Exploration du phénomène d'heterosis chez deux espèces de levure d'oenologie : Saccharomyces cerevisiae et S. uvarum

Telma da Silva

Université Paris-Sud

UFR Scientifique d'Orsay

THESE DE DOCTORAT

Spécialité : Sciences de la Vie
Ecole doctorale : Gènes, Génomes, Cellules

Présentée par

Telma da Silva

pour obtenir le grade de Docteur en Sciences de l'Université Paris-Sud

EXPLORATION DU PHENOMENE D'HETEROSIS CHEZ DEUX ESPECES DE LEVURE D'ŒNOLOGIE, *SACCHAROMYCES CEREVISIAE* ET *SACCHAROMYCES UVARUM*

Soutenue publiquement le 11 juin 2014 devant le jury composé de :

SYLVAIN BILLIARD, Maître de conférences à l'Université Lille 1	Examinateur
OLIVIER DAVID, Chargé de recherches INRA	Examinateur
CECILE FAIRHEAD, Professeur à l'Université Paris-Sud	Examinatrice
PHILIPPE MARULLO, Chargé de recherches BIOLAFFORT	Examinateur
ISABELLE MASNEUF-POMAREDE, Professeur à l'ENITA	Examinatrice
JOËLLE RONFORT, Directrice de recherches INRA	Rapporteur
REINER VEITIA, Professeur à l'Université Paris-Diderot	Rapporteur
DOMINIQUE DE VIENNE, Professeur à l'Université Paris-Sud	Directeur de thèse

REMERCIEMENTS

Même si je sais que ces quelques lignes ne suffiront pas pour résumer les remerciements de presque quatre années de thèse…

Je tiens tout d'abord à remercier Dominique de Vienne, mon directeur de thèse, qui a accepté de me faire confiance et de me confier ce beau projet de thèse. Je revois encore notre première rencontre et mon enthousiasme à l'idée d'être choisie pour mener à bien ce travail. Merci pour votre disponibilité et votre précieuse aide malgré un planning toujours chargé. Votre soutien amical a été décisif dans la réalisation de ma thèse.

Un grand merci à Joëlle Ronfort et à Reiner Veitia d'avoir accepté instantanément d'être rapporteurs de mon mémoire. Je suis consciente des efforts nécessaires pour lire et rapporter sur un tel travail en un temps si court. Je remercie également très sincèrement tous les autres membres du jury.

Warren Albertin, Philippe Marullo, Marina Bely et Chantal Mansour, vous êtes ma première famille scientifique. Sincèrement merci. Je n'oublierai pas tout ce que vous m'avez apporté, tant sur le plan scientifique que personnel.

Je remercie bien sûr Christine Dillmann, Delphine Sicard et Sylvie Huet, qui ont ensuite pris la relève. Grâce à vous, j'ai pu m'épanouir dans les biostatistiques. Christine, tu m'as appris la quasi-intégralité de ce que je sais ! Merci infiniment pour ta patience et ton sens pédagogique.

J'exprime ma plus profonde et sincère amitié à tous mes copains de thèse : Adrien Zimmer, Maria de Araujo, Sophie Tempère, Philippe-Emmanuel Renault, Pierre Montalent, Paulina Martinez, Stéphanie Thépot, et bien d'autres. Vous m'aurez beaucoup fait rire, même dans les moments de découragement.

Enfin, je remercie ma Maman, mes deux petit-frères, Fabio et Alexandre, ainsi que ma sœur jumelle Tania, pour avoir toujours été présents pour moi. Et toi, Guillaume, des mots ne suffiraient pas à te dire ce que ton soutien m'a apporté. Tu vois, cette fois c'est presque fini. POUR DE VRAI !

SOMMAIRE

INTRODUTION Page 7

CHAPITRE 1
The Mitochondrial Genome Impacts Respiration
but Not Fermentation in Interspecific *Saccharomyces* Hybrids Page 39

CHAPITRE 2
Multi-trait phenotyping of yeast strains in a diallel design reveals
complex genetic and plastic variations and homeostasis
for temperature in winemaking conditions Page 55

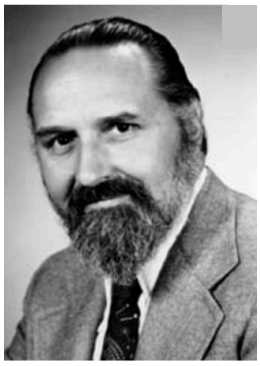
CONCLUSION – PERSPECTIVES Page 101

REFERENCES BIBLIOGRAPHIQUES Page 105

ANNEXE Page 111

Joseph Kölreuter

1733 - 1806



George Harrison Shull

1874 - 1954

Edward Murray East

1879 - 1938

L'hétérosis

La levure œnologique

Louis Pasteur

1822 - 1895

Louis Joseph Gay-Lussac

1778 - 1850

EXPLORATION DU PHENOMENE D'HETEROSIS

CHEZ DEUX ESPECES DE LEVURE D'ŒNOLOGIE,

SACCHAROMYCES CEREVISIAE* ET *SACCHAROMYCES UVARUM

INTRODUCTION

L'hétérosis, connu comme étant la supériorité d'un hybride par rapport au meilleur de ses parents, ou à la moyenne de ses parents, occupe depuis longtemps une place particulière en génétique, en raison notamment de son importance majeure en amélioration des espèces. Il fait l'objet de très nombreuses recherches, en particulier dans le domaine végétal, mais a rarement été étudié, et encore moins exploité, chez les levures, espèces d'intérêt agronomique et biotechnologique majeur. Ce travail de thèse a pour ambition de commencer à combler cette lacune. Dans cette introduction je présenterai les notions d'hybridation et d'hétérosis, puis l'intérêt du modèle « levure » en me focalisant sur les levures d'intérêt œnologique, et enfin je donnerai les objectifs principaux de ce travail.

I. Hybridation et hétérosis

1 - Hybridation

De par son étymologie (en latin *hybrida* signifie « sang mêlé »), le terme hybridation désigne le croisement entre deux parents, issus d'espèces ou de variétés différentes, à l'origine de la production d'hybrides viables réunissant, à un degré plus ou moins marqué, des caractères spécifiques des parents (Mallet 2005). Lorsque les parents sont des lignées pures, leurs descendants constituent des hybrides de première génération (F1) et sont qualifiés d'hybrides simples. On ne considèrera dans ce manuscrit que ce type d'hybride. Les hybrides intraspécifiques sont fertiles et peuvent se croiser entre eux pour produire des hybrides de deuxième génération, ou hybrides doubles (F2). Quant aux hybrides interspécifiques, ils sont souvent stériles en raison d'incompatibilités génomiques et de méioses irrégulières résultant de la divergence des génomes parentaux (Buerkle et al. 2000). Le mécanisme d'hybridation peut être spontané, lorsque le croisement parental ne dépend pas de l'Homme, ou artificiel, lorsque l'hybride résulte de la reproduction entre deux espèces qui ne croisent généralement pas dans le milieu naturel.

Selon les caractéristiques génétiques des individus croisés, les hybrides peuvent présenter des phénotypes plus ou moins différents de ceux des parents. On parle d'*additivité* lorsque l'hybride présente, pour un caractère quantitatif donné, une valeur phénotypique égale à la valeur moyenne des parents, sinon on parle d'*écart à l'additivité* (Fig. 1). L'écart à l'additivité peut être positif ou négatif :

- Lorsque l'hybride présente une valeur qui augmente la fitness et/ou qui présente un avantage économique, on parle d'hétérosis et on distingue deux cas :
  - L'hétérosis « parent-moyen » (*mid-parent heterosis,* ou MPH) lorsque l'hybride présente une valeur phénotypique comprise entre la valeur parentale moyenne et la valeur du meilleur parent.
  - L'hétérosis « meilleur parent » (*best-parent heterosis,* ou BPH) lorsque l'hybride présente une valeur plus élevée que celle du meilleur parent.
- Lorsque l'hybride présente une valeur qui diminue la fitness et/ou qui présente un désavantage économique, on parle de :
  - *Dépression de consanguinité* lorsque l'hybride est issu d'un croisement entre apparentés.
  - *Dépression hybride* lorsque l'hybride est issu d'un croisement entre groupes génétiquement différents. L'expression *hétérosis négatif* est parfois utilisée, et on peut alors distinguer le *negative mid-parent heterosis* et le *worst-parent heterosis*.

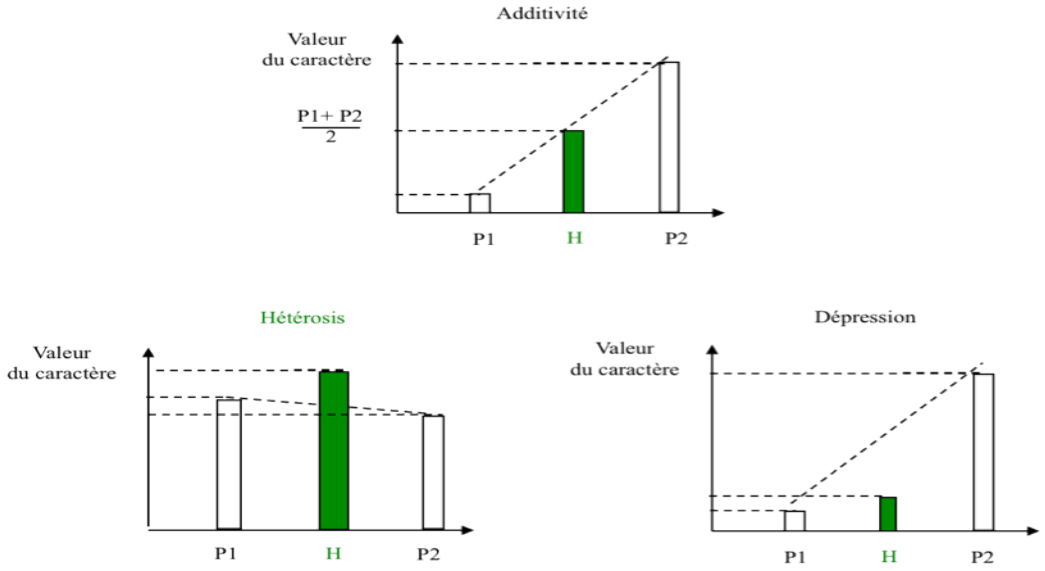


Fig. 1 – Les différents scénarios pouvant décrire la relation entre la valeur phénotypique d'un hybride et celles de ses parents (H, hybrides. P1 et P2, parents).

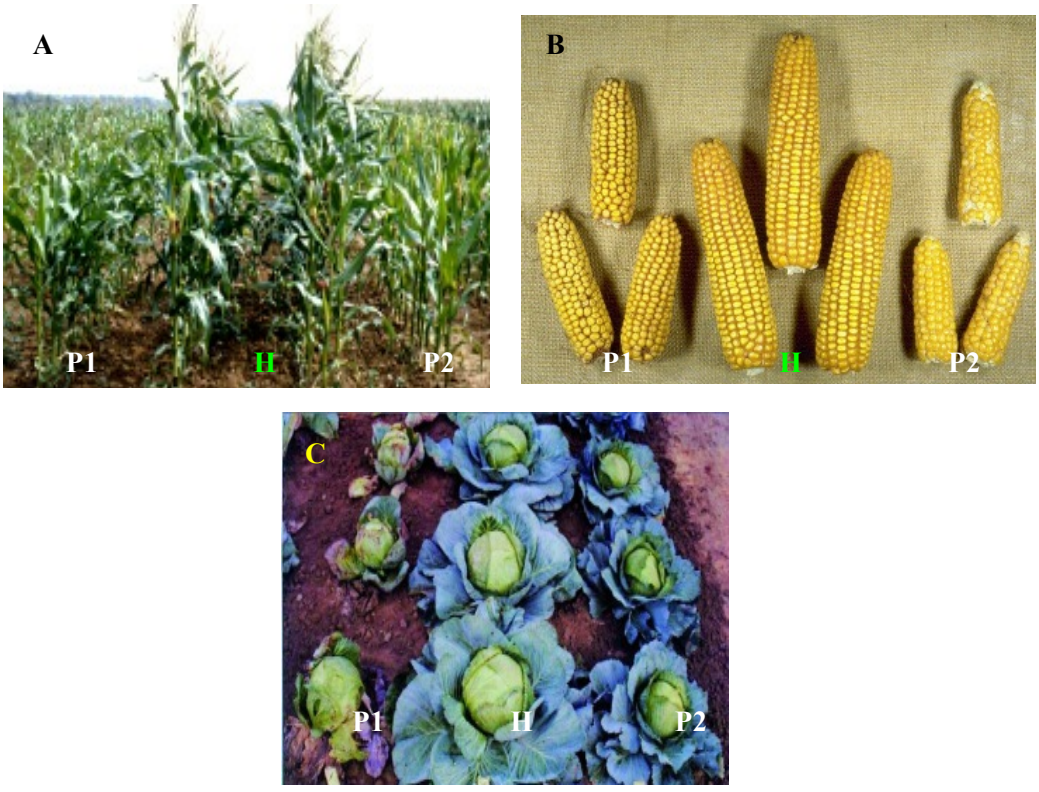


Fig. 2 – Exemples d'hétérosis. Comparaison d'un hybride H et de ses parents P1 et P2. A, pour la taille de la plante et la précocité chez le maïs (P. Bertin, Inra, Moulon). B, pour la taille des épis de maïs (A. Gallais, AgroParisTech). C, pour la taille chez le chou pommé (Inra, Rennes). D'après Gallais 2009.

Il faut noter que par défaut, on considère le plus souvent qu'une forte valeur du caractère est avantageuse pour les individus, ce qui n'est pas toujours le cas (ex. temps de latence dans une croissance microbienne).

Le phénomène d'hétérosis passionne. En 1947, sa « puissance » fut même comparée à celle de la bombe atomique par Henri A. Wallace (cité par Berlan 2002) : « De nos jours, nous entendons beaucoup parler de l'énergie atomique. Je suis pourtant convaincu que les historiens tiendront pour aussi important le développement et l'utilisation de la puissance hybride ». Et quelques années plus tard, Mangelsdorf (1951, cité par Berlan 2002) affirmait « Le temps approche rapidement où la majorité de nos plantes cultivées et animaux domestiques seront des hybrides. L'Homme a seulement commencé à exploiter les généreux cadeaux de l'hybridation ».

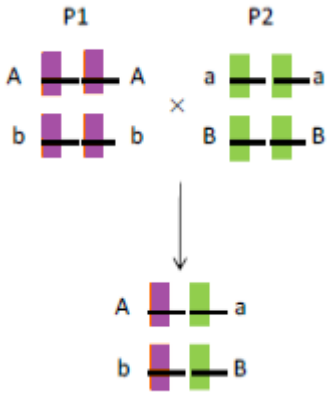
2 - Hétérosis

2.1 - Historique

Tout a commencé il y a 250 ans (en 1764), lorsque le botaniste allemand Joseph Kolreüter croisa différentes espèces de tabacs, d'œillets, de molènes, d'ancolies, de daturas et d'hibiscus, et sema les graines des hybrides interspécifiques ainsi créées (cité par De Wit et Baudière 1993). Il observa que la descendance présentait des caractéristiques communes : " *A more rapid growth, the accelerated, earlier, and prolonged time of flowering, the development of young shoots in autumn from the roots, as well as from the stem, and a longer duration of the plants* ".

Il fallut ensuite attendre le XIX^{ème} siècle pour que des études plus approfondies soient menées. Charles Darwin fut le premier scientifique à détailler et à quantifier les effets du croisement et de la dépression de consanguinité chez les plantes : il compara de façon précise les performances de la descendance en autofécondation et en allofécondation chez 57 espèces. Cette expérimentation lourde lui permit d'appuyer son hypothèse de départ et de conclure que " *The first and most important conclusion which may be drawn by observations given in this volume is that cross-fertilization is generally beneficial and self-fertilization injurious* " (Darwin 1876).

Au début du XX^{ème} siècle, ce fut au tour de l'américain George H. Shull d'explorer le phénomène chez le maïs. Il observa, lui aussi, que l'avantage hybride était particulièrement marqué, tout comme la dépression consanguinité. En 1914, il proposa le terme *heterosis* (en grec *heterôsis* signifie « changement »), pour faire référence au phénomène empirique décrit par ses prédécesseurs et par lui-même, et pour remplacer les expressions telles que *heterozygotic stimulation* ou *the stimulating effects of hybridity*. Plus tard, en 1948, il définit l'hétérosis comme étant " *The greater vigor or capacity for growth frequently displayed by crossbred animals or plant as compared with those resulting from inbreeding ". G. Shull insista sur le fait que le terme hétérosis et sa définition n'impliquaient aucun mécanisme particulier.*



Individu	Génotype	Phénotype
P1	*Ab/Ab*	[*Ab*]
P2	*aB/aB*	[*aB*]
Hybride	*Ab/aB*	[*AB*]

Fig. 3 – Dominance complémentaire.

Table 1 – Illustration du mécanisme de l'épistasie (Gallais 2009).

	Locus 2		
Locus 1	*BB*	*Bb*	*bb*
AA	6	4	**2**
Aa	4	**2.5**	1
aa	**2**	1	0

2.2 - Modèles génétiques de l'hétérosis

Les causes génétiques, non mutuellement exclusives, avancées aujourd'hui pour expliquer le phénomène d'hétérosis peuvent être séparées en deux groupes, selon que plusieurs locus, ou un seul, sont impliqués :

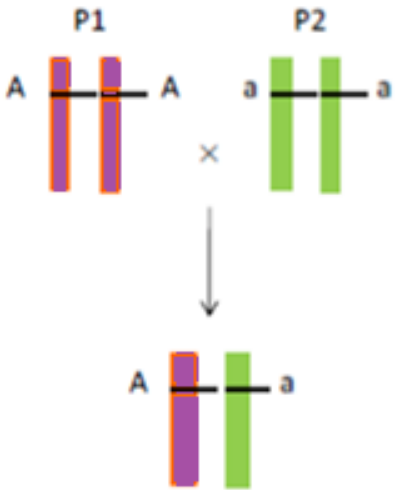
- La dominance complémentaire et l'épistasie impliquent au moins deux locus.
- La superdominance en implique un seul.

La dominance complémentaire

L'hétérosis correspond, dans ce cas, à la complémentation, chez l'hybride, des allèles dominants favorables de différents locus (Davenport 1908, Bruce 1910, Keeble et Pellew 1910, Jones 1917). Le mécanisme de la dominance complémentaire repose ainsi sur l'hypothèse que les caractères favorables sont habituellement gouvernés par des allèles dominants et les caractères défavorables généralement par des allèles récessifs (Davenport 1908, Bruce 1910). Considérons le croisement d'un parent P1 de génotype *Ab*/*Ab* par un autre parent P2 de génotype *aB*/*aB*, les allèles *A* et *B* étant respectivement dominants ou partiellement dominants sur les allèles *a* et *b*, défavorables pour le caractère considéré (Fig. 3). L'hybride obtenu, de génotype *Ab*/*aB*, aura alors une valeur phénotypique plus élevée que celles des homozygotes parentaux ([*AB*] > [*Ab*] et [*aB*]). Dès 1910, Keeble et Pellew publiaient un exemple d'hétérosis dû à la dominance complémentaire chez le pois *Pisum sativum*. Les deux lignées « Autocrat » et « Bountiful », dont les tiges mesurent environ 1,8 m, produisent en croisement un hybride dont les tiges atteignent 2,4 m. Le déterminisme génétique est ici très simple. La longueur des entre-nœuds est monogénique, avec les allèles *L* (longs) et *l* (courts). Le nombre d'entre-nœuds est également monogénique, avec les allèles *N* (nombreux) et *n* (peu nombreux). Les lignées parentales étant de génotype *lN*/*lN* et *Ln*/*Ln*, leur hybride, de génotype *lN*/*Ln*, a des entre-nœuds à la fois longs et nombreux en raison de la dominance de *L* sur *l* et de *N* sur *n*, d'où la grande taille de leur tige. Il s'agit dans ce cas d'hétérosis « meilleur parent ». Toutefois, en cas de dominance partielle, une telle situation pourrait engendrer de l'hétérosis « parent-moyen ».

L'épistasie

Dans sa définition la plus générale, l'épistasie est l'interaction entre gènes non allèles. Plus concrètement, la présence d'épistasie implique que l'effet d'une substitution allélique dépend du fonds génétique, pouvant ainsi engendrer de l'hétérosis (Jones 1945). Prenons un exemple. Soit *A* et *a* les allèles à un locus et *B* et *b* les allèles à l'autre locus, avec additivité à chaque locus. Supposons que les valeurs phénotypiques à un locus dépendent du génotype à l'autre locus comme indiqué Table 1. Dans ce cas le croisement des génotypes *Ab*/*Ab*, de valeur phénotypique égale à 2, et *aB*/*aB*, de valeur phénotypique également égale à 2, conduit à un génotype *Aa*/*Bb* de valeur 2,5 (valeurs entourées). C'est une situation d'épistasie sans dominance. Lorsque le croisement implique des génotypes avec dominance aux deux locus, l'hétérosis résulte à la fois des effets de dominance et d'épistasie. Une situation bien connue, où l'épistasie et la dominance interviennent conjointement, est celle des caractères liés à des flux à travers des chaînes métaboliques. Considérons un processus métabolique contrôlé par deux locus : le locus 1 (allèles *A* et *a*) contrôle le passage d'un substrat S1 à un autre substrat S2, et le locus 2 (allèles *B* et *b*) contrôle le passage de S2 à S3. Si une plante est homozygote *aa* au locus 1, le flux métabolique est arrêté ou ralenti. Si une plante est homozygote *bb* au locus 2, le même problème survient. Il en résulte que deux parents *Ab*/*Ab* et *aB*/*aB* ont un flux métabolique arrêté ou ralenti, tandis que l'hybride Aa/Bb voit son flux rétabli. C'est une situation d'hétérosis qui fait intervenir à la fois les mécanismes de dominance (A domine a et B domine b) et d'épistasie (l'effet d'une substitution en locus dépend du génotype à l'autre locus). Ce schéma typique a été décrit il y a déjà longtemps pour la teneur en anthocyanes chez diverses espèces (Dooner et al. 1991).

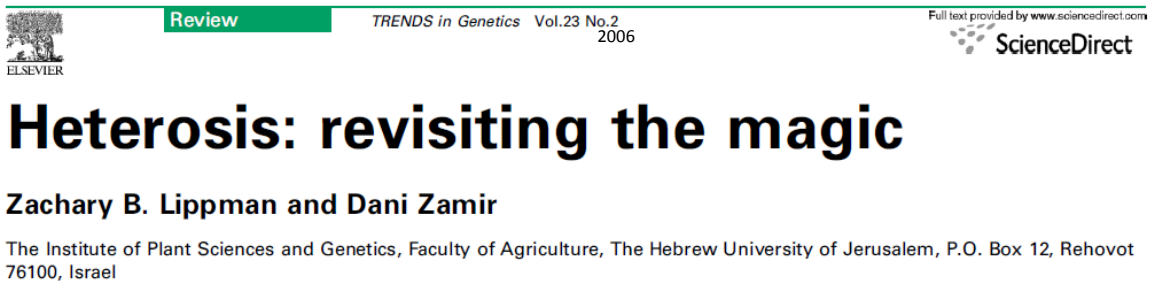


Individu	Génotype	Phénotype
P1	*A*/*A*	[*A*]
P2	*a*/*a*	[*a*]
Hybride	*A*/*a*	**[*Aa*]** > [*AA*]

Fig. 4 – Superdominance.

Table 2 – Cas de l'anémie falciforme chez l'homme.

GENOTYPE / CARACTERE	*HbA*/*HbA*	*HbS*/*HbA*	*HbS*/*HbS*
Anémie	Non	Faible (récessive)	Oui
Résistance paludisme	Non	Oui (dominante)	Oui
Survie	Non	**Oui**	Non


Review TRENDS in Genetics Vol.23 No.2 2006

Full text provided by www.sciencedirect.com ScienceDirect

Heterosis: revisiting the magic

Zachary B. Lippman and Dani Zamir

The Institute of Plant Sciences and Genetics, Faculty of Agriculture, The Hebrew University of Jerusalem, P.O. Box 12, Rehovot 76100, Israel

Fig. 5 – Evocation de l'hétérosis comme un phénomène inexpliqué.

