous enologically important properties of wine yeasts segregate in this manner, producing a broad variety of spore clones. A deviation from the 2:2 pattern indicates that the trait is under polygenic control and that the sporulating strain is heterozygous at more than one gene. Alcohol tolerance, hydrogen sulphide production, and the production of metabolites such as acetaldehyde, acetic acid, ethyl acetate, n-propanol, isobutanol, isoamyl-alcohol are polygenic traits, so they rarely show 2:2 segregation (Romano et al. 1985, 2003; Johnston et al. 2000; Sipiczki et al. 2001, 2004; Marullo et al. 2004, 2007).

The mechanism of genetic changes

The exact nature of the modifications in the genome has not been precisely defined, and the underlying molecular mechanisms are still largely unknown.

Possible mechanisms accounting for chromosome-length polymorphism can be ectopic reciprocal (crossing-over) and nonreciprocal (gene conversion) recombination between non-allelic loci (Nadal et al. 1999; Puig et al. 2000), Ty transposon-mediated chromosomal translocations (Rachidi et al. 1999; Dunham et al. 2002) and recombination between repetitive sequences of subtelomeric regions (Carro et al. 2003b; Carreto et al. 2008). Some of these processes can occur more often than the other events. The frequency of mitotic gene conversion was estimated to range between 1×10^{-5} and 3×10^{-5} per generation and was frequently associated with rearrangements of chromosomal structures (Puig et al. 2000).

Repetitive sequences interspersed in the yeast genome are thought to be a major source of genome instability. The Ty1 elements are dispersed retrotransposons and are able to promote chromosomal translocations by ectopic recombination (for a review see Mieczkowski et al. 2006). Each Ty1 element is about 6 kb in length, including long terminal repeats or delta sequences of about 340 bp. There are many more solo delta elements in the genome than complete Ty1 elements. The solo deltas presumably were derived from complete Ty1 elements by recombination events between their lateral deltas. Recombinations can also occur between Ty1 elements and delta sequences that are located far apart on the same chromosome, leading to large deletions or duplications. Recombinations between Ty or delta sequences located on nonhomologous chromosomes generates interchromosomal translocations. The “breakpoints” of many rearrangements of experimental and wine strains coincided with the positions of transposons and transposon fragments (Rachidi et al. 1999; Dunham et al. 2002; Infante et al. 2003; Carreto et al. 2008). In contrast, Nadal et al. (1999) found no relationship between the distribution of Ty elements and the rate of changes in the chromosomes.

Fine genetic changes that do not cause spectacular rearrangements in the genome can also significantly contribute to genetic instability of wine yeasts. For example, the high frequency LOH observed in heterozygous cycloheximide-resistant wine strains is probably attributable to FGD events. Its exact mechanism is unknown but its frequency is much higher than the calculated gene conversion rate in the chromosomal region involved (Ramirez et al. 2004).

Chromosomal sequences of foreign origin may also be a source of genetic instability. For example, the *Zygosaccharomyces* sequences detected in the genomes of certain wine yeast strains vary in size, presumably because they are still unstable in the *S. cerevisiae* genome (Novo et al. 2009).

Genetic stabilization of technological strains

Genetic instability may alter useful properties of wine yeasts, resulting in problems in fermentation or lower quality of wine. Thus, obtaining genetically stable strains with good fermentation properties is of great importance for technologies based on inoculated fermentation.

The benefit of diploidy and aneuploidy is that the presence of two or more homologous chromosomes masks the harmful recessive mutations. However, this masking effect provides only a short-term advantage because the growing load of harmful mutations is associated with the growing risk of segregation. The yeast genome can get rid of these mutations at meiotic division, which abolishes the heterozygotic state. The haploid products (spores) of meiosis have only single copies of each chromosome and,

if they undergo autodiploidisation, produce homozygous diploid clones with two identical sets of chromosomes (Mortimer et al. 1994). A consequence of this process is that karyotypically unstable strains produce karyotypically (more) stable meiotic products (Miklos et al. 1997; Carro and Pina 2001). If the sporulating wine strain was heterozygous for deleterious mutations, its meiotic progeny will contain segregants with poor enological properties and segregants that received favourable sets of alleles (see above). The autodiploids of the latter group will exhibit greater fitness and will be able to outgrow the other autodiploids. From this it follows that meiosis has a genome purification effect and that the sexual cycle is in fact a natural genome stabilisation mechanism.

Although genome stability is significantly improved in the autodiploidised spore clones (F1 generation), one meiosis may not eliminate all genetic instability. Certain F1 spore clones may further segregate, producing F2 spore clones still differing in the length of certain chromosomes and the production of certain metabolites (Sipiczki et al. 2001, 2004). This residual post-meiotic instability can be eliminated by producing additional filial generations.