La superdominance

La superdominance correspond à la supériorité en soi de l'hétérozygote à un locus donné (Shull 1908, Hull 1946, Crow 1948). Dans ce modèle le génotype *A*/*a* présente une valeur phénotypique plus élevée que celles des homozygotes parentaux ([*Aa*] > [*AA*] > [*aa*]) (Fig. 4). En regard des efforts déployés depuis plus d'un siècle de génétique pour trouver des cas de superdominance, leur nombre reste très restreint. L'archétype en est celui de l'anémie falciforme chez l'homme, maladie génétique causée par la mutation *HbS* du gène de l'hémoglobine *HbA* (Table 2). Dans les régions impaludées, les hétérozygotes *HbA*/*HbS* résistent au paludisme, contrairement aux homozygotes *HbA*/*HbA*, tandis que les individus *HbS*/*HbS* souffrent d'une anémie qui réduit fortement leur espérance de vie. Il y a un donc un avantage de l'hétérozygotie qui explique la fréquence élevée de l'allèle mutant dans les régions impaludées. En réalité, cet avantage est lié au fait que le gène en question est à effet pléiotrope, et qu'il y a inversion de la dominance pour les deux caractères affectés : le niveau d'anémie et la résistance au paludisme. Comme l'allèle *HbS* est pratiquement récessif pour l'anémie et dominant pour la résistance au paludisme, les individus *HbA*/*HbS* sont avantagés en milieu impaludé. La plupart des autres cas de superdominance répertoriés (moins d'une dizaine) semblent répondre à ce mécanisme, à savoir que l'avantage de l'hétérozygote vient du fait que plusieurs caractères sont affectés par la mutation. Lorsque des gènes sont liés en répulsion, c'est-à-dire lorsqu'un gène dominant favorable à un locus est lié à un gène récessif défavorable à un autre locus très proche du premier, on peut obtenir un hybride qui semble présenter de la superdominance pour un locus donné. Il s'agit en réalité de dominance complémentaire à deux locus et non de superdominance : on parle ici de pseudo-superdominance.

La dominance et l'épistasie sont des phénomènes extrêmement répandus, et un seul de ces deux effets génétiques est suffisant pour engendrer de l'hétérosis. Comme nous venons de le voir, il existe d'ailleurs de nombreux exemples d'hétérosis expliqué au niveau génétique (voir revue de Gallais 2009) et même moléculaire (*i.e.* Sinha et al. 2006, chez la levure). Il est donc intriguant de remarquer que ce phénomène est encore régulièrement qualifié de « magique » (Fig. 5) ou d'inexpliqué et inexplicable (Goldman et Hallauer 1997), si bien que divers « principes unificateurs » (« *unyfing principles* », Birchler et al. 2003) sont régulièrement proposés. Ceux-ci concernent des processus très divers : interactions nucléo-cytoplasmiques (Jones 1952, Lintz 1963, Srivastava 1981), balance hormonale (Rood et al. 1988), métabolisme de l'horloge circadienne (Chen 2010), turnover des protéines (Goff, 2010), effet de dosage dans les complexes macromoléculaires (Veitia et Vaiman, 2011), méthylation de l'ADN (Shen et al. 2012) et rôle des petits ARN et de la régulation épigénétique (Ha et al. 2009, Groszmann et al. 2011, Ng et al. 2012). Ces mécanismes sont certainement à l'œuvre mais, comme l'ont souligné Flint-Garcia et al. (2009), s'il existait une explication globale du phénomène, les degrés d'hétérosis des différents caractères seraient très corrélés, ce qui n'est pas observé.

Outre son caractère parfois spectaculaire, on peut avancer deux raisons au prétendu mystère de l'hétérosis. La première est d'ordre méthodologique : les parts relatives de la dominance, de la superdominance et de l'épistasie sont difficiles à évaluer, et doivent l'être au cas par cas, c'est-à-dire pour chaque caractère dans chaque fonds génétique. La seconde raison n'est pas scientifique : il peut être utile, pour obtenir des moyens de recherche, d'entretenir l'idée qu'il s'agit d'un phénomène complexe et mal compris, mais très prometteur pour nourrir l'humanité...

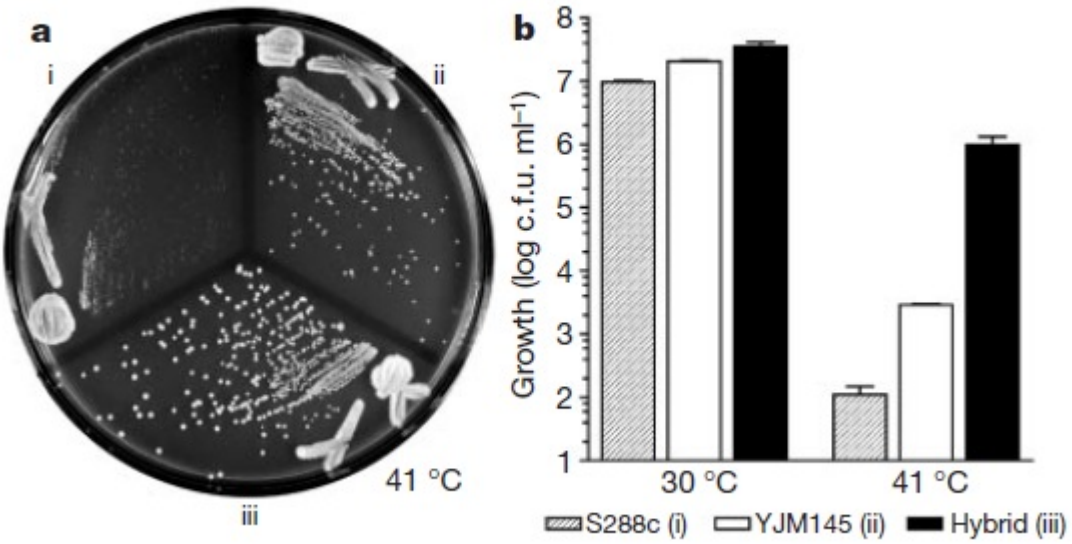


Fig. 6 – Illustration du phénomène d'hétérosis chez la levure *Saccharomyces cerevisiae* (Steinmetz et al. 2002). L'échelle est logarithmique. A 41 °C, la vitesse de croissance de la souche hybride est environ 15 fois plus élevée que celle de la meilleure souche parentale.

3 - Quel modèle pour progresser dans la connaissance et la compréhension de l'hétérosis ?

La levure est un eucaryote unicellulaire, au génome de petite taille. La disponibilité d'outils génétique et génomique chez cet organisme, ainsi que l'importance de la communauté des « levuristes », en font un excellent modèle pour les études fonctionnelles (Dujon 1996). Au début de ma thèse, seuls quelques rares cas d'hétérosis avaient déjà été observés et décrits, chez l'espèce *Saccharomyces cerevisiae*. La figure 6 en montre un exemple spectaculaire, où la vitesse de croissance de la souche hybride est d'environ 15 fois celle de la souche parentale la plus rapide. D'autres articles, plus récents, seront évoqués dans la partie Contexte et question de ma thèse ».

La levure, et notamment *S. cerevisae*, est très utilisée dans l'industrie biotechnologique. Utilisée inconsciemment par l'homme depuis des millénaires pour la production de vin, de bière et de pain, elle est aussi largement employée comme « usine cellulaire » pour différentes applications, comme la production de protéines recombinantes d'intérêt pharmaceutique, de divers produits chimiques et plus récemment pour la production de bioéthanol. Développer des souches de levures plus performantes, pour répondre aux besoins de l'industrie, constitue un enjeu actuel. En réponse à la réticence publique grandissante face à la construction d'organismes génétiquement modifiés (OGM), des stratégies de sélection génétique pour améliorer les levures industrielles se sont fortement développées. La sélection d'individus plus performants, par des techniques de croisements assistés par marqueurs (Marullo et al. 2007) ou par des approches d'évolution dirigée, se développe fortement (Steensels et al. 2014). Travailler avec des levures hybrides, en exploitant le potentiel du phénomène d'hétérosis, constitue une stratégie prometteuse.

C'est dans ce contexte que la levure a été choisie comme modèle pour progresser dans la connaissance et la compréhension du phénomène.

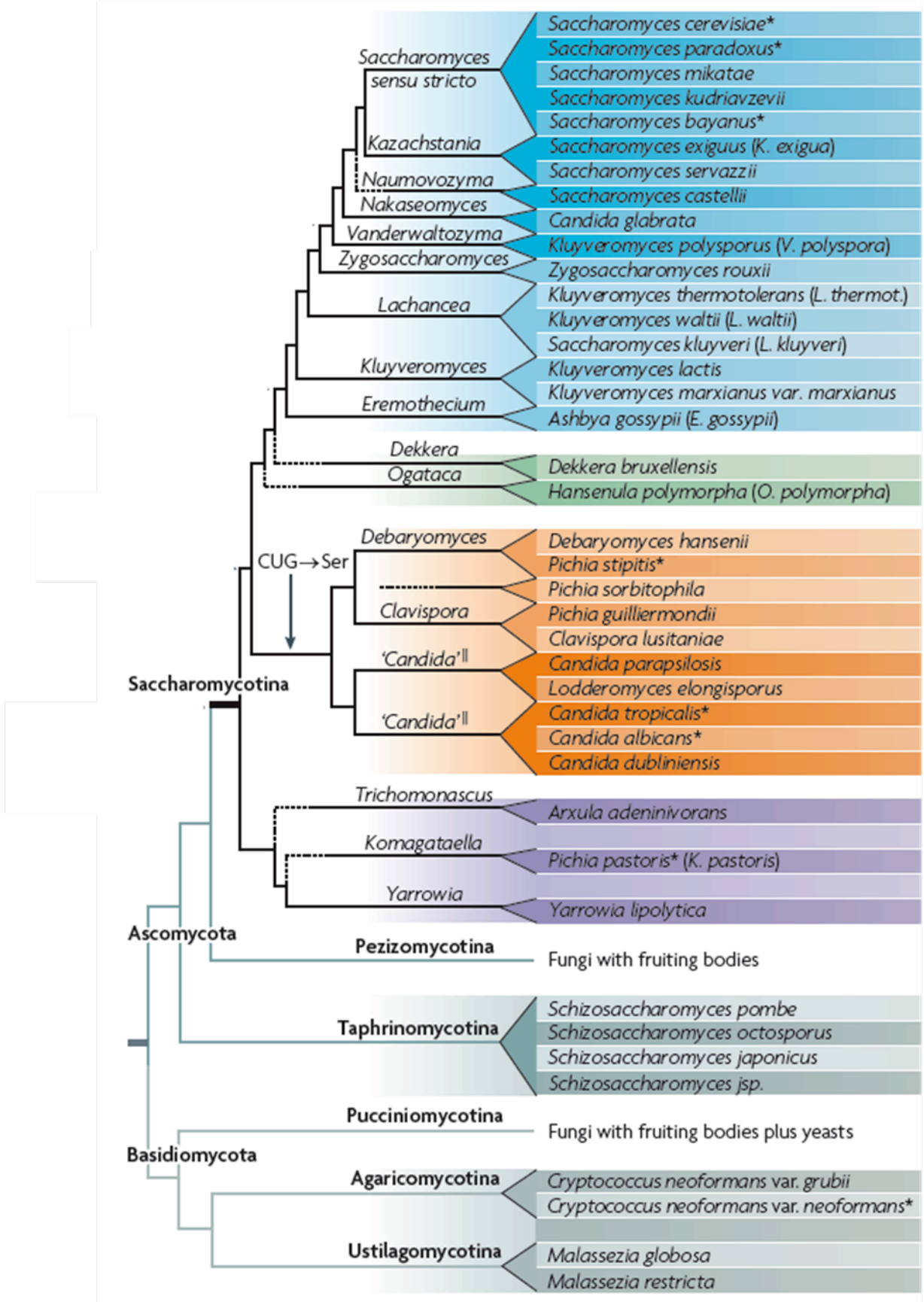


Fig. 7 – Souches de levures dont les génomes ont été séquencés (d'après Dujon 2010).

II. Les Levures

1 - Généralités

Les levures constituent un vaste groupe de champignons unicellulaires très répandus dans la nature. Leur classification n'a cessé d'évoluer depuis la première proposition d'Hansen du début du XX^{ème} siècle car les critères de délimitation des espèces ont changé au cours des années (cité par Ribéreau-Gayon et al. 2004). Le premier critère de démarcation sur lequel se basa Hansen fut l'aptitude des levures à former des spores. Puis de nouveaux caractères morphologiques et physiologiques furent considérés, comme la forme et la dimension des cellules, la capacité fermentaire ou encore l'assimilation des nitrates. Toutefois, les scientifiques comprirent que la différentiation des espèces ne pouvait se baser sur ces seuls critères, compte tenu de leur variabilité intraspécifique. Progressivement une délimitation fondée sur la signification biologique et génétique de la notion d'espèce s'est imposée (Ribéreau-Gayon et al. 2004). Le concept de l'espèce « biologique » (Mayr 1996) stipule qu'une espèce représente une communauté d'êtres vivants interféconds pouvant échanger du matériel génétique et produisant des descendants eux-mêmes féconds, c'est-à-dire capables de donner des spores viables. Cependant, cette définiton est aussi confrontée à des limites majeures, comme l'inaptitude de certaines souches à sporuler, rendant ainsi complexe l'utilisation du critère d'interfertilité des souches. Pour pallier ces difficultés s'est finalement développée une classification moléculaire fondée sur la similitude de la composition en bases de l'ADN des souches de levures (Barnett 2000). Une accélération spectaculaire, avec le séquençage du génome de *S. cerevisiae* en 1996 (Goffeau et al. 1996), posa les bases de nouvelles potentialités. Depuis, les progrès des techniques furent tels que les scientifiques parvinrent à séquencer l'intégralité du génome de plusieurs espèces de levures, pouvant ainsi prétendre à une classification plus précise (Fig. 7). Ils permirent de démontrer que le génome des espèces de levure n'évoluait pas de façon totalement indépendante. De récents travaux de génomique ont montré que le clade des *Saccharomyces* présentait une évolution fortement réticulée (Albertin et al. 2012, Morales et al. 2012, Hittinger 2013). Des hybridations interspécifiques et des transferts horizontaux ont eu lieu entre espèces éloignées. C'est par exemple le cas des souches d'œnologie de *S. cerevisiae* qui ont acquis aux moins trois régions génomiques par transferts horizontaux, provenant de *Zygosaccharomyces balii* et d'espèces du clade des *Torulaspora* (Novo et al. 2009).

2 - Les levures d'intérêt œnologique

Le vin est un des produits de notre alimentation ayant focalisé l'attention de nombreux scientifiques. La production de cette boisson s'obtient en trois étapes : la culture de la vigne qui conduit à la vendange, la phase fermentaire pendant laquelle ont lieu les fermentations alcoolique et malo-lactique, enfin le traitement post-fermentaire avec l'élevage du vin, sa mise en bouteille et son vieillissement. Les levures d'intérêt œnologique interviennent en phase fermentaire et plus précisément lors de la fermentation alcoolique. Le rôle des microorganismes dans la transformation du jus de raisin (ou moût) en vin n'a été définitivement établi qu'au milieu du XIX^{ème} siècle par Louis Pasteur. Les levures furent reconnues dès lors comme l'élément déclencheur de la conversion du sucre du raisin en éthanol. Lors de la fermentation alcoolique, une centaine de composés issus du métabolisme de la levure sont également produits. Certains d'entre eux peuvent avoir un impact organoleptique sur la qualité du vin, positif ou négatif. Les conditions physicochimiques de la fermentation alcoolique (compositions du milieu en nutriments, température, etc.), mais aussi les espèces et les souches de levures associées, influencent directement la quantité des métabolites secondaires produits.

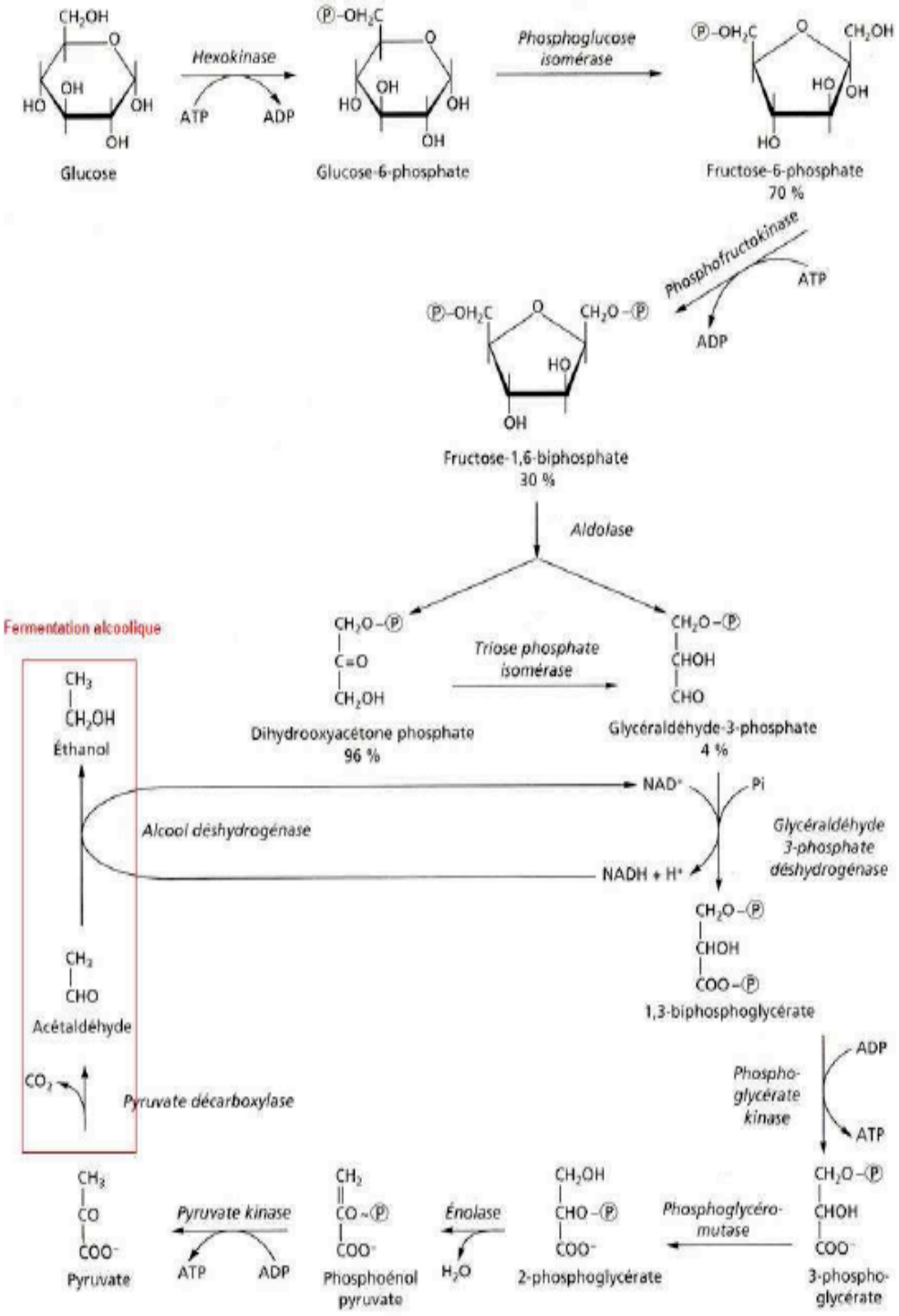


Fig. 8 – Voies de la glycolyse et de la fermentation alcoolique.

2.1 - Métabolismes du carbone et de l'azote

2.1.1 - Les voies réactionnelles de la dégradation des sucres et leur régulation

La levure *S. cerevisiae* peut dégrader les sucres en utilisant deux voies métaboliques, la respiration et la fermentation alcoolique. L'activation de l'une de ces deux voies dépend principalement de la teneur en oxygène dissous dans le milieu réactionnel ainsi que de la quantité de source carbonée disponible. Dans les deux cas les sucres sont préalablement métabolisés par la voie commune de la glycolyse.

2.1.1.1 - La glycolyse

La voie réactionnelle de la glycolyse (Fig. 8) a été complètement décrite en 1940 par Embden, Meyerhoff et Neuberg, et est donc parfois appelée la « voie d'Embden-Meyerhoff ». L'assimilation des sucres d'un milieu par la cellule repose sur le transport de ceux-ci à travers la membrane plasmique par un système de transporteurs protéiques. Aucune dépense d'énergie n'est jusque-là requise puisque le mouvement du soluté s'effectue du milieu réactionnel concentré au milieu cellulaire dilué. La glycolyse commence ainsi dans le cytosol de la levure, et compte deux phases principales :

- La dégradation du glucose en glycéraldéhyde-3-phosphate (GAP) qui met en jeu une chaîne de quatre réactions enzymatiques et qui implique la dégradation de deux molécules d'adénosine triphosphate (ATP) en adénosine diphosphate (ADP).

$$C_6H_{12}O_6 + 2\ ATP \Rightarrow 2\ GAP + 2\ ADP \qquad [1]$$

- La conversion du GAP en pyruvate qui permet la récupération d'une partie de l'énergie du GAP sous forme d'ATP avec formation de NADH.

$$2\ GAP + 4\ ADP + 2\ H_2PO_4^- + 2\ NAD^+ \Rightarrow 2\ CH_3COCOO^- + 4\ ATP + 2\ NADH + 2\ H^+ + 2\ H_2O \qquad [2]$$

D'après [1] et [2], le bilan de la glycolyse s'écrit :

$$C_6H_{12}O_6 + 2\ ATP + 2\ ADP + 2\ H_2PO_4^- + 2\ NAD^+ \Rightarrow 2\ CH_3COCOO^- + 4\ ATP + 2\ NADH + 2\ H^+ + 2\ H_2O$$

Du point de vue énergétique, la glycolyse se solde donc par la balance positive de deux molécules d'ATP qui sont immédiatement utilisées pour dégrader une nouvelle molécule de glucose. Cette observation s'accorde avec le caractère « chimio-organotrophe » des levures qui tirent l'énergie qui leur est nécessaire, en l'occurrence l'ATP, de la dégradation de molécules organiques, ici du GAP. Par ailleurs, le déroulement de cette voie métabolique dépend également de la présence dans le milieu réactionnel de deux molécules de NAD^+ (nicotinamide adénine dinucléotide), cofacteur d'une enzyme impliquant la transformation du GAP. Ainsi le coenzyme réduit NADH doit être continuellement oxydé en NAD^+ pour assurer le fonctionnement de la glycolyse. La différence entre les voies oxydative et fermentaire réside dans le devenir du pyruvate.

2.1.1.2 - La fermentation alcoolique

En anaérobiose, *S. cerevisiae* ne peut pas respirer et présente un métabolisme uniquement fermentaire. Le pyruvate issu de la glycolyse est réduit et il y a production d'acétaldéhyde. Ce dernier est l'accepteur d'électrons participant à la réaction d'oxydation du NADH. L'étape finale est alors la production d'éthanol (Fig. 8), d'où l'expression « fermentation alcoolique ». L'équation globale de la fermentation alcoolique a été décrite dès 1815 par Louis Joseph Gay-Lussac :

$$C_6H_{12}O_6 + 2\ ADP \Rightarrow 2\ C_2H_5OH + 2\ CO_2 + 2\ ATP$$

Le rendement théorique maximal en éthanol (rendement de Gay-Lussac) est de 0,511 grammes d'éthanol par gramme de glucose consommé. Cependant les réactions de maintenance, de synthèse des

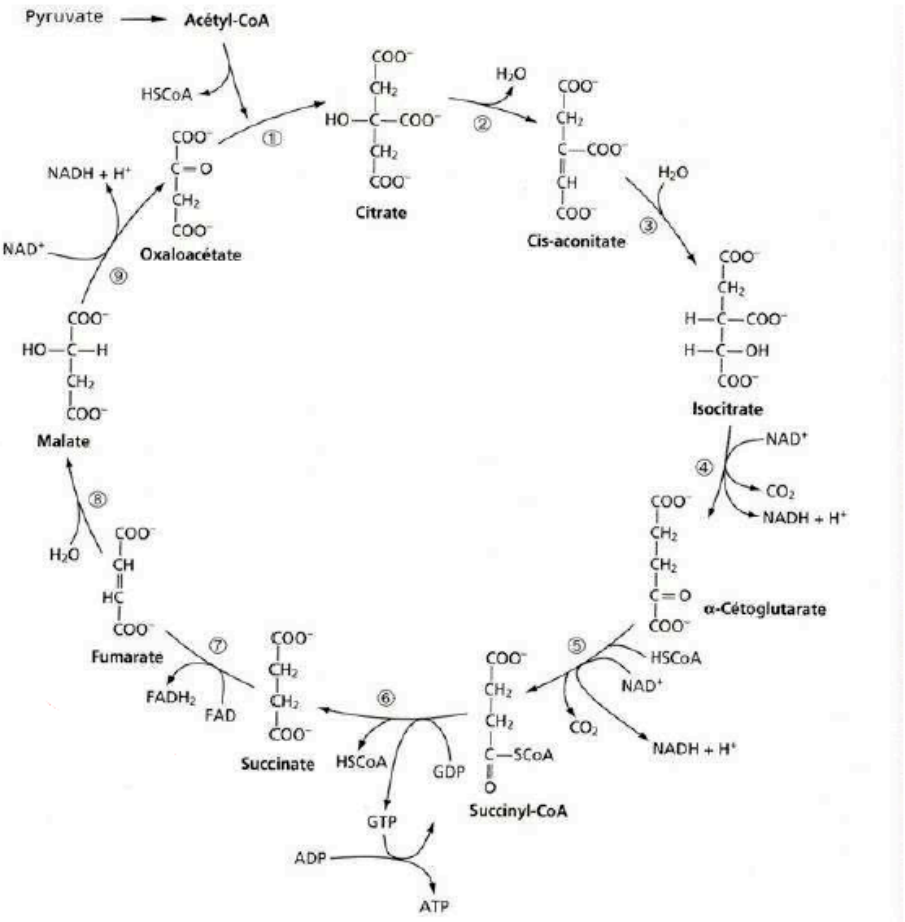


Fig. 9 – Cycle de Krebs.

infrastructures cellulaires et la formation des composés secondaires (glycérol, acide acétique, etc.) limitent ce rendement à 80-90 % de sa valeur théorique.

2.1.1.3 - La respiration

Lorsque le sucre est utilisé par la voie respiratoire (Fig. 9), la réoxydation du coenzyme réduit NADH, produit par la glycolyse, s'opère dans les mitochondries par phosphorylation oxydative. Ce processus implique la libération des électrons et leur transport jusqu'à l'oxygène qui en est l'accepteur final. Il s'accompagne de la production d'ATP. L'acide pyruvique provenant de la glycolyse peut ainsi subir une décarboxylation oxydative en présence de coenzyme A (CoA) et de NAD^+, produisant du CO_2, du NADH et de l'acétyl-CoA, lequel est ensuite complètement oxydé dans les mitochondries en CO_2 et H_2O par les réactions du cycle de Krebs. Le bilan énergétique de la respiration d'une molécule de glucose est de 36 ou 38 molécules d'ATP. Deux proviennent du bilan net de la glycolyse, 4 à 6 résultent de la phosphorylation oxydative à partir des 2 molécules de NADH issues de la glycolyse (selon le système de navette utilisée pour faire passer les électrons du NADH cytosolique à la chaîne respiratoire dans les mitochondries), 28 de la phosphorylation oxydative à partir du NADH et du $FAFH_2$ produits par le cycle de Krebs, et enfin 2 de la phosphorylation au niveau du substrat lors de la formation du succinate. La respiration d'une molécule de sucre met ainsi à la disposition de la levure 18 à 19 fois plus d'énergie biologiquement utilisable que la fermentation. Cette énergie fournie par la chaîne respiratoire est utilisée pour l'élaboration de nouvelles cellules, et est exploitée pour la production industrielle de levures. Le rendement théorique en biomasse en conditions oxydatives est d'environ 0,5 $g.g^{-1}$ de glucose (Kappeli 1986).

2.1.1.4 - Régulation de la fermentation/respiration

En anaérobiose la voie fermentaire est directement activée. En présence d'oxygène la respiration devient possible, ce qui permet aux levures d'oxyder complètement le glucose par la voie oxydative. Cependant, si le glucose est en excès dans le milieu, il y a inhibition de la respiration (« effet Crabtree » [Crabtree 1929]), et ce d'autant plus que la concentration en glucose est élevée, jusqu'à l'inhibition complète de la respiration. Les voies oxydative et fermentaire peuvent donc fonctionner simultanément, avec à la fois production d'éthanol et activité respiratoire (régime oxydo-fermentaire). La concentration seuil en glucose activant cette répression dépend des espèces de levures, mais elle est similaire pour des souches de la même espèce. L'effet Crabtree se traduit chez la levure par une dégénérescence des mitochondries, une diminution du taux de stérols et d'acides gras cellulaires et une répression de la synthèse des enzymes mitochondriales du cycle de Krebs et des constituants de la chaîne respiratoire (Ribéreau-Gayon et al. 2004). Une levure est dite « Crabtree positive » si elle réalise la fermentation alcoolique d'un milieu réactionnel riche en glucose en conditions aérobies. Elle est « Crabtree négative » si elle est susceptible de fonctionner en métabolisme oxydatif et ce même à de très fortes teneurs en sucres (Urk (1989, Fiechter et Seghezzi 1992). Une des différences majeures entre les levures Crabtree positives et négatives est le contrôle du transport des sucres : en présence de glucose, les levures Crabtree positives auraient un flux important de glucose avec un transfert dans la cellule par diffusion facilité alors que les levures Crabtree négatives réguleraient l'entrée de glucose et contrôleraient ainsi le flux de glucose à un niveau qui n'excède pas les capacités d'oxydation de la cellule.

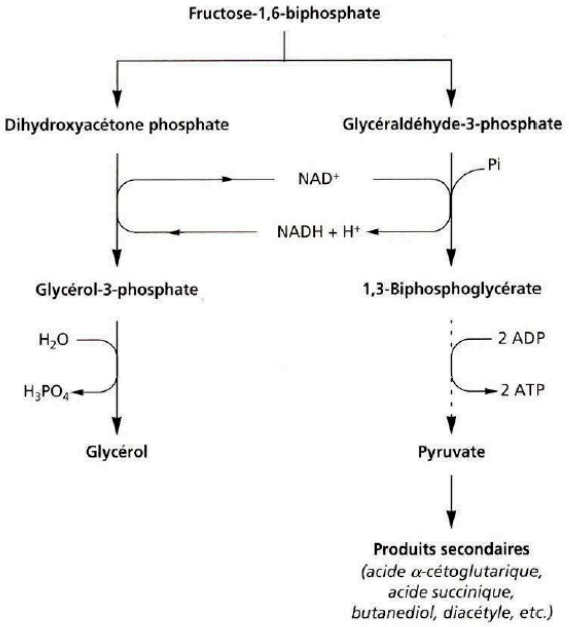


Fig. 10 – Fermentation glycéro-pyruvique.

Toutes les levures du clade des *Saccharomyces sensu stricto* sont Crabtree positives, dont les espèces *S. cerevisiae* et *S. uvarum* utilisées en œnologie (Hagman et al. 2011). Elles présentent un métabolisme oxydatif pour de faibles concentrations de glucose en présence d'oxygène, un métabolisme respiro-fermentaire lorsque la concentration en glucose résiduel augmente et un métabolisme fermentaire en condition anaérobie. L'effet Crabtree se manifeste chez *S. cerevisiae* pour des concentrations en glucose supérieures à 1 g/L. Par conséquent la répression exercée sur les levures œnologiques dans le moût de raisin est très forte puisque celui-ci présente des teneurs en sucres réducteurs proches de 200 g/L. Dans ces conditions la fermentation est la seule voie métabolique utilisée au cours de l'élaboration du vin.

2.1.1.5 - La fermentation glycéro-pyruvique

En conditions d'anaérobiose, l'ensemencement d'un moût avec des levures ayant subi une pré-culture en conditions aérobie implique une faible expression de la pyruvate décarboxylase et de l'alcool déshydrogénase au cours des premières heures de la fermentation alcoolique (Sharma et Tauro 1986). Il en résulte une faible production d'acétaldéhyde associée à une faible réoxydation du NADH. De plus différentes réactions de biosynthèse nécessaires pour le métabolisme cellulaire, en particulier les synthèses d'acides aminés et les oxydations génératrices des produits secondaires, produisent un nouvel excès de NAD(P)H que la cellule doit réoxyder pour maintenir son activité (Ribéreau-Gayon et al. 2004). Comme il n'y a pas d'activité respiratoire, la régénération du NAD nécessaire à la glycolyse ne peut se faire par la phosphorylation oxydative. Cette réaction est alors assurée par la fermentation glycéropyruvique (Fig. 10) au cours de laquelle le dihydroxyacétone phosphate (DHAP), métabolite intermédiaire de la glycolyse, est réduit en glycerol-3-phosphate (G3P) puis est déphosphorylé et devient du glycérol, polyol inodore et incolore. La majorité du glycérol est produite au cours de la première partie de la fermentation alcoolique. Le rôle du glycérol dans le maintien de la balance rédox, par la régulation du rapport $NADH/NAD^+$ et non par la production d'énergie en anaérobiose, est mis en évidence (Weusthuis 1994). Il en résulte qu'en conditions anaérobies, 92 % des sucres fermentescibles sont transformés en éthanol et les 8 % restant en glycérol, pyruvate et autres produits secondaires dérivants de ce dernier. Sur le plan organoleptique certains de ces composés carbonés ont un impact sensoriel. En raison de leur forte concentration dans les vins et de leur faible saveur sucrée, leur importance dans la structure des vins a été soulevée (Noble et Bursick 1984). Cependant cette contribution a certainement été surestimée au regard de travaux plus récents (Marchal et al. 2011). En revanche l'acide acétique, l'acétoïne et le diacétyle sont des composés volatiles affectant très négativement la qualité des vins. D'autres comme l'acide succinique et le butanediol sont relativement neutres et participent à la note vineuse générale (Ribéreau-Gayon et al. 2004).

2.1.2 - Le métabolisme des constituants azotés

L'azote joue un rôle capital car il entre dans la constitution de molécules simples (nucléotides, acides aminés, coenzymes et vitamines) indispensables au fonctionnement cellulaire. L'essentiel de l'alimentation azotée de la levure est fourni par les acides aminés et par l'ammonium contenu dans le moût de raisin. Nous nous attacherons dans cette partie à la description des mécanismes de formation des arômes fermentaires issus du métabolisme de l'azote : alcools supérieurs et esters.

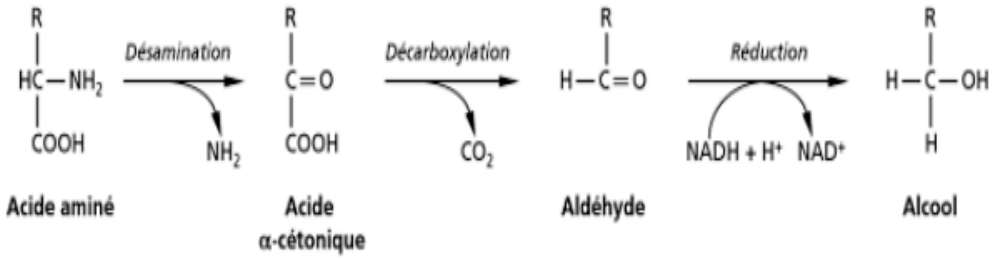


Fig. 11 – Formation des alcools supérieurs à partir des acides aminés.

Table. 3 – Principaux alcools supérieurs du vin et acides aminés pouvant en être les précurseurs.

Alcool supérieur	*Descripteur aromatique*	*Conc. mg/L et (seuil aromatique mg/L) dans le vin*	*Acide aminé précurseur*
3-méthyl-butan-1-ol ou acool isoamylique	Vernis Frangipane	80 - 300 (65)	*Leucine*
2-méthyl-butan-1-ol ou acool isoamylique actif	Vernis, âpre	30 - 100 (30)	*isoleucine*
2-methyl-propan-1-ol	Fusel, spiritueux	50 - 150 (40)	*Valine*
Phenylethanol	Floral, rose	10 - 100 (10)	*Phénylalanine*
Tyrosol	Amère ou âpre chimique	20 - 50	*Tyrosine*
Tryptophol	Amande, solvant	0 - 1	*Tryptophane*
γ-Butyrolactone	Vernis ou époxy	0 - 5	*Acide glutamique*
Méthionol	Chou-fleur, chou	0 - 5 (0,5)	*Méthionine*

2.1.2.1 - Production d'alcools supérieurs

Les alcools supérieurs contiennent au moins deux atomes de carbone dans leurs molécules. Leur biogenèse s'effectue selon deux voies métaboliques distinctes. Il y a, premièrement, la voie catabolique d'Ehrlich, liée à la désamination des acides aminés (Figure 11). Dans ce cadre, la désamination en acide α-cétonique est suivie par une décarboxylation pour former un aldéhyde qui est ensuite réduit en alcool. Les principaux alcools supérieurs du vin et les acides aminés pouvant en être les précurseurs (Ribéreau-Gayon et al. 2004) sont présentés Table 3. Il existe par ailleurs la voie anabolique des acides aminés à partir des sucres, pour les alcools supérieurs n'ayant pas de précurseurs parmi les acides aminés. On peut citer le cas du propan-1-ol qui provient de l'α-cétobutyrate qui peut être formé à partir du pyruvate de l'acétyl-CoA. Les proportions relatives de chacune des voies sont respectivement de 25 et 75 %, sachant que ces valeurs varient en fonction du taux d'azote assimilable et de la teneur en sucres fermentescibles du moût. Quantitativement, les principaux alcools supérieurs sont les 2- et 3-méthylbutanol, le propanol, le 2-méthylpropanol, le butanol, le pentanol, le 2-phényléthanol, le 3-méthylthiopropanol, le tyrosol et le tryptophol. Ces alcools supérieurs peuvent avoir des impacts positifs ou négatifs sur les arômes du vin. A un niveau de concentration faible (moins de 300 mg/L), ils contribuent au caractère floral ou fruité du vin. Par exemple le phényléthanol apporte des notes florales (odeur de rose). Au-delà de cette valeur, ces composés entrainent des défauts d'odeur. Globalement, les alcools supérieurs sont peu favorables à l'arôme du vin (Ribéreau-Gayon et al. 2004).

2.1.2.2 - Production d'esters

On considère les esters comme la famille principale qui marque l'arôme fruité des vins jeunes (Ribéreau-Gayon et al. 2004). Ils sont présents également dans de nombreux produits fermentés (Sumby et al. 2010). Les esters ayant un impact aromatique se divisent en deux principales familles. Les esters éthyliques d'acide gras sont formés en grande partie durant la fermentation alcoolique et sont issus du métabolisme des levures (Saerens et al. 2006, Saerens et al. 2008). La synthèse de ces esters est très dépendante de la souche de levure ainsi que de la disponibilité des acides gras précurseurs, de la température de la fermentation alcoolique, de la composition nutritive du milieu (éléments azotés, particules solides du moût), du niveau d'oxygène et de la teneur en acides gras insaturés et en stérols (Saerens et al. 2008, Sumby et al. 2010). Les acétates d'alcools supérieurs possèdent des odeurs un peu plus lourdes que les esters éthyliques. En quantité élevée, ils peuvent masquer l'arôme variétal du vin comme décrit pour l'acétate d'isoamyle par Ribéreau-Gayon et al. (2004).

Comme les esters éthyliques d'acides gras, ces esters, produits en grande majorité par les levures au cours de la fermentation alcoolique, sont hydrolysés au cours du vieillissement du vin (Sumby et al. 2010).

2.1.3 - Le métabolisme des composés soufrés

2.1.3.1 - Composés soufrés issus du métabolisme de la levure

Au cours de la fermentation alcoolique, le métabolisme de la levure produit un nombre important de composés soufrés ayant un impact organoleptique négatif sur le vin. Parmi ces composés, le sulfure d'hydrogène H_2S présente une odeur nauséabonde d'œuf pourri. Sa production résulte de la réduction des sulfates (SO_4^{2-}) et des sulfites (HSO_3^-) présents dans le moût, et implique le complexe enzymatique de la sulfite réductase (Jiranek et al. 1995). Cette réduction conduit à la formation d'ions

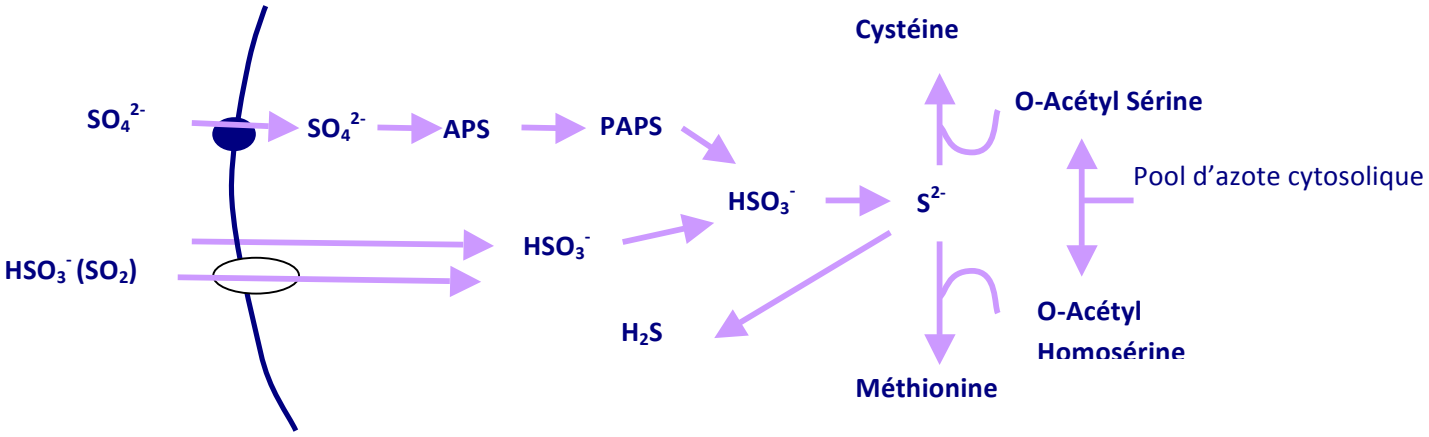


Fig. 12 – Voie d'assimilation du soufre et de formation de l'H_2S chez la levure œnologique. Les principales étapes de la voie d'assimilation du soufre chez la levure sont présentées. APS, adenosyl 5'-phosphosulfate. PAPS, 3'phosphoadenosyl-5'phosphosulfate. S^{2-}, ions sulfides. SO_4^{2-}, ions sulfates. HSO_3^-, ions sulfites. H_2S, sulfure d'hydrogène. SO_2, dioxyde de soufre (Jiranek et al. 1995).

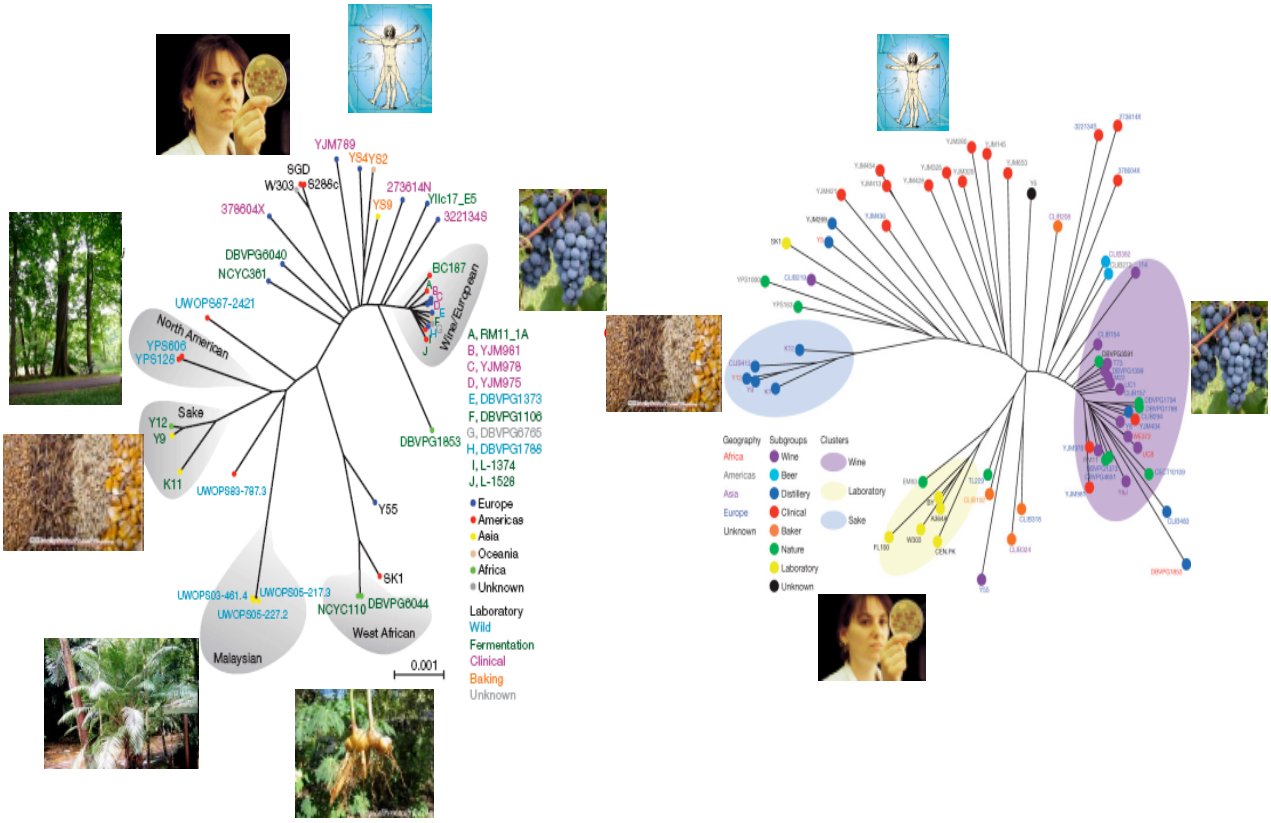


Fig. 13 – Arbres phylogénomiques construits avec la méthode de Neighbour-Joining sur les différences nucléotidiques (SNP) entre génomes de *S. cerevisiae* (d'après Liti et al. 2009, à gauche, et Schacherer et al. 2009, à droite). Les photos montrent le type d'habitat d'origine (tubercule, palmier, grains de céréales, forêt, laboratoire ou œnologie).

sulfides (S^{2-}) partiellement destinés à la synthèse des acides aminés soufrés (Fig. 12). Malgré sa place centrale dans cette voie métabolique, la sulfite réductase n'est pas le seul effecteur de la production d'H_2S. En effet il existe d'importantes connexions entre le métabolisme d'H_2S et le métabolisme des acides aminés soufrés (Jiranek et al. 1996). D'autres composés tels que le méthionol, le méthane thiol et l'éthanethiol, dérivent de ce métabolisme. Ils confèrent aux vins des notes lourdes de choux cuits et de réduction qui sont peu qualitatives (Ribéreau-Gayon et al. 2004).

2.1.3.2 - Composés soufrés issus de la bioconversion de précurseurs présents dans le moût

Les arômes variétaux, spécifiques au cépage, sont principalement responsables de la typicité du vin. Certains d'entre eux, appartenant à la catégorie des thiols, sont révélés par la levure à partir de précurseurs inodores et non volatils contenus dans le moût. Des précurseurs d'arômes conjugués à la cystéine et au glutathion ont été identifiés chez plusieurs cépages et notamment chez le sauvignon blanc (Tominaga et al. 1998, Subileau et al. 2008, Peyrot et al. 2000). Il a été démontré que certaines souches de levures révélaient mieux que d'autres le potentiel aromatique des moûts (Masneuf et al. 2002, Howell et al. 2004) et que cette différence était liée à des aptitudes enzymatiques ainsi qu'à des régulations du métabolisme azoté (Thibon et al. 2008, Dufour et al. 2013).

2.2 - Diversité génétique des levures d'œnologie

Les espèces de levures les plus connues et répandues au cours de la fermentation alcoolique en conditions œnologiques appartiennent au clade des *Saccharomyces sensu stricto*. Parmi elles on compte *S. cerevisiae,* reconnue pour ses bonnes capacités fermentaires, et *S. uvarum* que l'on retrouve naturellement dans les régions septentrionales et qui confère au vin des notes florales particulières (Tosi et al. 2009, Masneuf-Pomarède et al. 2010). *S. uvarum* est également connue pour son aptitude à fermenter le jus de pomme. Elle est plus cryo-tolérante (Kishimoto et Goto 1995, Naumov 1996, Belloch et al. 2008) et produit moins d'acide acétique que *S. cerevisiae* (Castellari et al. 1994, Blank et al. 2005).