Genetic stabilization through meiosis and autodiploidisation is possible only in homothallic strains because only the haploid cells produced by a germinating homothallic spore can switch their mating type and conjugate with each other. In heterothallic strains, the mating type is stable, so a heterothallic spore clone cannot autodiploidise. Its cells can diploidise only by conjugating with haploid cells of a different spore clone. These "heterodiploids" are rarely homozygous. Therefore the karyotypes of the meiotic descendants can be more polymorphic in a heterothallic strain than in a homothallic strain (Miklos et al. 1997). Semi-homothallic strains (e.g. certain flor yeasts) are also poor at autodiploidisation because they segregate into homothallic and heterothallic spores, neither of which can restore the semi-homothallic sexual type by selfconjugation (Guijo et al. 1997; Budroni et al. 2000; Naumov et al. 2000). However, as only about 10% of wine strains are heterothallic (Mortimer 2000) and even fewer strains are semi-homothallic, autodiploidisation after sporulation may be an important mode of reduction of heterozygosity (Mortimer et al. 1994).

Genome purification by meiosis is associated with the risk that none of the segregants will be better than the original strain. This risk is obviously lower in strains that are sterile or poor at sporulation. Low sporulation activity is frequently observed in association with aneuploidy and chromosomal translocations that impair homologous pairing of chromosomes in prophase-I of meiosis (e.g. Martinez et al. 1995; Ibeas and Jimenez 1996). The cost of this protection is, however, the lack of (or poor) sporulation at the end of fermentation, which not only prevents genome purification but also drastically reduces the strain's chance of survival until the next vintage (Ramirez and Ambrona 2008). Apomixis, a sexual aberration, which makes the cell skip one of the divisions in meiosis and prevents karyotype segregation (Castrejon et al. 2004) seems to be a possibility for ensuring both low meiotic segregation and producing spores for survival in the harsh conditions of the inter-vintage periods.

Mitotic recombination (particularly gene conversion) can also reduce the level of heterozygosity (Puig et al. 2000) and probably also eliminates deleterious mutations from the genome. In strains with low sporulation efficiency this may be the major stabilisation mechanism. Genetic stabilisation can also be achieved by reducing heterozygosity through the elimination of individual chromosomes with the aid of drugs (e.g. benomyl) that interact with components of the division machinery (Blasco et al. 2008). Modification of the recombinational machinery may also have a stabilising effect. Carro et al. (2003a) found that the inactivation of the recombinational gene RAD52 partially stabilised the karyotypes in a hypervariable strain.

Fast adaptive genome evolution during wine fermentation

The experimental results summarised in this review demonstrate that the physiological and genetic properties of the *S. cerevisiae* population are fairly unstable, and allow frequent genetic/genomic changes during the fermentation process. A model can be proposed for the role of this changeability in the remarkably fast adaptation of the yeast population to the continuously changing and rather harsh fermentation environment (Fig. 1). The physiological adaptation of the cells is based mainly on transcriptional up- and down-regulation of genes (e.g. Ferea et al. 1999; Backhus et al. 2001; Erasmus et al. 2003; Marks et al. 2003; Rossignol et al. 2003; Townsend et al. 2003; Varela et al. 2005; Pizarro et al. 2008), which, however, may have limits. Therefore changes in the genome are also necessary to make adaptation faster and more efficient (adaptive genome evolution). Due to its unique plasticity, the wine yeast genome can change easily both during vegetative propagation (mitotic divisions) and in the sexual cycle (meiosis-sporulation-conjugation). During fermentation, the genomes of certain vegetatively propagating yeast cells undergo multiple, successive mutations and gross genomic rearrangements, resulting in a variety of clones with different genomes. These changes occur spontaneously but certain components of the environment, such as ethanol and acetaldehyde, can induce modification events related to recombination (Ristow et al. 1995). In each phase of fermentation, the clone(s) with the highest fitness outgrow