La domestication de *S. cerevisiae* est étudiée de façon intensive depuis quelques années (Fay et Benavides 2005, Legras et al. 2007, Liti et al. 2009, Schacherer et al. 2009, Wang et al. 2011, Magwene et al. 2011, Sicard et Legras 2011). Les souches domestiquées diffèrent des souches isolées d'environnements naturels (Fig. 13). Les souches domestiquées se regroupent selon le type de fermentation auquel elles sont associées (boulangerie, œnologie, saké, fromage), indiquant que l'homme a façonné de manière inconsciente leur diversité. La majorité des souches d'œnologie appartiennent au même groupe génétique. Elles seraient issues d'un évènement majeur de domestication en Mésopotamie suivi d'une diffusion par au moins deux routes de migration, l'une en Europe de l'ouest, l'autre en Europe de l'Est (Legras et al. 2007, Sicard et Legras 2011). Les souches d'oenologie présentent un niveau d'hétérozygotie supérieur aux souches issues d'habitats naturels, suggérant que leurs cycles de reproduction sexuée sont plus fréquents et/ou que les hétérozygotes sont avantagés dans le milieu domestiqué (Magwene et al. 2011, Hittinger 2013).

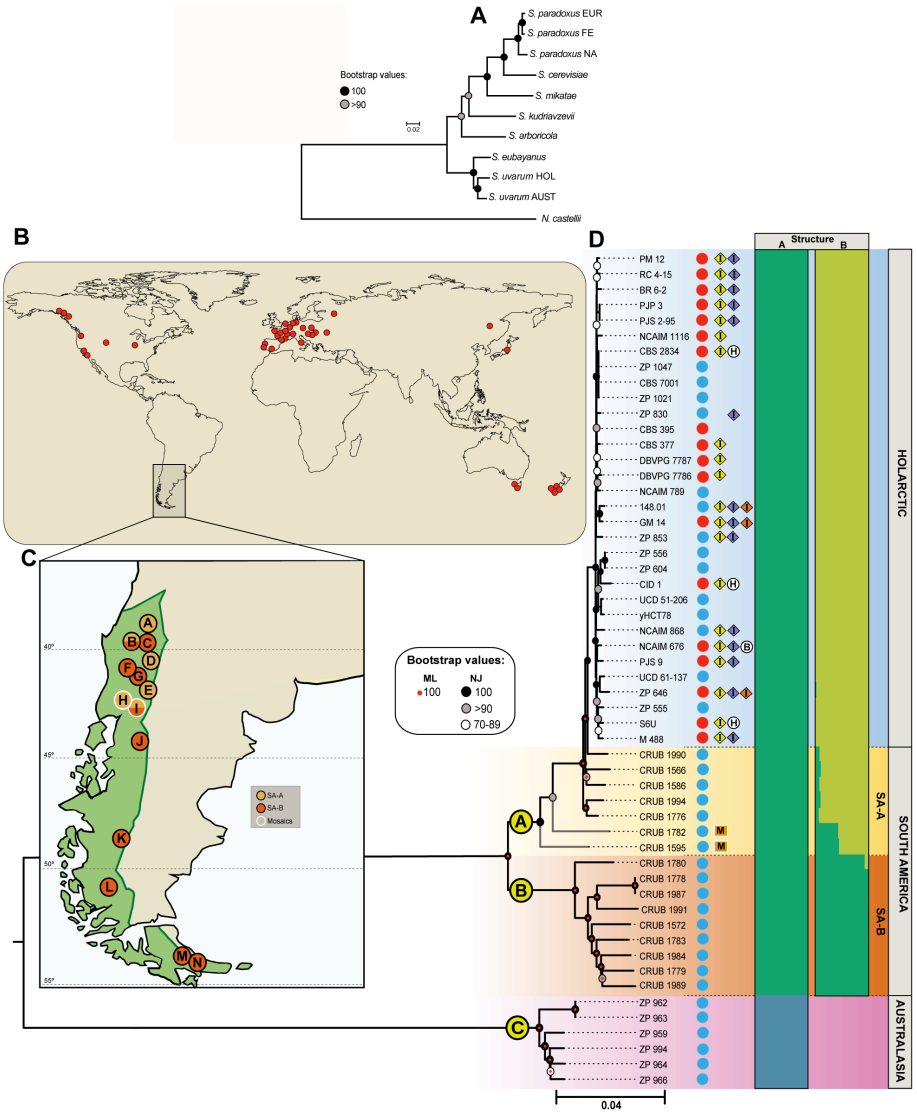


Figure 14. Distribution géographique, phylogénie et structure des populations de *S. uvarum*. A, phylogénie du genre *Saccharomyces*. B et C, origine géographique de diverses souches de *S. uvarum*. D, phylogénie NJ de 54 souches établie avec 129096 SNP (Almeida et al. 2014).

L'histoire évolutive de *S. uvarum* a été beaucoup moins étudiée. Une récente étude phylogénomique montre que *S. uvarum* est issue du pancontinent Gondwana situé dans l'hémisphère sud (Libkind et al. 2011, Almeida et al. sous presse). Dans l'hémisphère sud actuel, cette espèce est principalement inféodée aux arbres de la famille des *Nothofagaceae* proche des hêtres (*Fagus*). On la retrouve dans l'hémisphère nord sur le genre *Quercus* (chêne) mais aussi associée à des milieux anthropiques tels que le vin et le cidre. Il existe au sein de l'espèce *S. uvarum* trois sous-groupes avec des différences génétiques importantes : un groupe australasien montrant une différentation allopatrique évidente, un groupe sud américain et un groupe retrouvé dans l'hémisphère nord dit groupe holoarctique (Fig. 14). Dans le groupe nordique les différences génétiques entre les individus sont très faibles. Par contre on retrouve des traces récentes d'hybridation entre l'espèce *S. uvarum* et d'autres espèces issues d'environnements industriels du genre *Saccharomyces*. Ces événements d'hybridation visibles sous la forme d'introgressions chromosomiques sont notamment important avec une nouvelle espèce du genre *Saccharomyces* (*S. eubayanus*) récemment decouverte en Patagonie (Libkind et al. 2011).

De manière plus générale l'hybridation interspécifique est un phénomène commun parmi les levures du clade des *Saccharomyces sensu stricto*, en particulier parmi les souches domestiquées (Sicard et Legras 2011, Libkind et al. 2011). Un exemple frappant est celui de la levure de bière *S. pastorianus* issue de l'hybridation de *S. eubayanus* et de *S. cerevisiae* (Libkind et al. 2011). Concernant les levures isolées d'un environnement viticole, des observations, relativement récentes, rapportent la présence d'hybrides naturels entre *S. cerevisiae et S. uvarum* (Giudici et al. 1998, Le Jeune et al. 2007) ou entre *S. cerevisiae* et *S. kudriavzevii* (Erny et al. 2012). En ce qui concerne les espèces *S. cerevisiae* et *S. uvarum*, elles sont abondamment présentes dans les chais (Ribéreau-Gayon et al. 2004). Toutefois l'étude de l'avantage sélectif de ces hybrides interspécifiques n'a été initiée que récement (Dunn et al. 2013), et le phénomène de vigueur hybride reste inexploré.

Table 3 – Synthèse bibliographique des études sur l'hétérosis chez *S. cerevisiae*. L'origine du matériel génétique, les conditions environnementales et les caractères étudiés sont présentés ainsi que le message de chacun des articles.

Origine	Souches	Milieux	Caractère	BPH	MPH	Message	Ref.
Laboratoire	3 parents 1 hybride	YEPD 19 °C, 37 °C	Resistance à l'allyl-alcool	+	–	Hétérosis pour la résistance à l'allyl-alcool à 37 °C (pas à 19 °C). Cas d'hétérosis meilleur-parent expliqué par de la superdominance au locus ADH1.	(1)
Clinique, laboratoire	2 parents 1 hybride	YPD 30 °C, 41 °C	Taux de croissance	+	–	Hétérosis pour la croissance à 41 °C. Cas d'hétérosis meilleur-parent expliqué au niveau moléculaire par de la dominance complémentaire et de l'épistasie (Sinha et al. 2006)	(2)
Vin, raisin, sol, palmier, fruit, chêne	16 parents 120 hybrides	YPD	- Production d'éthanol -Tolérance à l'éthanol - Croissance à des températures extrêmes - Capacité à produire des spores viables	–	±	Les hybrides ont le même comportement moyen que leurs parents pour les quatre caractères étudiés. Quelques hybrides présentent de l'hétérosis.	(3)
Laboratoire, vin, saké, chêne, palmier, fruit, clinique	9 parents 34 hybrides	SD 56 conditions	- Taux de croissance - Biomasse - Temps de latence	+	++	30 % des hybrides présentent de l'hétérosis parent-moyen. L'hétérosis meilleur-parent est très rare. L'hétérosis est associée à la distance phénotypique entre les parents mais pas à la distance génétique. La dominance complémentaire peut expliquer ce pattern d'hétérosis.	(4)
Laboratoire, vin, saké, chêne, palmier, fruit, clinique	22 parents 231 hybrides	YPD 11 conditions	Taux de croissance	+	+++	Plus d'hétérosis (parent-moyen et meilleur-parent) chez les souches hybrides domestiquées (82 %) que chez les souches hybrides non domestiquées. Plus d'hétérosis parent-moyen que d'hétérosis meilleur-parent.	(5)
Laboratoire, vin, saké, chêne, palmier, fruit, clinique	16 parents 120 hybrides	YPD 5 conditions	Taux de croissance	++	+++	72 % des hybrides présentent de l'hétérosis (parent-moyen et meilleur-parent). 35 % présentent de l'hétérosis meilleur-parent. Pas de lien entre hétérosis et distance génétique entre les parents.	(6)

(1) Hall et Wills 1987, (2) Steinmetz et al. 2002, (3) Timberlake et al. 2010, (4) Zörgö et al. 2012, (5) Plech et al. 2014, (6) Shapira et al. 2014.

III. Contexte et questions de ma thèse

Bien que la levure soit à la fois une espèce modèle et une espèce d'intérêt agronomique et technologique majeure, nous n'avons trouvé dans la bibliographie que six articles portant explicitement sur l'hétérosis chez cette espèce, les quatre plus récents (postérieurs à 2010) visant à étudier le phénomène à partir d'un dispositif diallèle (Table 3). D'autres articles l'évoquent dans le cadre de programmes de croisements en biotechnologie (Giudici et al. 2005, Marullo et al. 2006), et une étude mentionne la dépression hybride (Dettman et al. 2007).

Le plus ancien des articles s'intéressant à l'hétérosis chez la levure décrit l'un des rares exemples attestés de superdominance, au niveau du gène ADH1 (Hall et Wills 1987). Plus récemment une étude de cartographie de QTL a démontré un cas de pseudo-superdominance lié à l'association de trois gènes en répulsion (Steinmetz et al. 2002), dont les bases moléculaires ont ensuite été analysées (Sinha et al. 2006).

Timberlake et al. (2010) ont été les premiers à construire un dispositif diallèle complet chez la levure, dont les 16 souches parentales provenaient de la collection de Liti et al. (2009). Les 120 hybrides intraspécifiques ont été obtenus en utilisant des marqueurs d'auxotrophie. Bien que ce travail utilise une collection d'hybrides importante, il n'analyse le phénomène d'hétérosis que de manière assez sommaire. Les phénotypes mesurés concernent la production d'éthanol et la tolérance à l'éthanol, et la croissance à différentes températures. Trois autres équipes ont récemment étudié la même population de manière indépendante en réalisant des mesures de phénotype similaires (Table 3)

Zörgö et al. (2012) ont construit un diallèle de 9 parents (34 hybrides) et mesuré trois paramètres de croissance (phase de latence, taux de croissance et population maximale) dans 56 conditions de culture dont un bon nombre comprenant des substances toxiques. Leur analyse montre que 30 % des hybrides présentent du MPH. De plus on observe un fort biais d'additivité positive assimilée à une dominance du meilleur des parents. Seuls quelques rares cas de BPH (6 %) ont été trouvés dans ces conditions. Enfin une corrélation entre le niveau de MPH et la distance génétique entre les souches parentales a pu être établie. Selon les auteurs, ces résultats suggèrent que la complémentation d'allèles délétères serait chez la levure la principale cause de l'hétérosis observée.

De manière surprenante, en partant de la même collection enrichie de parents supplémentaires, deux études mènent à des conclusions différentes. D'une part Shapira et al. (2014), dont le diallèle comprenait 16 parents et 120 hybrides (dont 15 parents communs avec ceux de l'étude de Zörgö et al. 2012), ont montré qu'environ 35 % des hybrides étudiés présentaient du BPH pour le taux de croissance dans cinq milieux riches, et n'ont pas observé de corrélation entre hétérosis et distance génétique entre les parents. Ils rejettent l'hypothèse de la complémentation comme principale source de l'hétérosis, et proposent que la nature plus complexe des milieux de culture et le mode de calcul de l'hétérosis expliquent en partie les différences observées entre les deux études. Parallèlement une autre équipe a utilisé 22 souches parentales issues de la même collection et créé un diallèle de 231 hybrides (Plech et al. 2014). La proportion d'hétérosis était fortement dépendante de l'origine des souches parentales, puisqu'il y avait plus d'hétérosis chez les hybrides issus de parents dont l'origine était liée à la domestication par l'homme. La fréquence cumulée du MPH et du BPH était supérieure à 80 % chez les hybrides issus de souches « domestiquées ». Ces auteurs montrent également une corrélation entre distance génétique et hétérosis au sein des hybrides issus de souches domestiquées. Mais dans

cette étude les valeurs des hybrides ne semblent pas avoir été comparées par des tests statistiques aux valeurs parentales, ce qui limite considérablement la portée des résultats.

En conclusion ces études récentes montrent que la nature du diallèle utilisé et les phénotypes étudiés peuvent faire varier de manière importante les proportions d'hétérosis observées. De plus elles ne concernent que l'espèce *S. cerevisiae* et s'intéressent à un nombre très limité de caractères. Enfin elles permettent difficilement d'envisager d'éventuelles applications en œnologie. Il nous a donc semblé essentiel : 1) d'élargir la base génétique des parents des hybrides en s'intéressant à des hybrides interspécifiques ; 2) de choisir des milieux que les levures peuvent rencontrer en dehors du laboratoire ; 3) d'augmenter le nombre de caractères, d'une part en privilégiant ceux qui ont un intérêt industriel, d'autre part en ciblant plusieurs niveaux d'intégration (métabolique, protéomique, populationnel) pour accéder à une meilleure compréhension des bases du phénomène.

Ma thèse a porté sur l'étude de l'hétérosis chez deux espèces de levures d'intérêt œnologique, *S. cerevisiae* et *S. uvarum*, cultivées dans des conditions proches de celles de l'œnologie. Nous avons construit un diallèle à partir de 11 souches parentales (7 *S. cerevisiae* et 4 *S. uvarum*). Les 11 souches parentales et leurs 55 hybrides ont été cultivés dans du moût à 18 °C et à 26 °C. Au total, 396 fermentations alcooliques ont été réalisées. Nous nous sommes interessés à un ensemble de caractères qui décrivent la dynamique de la population, les capacités fermentaires et aromatiques. Nous avons cherché à répondre aux questions suivantes :

1) Y a-t-il une influence des interactions nucléo-cytoplasmique chez les hybrides interspécifiques sur les performances fermentaires, métaboliques et sur la croissance ?
2) Peut-on développer des modèles statistiques qui permettent d'estimer des caractères pertinents pour résumer la dynamique des populations et les capacités fermentaires des hybrides intra- et interspécifiques ?
3) Quelles sont les sources de la variance phénotypique (génétique, environnementale, interactions G x E) des caractères fermentaires, métaboliques et populationnels chez les parents et les hybrides ?
4) Les hybrides sont-ils plus robustes que les parents vis-à-vis du changement de température du milieu ?
5) Quels sont les caractères qui présentent de l'hétérosis ?
6) Les hybrides interspécifiques présentent-ils le même niveau d'hétérosis que les hybrides intraspécifiques ?

Cette thèse comporte deux chapitres principaux :

Le premier correspond à un article publié, dont je suis le co-premier auteur (Albertin, da Silva et al. 2013). Ce travail, qui décrit en détail le protocole de création des hybrides, a permis d'étudier l'influence du génome mitochondrial sur les phénotypes respiratoire et fermentaire d'hybrides *S. cerevisiae* * *S. uvarum* isogéniques pour l'ADN nucléaire mais possédant soit le patrimoine mitochondrial de *S. cerevisiae*, soit celui de *S. uvarum*. Nous avons notamment montré qu'en conditions fermentaires l'origine du cytoplasme n'avait pas d'influence, ni sur la cinétique fermentaire

ni sur la production de métabolites œnologiques de base. Nous nous sommes ainsi assurés que les mesures sur les hybrides du diallèle ne seraient pas biaisées par l'éventuels effets cytoplasmiques.

Le deuxième chapitre, qui a la forme d'un projet d'article, décrit en détail la création du dispositif diallèle), le protocole expérimental de fermentation et explicite les 35 variables mesurées ou estimées chez les parents et les hybrides du diallèle. Les résultats portent sur la comparaison globale, par des analyses univariées ou multivariées, des trois types d'hybrides du diallèle, à savoir les hybrides intraspécifiques *S. cerevisiae* * *S. cerevisiae*, les hybrides intraspécifiques *S. uvarum* * *S. uvarum* et les hybrides interspécifiques *S. cerevisiae* * *S. uvarum*. (L'analyse détaillées des résultats de tous les croisements fera l'objet d'un autre article, qui devait initialement faire partie de cette thèse, mais que faute de temps je n'ai pas pu terminer.) La partie expérimentale de ce travail de thèse a produit un jeu de données de phénotypage de très grande taille. Certains caractères ont été directement mesurés, d'autres sont des paramètres estimés à partir de modèles mathématiques d'ajustement des données. Dans tous les cas des analyses statistiques et des analyses multivariées ont été nécessaires pour tirer de la masse des données des informations biologiquement pertinentes. Les modèles élaborés pour l'estimation de divers paramètres de fermentation sont originaux et ont une valeur générique pour les processus de croissance de populations microbiennes. Même si, pour éviter des redondances, ils n'apparaissent que dans les Matériels et Méthodes de ce deuxième chapitre, ils doivent être considérés comme faisant partie des résultats de cette thèse.

Par ailleurs j'ai mis en annexe un article dont je suis co-auteur et qui porte sur l'analyse de l'hétérosis sur près de 1400 protéines quantifiées par spectrométrie de masse sur l'ensemble des souches du diallèle (Blein-Nicolas et al. article soumis).

Enfin la partie Conclusion et Perspectives discute brièvement l'ensemble des résultats et résume les analyses qui restent à faire, à savoir l'étude de l'hétérosis hybride par hybride et la recherche de relations éventuelles avec les données protéomiques.

CHAPITRE 1

The Mitochondrial Genome Impacts Respiration but Not Fermentation in Interspecific *Saccharomyces* Hybrids

Albertin W, da Silva T, Rigoulet M, Salin B, Masneuf-Pomarede I, et al. (2013) The Mitochondrial Genome Impacts Respiration but Not Fermentation in Interspecific *Saccharomyces* Hybrids. PLoS ONE 8(9): e75121.

OPEN ACCESS Freely available online

PLOS ONE

The Mitochondrial Genome Impacts Respiration but Not Fermentation in Interspecific *Saccharomyces* Hybrids

Warren Albertin[1,2☯], Telma da Silva[3☯], Michel Rigoulet[4,5], Benedicte Salin[4,5], Isabelle Masneuf-Pomarede[1,2], Dominique de Vienne[6], Delphine Sicard[6], Marina Bely[1], Philippe Marullo[1,7]*

1 Univ. de Bordeaux, ISVV, EA 4577, Unité de recherche CEnologie, Villenave d'Ornon, France, **2** Bordeaux Sciences Agro, Gradignan, France, **3** INRA, UMR 0320/UMR 8120 Génétique Végétale, Gif-sur-Yvette, France, **4** CNRS, UMR 5095, Institute of Biochemistry and Genetics of the Cell, Bordeaux, France, **5** Univ. de Bordeaux, IBGC, UMR 5095, Bordeaux, France, **6** Univ Paris-Sud, UMR 0320/UMR 8120 Génétique Végétale, Gif-sur-Yvette, France, **7** BIOLAFFORT, Bordeaux, France

Abstract

In eukaryotes, mitochondrial DNA (mtDNA) has high rate of nucleotide substitution leading to different mitochondrial haplotypes called mitotypes. However, the impact of mitochondrial genetic variant on phenotypic variation has been poorly considered in microorganisms because mtDNA encodes very few genes compared to nuclear DNA, and also because mitochondrial inheritance is not uniparental. Here we propose original material to unravel mitotype impact on phenotype: we produced interspecific hybrids between *S. cerevisiae* and *S. uvarum* species, using fully homozygous diploid parental strains. For two different interspecific crosses involving different parental strains, we recovered 10 independent hybrids per cross, and allowed mtDNA fixation after around 80 generations. We developed PCR-based markers for the rapid discrimination of *S. cerevisiae* and *S. uvarum* mitochondrial DNA. For both crosses, we were able to isolate fully isogenic hybrids at the nuclear level, yet possessing either *S. cerevisiae* mtDNA (Sc-mtDNA) or *S. uvarum* mtDNA (Su-mtDNA). Under fermentative conditions, the mitotype has no phenotypic impact on fermentation kinetics and products, which was expected since mtDNA are not necessary for fermentative metabolism. Alternatively, under respiratory conditions, hybrids with Sc-mtDNA have higher population growth performance, associated with higher respiratory rate. Indeed, far from the hypothesis that mtDNA variation is neutral, our work shows that mitochondrial polymorphism can have a strong impact on fitness components and hence on the evolutionary fate of the yeast populations. We hypothesize that under fermentative conditions, hybrids may fix stochastically one or the other mt-DNA, while respiratory environments may increase the probability to fix Sc-mtDNA.

Citation: Albertin W, da Silva T, Rigoulet M, Salin B, Masneuf-Pomarede I, et al. (2013) The Mitochondrial Genome Impacts Respiration but Not Fermentation in Interspecific *Saccharomyces* Hybrids. PLoS ONE 8(9): e75121. doi:10.1371/journal.pone.0075121

Editor: Dan Mishmar, Ben-Gurion University of the Negev, Israel

Received June 28, 2013; **Accepted** August 8, 2013; **Published** September 23, 2013

Funding: This work was founded by the Agence Nationale de la Recherche (ANR-08-ALIA-09 HeterosYeast). The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The co-author Philippe Marullo is affiliated with BIOLAFFORT. This does not alter the authors' adherence to all the PLOS ONE policies on sharing data and materials.

* E-mail: philippe.marullo@u-bordeaux2.fr

☯ These authors contributed equally to this work.

Introduction

Eukaryotes possess a cytoplasmic organelle called mitochondrion, either fully functional or vestigial [1,2]. Mitochondria are thought to originate from endosymbiosis between eukaryote's ancestry and α-proteobacteria. This endosymbiotic event, first proposed by Wallin [3] and popularized by Sagan [4], may have arisen more than two billion years ago [5]. However, nowadays mitochondrial genomes contain far less genes than the genomes of α-proteobacteria [6]. Following endosymbiosis, most of the genes of the endosymbiote were either lost or transferred to the host cell genome during evolution [7]. While mitochondria are complex organelles requiring several hundred proteins to function properly, most of them (>99%) are now the product of nuclear genes. Mitochondrial genomes encode very few genes, between 3 and 96 genes in animals, plants, fungi and protists [7,8] and the proportion of genes encoded by mtDNA in Eukaryotes usually represents less than 0.5% of the total number of genes.

Mitochondrial gene content varies in a large extent among eukaryotes, with several lineage-specific variations in rates of gene loss. For example, 5 S rRNA is present only in land plants, some green algae, red algae, brown algae and protists [6], implying many independent and repeated losses of the 5 S rRNA gene across eukaryotic evolution. Identically, the number of tRNA genes encoded by mtDNA varies greatly across eukaryotes, ranging from none to around 30 tRNAs genes. In contrast, two major sets of mitochondrial genes are remarkably well conserved, those involved in respiration and in protein synthesis [6].

Unlike nuclear DNA (nuDNA), mtDNA has high rate of nucleotide substitution [9,10], so that several mitochondrial haplotypes (so-called mitotypes) coexist within species. The analysis of mitochondrial genetic diversity is widely used in population genetics to follow uniparental transmitted markers. However, the importance of mitochondrial genetic variation on phenotypic variation is scarcely considered, firstly because mtDNA encodes very few genes compared to nuDNA, and because mtDNA genetic variation has long been thought to be neutral [11,12]. In recent years, several studies revisited this longstanding view and showed that mtDNA variation might impact various

phenotypic traits [13]. For example, in human, two mtDNA haplotypes were shown to be associated with human survival [14]. Other association studies showed that specific mtDNA mutations in humans are associated with oxygen consumption [15], athletic performance [16], sperm motility [17], Parkinson disease [18], adaptation to diet change and climate [19]. In other animals, cold acclimation was also shown to be associated with mitotypes in the greater white-toothed shrew, *Crocidura russula* [20]. Mitochondrial polymorphism is associated with muscle composition in pig [21] or with resistance to insecticide in an arthropod pest (*Tetranychus urticae*) [22]. Nearly isogenic lines of *Drosophila simulans*, differing for mtDNA, showed important variations for fitness traits (longevity, activity, oxygen consumption, etc) [23–25]. In mice, 'transmitochondrial cybrids', resulting from the transfer of mitochondria to a mtDNA-less receptor cell line, varied for oxidative phosphorylation performances [26]. In plants and fungi also, cytoplasmic variants are related to fitness traits like in *Silene vulgaris* [27] or in the common button mushroom *Agaricus bisporus* [28].

However, most of these studies were performed at the population level or involved nearly isogenic lines. Indeed, it is very difficult to establish that mtDNA variants are actually associated with phenotype, essentially because nuDNA variations may also be involved. To overcome such difficulties, it is possible to study reciprocal hybrids. Since cytoplasmic organelles are mainly maternally inherited, reciprocal hybridization between two parental lines (♀A x ♂B and ♀B x ♂A) allows the recovery of hybrids displaying identical nuDNA, but differing for organelles' DNA. Reciprocal hybrids are easily produced for many plants and frequently showed asymmetric phenotypes [29–32], but the hybrids differ for both mtDNA and chloroplastic DNA. Thus, assessing unambiguously the role of mtDNA alone requires the use of reciprocal hybrids of non-photosynthetic organisms. This was done for example in *Drosophila* species, where reciprocal hybrids displayed different longevity [33], in reciprocal hybrids of stonechat bird (*Saxicola torquata spp.*) that differ for basal metabolic rate [34], while reciprocal centrarchid fish hybrids had asymmetrical viabilities [35]. However, in most cases, the phenomenon of parental genomic imprinting may be confounded with the effect of mtDNA variability by itself [36].

In this work we took advantage of the particular mitochondrial inheritance of the *Saccharomyces* species [37]. *Saccharomyces* zygotes result from the fusion of two parental cells, each having its own mitochondrial DNA. Thus, in the very first generations after hybridization, hybrids possess both parental mtDNA, which is called heteroplasmy [38]. This heteroplasmic status is only transient and after a few generations (less than 20 divisions), homoplasmic cells harboring only one parental mtDNA are recovered [39]. In some cases, recombination between parental mtDNA may arise [40], yet only one recombined mitotype (homoplasmy) is recovered after a few generations. The transition from heteroplasmy to homoplasmy can be stochastic [41,42] or non-stochastic [38,43]. Thus, it is theoretically possible to obtain fully isogenic hybrids resulting from the same cross, but harbouring one or the other of the two parental mtDNA.

In a previous work, Solieri et al. [38] showed that interspecific hybrids between *S. cerevisiae* and *S. uvarum* may have increased respiratory ability when harbouring *S. cerevisiae* mtDNA compared to *S. uvarum* one. However, the synthetic interspecific hybrids tested differed regarding both mtDNA and nuDNA, so that it was difficult to assess whether differences in fermentative and respiratory performances were actually due to mtDNA by itself.

In this work, we produced interspecific hybrids between *S. cerevisiae* and *S. uvarum* species, using fully homozygous diploid parental strains. For two different interspecific crosses involving different parental strains, we recovered 10 independent hybrids per cross, and allowed mtDNA fixation after around 80 generations. For both crosses, we were able to isolate fully isogenic hybrids at the nuclear level, yet possessing either *S. cerevisiae* mtDNA (Sc-mtDNA) or *S. uvarum* mtDNA (Su-mtDNA). These hybrids were used to test the phenotypic impact of mitochondrial inheritance under respiratory conditions. In addition, even though it has long been suggested that mtDNA do not play any role in fermentation, indirect evidences suggested that actually they could [44]. Accordingly Sc-mtDNA and Su-mtDNA hybrids were also compared under fermentative conditions.

Materials and Methods

Yeast Strains and Culture Conditions

Eleven strains of *Saccharomyces cerevisiae* and four strains of *S. uvarum* were selected (Table 1). Monosporic clones were isolated from all these strains using a micromanipulator (Singer MSM Manual; Singer Instrument, Somerset, United Kingdom). All strains but Alcotec 24 and NRRL-Y-7327 were homothallic (*HO/HO*), so that the monosporic derivates were fully homozygous diploid. For Alcotec 24 and NRRL-Y-7327 (*ho/ho*), the isolated haploid meiospore were diploidized via transient expression of the HO endonuclease (see Albertin et al., 2009 [45]). These fully homozygous diploid strains, called W1–W2, D1–D2, B1–B2, E1–E5 for *S. cerevisiae* and U1–U4 for *S. uvarum* were used for subsequent analysis of the genetic diversity of mitochondrial DNA and for interspecific hybrid construction.

All strains were usually grown at 24°C in YPD medium containing 1% yeast extract (Difco Laboratories, Detroit, MI), 1% Bacto peptone (Difco), and 6% glucose, supplemented or not with 2% agar. When necessary, antibiotic concentration was as followed: 100 µg/mL for G418 (Sigma, France), 300 µg/mL for hygromycin B (Sigma, France), and 100 µg/mL for nourseothricin (Sigma, France).

For a quick assessment of respiratory-ability, cells were plated on YPGly medium, containing glycerol as unique source of carbon: 1% yeast extract (w/v, Difco Laboratories, Detroit, MI), 1% Bacto peptone (w/v, Difco), 2% (v/v) glycerol and 2% (w/v) agar.

Mitochondrial DNA Sequence

Genomic DNA extraction were performed as described by Albertin *et al* [46] or by using FTA® CloneSaver ™ Card (Whatman®BioScience, USA). Three mitochondrial loci, *COX3, COX2* and *ATP6,* were sequenced in 11 *S. cerevisiae* and 4 *S. uvarum* fully homozygous strains. An additional locus *VAR1* was sequenced only for *S. cerevisiae* strains. Both strands of PCR products were sequenced using Sanger method (GATC biotech, Germany). The sequences were aligned with ClustalW using the BioEDIT program [47]. Aligned fragments were deposited in EMBL (accession numbers HF951715–HF951770). The genetic distance between sequences (number of differences per base) was estimated using MEGA 5 software [48]. Phylogenic trees were build using the Neighbor-Joining method [49] with bootstrap implementation (500 iterations).

Mitochondrial Genotyping

The three loci *COX2*, *COX3* and *ATP6* were used to design degenerated primers able to amplify in a single PCR reaction the *S cerevisiae* and *S uvarum* alleles. The *COX2* primers used were previously described by Belloch *et al.* [50] and required the digestion of PCR fragment by the endonuclease *Sfc*I. The *ATP6* and *COX3* primers allow differentiating *S. uvarum* and *S. cerevisiae* by

Table 1. Characteristics of *Saccharomyces cerevisiae* and *S. uvarum* strains used.

Species	Strain	Genotype	Ploidy	Collection/ supplier[a]	Origin	Reference
S. cerevisiae	YSP128	*HO/HO*	diploid	SGRP	Forest Oak exudate, Pennsylvania, USA	Liti et al., 2009 [60]
S. cerevisiae	UWOPS83-787.3	*HO/HO*	diploid	SGRP	Fruit *Opuntia stricta*, Bahamas	Liti et al., 2009 [60]
S. cerevisiae	Alcotec 24	*ho/ho*	diploid	Hambleton Bard	Distillery, UK	Albertin et al., 2011 [46]
S. cerevisiae	CLIB-294	*HO/HO*	diploid	CIRM-Levures	Distillery, Cognac, France	Albertin et al., 2011 [46]
S. cerevisiae	CLIB-328	*HO/HO*	diploid	CIRM-Levures	Enology, UK	Albertin et al., 2011 [46]
S. cerevisiae	CLIB-382	*HO/HO*	diploid	CIRM-Levures	Brewery, Japan	Albertin et al., 2011 [46]
S. cerevisiae	VL1	*HO/HO*	diploid	Laffort Œnologie	Enology, Bordeaux, France	Marullo et al., 2006 [88]
S. cerevisiae	F10	*HO/HO*	diploid	Laffort Œnologie	Enology, Bordeaux, France	Marullo et al., 2009 [89]
S. cerevisiae	VL3c	*HO/HO*	diploid	Laffort Œnologie	Enology, Bordeaux, France	Marullo et al., 2004 [90]
S. cerevisiae	BO213	*HO/HO*	diploid	Laffort Œnologie	Enology, Bordeaux, France	Marullo et al., 2006 [88]
S. cerevisiae	NRRL-Y-7327	*ho/ho*	diploid	NRRL	Brewery, Tibet	Albertin et al., 2009 [45]
S. uvarum	PM12	*HO/HO*	diploid	ISVV	Grape must, Jurançon, France	Naumov et al., 2000 [91]
S. uvarum	PJP3	*HO/HO*	diploid	ISVV	Grape must, Sancerre, France	Naumov et al., 2000 [91]
S. uvarum	Br6.2	*HO/HO*	diploid	ADRIA Normandie	Cider fermentation, Normandie, France	
S. uvarum	RC4-15	*HO/HO*	diploid	ISVV	Grape must, Alsace, France	Demuyter et al., 2004 [92]
S. cerevisiae	W1	monosporic clone of YSP128, HO/HO	diploid	ISVV		Blein et al., 2013 [62]
S. cerevisiae	W2	monosporic clone of UWOPS83-787.3, HO/HO	diploid	ISVV		this work
S. cerevisiae	D2	monosporic clone of Alcotec 24, ho/ho	diploid	ISVV		Albertin et al., 2011 [46]
S. cerevisiae	D1	monosporic clone of CLIB-294, HO/HO	diploid	ISVV		Albertin et al., 2011 [46]
S. cerevisiae	E1	monosporic clone of CLIB-328, HO/HO	diploid	ISVV		Albertin et al., 2011 [46]
S. cerevisiae	B1	monosporic clone of CLIB-382, HO/HO	diploid	ISVV		Albertin et al., 2011 [46]
S. cerevisiae	E3	monosporic clone of VL1, HO/HO	diploid	ISVV		Albertin et al., 2011 [46]
S. cerevisiae	E4	monosporic clone of F10, HO/HO	diploid	ISVV		Albertin et al., 2011 [46]
S. cerevisiae	E5	monosporic clone of VL3c, HO/HO	diploid	ISVV		Blein et al., 2013 [62]
S. cerevisiae	E2	monosporic clone of SB, HO/HO	diploid	ISVV		Marullo et al., 2009 [89]
S. cerevisiae	B2	monosporic clone of NRRL-Y-7327, ho/ho	diploid	ISVV		Blein et al., 2013 [62]
S. uvarum	U1	monosporic clone of PM12, HO/HO	diploid	ISVV		Blein et al., 2013 [62]
S. uvarum	U2	monosporic clone of PJP3, HO/HO	diploid	ISVV		Blein et al., 2013 [62]
S. uvarum	U3	*monosporic clone of Br6.2, HO/HO*	diploid	ISVV		Blein et al., 2013 [62]
S. uvarum	U4	*monosporic clone of RC4-15, HO/HO*	diploid	ISVV		this work
S. cerevisiae	D2-3A-HYG	*ho::hygR, MATα*	haploid	ISVV		this work
S. cerevisiae	W1-NAT-1B	*ho::natR, MATa*	haploid	ISVV		this work
S. uvarum	U2-KAN-3B	*Suho::kanR, MATα*	haploid	ISVV		this work
S. uvarum	U3-KAN-3A	*Suho::kanR, MATa*	haploid	ISVV		this work

[a]Laffort CEnologie: http://www.laffort.com; CIRM-Levures (Centre International de Ressources Microbiennes): http://www.inra.fr/internet/Produits/cirmlevures; NRRL (Northern Regional Research Laboratory, now Agricultural Research Service Culture Collection): http://nrrl.ncaur.usda.gov; Hambleton Bard: http://www.hambletonbard.com; ISVV (Institut Scientifique de la Vigne et du Vin): http://www.oenologie.u-bordeaux2.fr/; ADRIA Normandie: http://www.adria-normandie.com; SGRP (Saccharomyces Genome Resequencing Project): http://www.sanger.ac.uk/research/projects/genomeinformatics/sgrp.html.

doi:10.1371/journal.pone.0075121.t001

the PCR product length (Table S1). An additional locus, *VAR1* can be used to discriminate *Saccharomyces cerevisiae* strains by an RFLP approach (Table S1). The PCR reactions were carried out with 2–6 ng of genomic DNA extract as template, 1X Taq-&GO master mix for PCR (Qbiogene), in 20 µL final volume. PCR fragment sizes were analyzed by capillary electrophoresis with a multi NA apparatus (Shimatzu, Germany) using the 1000 pb gel kit.

Hybrid Construction

In order to produce interspecific hybrids, two diploid parental strains per species (W1 and D2 for *S. cerevisiae*, U2 and U3 for *S. uvarum*) were transformed with a cassette containing the *HO* allele disrupted by a gene resistance to either G418 (*ho::KanR*), hygromycin B (*ho::HygR*) or nourseothricin (*ho::NatR*). For *S. cerevisiae* strains, the *ho::KanR*, *ho::HygR* and *ho::NatR* cassettes were respectively amplified by PCR using the following primers p25: TGGTTTACGAAATGATCCACG, p26: AAATCGAAGACC-CATCTGCT and the genomic DNA of the strains BY4741 (Euroscarf, Franckfurt, Germany), RG1 and RG13 (kindly given by Professor Richard Gardner, Auckland, New Zealand). For *S. uvarum* strains, the *Suho::KanR* cassette containing the KanMX4 coding sequence (1506 pb) flanked on 5′ and 3′ by 500 pb flanking- sequence of *S. uvarum HO* gene was synthesized by Genscript and cloned in the pUC57 vector. This cassette was then amplified by PCR using the primers p599 TACCAC-GAAAAACTGATGTAATGG and p600 CTTTATCTGACGC-TATGGCCG. For all ho-disruption cassettes amplification, the PCR mix contained 100–600 ng of DNA template, 0.1 mM of each primer, 1X Taq-&GO master mix for PCR (Qbiogene), in 100 µl final volume. The PCR reaction was as followed: 3 minutes at 94°C, followed by 35 cycles –30 seconds at 94°C, 30 seconds at 54°C or 55°C (for *S. cerevisiae* and *S. uvarum* cassettes respectively), 3 minutes at 72°C – and a final elongation step of 5 minutes at 72°C.

Strains were transformed using the lithium acetate protocol described by Gietz and Schiestl [51] for all *S. cerevisiae* strains and for U4, and alternatively using the frozen yeast TRAFO protocol [52] for U1, U2 and U3. After transformation, monosporic clones were isolated, and the mating-type (*MATa* or *MATα*) of antibiotic-resistant clones was determined using testers of well-known mating-type. Strain transformation allowed (i) conversion to heterothallism for the homothallic strains (all but B2 and D2, see Table 1) and (ii) antibiotic resistance allowing easy hybrid production.

For DU23 hybrids, the parental strains D2-3A-HYG (*MATα*) and U3-KAN-3A (*MATa*) were pulled in contact two to four hours in YPD medium at room temperature, and then plated on YPD-agar with G418 and hygromycinB. The same procedure was applied for WU12 hybrids whose parental strains were W1-NAT-1B (*MATa*) and U2-KAN-3B (*MATα*) and were thus selected on YPD-agar added with G418 and nourseothricin. Ten independent hybrids per cross were recovered. Recurrent cultures on YPD-agar (24°C), each from one colony, which corresponded to ~80 generations, were made in order to allow mitochondrial fixation (homoplasmy) and to assess hybrids chromosomal stability through multiple generations.

Hybrid Characterization

Karyotype analysis of the hybrids and their corresponding progenitors was carried out using pulse-field gel electrophoresis (PFGE). Briefly, chromosomal DNA was prepared from overnight cultures in agarose plugs as described by Bellis et al. [53]. Chromosomes were separated with a CHEF DRII apparatus (Bio-Rad, Richmond, CA, USA) on a 1% agarose gel (Qbiogene, Carlsbad, CA, USA) and using TBE as running buffer. Electrophoresis was carried out at 200 V and 10°C for 16 h with a switching time of 60 ms, and then for 10 h with a switching time of 105 ms. DNA was bound by bromide ethidium staining (30 minutes).

In addition to PFGE, hybrids were characterized by PCR ribotyping (5.8S-ITS rDNA amplification followed by *Hae*III restriction) allowing discrimination between *S. cerevisiae* and *S. uvarum* strains [38,54,55].

Fermentation Assays

White grape must was obtained from Sauvignon grapes, harvested in vineyards in Bordeaux area (2009 vintage). Tartaric acid precipitation was stabilized and turbidity was adjusted to 100 NTU (Nephelometric Turbidity Unit) before long storage at –20°C. Sugar concentration was 188 g $L^{\times 1}$, and the indigenous yeast population, estimated by YPD-plate counting after must thawing, was low, *i.e.* less than 20 CFU (colony-forming unit) *per* mL.

Pre-cultures were run in half-diluted must filtered through a 0.45 µm nitrate-cellulose membrane, during 24 h, at 24°C with orbital agitation (150 rpm). Population size was measured using a flow cytometer (see below). Sauvignon must was inoculated at 10^6 viable cells *per* mL. Fermentation triplicates were run in closed 125 mL glass-reactors, locked to maintain anaerobiosis, with permanent stirring (300 rpm) at 18°C. The CO_2 released was allowed by a needle and was determined by measurement of glass-reactor weight loss regularly and the CO_2max was calculated as the maximal CO_2 released in g L^{-1}. The fermentation kinetics data were fitted with logistic model allowing the calculation of several kinetics parameters: *lag phase time* (h) was the time between inoculation and the beginning of CO_2 release. *AF time* (h) was the time to complete alcoholic fermentation (without lag-phase). *Vmax* was the maximal rate of CO_2 release in g L^{-1} h^{-1}.

At the end of the alcoholic fermentation, ethanol concentration (percent volume) was determined by infrared reflectance (Infra-Analyzer 450; Technicon, Plaisir, France), acetic acid production (g L^{-1}) were measured by colorimetry (*A*460) in continuous flux (Sanimat, Montauban, France) and both residual D-glucose and D-fructose (g L^{-1}) were quantified using an enzymatic method (Kit D glucose/D fructose Boehringer, Germany) in the supernatant.

External glycerol (g L-1) was assayed by the enzymatic method (Boehringer kits 10 148 270 035, R-Biopharm, Darmstadt, Germany).

Cell Growth Conditions for Respiratory Assays

Respiratory growth was assessed on YPEG medium containing 1% yeast extract (w/v, Difco Laboratories, Detroit, MI), 1% Bacto peptone (w/v, Difco, Detroit, MI), 3% ethanol (v/v) and 3% glycerol (v/v). Pre-cultures were run in half-diluted YPEG medium during 24 h, at 28°C with orbital agitation (150 rpm). Population size was measured using flow cytometry (see below) to inoculate YPEG at 10^6 viable cells *per* mL. Triplicates were run in 200 mL Erlenmeyers containing 50 mL YPEG medium, with high permanent stirring (900 rpm) to favour oxygenation at 28°C.

Population Dynamics Using Flow Cytometry

Regularly, cells were sampled and population size was estimated using a flow cytometer (Quanta SC MPL, Beckman Coulter, France), equipped with a 488 nm laser and a 670 nm long-pass filter, at 22 mW. Samples were diluted in McIlvaine buffer pH4 (0.1 M citric acid, 0.2 M sodium phosphate dibasic) added with propidium iodide (0.3% v/v) in order to stain dead cells (red fluorescence measure in FL3 channel). The experimental points were fitted with a logistic model [46] that allowed estimation of the

carrying capacity (maximum population size, *K*, cells per mL) and the intrinsic growth rate *r* (number of divisions *per* hour).

Oxygen Consumption Assays

WU12-8 (Sc-mtDNA) and WU12-1 (Su-mtDNA) were grown aerobically in YPEG liquid medium, at 28°C. During exponential phase, the oxygen consumption was measured polarographically at 28°C using a Clark oxygen electrode in a 1-mL thermostatically controlled chamber. Distinct respiratory rates were considered: spontaneous respiratory rate ($\mathcal{J}O_2$, which is oxygen uptake during growth conditions), uncoupled respiratory rate ($\mathcal{J}O_2max$), which is measured in the presence of 1 µM of the protonophoric uncoupler CCCP (carbonyl cyanide m-chlorophenylhydrazone, Sigma, France) and is an indication of the maximal respiratory rate achieved by the cells [56], and finally none-phosphorating respiratory rate (*basal* $\mathcal{J}O_2$) which is the residual respiratory rate measured when ATPsynthase is inhibited in presence of 200 µM of TET (Tri Ethyl Tin chloride, Alfa Aesar, USA). The ATP respiratory rate ($\mathcal{J}O_2ATP$) was calculated as the difference between spontaneous $\mathcal{J}O_2$ and *basal* $\mathcal{J}O_2$, and the percentage of spontaneous respiration due to ATPase activity was estimated ($\mathcal{J}O_2ATP/\mathcal{J}O_2$). All respiratory rates were determined from the slope of a plot of O_2 concentration versus time and were expressed as nmol O_2/min/10^{e6} cells, population size being measured by flow cytometry. Four measures of all three respiratory rates were performed during exponential growth, and the experiment was performed in duplicate.

Cytochrome Content Determination

The cellular content of *c+c1*, *b*, and *a+a3* hemes was calculated as described by Dejean et al. [57], taking into account the respective molar extinction coefficient values and the reduced minus oxidized spectra recorded using a dual beam spectrophotometer (Aminco DW2000). Two to four measures were made, and cytochrome content was expressed in pmol/mg dry weight of cells.

Electronic Microscopy

Yeast pellets (after YPEG overnight growth) were placed on the surface of a copper EM grid (400 mesh) that had been coated with formvar. Each grid was very quickly submersed in liquid propane pre-cooled and held at −180°C by liquid nitrogen. The loops were then transferred in a pre-cooled solution of 4% osmium tetroxide in dry acetone in a 1.8 ml polypropylene vial at −82°C for 72 h (substitution), warmed gradually to room temperature, followed by three washes in dry acetone. Specimens were stained for 1 h in 1% uranyl acetate in acetone at 4°C, blackroom (epoxy resin Fluka). Ultrathin sections were contrasted with lead citrate. Specimens were observed with a HITACHI 7650 (80 kV) electron microscope (PIE, BIC, Bordeaux Segalen University).

Statistical Analysis

Within each cross (WU12 and DU23), the variation of each trait was investigated using the lm function (R program), through the following model of ANOVA:

$$Z = \mu + strain_i + \varepsilon_i$$

where Z is the variable, *strain* is the strain effect ($i = 1, 2, 3, 4$) and ε is the residual error. Within each cross, the four strains corresponded to two independent strains with Sc-mtDNA and two independent strains with Su-mtDNA. Since several traits were tested, *P* values were adjusted for multiple testing using Benjamini-Hochberg methods by means of R's language, version 2.14.1 [58]. For each variable, the homogeneity of the variance was assessed using a Levene test by means of R's *car* package version 2.14.1 [58], as well as the normality of residual distribution using a Shapiro test [58]. Duncan's multiple comparison was used to determine which means differ significantly (Duncan's multiple comparison, $p<0.05$).