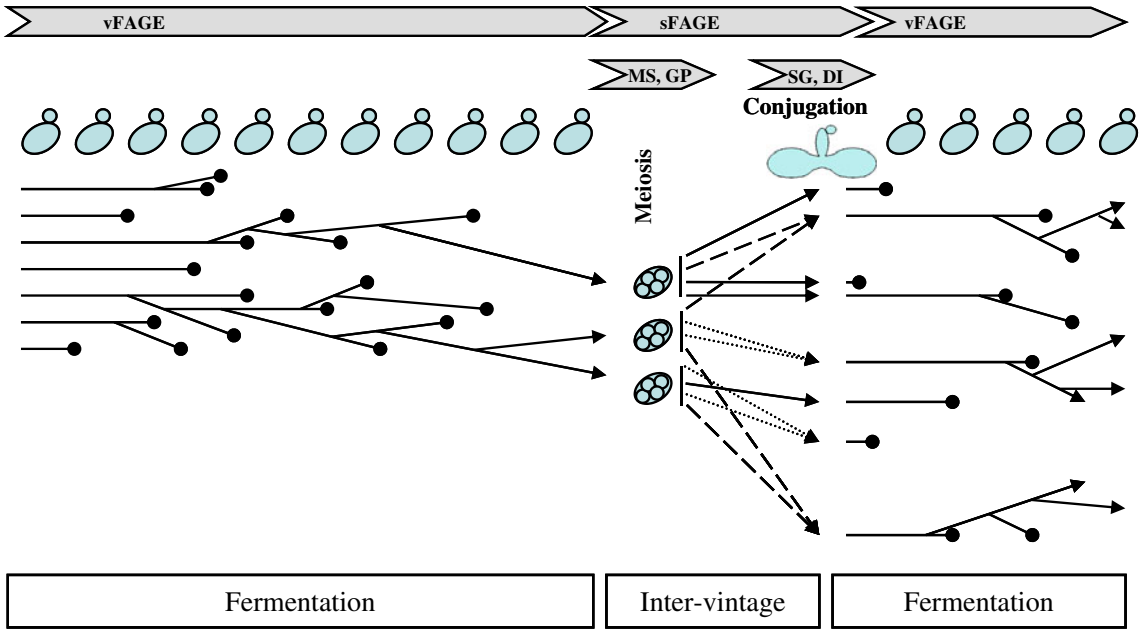

Fig. 1 Hypothetical model of fast adaptive genome evolution (FAGE) of wine yeasts. Each *line* represents a clone of cells. Less competitive clones are gradually outcompeted and vanish from the population (*lines ending with full circles*). Clones living in the last phase of fermentation are marked with *arrowheads*. These will sporulate and their spores will germinate in the next vintage. *Solid-line arrow* Autodiploidisation, *dotted-line arrow* heterodiplodisation by sister-spore conjugation, *broken-line arrow* heterodiploidisation by non-sister conjugation, *MS* meiotic segregation, *GP* genome purification, *SG* spore germination, *DI* diploidisation, *vFAGE* fast adaptive evolution during vegetative growth, *sFAGE* fast adaptive evolution in the sexual stage

(s) the other clones, but to cope with the even harsher conditions of the next phase, its/their cells will have to modify their genomes further. The cost of this process is a concomitant accumulation of recessive lethal and deleterious alleles in the heterozygous state. Upon completion of fermentation, starvation triggers meiosis (and sporulation) in the yeast cells, which results in the segregation of their heterozygous genomes. Many spores that received the deleterious alleles will die; their death eliminates most of the deleterious mutations (genome purification by meiosis-sporulation). The spores that survive until the next vintage will germinate in the fresh must to produce vegetative cells capable of conjugation. Conjugation of sister cells results in homozygous diploids (autodiploidisation, "genome renewal"; Mortimer et al. 1994), whereas conjugation of non-sister cells generates new genomes by combining the genomes of two different spores (heterodiploidisation). These auto- and heterodiploids will then compete with each other and evolve during the new fermentation.

Although it is likely that most genetic changes are neutral or harmful, some them may be beneficial in one or the other phase of fermentation by modifying the activity of one or two genes or altering indirectly the expression of larger gene groups of enological importance (e.g. Cavalieri et al. 2000). The translocation of the SSU1 gene (conferring sulfite resisitance; Goto-Yamamoto et al. 1998; Perez-Ortin et al. 2002) in certain wine yeasts, the amplification of genes coding for alcohol dehygrogenase (reducing the acetaldehyde level; Guijo et al. 1997), and genes facilitating velum formation in flor yeasts (Fidalgo et al. 2006) are examples of beneficial changes that have become fixed in the evolution of certain populations. Large-scale alterations such as polyploidisation and aneuploidisation can increase the number of beneficial genes (gene dosage; e.g. Salmon 1997).

This model is consistent with results of comparative studies on yeast populations of consecutive years in wineries (Frezier and Dubourdieu 1992; Vezinhet et al. 1992; Izquierdo Canas et al. 1997; Sabate et al. 1998; Gutierrez et al. 1999). For example, when the composition of the yeast microflora of spontaneous fermentation in a La Rioja winery was studied by DNA fingerprinting during five consecutive years (Gutierrez et al. 1999), strains showing identical patterns and strains showing different patterns were detected for each vintage. The former might have been clones derived from audodiploidisation events, whereas the latter might have been clones evolved from heterodiploidisation or postzygotic genetic changes occurring in the fermenting population. This may hold even if part of the fermenting *S. cerevisiae* population originates in the vineyard (Mortimer and Polsinelli 1999) because at least part of the vineyard yeast population may consist of winery yeasts (Schuller et al. 2007) probably disseminated by insects (e.g. *Drosophila*), which are abundant in the vicinity of wineries.

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