In silico Competition between Mitotypes

Modeling population growth was made using the kinetics parameters calculated under respiratory conditions (YPEG medium) using a logistic model: $K = 3.63.10^8$ cells per mL for both WU12 Sc-mtDNA and Su-mtDNA, $r = 0.222$ and 0.196 division per hour for WU12 Sc-mtDNA and Su-mtDNA respectively; $K = 3.29.\ 10^8$ cells per mL for both DU23 Sc-mtDNA and Su-mtDNA, $r = 0.207$ and 0.176 division per hour for DU23 Sc-mtDNA and Su-mtDNA respectively. The initial mixed population was of 10^6 cells per mL (ratio 1:1 Sc-mtDNA:Su-mtDNA). When the maximal population size was reached (*K*), a new *in silico* culture was inoculated at 10^6 cells per mL, using the ratio of mitotypes (Sc-mtDNA:Su-mtDNA) calculated at the end of the preceding culture.

Results

Mitochondrial Sequence Analysis in *S. cerevisiae* and *S. uvarum*

In order to develop polymorphic mitochondrial markers for both *S. cerevisiae* and *S. uvarum* species, we sequenced three mitochondrial genes (*COX3, COX2 and ATP6)* for 11 *S. cerevisiae* and 4 *S. uvarum* strains. An additional loci *VAR1* was sequenced only for *S. cerevisiae* strains. To maximize the chance to find polymorphism, intergenic segments were amplified from flanking coding regions. This dataset allows a first study of the intra-specific variability of mtDNA within natural populations (Table 2). For *S. cerevisiae*, sequence alignments of *COX2*, *ATP6*, *COX3,* and *VAR1* were performed for 12 strains, including the reference strain S288C. Depending on the gene, we identified 5 to 11 allelic forms. The genetic polymorphism varied greatly depending on the locus and the strain with an average of 2.33% nucleotide difference within the 12 strains. The *COX2* and *VAR1* coding sequences display low polymorphism (0.34% and 0.43% nucleotide difference). By contrast, the promoters of *ATP6* and *COX3* promoter harbored more nucleotide polymorphism between strains (9.75% and 0.66% nucleotide difference, respectively). The promoter region of *ATP6* was found to be particularly polymorphic due to the insertion of two CG clusters at different position defining two groups of strains. A multi-locus analysis was carried out concatenating these sequences (2650 positions). Wine yeasts were grouped together as illustrated by the phylogenic three presented in Figure 1, which is congruent with previous work studying nuclear DNA polymorphism [45,59,60].

For *Saccharomyces uvarum*, there is no published mitochondrial genome. So we used *S. pastorianus* mtDNA genome as reference: *S. pastorianus* is an allotetraploid whose progenitors are *S. cerevisiae* and *S. eubayanus*, a newly-described species phylogenetically closed to *S. uvarum*. *S. pastorianus* inherited the mitochondrial DNA from S. *eubayanus* [61]. Regarding the three loci analyzed (*COX2*, *COX3* and *ATP6*), the *S. eubayanus* mtDNA sequence is divergent from the four *S. uvarum* sequences with an average of 8.8% nucleotide difference for 1454 positions, while within *S. uvarum* few allelic variations were detected (0.30% nucleotide difference). Such a low genetic variability within *S. uvarum* in comparison to *S. cerevisiae* is

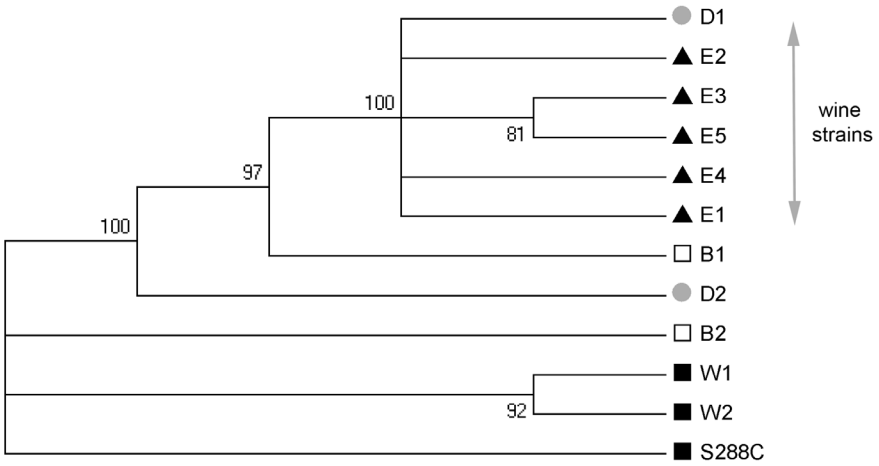


Figure 1. Evolutionary relationships *of Saccharomyces cerevisiae* strains for mtDNA. The phylogenic tree was inferred by using the Maximum Likelihood method based on the Tamura-Nei model with bootstrapping (500 iterations). Branches corresponding to partitions reproduced in less than 80% bootstrap replicates are collapsed. The analysis involved 12 nucleotide sequences representing the concatenation of 4 mitochondrial loci (*COX2*, *COX3*, *VAR1* and *ATP6*). All positions containing gaps and missing data were eliminated. There were a total of 2719 positions in the final dataset. Evolutionary analyses were conducted in MEGA5. Label describes the origin of the strains: natural isolates■, distillery●, brewing□, wine▲.
doi:10.1371/journal.pone.0075121.g001

consistent with the results of a recent multilocus genotyping experiment carried out on six nuclear genes [62].

Development of Co-dominant Mitochondrial Markers for *S. cerevisiae* and *S. uvarum* Species

To have a readily and economic mtDNA genotyping, co-dominant mitochondrial markers were developed using either variation in length PCR-amplicon (PCR-LP) or PCR followed by RFLP. Although numerous nucleotide polymorphisms were found by sequencing, a relative few number of restriction sites were observed. For inter-specific discrimination, three markers were developed (*COX3*, *COX2* and *ATP6*) that allowed a clear discrimination between *S. cerevisiae* and *S. uvarum* (Figure 2). In addition, *ATP6* and *VAR1* loci displayed intra-specific polymorphism within *S. cerevisiae* species when the PCR product was digested with *Bpl*I and *Btg*I respectively. When combined together, those loci allowed differentiating five of the 11 mtDNA of the *S. cerevisiae* strains analyzed (Figure 2). By contrast, the very low polymorphism of *S. uvarum* species prevents the use of these mtDNA markers to discriminate *S. uvarum* strains (Table S1).

Interspecific Hybrid Construction and Characterization

Interspecific hybridization between *S. cerevisiae* and *S. uvarum* was performed, allowing us to get the hybrids DU23 (D2×U3) and WU12 (W1×U2). For each cross, ten independent hybrids were isolated and confirmed by amplification of the rDNA *NTS2* region followed by *Hae*III restriction [63]. Recurrent cultures were then made, corresponding to 80 generations. Pulse-field gel electrophoreses were run to determine whether the hybrids actually possessed both parental chromosome sets. All 20 hybrids displayed additive karyotype, except DU23-2 (Figure 3) that presented large chromosomal rearrangements with additional and missing parental chromosome bands. This result indicated that inter-specific hybridization was relatively stable at the chromosomal level, even after 80 generations.

Mitochondrial inheritance was then assessed for these 20 interspecific hybrids to determine whether the different hybrids

Table 2. Genetic diversity of *COX2, COX3*, *ATP6* and *VAR1* mtDNA loci.

Locus	Species (# strains)	Alignment size	Alleles number	Nucleotide difference range[c]	Description	EMBL access
COX2	*S cerevisiae* (12) [a]	527	5	0–4	*COX2* coding sequence	HF951745-48
	S uvarum (4) [b]	561	2	0		HF951749-60
COX3	*S cerevisiae* (12)	630–749	7	0–78	*COX3* promoter	HF951734-44
	S uvarum (4)	704-507	3	0–6		HF951730-33
ATP6	*S cerevisiae* (12)	692–743	11	12–366	*ATP6* promoter	HF951719-29
	S uvarum (4)	450–480	4	0–7		HF951715-18
VAR1	*S cerevisiae* (12)	971–1068	7	0–145	*VAR1* coding sequence	HF951760-70
	S uvarum	ND	ND	ND		

[a]For *S. cerevisiae*, 12 sequences (11 strains+reference strain) were analyzed.
[b]For *S. uvarum*, 4 sequences were analyzed, the sequence of the strain PM12 was used as reference.
[c]Number of base differences per sequence respect to the reference. Results are based on the pairwise analysis conducted in MEGA5; all positions containing alignment gaps and missing data were eliminated only in pairwise sequence comparisons.
doi:10.1371/journal.pone.0075121.t002

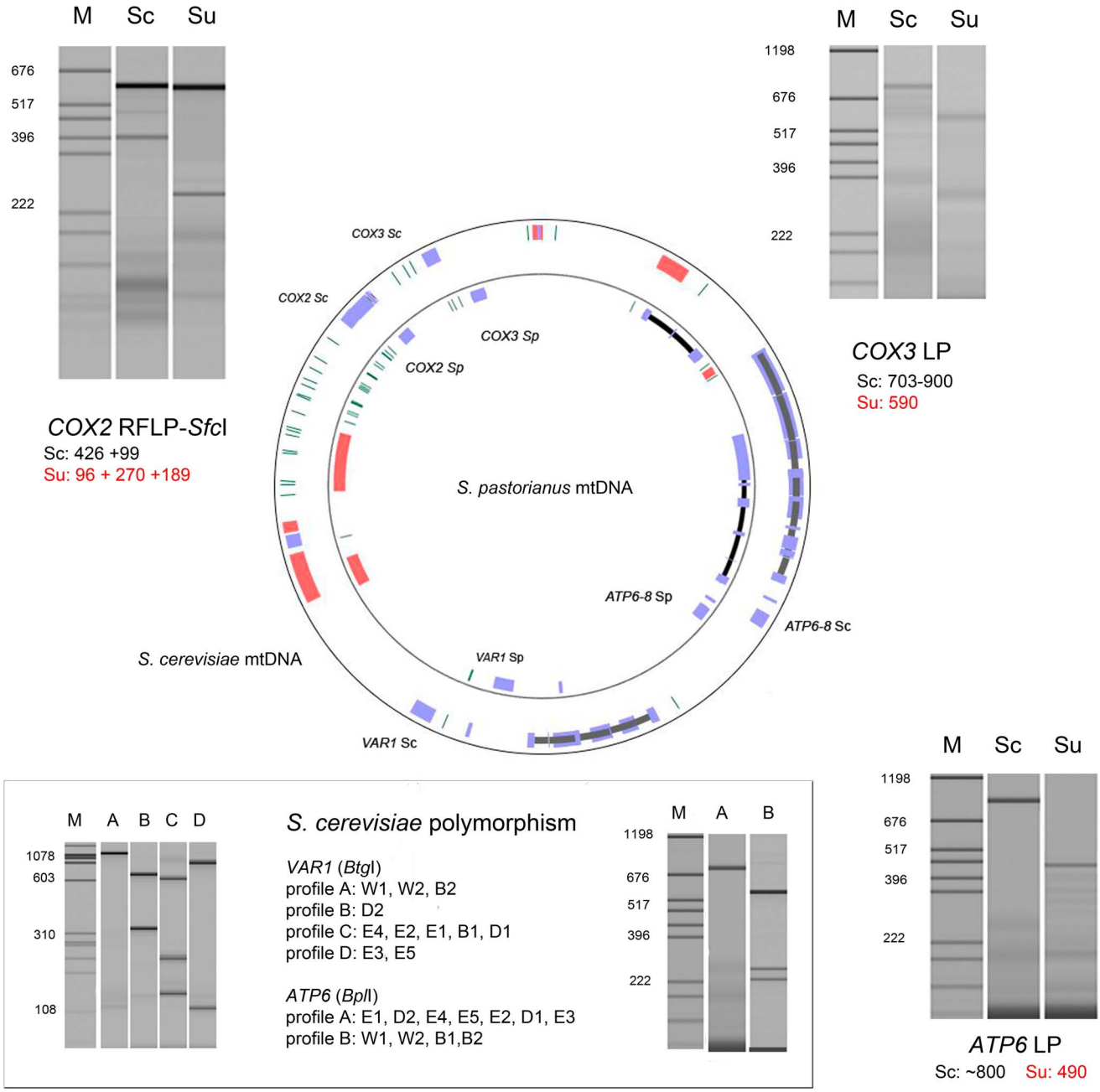


Figure 2. Molecular markers for typing intra and interspecific variability of mtDNA in *S. cerevisiae* and *S. uvarum* species. Three interspecific markers (*S. cerevisiae* vs. *S. uvarum*) and two intra *S. cerevisiae* markers were developed using PCR and enzymatic restriction. The interspecific markers *ATP6* and *COX3* allowed the rapid identification of mitotypes by length polymorphism after PCR. The *COX2* marker required the digestion of PCR fragments by the enzyme *Sfc*I to discriminate the two species mitotypes. For the identification of mtDNA within *S. cerevisiae* strains the *ATP6* and *VAR1* PCR fragments were digested with the restriction enzymes *Bpl*I and *Bgt*I, respectively. Combining both markers, five mitotypes could be identified.
doi:10.1371/journal.pone.0075121.g002

had recovered Sc-mtDNA or Su-mtDNA. The mtDNA was genotyped after 20 and 80 generations (Table S2). Depending on the interspecific cross, the results varied: after 20 generations, only one case of heteroplasmy was detected among 10 independent WU12 hybrids, and after 80 generations all 10 hybrids had fixed either Sc-mtDNA (7/10 hybrids) or Su-mtDNA (3/10 hybrids). All these interspecific hybrids were able to grow on YPGly petri plate, containing glycerol as carbon source, indicating efficient respiration metabolism. By contrast, for DU23 background, two heteroplasmic hybrid strains were observed after 20 generations, as well as three hybrids with mtDNA recombination, most of them being respiratory-defective (unable to grow on YPGly petri plate). After 80 generations, four inter-specific hybrids displayed partial or complete mtDNA loss, associated with inability to grow on YPGly petri plate. Two hybrids with mtDNA recombination were observed, of which only one was able to respire. The four remaining inter-specific hybrids were homoplasmic, two of them with Sc-mtDNA, and two with Su-mtDNA.

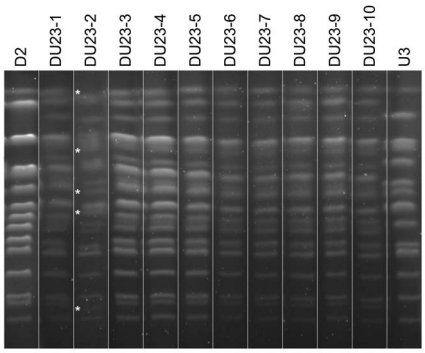


Figure 3. Karyotype analysis of the *S. cerevisiae* strain D2, *S. uvarum* strain U3 and their interspecific hybrids DU23. Pulse field gel electrophoresis was performed on 10 independent DU23 interspecific hybrids. Stars indicate absent parental chromosomes or chromosomes of unexpected size for DU23-2 interspecific hybrid.
doi:10.1371/journal.pone.0075121.g003

Hybrids with Sc-mtDNA have Higher Growth Performance Under Respiratory Conditions

The possibility to obtain readily numerous inter-specific hybrids by antibiotic selection and the development of molecular test for assessing mitochondrial inheritance pave the way to investigate the phenotypic impact of mitochondrial inheritance in an isogenic context. This unique genetic material allows evaluating the impact of natural genetic variations of mtDNA on the fitness of inter-specific hybrids. For each interspecific cross (WU12 and DU23), we chose two independent homoplasmic hybrids with either Sc-mtDNA (WU12-8, WU12-9, DU23-1 and DU23-9) or Su-mtDNA (WU12-1, WU12-2, DU23-3 and DU23-4). These hybrids are thus fully identical at the nuclear level and differ only for mitochondrial DNA, allowing reliable study of the impact of mtDNA inheritance alone on phenotype. As the foremost function of mitochondria in yeast is glucose oxidation through cellular respiration, we first analyzed cell growth under respiratory conditions. The interspecific hybrids were grown in YPEG medium associated with strong permanent stirring, and population was followed by flow cytometry analysis. For both crosses, interspecific hybrids having Su-mtDNA (WU12-1, WU12-2, DU23-3 and DU23-4) had apparent lower population size until the carrying capacity (maximal population size) was reached (Figure 4). Growth kinetics were fitted on logistic function to determine the lag phase time, the maximal population size K, and the intrinsic growth rate r. Variance analysis (ANOVA) revealed that interspecific hybrids reached similar maximal population size within each cross, indicating that mtDNA inheritance had no impact on final carrying capacity in interspecific hybrids (Table 3). By contrast, lag phase time and intrinsic growth rate were strongly affected: for WU12 cross, hybrids with Su-mtDNA had increase lag phase time (15.4 and 16.1 hours for WU12-1 and WU12-2 respectively) than hybrids with Sc-mtDNA (13.2 and 13.8 hours for WU12-8 and WU12-9, respectively). In addition, Su-mtDNA hybrids showed lower intrinsic growth rate than hybrids with Sc-mtDNA (0.201 and 0.191 division per hour for WU12-1 and WU12-2 respectively, compared to 0.224 and 0.221 division per hour for WU12-8 and WU12-9, respectively). The same features were observed for DU23 cross, with Su-mtDNA hybrids having higher lag phase time (around 16.6 hours) and lower growth rate (around 0.175) compared to Sc-mtDNA hybrids (14 hours of lag phase and 0.207 division per hour). In both interspecific crosses, Su-mtDNA inheritance was associated with delayed and slower growth compared to Sc-mtDNA.

Hybrids having Sc-mtDNA have Higher Respiratory Rate

To go further, the respiratory ability of WU12 hybrids was investigated. Four different respiratory rates were measured: the spontaneous respiratory rate ($\mathcal{J}O_2$, which is oxygen uptake under non-limiting growth conditions), the uncoupled respiratory rate, a proxy for the maximal respiratory rate ($\mathcal{J}O_2max$) achieved by the cells [56], the none phosphorylating respiratory rate (*basal* $\mathcal{J}O_2$) which is the residual respiratory rate measured when ATPsynthase is inhibited and finally the ATPase respiratory rate coupled to ATP synthesis ($\mathcal{J}O_2\,ATP$), which is the respiratory rate due to ATP synthesis functioning. For all respiratory rates, the hybrid having Sc-mtDNA (WU12-8) showed higher respiratory ability (Table 3), with a similar increase of 60% compared to Su-mtDNA (WU12-1). By contrast, the proportion of respiratory rate associated with ATPase functioning was identical (73–76%) in both hybrids. Such a large increase in respiratory rates could be due either to differences in mitochondria number and/or volume, or to variation in intrinsic mitochondrial respiratory abilities. To test these hypotheses, we first run electron microscopy of both hybrids (Figure S1). There was no evident difference in the number of mitochondria, their volume and the number of observed cristae, indicating that both hybrids displayed similar qualitative and quantitative mitochondrial content, independent of mtDNA heredity. We then measured the cellular content in cytochromes *c+c1*, *b* and *a+a3*. WU12-8 Sc-mtDNA showed significant lower content in cytochrome c+c1 cytochromes, as well as significant higher content in cytochrome *a+a3*, in comparison with WU12-1 Su-mtDNA. Interestingly, for cytochrome *a+a3*, a similar trend was observed for DU23 hybrids: DU23-1 Sc-mtDNA harboured higher yet not significant *a+a3* cytochrome content (10.6 pmol/mg dry weight) compared to DU23-4 Su-mtDNA (7.4 pmol/mg dry weight). The cytochrome content of the parental strains revealed that both *S. cerevisiae* parental strains (W1 and D2) had significant higher content in *a+a3* cytochromes (10.0 pmol/mg dry weight) compared to *S. uvarum* strains U2 and U3 (6.2 pmol/mg dry weight), suggesting that variation in *a+a3* cytochrome content might be related to the mitotype. It has been shown in yeast that the respiratory rate is mainly controlled by cytochrome oxidase activity [64] and that during growth on none-fermentable carbon source, cell respiratory rate is directly proportional to cytochromes a+a3 content [65]. Thus, the fact that WU12-8 Sc-mtDNA harboured higher *a+a3* cytochrome content than WU12-8 Su-mtDNA explains the difference observed in respiratory rate between both hybrids during growth.

Mitotype has no Phenotypic Impact on Fermentation Kinetics and Products

For a long time, mitochondrion was thought to be useless under fermentative conditions, mainly because cells with defective respiration were able to ferment normally [66]. In addition, many genes encoding mitochondrial proteins are repressed under fermentative conditions associating high glucose content and anaerobia [67,68]. However, several authors suggested that mitochondria may be critical for yeast fermentative performance [44,69]. Therefore we assessed the possible effect of mitochondrial genotype under fermentative conditions. Alcoholic fermentations were run in grape must, and parameters related to fermentation kinetics (Figure 5) were measured (*lag phase time*, *AF time*, CO_2max, *Vmax*). In addition, at the end of the fermentation, the main products (ethanol, acetic acid, glycerol) were measured, as well as the residual sugar (Table 4). Within each cross, all four strains,

Table 3. Results of the ANOVAs: F values and Mean values for respiration parameters.

	WU12 interspecific cross						DU23 interspecific cross					
	ANOVA		Mean value +/− SD (Duncan's class)				ANOVA		Mean value +/− SD (Duncan's class)			
Parameters	Fvalue	df	WU12-1 Su	WU12-2 Su	WU12-8 Sc	WU12-9 Sc	Fvalue	Df	DU23-1 Sc	DU23-3 Su	DU23-4 Su	DU23-9 Sc
K	3,34	3	3.96e+08+/− 1.9e+07	3.91e+08 +/−2.8e+07	3.51e+08 +/−1.4e+07	3.75e+08 +/−1.2e+07	1,92	3	3.44e+08 +/−5e+06	3.49e+08 +/−2.5e+07	3.16e+08 +/−2.6e+07	3.13e+08 +/−2.9e+07
R	9,38**	3	0.201+/− 0.006(a)	0.191+/− 0.007(a)	0.224+/− 0.008(b)	0.221+/− 0.013(b)	7,85*	3	0.204+/− 0.01(b)	0.178+/− 0.009a	0.173+/− 0.002(a)	0.211+/− 0.019(b)
lag-phase	9,22**	3	15.38+/− 0.01(b)	16.07+/− 0.01(b)	13.25+/− 0.01(a)	13.79+/− 0.01(a)	11,45*	3	14.52+/− 0.01(a)	16.65+/− 0.01(b)	16.57+/− 0(b)	13.59+/− 0.02(a)
JO_2	181,55***	1	1.07+/− 0.13(a)	ND	3.03+/− 0.39(b)	ND	ND	ND	ND	ND	ND	ND
JO_2max	66,10***	1	1.67+/− 0.26(a)	ND	3.67+/− 0.64(b)	ND	ND	ND	ND	ND	ND	ND
JO_2ATP	155,63***	1	0.78+/− 0.15(a)	ND	2.31+/− 0.32(b)	ND	ND	ND	ND	ND	ND	ND
basal JO_2	35,87***	1	0.29+/− 0.06(a)	ND	0.72+/− 0.19(b)	ND	ND	ND	ND	ND	ND	ND
JO_2ATP/JO_2	1,37	1	0.73+/− 0.07	ND	0.76+/− 0.05	ND	ND	ND	ND	ND	ND	ND
c+c1	133,9***	1	58+/− 0.82(b)	ND	37.75+/− 3.4(a)	ND	42,88*	1	30.2+/− 0.28(b)	ND	24.8+/− 1.13(a)	ND
b	0,01	1	14.12+/− 2.02	ND	14.25+/− 0.96	ND	256*	1	10.2+/− 0.28	ND	7+/− 0	ND
a+a3	7,63*	1	6.38+/− 1.8(a)	ND	10.62+/− 2.5(b)	ND	16,79	1	10.6+/− 0.85	ND	7.4+/− 0.71	ND

Significance of the ANOVA (strain effect) is indicated as follow: * significant at 5%; ** significant at 1%; *** significant at 0.1% (Benjamini-Hochberg correction for multiple testing). df stands for degree of freedom. When ANOVA is significant, Duncan's class for each strain is noted in bracket. The units are as follow: *K* in cells mL^{-1}, *r* in division h^{-1}, *lag-phase time* in h, the respiratory rates JO_2, JO_2max, *basal* JO_2, JO_2ATP in nmol of O_2 consumption per minute per 10^{e6} cells, JO_2ATP/JO_2 in % JO_2 due to ATPase, cytochromes *c+c1*, *b* and *a+a3* in pmol/mg dry weight.

doi:10.1371/journal.pone.0075121.t003

whatever the mtDNA genotype, harboured similar fermentative features for all ten fermentative parameters, suggesting that mitochondrial genotype has a negligible effect, if any, in fermentation conditions.

Discussion

Mitochondrial PCR-based Markers: A Useful Tool for Future Research

Previous mitochondrial genotyping in yeast was based mostly on mtDNA restriction patterns, which is time-consuming and unsuitable for phylogenic comparison and recombination studies [70–72]. Only one mtDNA PCR-based marker was available (*COX2*) [50], mainly due to the nature of *Saccharomyces* mtDNA showing long AT stretches and short GC clusters [73–75], thus limiting the use of PCR approaches. Here, we developed three additional PCR-based markers, two allowing rapid discrimination between Sc-mtDNA and Su-mtDNA (*ATP6* and *COX3*), and two displaying intra-Sc-mtDNA variation (*ATP6* and *VAR1*). Genotyping mtDNA of inter-specific independent hybrids revealed a few events of mtDNA recombination: while for one inter-specific hybrid (WU12) no recombinant mtDNA was found, DU23 hybrid was associated with two stable cases of mtDNA recombination. Although the number of tested hybrids is too low to compare accurately the probability of mtDNA recombination between crosses, these results suggest that mtDNA recombination may vary depending on the parental strains. In any case, our work provides new molecular tools (PCR-based markers) that will be useful to determine the level of mtDNA recombination. Mitochondrial DNA genotyping could now be applied to other hybrids including other *Saccharomyces* inter-specific hybrids but also intra-specific hybrids of *S. cerevisiae*, in order to assess the mtDNA variation according to the genetic backgrounds. In addition, the use of these PCR-based markers may be useful to definitely resolve whether the fixation of one mitotype is stochastic or not in yeast, as different works suggested either random mitochondrial inheritance [41,42] or non-stochastic one [38,43].

Isogenic Yeast Strains Differing Only for mtDNA: An Original Material to Unravel Nucleo-mitochondrial Interactions and Mitochondrial Impact

Previous work addressed the relationships between mtDNA variation and phenotypic traits through the study of reciprocal hybrids in various organisms such as plants [29–32], insects [33], birds [34]and fishes [35]. However, in most of these cases, the phenomenon of parental genomic imprinting may be confounded with the effect of mtDNA variability [36]. Here, we exploited the peculiar mtDNA inheritance in yeast to produce hybrids being fully isogenic at the nuclear level, but possessing either Sc-mtDNA or Su-mtDNA. Such a biological material is particularly appropriate for the proper testing of the phenotypic impact of mtDNA polymorphism, in absence of reciprocal parental imprinting. In addition, hybrids differing only for mtDNA could be useful for future investigations regarding nucleo-cytoplasmic interactions. Previous works in yeast revealed nucleo-mitochondrial epistasis in yeast, with phenotypic effect on fitness [76]. Incompatibility between *S. cerevisiae* mitochondria and a nuclear gene of *S. bayanus*

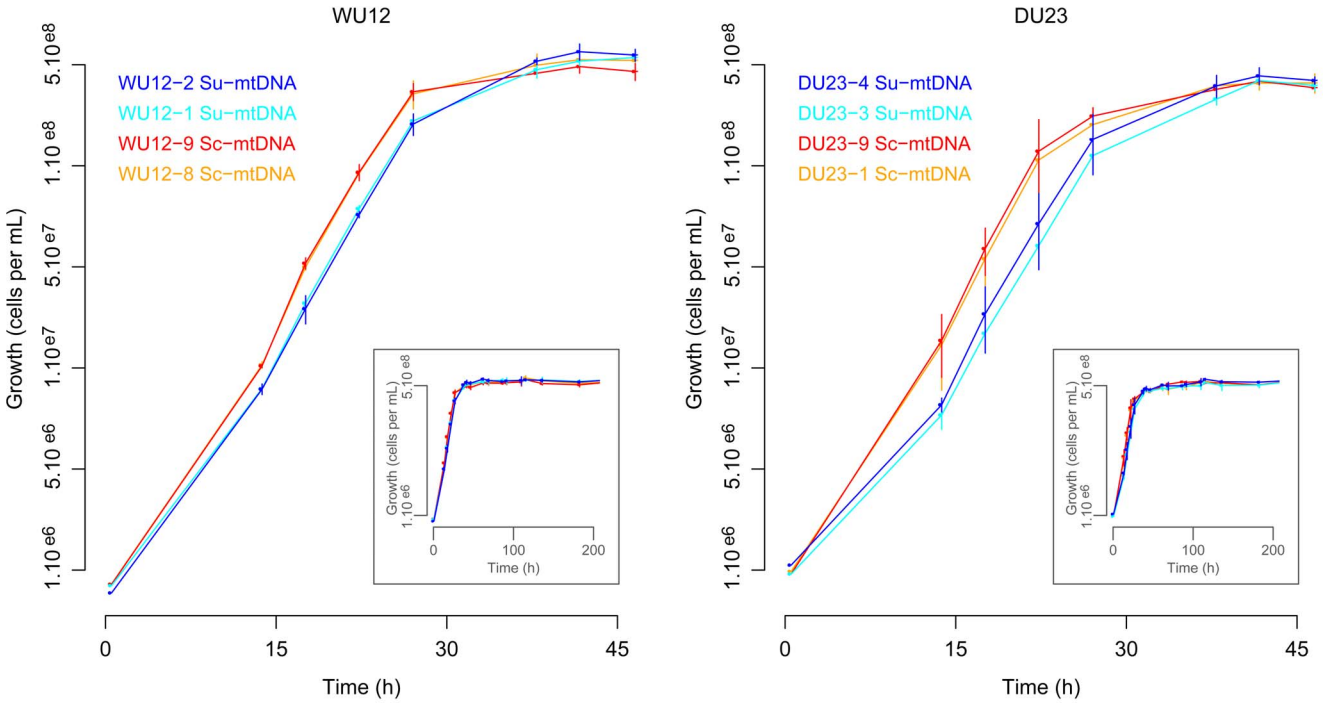


Figure 4. Growth dynamics under respiratory conditions for WU12 and DU23 interspecific hybrids. Population growth was assessed on YPEG medium, using flow cytometry. For each strain, triplicates were made and error bars show standard deviations. The growth kinetics are represented in small captions, while large captions focus on the first part of growth dynamics.
doi:10.1371/journal.pone.0075121.g004

AEP2 was shown to be responsible for hybrid sterility [77]. Additional 'incompatibility' genes were further identified within *Saccharomyces* hybrids of *S. cerevisiae*, *S. bayanus* and *S. paradoxus* [78]. The relationship between cytonuclear incompatibilities and hybrid sterility suggests that this mechanism may be involved in reproductive isolation and subsequently in speciation [79].

Table 4. Results of the ANOVAs: F values and Mean values for fermentation parameters.

	WU12 interspecific cross						DU23 interspecific cross					
	ANOVA		Mean value +/− SD (Duncan's class)				ANOVA		Mean value +/− SD (Duncan's class)			
	Fvalue	df	WU12-1 Su	WU12-2 Su	WU12-8 Sc	WU12-9 Sc	Fvalue	df	DU23-1 Sc	DU23-3 Su	DU23-4 Su	DU23-9 Sc
Ethanol	0,49	3	10.97+/− 0.08	11.07+/− 0.15	10.94+/− 0.2	11.03+/− 0.12	1,91	3	11,00+/− 0.17	10.88+/− 0.11	11.15+/− 0.17	11.1+/− 0.14
residual sugar	0,21	3	2,00+/− 2.77	1.47+/− 1.29	0.77+/− 0.64	1.67+/− 2.37	2,92	3	3.93+/− 4.11	6.37+/− 3.12	0.53+/− 0.42	1.37+/− 1.36
acetic acid	0,81	3	0.05+/− 0.03	0.07+/− 0.06	0.09+/− 0.01	0.09+/− 0.02	7,44	3	0.08+/− 0.03	0.04+/− 0.03	0.05+/− 0.04	0.17+/− 0.05
Glycerol	0,35	3	11.2+/− 0.8	10.9+/− 0.6	11.2+/− 0.6	11.4+/− 0.7	0,83	3	9.7+/− 0.9	9.3+/− 0.8	10.1+/− 1.0	10.3+/− 0.6
CO_2max	0,63	3	86.78+/− 2.04	86.55+/− 0.28	87.89+/− 1.17	87.25+/− 1.04	4,61	3	86.35+/− 2.97	84.4+/− 0.26	88.89+/− 0.75	87.95+/− 0.77
lag-phase	6,07	3	38.0+/− 1.8	42.5+/− 4.3	35.9+/− 5.8	40.7+/− 3.3	1,94	3	26.4+/− 2.1	27.6+/− 2.2	28.2+/− 0.3	24.3+/− 2.2
AF time	2,12	3	126,0+/− 5.0	117.5+/− 3.4	110.2+/− 5.6	106.2+/− 14.6	0,84	3	158,0+/− 15.9	150.3+/− 0.3	165.5+/− 8.6	160.6+/− 2.4
Vmax	1,39	3	1.26+/− 0.02	1.41+/− 0.15	1.30+/− 0.04	1.41+/− 0.09	2,08	3	1.19+/− 0.08	1.14+/− 0.06	1.05+/− 0.03	1.14+/− 0.05

Significance of the ANOVA (strain effect) is indicated as follow: * significant at 5%; ** significant at 1%; *** significant at 0.1% (Benjamini-Hochberg correction for multiple testing). df stands for degree of freedom. When ANOVA is significant, Duncan's class for each strain is noted in bracket. The units are as follow: *ethanol* in percent volume, *residual sugar* in g L^{-1}, *acetic acid* in g L^{-1}, *glycerol* in g L^{-1}, *CO_2max* in g L^{-1}, *lagphase* and *AF time* in h, *Vmax* in g CO_2 L^{-1} h^{-1}.
doi:10.1371/journal.pone.0075121.t004

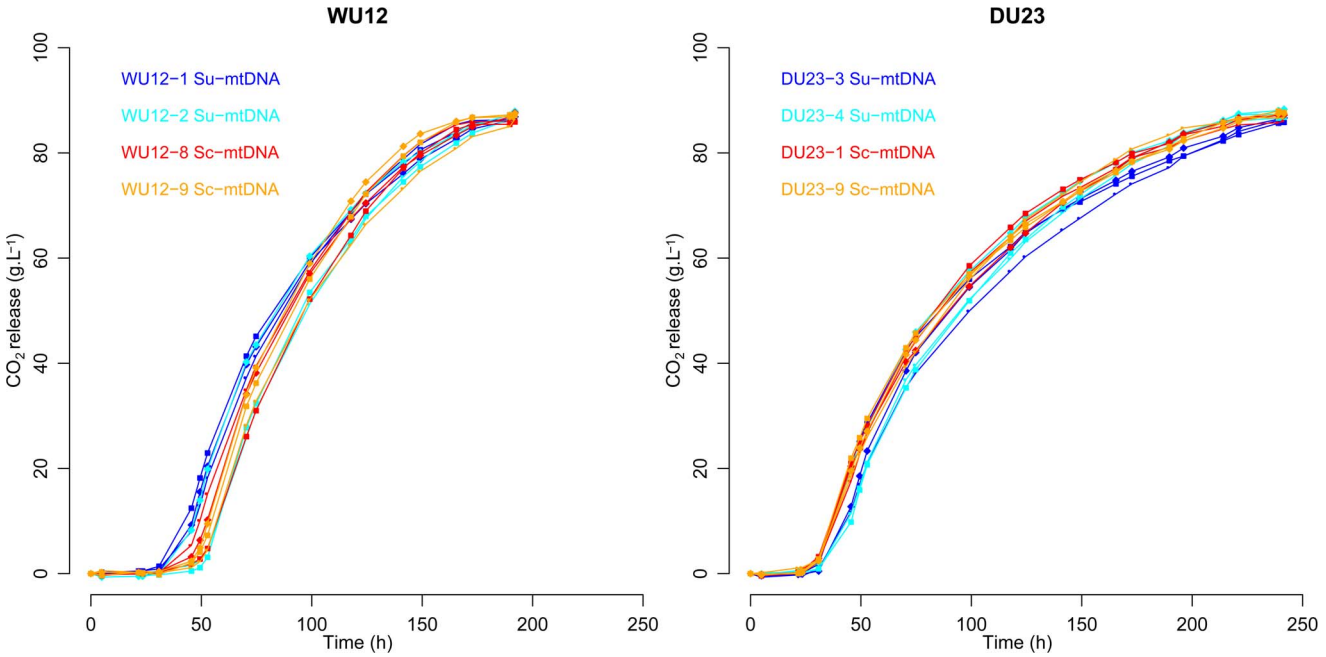


Figure 5. Fermentation kinetics in Sauvignon grape must for WU12 and DU23 interspecific hybrids. Fermentations were performed in 2009 Sauvignon grape must at 18°C in 125 mL bioreactors. CO_2 release ($g.L^{-1}$) was measured through weight loss. For each strain, the three replicates are represented.
doi:10.1371/journal.pone.0075121.g005

Nucleo-mitochondrial interactions (also designed as mitonuclear interactions) may have also played a major role in other evolutionary processes, like in the evolution of sex [80]. Therefore, hybrids differing only for mtDNA may help understanding the role played by cytonuclear interactions in yeast evolution and adaptive ability.

Mitochondrial DNA Polymorphism has a Phenotypic Impact on Respiration, not on Fermentation

We showed that under fermentative conditions, no phenotypic differences were observed between hybrids having either Sc-mtDNA or Su-mtDNA. It was suggested that mitochondria may play a role in fermentation, in particular because trace amounts of oxygen are necessary for completing fermentation [44], particularly under high sugar concentrations. However, it has been shown that under these conditions, oxygen was not consumed by mitochondria but used for sterol biosynthesis and NADPH-dependent systems localized in microsomal membranes [81]. It should be noted that the fermentative conditions used here were permissive (for oenological conditions), with normal-to-low sugar content (188 g/L). It is possible that under harsher fermentative conditions we may have observed significant differences between hybrids having different mitotypes. Additional analyses under various fermentative environments, from permissive to harsh, will help determining whether mtDNA variation may affect fermentation parameters.

By contrast, under respiratory growth conditions, large differences were associated with the mitotypes. This result is not surprising, knowing that the replacement of the mitochondria of one *Saccharomyces* species by another is usually associated with variation in traits related to respiration [75,82,83]. Here, we showed that hybrids having Sc-mtDNA start to grow earlier and faster than their counterparts with Su-mtDNA. The differences in population growth could be related to the respiratory rate (the higher respiratory rate $\mathcal{J}O_2$, the higher the intrinsic growth rate r and the lower the lag-phase time). Accordingly, previous work showed that the respiratory rate varied greatly from one strain to another and was related to cell growth in *S. cerevisiae* species [65]. In addition, the differences in respiratory rates between hybrids harbouring either Sc-mtDNA or Su-mtDNA were associated with cytochrome contents variation, particularly with *a+a3* content which appears to be higher for Sc-mtDNA than for Su-mtDNA. It has been shown that electron transfer through cytochrome *a+a3* is a main controlling step in mitochondrial oxidative phosphorylation in yeast [64,84]. Thus, an increase in cell cytochrome *a+a3* content induce a nearly proportional increase in cell respiration during growth. From a bioprocess point of view, the mtDNA inheritance of interspecific hybrids has to be taken into account for selection. In fact, although some industrial starters used in brewing [85] or winemaking [86] are interspecific hybrids, few studies have investigated the role of mtDNA on their aerobic propagation [87]. The respiratory rate discrepancy observed here between Sc-mtDNA and Su-mtDNA is a key factor that likely affects biomass yield of interspecific hybrids and therefore their subsequent development for industry.

Whatever the molecular mechanisms underlying differences in cytochrome contents and thus in respiratory rates, we demonstrated clearly that mitotypes strongly impact cell growth in yeast, and potentially subsequent fitness. To test this last hypothesis, we predicted the evolution of a yeast population initially composed of 1:1 ratio of Sc-mtDNA:Su-mtDNA cells. Using the cell growth parameters calculated through logistic fit, we showed that after four recurrent *in silico* cultures with initial population size of 10^{e6} cells *per* mL, the Sc-mtDNA mitotype outcompeted Su-mtDNA mitotype and represented 92.9% of the total population for WU12 and 96.5% for DU23 respectively (Figure 6). Far from the hypothesis that mtDNA variation is neutral, our work shows that mitochondrial polymorphism can have strong impact on fitness components and hence on the evolutionary fate of the yeast

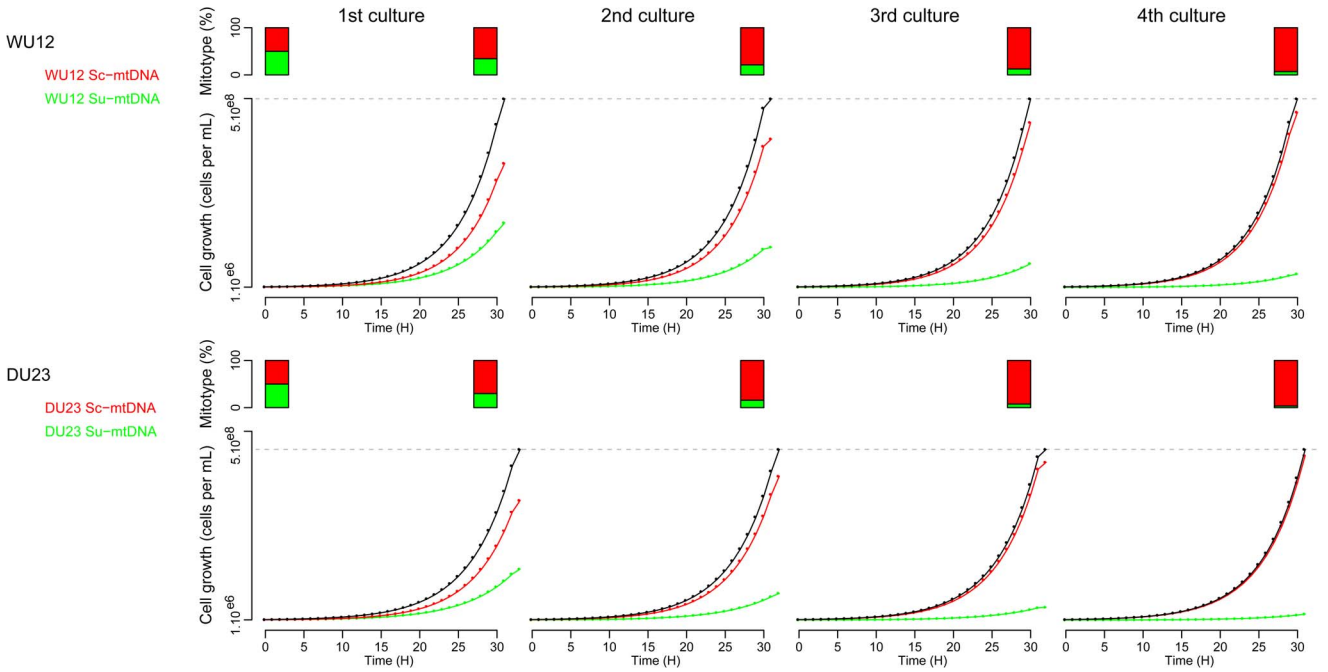


Figure 6. Theoretical evolution of mixed-populations with different mitotypes under respiratory conditions. Modeling population growth was made using the kinetics parameters (maximal population size *K*, intrinsic growth rate *r* and lag-phase) calculated under respiratory conditions (YPEG medium). The initial mixed population contained 10^6 cells per mL (ratio 1:1 Sc-mtDNA:Su-mtDNA). When the maximal population size was reached (grey dashed line), the next cycle started with 10^6 cells per mL. After four cycles, the Sc-mtDNA mitotype represented 92.9% of the total population for WU12, and 96.5% for DU23.
doi:10.1371/journal.pone.0075121.g006

populations. From these results, we can hypothesize that the environmental conditions could influence mitochondrial inheritance in interspecific hybrids: under fermentative conditions, hybrids may fix stochastically one or the other mt-DNA, while respiratory environments may increase the probability to fix Sc-mtDNA. The interaction with environments may explain why mitochondrial inheritance was described either as random [41,42] or non-stochastic [38,43] in previous works. In any case, our work provides both the biological material and the genetic markers necessary to elucidate the mechanisms of mitochondrial inheritance.

Supporting Information

Figure S1 Microscopy of WU12 interspecific hybrids harboring either Sc-mtDNA or Su-mtDNA. Several mitochondria per cell are observable (black arrows). The number of mitochondria, their volume, and the number of cristae are similar for both mitotypes. (TIF)

Table S1 Development of polymorphic mitochondrial markers. [a] SGD (http://www.yeastgenome.org); [b] GenBank: EU852811.1 [73]; [c] range observed for 12 *S. cerevisiae* strains; [d] range observed for 4 *S. uvarum* strains; [e] RFLP: Restriction Fragment Length polymorphism, LP: Length Polymorphism of amplicon.
(XLSX)

Table S2 mtDNA inheritance for two inter-specific crosses between *S. cerevisiae* x *S. uvarum*. ND: not detected.
(XLSX)

Acknowledgments

We thank Eoin Anthony Moynihan for its corrections that helped improving the manuscript.

Author Contributions

Conceived and designed the experiments: WA IMP DdV DS MB PM. Performed the experiments: WA TdS MR BS PM. Analyzed the data: WA MR PM. Wrote the paper: WA MR IMP DdV DS PM.

References

1. Tovar J, Leon-Avila G, Sanchez LB, Sutak R, Tachezy J, et al. (2003) Mitochondrial remnant organelles of Giardia function in iron-sulphur protein maturation. Nature 426: 172–176.
2. Emelyanov VV (2003) Mitochondrial connection to the origin of the eukaryotic cell. European Journal of Biochemistry 270: 1599–1618.
3. Wallin IE (1923) The mitochondria problem. The American Naturalist 57: 255–261.
4. Sagan L (1967) On the origin of mitosing cells. Journal of Theoretical Biology 14: 225-IN226.
5. Feng D-F, Cho G, Doolittle RF (1997) Determining divergence times with a protein clock: Update and reevaluation. Proceedings of the National Academy of Sciences 94: 13028–13033.
6. Adams KL, Palmer JD (2003) Evolution of mitochondrial gene content: gene loss and transfer to the nucleus. Mol Phylogenet Evol 29: 380–395.
7. Timmis JN, Ayliffe MA, Huang CY, Martin W (2004) Endosymbiotic gene transfer: organelle genomes forge eukaryotic chromosomes. Nat Rev Genet 5: 123–135.
8. Burger G, Gray MW, Forget L, Lang BF (2013) Strikingly Bacteria-Like and Gene-Rich Mitochondrial Genomes throughout Jakobid Protists. Genome Biol Evol 5: 418–438.
9. Johnson JA, Toepfer JE, Dunn PO (2003) Contrasting patterns of mitochondrial and microsatellite population structure in fragmented populations of greater prairie-chickens. Molecular Ecology 12: 3335–3347.
10. Burton RS, Byrne RJ, Rawson PD (2007) Three divergent mitochondrial genomes from California populations of the copepod Tigriopus californicus. Gene 403: 53–59.
11. Ballard JWO, Kreitman M (1995) Is mitochondrial DNA a strictly neutral marker? Trends in Ecology & Evolution 10: 485–488.

12. Dowling DK, Friberg U, Lindell J (2008) Evolutionary implications of non-neutral mitochondrial genetic variation. Trends in Ecology & Evolution 23: 546–554.
13. Ballard JWO, Melvin RG (2010) Linking the mitochondrial genotype to the organismal phenotype. Molecular Ecology 19: 1523–1539.
14. Yashin AI, De Benedictis G, Vaupel JW, Tan Q, Andreev KF, et al. (2000) Genes and longevity: lessons from studies of centenarians. J Gerontol A Biol Sci Med Sci 55: B319–328.
15. Marcuello A, Martínez-Redondo D, Dahmani Y, Casajús JA, Ruiz-Pesini E, et al. (2009) Human mitochondrial variants influence on oxygen consumption. Mitochondrion 9: 27–30.
16. Niemi A-K, Majamaa K (2005) Mitochondrial DNA and ACTN3 genotypes in Finnish elite endurance and sprint athletes. Eur J Hum Genet 13: 965–969.
17. Montiel-Sosa F, Ruiz-Pesini E, Enríquez JA, Marcuello A, Díez-Sánchez C, et al. (2006) Differences of sperm motility in mitochondrial DNA haplogroup U sublineages. Gene 368: 21–27.
18. van der Walt JM, Nicodemus KK, Martin ER, Scott WK, Nance MA, et al. (2003) Mitochondrial polymorphisms significantly reduce the risk of Parkinson disease. Am J Hum Genet 72: 804–811.
19. Mishmar D, Ruiz-Pesini E, Golik P, Macaulay V, Clark AG, et al. (2003) Natural selection shaped regional mtDNA variation in humans. Proc Natl Acad Sci U S A 100: 171–176.
20. Fontanillas P, Depraz A, Giorgi MS, Perrin N (2005) Nonshivering thermogenesis capacity associated to mitochondrial DNA haplotypes and gender in the greater white-toothed shrew, Crocidura russula. Molecular Ecology 14: 661–670.
21. Fernández AI, Alves E, Fernández A, de Pedro E, López-García MA, et al. (2008) Mitochondrial genome polymorphisms associated with longissimus muscle composition in Iberian pigs. Journal of Animal Science 86: 1283–1290.
22. Van Leeuwen T, Vanholme B, Van Pottelberge S, Van Nieuwenhuyse P, Nauen R, et al. (2008) Mitochondrial heteroplasmy and the evolution of insecticide resistance: Non-Mendelian inheritance in action. Proceedings of the National Academy of Sciences 105: 5980–5985.
23. James AC, Ballard JWO (2003) Mitochondrial Genotype Affects Fitness in Drosophila simulans. Genetics 164: 187–194.
24. Pichaud N, Ballard JWO, Tanguay RM, Blier PU (2012) Naturally Occurring Mitochondrial Dna Haplotypes Exhibit Metabolic Differences: Insight Into Functional Properties Of Mitochondria. Evolution 66: 3189–3197.
25. Pichaud N, Ballard JW, Tanguay R, Blier P (2012) Mitochondrial haplotype divergences affect specific temperature sensitivity of mitochondrial respiration. Journal of Bioenergetics and Biomembranes: 1–11.
26. Moreno-Loshuertos R, Acin-Perez R, Fernandez-Silva P, Movilla N, Perez-Martos A, et al. (2006) Differences in reactive oxygen species production explain the phenotypes associated with common mouse mitochondrial DNA variants. Nat Genet 38: 1261–1268.
27. McCauley DE, Olson MS (2003) Associations among cytoplasmic molecular markers, gender, and components of fitness in Silene vulgaris, a gynodioecious plant. Molecular Ecology 12: 777–787.
28. De La Bastide PY, Sonnenberg A, Van Griensven L, Anderson JB, Horgen PA (1997) Mitochondrial Haplotype Influences Mycelial Growth of Agaricus bisporus Heterokaryons. Applied and Environmental Microbiology 63: 3426–3431.
29. Burgess KS, Husband BC (2004) Maternal and paternal contributions to the fitness of hybrids between red and white mulberry (Morus, Moraceae). American Journal of Botany 91: 1802–1808.
30. Tiffin P, Olson MS, Moyle LC (2001) Asymmetrical crossing barriers in angiosperms. Proc Biol Sci 268: 861–867.
31. Sanford JC, Hanneman RE (1982) Large yield differences between reciprocal families of Solanum tuberosum. Euphytica 31: 1–12.
32. Sanford J, Hanneman R (1979) Reciprocal differences in the photoperiod reaction of hybrid populations *Solanum tuberosum*. American Journal of Potato Research 56: 531–540.
33. Rand DM, Fry A, Sheldahl L (2006) Nuclear-mitochondrial epistasis and drosophila aging: introgression of Drosophila simulans mtDNA modifies longevity in D. melanogaster nuclear backgrounds. Genetics 172: 329–341.
34. Tieleman BI, Versteegh MA, Fries A, Helm B, Dingemanse NJ, et al. (2009) Genetic modulation of energy metabolism in birds through mitochondrial function. Proceedings of the Royal Society B: Biological Sciences 276: 1685–1693.
35. Bolnick DI, Turelli M, López-Fernández H, Wainwright PC, Near TJ (2008) Accelerated Mitochondrial Evolution and "Darwin's Corollary": Asymmetric Viability of Reciprocal F1 Hybrids in Centrarchid Fishes. Genetics 178: 1037–1048.
36. Wang X, Sun Q, McGrath SD, Mardis ER, Soloway PD, et al. (2008) Transcriptome-Wide Identification of Novel Imprinted Genes in Neonatal Mouse Brain. PLoS ONE 3: e3839.
37. Dujon B (1981) Mitochondrial genetics and function. In: Strathern JN, Jones EW, Broach JR, editors. The Molecular Biology of the Yeast Saccharomyces: Life Cycle and Inheritance. New York: Cold Spring Harbor Laboratory Press. 505–635.
38. Solieri L, Antunez O, Perez-Ortin JE, Barrio E, Giudici P (2008) Mitochondrial inheritance and fermentative : oxidative balance in hybrids between Saccharomyces cerevisiae and Saccharomyces uvarum. Yeast 25: 485–500.
39. Solieri L (2010) Mitochondrial inheritance in budding yeasts: towards an integrated understanding. Trends Microbiol 18: 521–530.
40. Nunnari J, Marshall WF, Straight A, Murray A, Sedat JW, et al. (1997) Mitochondrial transmission during mating in Saccharomyces cerevisiae is determined by mitochondrial fusion and fission and the intramitochondrial segregation of mitochondrial DNA. Mol Biol Cell 8: 1233–1242.
41. Pulvirenti A, Caggia C, Restuccia C, Giudici P, Zambonelli C (2000) Inheritance of mitochondrial DNA in interspecific Saccharomyces hybrids. Annals of Microbiology 50: 61–64.
42. De Vero L, Pulvirenti A, Gullo M, Bonatti PM, Giudici P (2003) Sorting of mitochondrial DNA and proteins in the progeny of Saccharomyces interspecific hybrids. Annals of Microbiology 53: 219–231.
43. Marinoni G, Manuel M, Petersen RF, Hvidtfeldt J, Sulo P, et al. (1999) Horizontal transfer of genetic material among Saccharomyces yeasts. J Bacteriol 181: 6488–6496.
44. O'Connor-Cox ESC, Lodolo EJ, Axcell BC (1996) Mitochondrial Relevance To Yeast Fermentative Performance: A Review. J Inst Brew 102: 19–25.
45. Albertin W, Marullo P, Aigle M, Bourgais A, Bely M, et al. (2009) Evidence for autotetraploidy associated with reproductive isolation in *Saccharomyces cerevisiae*: towards a new domesticated species. J Evol Biol 22: 2157–2170.
46. Albertin W, Marullo P, Aigle M, Dillmann C, de Vienne D, et al. (2011) Population size drives the industrial yeast alcoholic fermentation and is under genetic control. Appl Environ Microbiol 77: 2772–2784.
47. Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl Acids Symp Ser 41: 95–98.
48. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, et al. (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28: 2731–2739.
49. Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4: 406–425.
50. Belloch C, Querol A, Garcia MD, Barrio E (2000) Phylogeny of the genus Kluyveromyces inferred from the mitochondrial cytochrome-c oxidase II gene. Int J Syst Evol Microbiol 50 Pt 1: 405–416.
51. Gietz RD, Schiestl RH (1991) Applications of high efficiency lithium acetate transformation of intact yeast cells using single-stranded nucleic acids as carrier. Yeast 7: 253–263.
52. Dohmen RJ, Strasser AWM, Honer CB, Hollenberg CP (1991) An Efficient Transformation Procedure Enabling Long-Term Storage of Competent Cells of Various Yeast Genera. Yeast 7: 691–692.
53. Bellis M, Pagès M, Roizès G (1987) A simple and rapid method for preparing yeast chromosomes for Pulse Field Gel Electrophoresis. Nucleic Acids Research 15: 6749.
54. McCullough MJ, Clemons KV, McCusker JH, Stevens DA (1998) Intergenic transcribed spacer PCR ribotyping for differentiation of Saccharomyces species and interspecific hybrids. J Clin Microbiol 36: 1035–1038.
55. Fernández-Espinar MT, Esteve-Zarzoso B, Querol A, Barrio E (2000) RFLP analysis of the ribosomal internal transcribed spacers and the 5.8 S rRNA gene region of the genus Saccharomyces: a fast method for species identification and the differentiation of flor yeasts. Antonie Van Leeuwenhoek 78: 87–97.
56. Beauvoit B, Rigoulet M, Bunoust O, Raffard G, Canioni P, et al. (1993) Interactions between glucose metabolism and oxidative phosphorylations on respiratory-competent Saccharomyces cerevisiae cells. European Journal of Biochemistry 214: 163–172.
57. Dejean L, Beauvoit B, Guerin B, Rigoulet M (2000) Growth of the yeast Saccharomyces cerevisiae on a non-fermentable substrate: control of energetic yield by the amount of mitochondria. Biochim Biophys Acta 1457: 45–56.
58. R Development Core Team (2010) R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.
59. Legras JL, Merdinoglu D, Cornuet JM, Karst F (2007) Bread, beer and wine: *Saccharomyces cerevisiae* diversity reflects human history. Mol Ecol 16: 2091–2102.
60. Liti G, Carter DM, Moses AM, Warringer J, Parts L, et al. (2009) Population genomics of domestic and wild yeasts. Nature 458: 337–341.
61. Groth C, Petersen RF, Piskur J (2000) Diversity in organization and the origin of gene orders in the mitochondrial DNA molecules of the genus Saccharomyces. Mol Biol Evol 17: 1833–1841.
62. Blein-Nicolas M, Albertin W, Valot B, Marullo P, Sicard D, et al. (2013) Yeast proteome variations reveal different adaptative responses to grape must fermentation. Mol Biol Evol 30: 1368–1383.
63. Pulvirenti A, Solieri L, De Vero L, Giudici P (2005) Limitations on the use of polymerase chain reaction - restriction fragment length polymorphism analysis of the rDNA NTS2 region for the taxonomic classification of the species Saccharomyces cerevisiae. Canadian Journal of Microbiology 51: 759–764.
64. Mazat J-P, Jean-Bart E, Rigoulet M, Guérin B (1986) Control of oxidative phosphorylations in yeast mitochondria. Role of the phosphate carrier. Biochimica et Biophysica Acta (BBA) - Bioenergetics 849: 7–15.
65. Devin A, Dejean L, Beauvoit B, Chevtzoff C, Avéret N, et al. (2006) Growth Yield Homeostasis in Respiring Yeast Is Due to a Strict Mitochondrial Content Adjustment. Journal of Biological Chemistry 281: 26779–26784.
66. Hutter A, Oliver SG (1998) Ethanol production using nuclear petite yeast mutants. Applied Microbiology and Biotechnology 49: 511–516.
67. DeRisi JL, Iyer VR, Brown PO (1997) Exploring the metabolic and genetic control of gene expression on a genomic scale. Science 278: 680–686.
68. Ter Linde JJ, Steensma HY (2002) A microarray-assisted screen for potential Hap1 and Rox1 target genes in Saccharomyces cerevisiae. Yeast 19: 825–840.

69. Kitagaki H, Shimoi H (2007) Mitochondrial dynamics of yeast during sake brewing. J Biosci Bioeng 104: 227–230.
70. Vezinhet F, Blondin B, Hallet J-N (1990) Chromosomal DNA patterns and mitochondrial DNA polymorphism as tools for identification of enological strains of Saccharomyces cerevisiae. Applied Microbiology and Biotechnology 32: 568–571.
71. Aigle M, Erbs D, Moll M (1984) Some molecular structures in the genome of larger brewing yeast. Journal of the American Society of Breweing Chemists 42: 1–7.
72. Dubourdieu D, Sokol A, Zucca J, Thalouarn P, Dattee A, et al. (1987) Identification des souches de levures isolees de vins par l'analyse de leur ADN mitochondrial. Connaissance de la Vigne et du Vin 21: 267–278.
73. Nakao Y, Kanamori T, Itoh T, Kodama Y, Rainieri S, et al. (2009) Genome sequence of the lager brewing yeast, an interspecies hybrid. DNA Res 16: 115–129.
74. Langkjaer RB, Casaregola S, Ussery DW, Gaillardin C, Piskur J (2003) Sequence analysis of three mitochondrial DNA molecules reveals interesting differences among Saccharomyces yeasts. Nucleic Acids Res 31: 3081–3091.
75. Procházka E, Franko F, Poláková S, Sulo P (2012) A complete sequence of Saccharomyces paradoxus mitochondrial genome that restores the respiration in S. cerevisiae. FEMS Yeast Research 12: 819–830.
76. Zeyl C, Andreson B, Weninck E (2005) Nuclear-Mitochondrial Epistasis for Fitness in Saccharomyces cerevisiae. Evolution 59: 910–914.
77. Lee H-Y, Chou J-Y, Cheong L, Chang N-H, Yang S-Y, et al. (2008) Incompatibility of Nuclear and Mitochondrial Genomes Causes Hybrid Sterility between Two Yeast Species. Cell 135: 1065–1073.
78. Chou JY, Hung YS, Lin KH, Lee HY, Leu JY (2010) Multiple molecular mechanisms cause reproductive isolation between three yeast species. PLoS Biol 8: e1000432.
79. Meadows R (2010) Genetic Mismatches Between Nuclei and Mitochondria Make Yeast Hybrids Sterile. PLoS Biol 8: e1000433.
80. Hadjivasiliou Z, Pomiankowski A, Seymour RM, Lane N (2012) Selection for mitonuclear co-adaptation could favour the evolution of two sexes. Proceedings of the Royal Society B: Biological Sciences 279: 1865–1872.
81. Rosenfeld E, Beauvoit B, Rigoulet M, Salmon J-M (2002) Non-respiratory oxygen consumption pathways in anaerobically-grown Saccharomyces cerevisiae: evidence and partial characterization. Yeast 19: 1299–1321.
82. Sulo P, Spirek M, Soltesova A, Marinoni G, Piskur J (2003) The efficiency of functional mitochondrial replacement in Saccharomyces species has directional character. FEMS Yeast Res 4: 97–104.
83. Spirek M, Horvath A, Piskur J, Sulo P (2000) Functional co-operation between the nuclei of Saccharomyces cerevisiae and mitochondria from other yeast species. Curr Genet 38: 202–207.
84. Rigoulet M, Guerin B, Denis M (1987) Modification of flow-force relationships by external ATP in yeast mitochondria. European Journal of Biochemistry 168: 275–279.
85. Rainieri S, Kodama Y, Nakao Y, Pulvirenti A, Giudici P (2008) The inheritance of mtDNA in lager brewing strains. FEMS Yeast Res 8: 586–596.
86. Borneman AR, Desany BA, Riches D, Affourtit JP, Forgan AH, et al. (2012) The genome sequence of the wine yeast VIN7 reveals an allotriploid hybrid genome with Saccharomyces cerevisiae and Saccharomyces kudriavzevii origins. FEMS Yeast Res 12: 88–96.
87. Serra A, Strehaiano P, Taillandier P (2005) Influence of temperature and pH on Saccharomyces bayanus var. uvarum growth; impact of a wine yeast interspecific hybridization on these parameters. Int J Food Microbiol 104: 257–265.
88. Marullo P, Bely M, Masneuf-Pomarede I, Pons M, Aigle M, et al. (2006) Breeding strategies for combining fermentative qualities and reducing off-flavor production in a wine yeast model. FEMS Yeast Res 6: 268–279.
89. Marullo P, Mansour C, Dufour M, Albertin W, Sicard D, et al. (2009) Genetic improvement of thermo-tolerance in wine *Saccharomyces cerevisiae* strains by a backcross approach. FEMS Yeast Res 9: 1148–1160.
90. Marullo P, Bely M, Masneuf-Pomarede I, Aigle M, Dubourdieu D (2004) Inheritable nature of enological quantitative traits is demonstrated by meiotic segregation of industrial wine yeast strains. FEMS Yeast Res 4: 711–719.
91. Naumov GI, Masneuf I, Naumova ES, Aigle M, Dubourdieu D (2000) Association of Saccharomyces bayanus var. uvarum with some French wines: genetic analysis of yeast populations. Res Microbiol 151: 683–691.
92. Demuyter C, Lollier M, Legras JL, Le Jeune C (2004) Predominance of Saccharomyces uvarum during spontaneous alcoholic fermentation, for three consecutive years, in an Alsatian winery. J Appl Microbiol 97: 1140–1148.

CHAPITRE 2

Multi-trait phenotyping of yeast strains in a diallel design reveals complex genetic and plastic variations and homeostasis for temperature in winemaking conditions

Telma da Silva*, Warren Albertin*, Christine Dillmann*, Marina Bely, Stéphane La Guerche, Christophe Giraud, Sylvie Huet, Delphine Sicard, Isabelle Masneuf-Pomarede, Dominique de Vienne and Philippe Marullo.

* These authors contributed equally to this work

INTRODUCTION

Heterosis, or hybrid vigor, is one of the most fascinating biological phenomena. It has been described 250 years ago (Kölreuter, 1764, in Roberts, 1965), and seems to be a universal phenomenon since it is observed in a very large range of species, from microorganisms such as yeast or *Neurospora crassa*, to animals (including humans) and plants. The hybrids commonly harbor non-additive inheritance for polygenic traits, with phenotypic values usually higher than the mean parental values. These "monsters" produced by hybridization (Newman 1917) have evolutionary implications (Baack et al. 2005, Mallet 2005) and are extensively exploited for producing improved crops. For instance in maize, F1 hybrids between homozygous lines show heterosis of 100 to 400% for grain yield (Hallauer and Miranda, 1981; Sprague, 1983), and many other complex traits, such as height, leaf area, grain size, germination rate, root growth and root nitrogen uptake, also display heterosis (Sarkissian *et al*., 1964; Mino, 1980, Coque and Gallais, 2008).

Heterosis affects not only the phenotypic values, but also their stability over environmental changes. Homeostasis, canalization or robustness – the term depends on the biological field Lerner 1954, Waddington 1942, Meyers and Bull 2002, Jarosz 2010)– allows the organisms to buffer the effects of external perturbations through metabolic, physiological or developmental adjustments, and thus to maintain fitness in diverse habitats. Homeostasis is usually higher in intra- or interspecific hybrids than in their parents, as shown for instance for yield, tolerance to soil acidity and to soil moisture stress in maize (Schnell and Beckert 1986, Welcker et al. 2005, Zaidi et al. 2007, Coque and Gallais 2008*,* Nguyen et al. 2011) or morphometric traits in mice (Leamy and Thorpe 1984).

Heterosis has been studied in a large range of wild as well as domesticated species, but has scarcely been studied and exploited in industrial eukaryotic micro-organisms such as yeast. Recently some authors investigated the heterosis phenomenon within natural and domesticated strains of *Saccharomyces cerevisiae* (Timberlake 2011, Zorgo 2012, Plech 2014, Shapira 2014). However these studies mainly investigated the cell growth in laboratory conditions.

In the *Saccharomyces sensu stricto* clade, yeast species are not in complete reproductive isolation and the prezygotic barrier can be easily bypassed leading to viable interspecific hybrids. Numerous interspecific hybrids between *S. cerevisiae* and psychrophilic species *S. uvarum* or *S. kudriavzevii* have been isolated in wine and natural environment (Masneuf 1998, Nguyen 2000, Antunovics 2005, Le Jeune 2007, Lopandic 2008, Gonzalez 2008, Lopes 2010, Peris 2011, Erny 2012). These natural hybrids have technological properties differing to their respective "parental" species, with sometimes better robustness (Belloch et al. 2008, Arroyo-Lopez et al. 2009, Tronchoni et al. 2009, Arroyo-Lopez et al. 2010, Gamero et al. 2013). Moreover some wine starters empirically selected proved to be interspecific hybrids (Borneman et al. 2012, Erny et al. 2012), promoting the idea that interspecific hybridization is a good way for obtaining valuable strains for wine fermentation.

However in the previous works the parental strains of the hybrids were not known, so it was not possible to state definitely that interspecific hybridization conferred heterosis and possibly better homeostasis. Moreover, these natural isolates can have gross chromosomal rearrangement (Piotrowski et al. 2012, Dunn et al. 2013), loss of heterozygosity (Gonzalez et al. 2008, Peris et al. 2012), particular mitotypes (de Barros et al. 2002) and aneuploidies (Peris et al. 2012) that can drastically affect their phenotype. Therefore the phenotypic characteristics of natural interspecific hybrids may be due to other causes than heterosis resulting from genome heterozygosity.

In order to assess rigorously the phenotypic impact of intra- and interspecific hybridization, the hybrids must be compared to their parental strains. As previously reviewed (Sipiczki 2008, Morales et al. 2012) various laboratories have produced such hybrids between *Saccharomyces* species (Masneuf et al. 2002, Sebastiani et al. 2002, Antunovics et al. 2005, Solieri et al. 2008). However only a few interspecific hybrids were compared to their parents, and for a quite small number of traits (Antonelli et al. 1999, Masneuf et al. 2002, Caridi et al. 2002). As the *Saccharomyces* strains harbor huge genetic and phenotypic diversity (Liti et al. 2009, Camarasa et al. 2011), the behavior of few hybrids is not sufficient to have an overall view on the effects of intraspecific hybridization.

In this work we examined the extent to which hybridization within and between *Saccharomyces* species modified a large series of traits measured during and at the end of fermentation at two temperatures, with particular attention to homeostasis.

We focused on *S. cerevisiae* and *S. uvarum* (formerly *S. bayanus* var. *uvarum* (Rainieri et al. 1999, Naumov 2000, Nguyen et al. 2000)), two related species naturally associated with wine fermentations (Torriani et a. 1999, Naumov et al. 2000, Naumov et al. 2002). *S. cerevisiae* is the main yeast able to achieve grape must fermentation, but *S. uvarum* can display similar fermentation performance, particularly at low temperature (Sipiczki 2008, Demuyter et al. 2004, Tosi et al. 2009, Blein-Nicolas et al. 2013). Although these sister species share large synteny, with 98 % of the genes in *S. uvarum* retaining the same neighboring relationships as in *S. cerevisiae* (Bon et al. 2000, Fischer et al. 2001), they differ for several technological traits. First, *S. cerevisiae* has a higher resistance to high temperature stress (up to 37 ºC) (Belloch et al. 2008) while *S. uvarum* is more tolerant to low temperatures (Rainieri et al. 1998). Second, *S. uvarum* exhibits a specific aromatic profile by producing higher amounts of 2-phenylethanol and 2-phenylethyl acetate than *S. cerevisiae* strains (Antonelli et al. 1999, Masneuf-Pomarède et al. 2010, Gamero et al. 2013). Finally, although *S. uvarum* harbors a high ethanol resistance (up to 15 % (Masneuf-Pomarède et al. 2010)), it produces lower ethanol than *S. cerevisiae* (Castellari et al. 1994). Several natural hybrids between these two species have been described (Masneuf et al. 1998, Belloch et al. 2008, Belloch et al. 2009), and the possibility to produce synthetic inter-specific hybrids (Albertin et al. 2013) established *S. cerevisiae* and *S. uvarum* as model systems for hybridization studies.

Measuring an unprecedented number of traits, we investigated the physiological and technological properties of a collection of four *S. uvarum* and seven *S. cerevisiae* parental strains and their 55 possible hybrids, namely 27 intraspecific hybrids and 28 inter-specific hybrids, under winemaking conditions at two temperatures.

We analyzed the sources of phenotypic variation – genetic and/or environmental – for various categories of traits (fermentation kinetics, life-history, wine composition and organoleptic quality), we compared the intra- and interspecific hybrids and assessed the extent to which hybridization increased homeostasis.

MATERIALS AND METHODS

PARENTAL STRAINS

The original strains of the experimental design were seven *S. cerevisiae* strains and four *S. uvarum* strains, associated to various food-processes (enology, brewery, cider fermentation and distillery) or isolated from natural environment (oak exudates) (Table 1). These strains could not be used as such as parents of the diallel design because they were supposed to be heterozygous at many loci. So we isolated monosporic clones by tetrad dissection from each of these strains using a micromanipulator (Singer MSM Manual; Singer Instrument, Somerset, United Kingdom). All original strains but Alcotec 24 were homothallic (*HO*/*HO*), therefore fully homozygous diploid strains were spontaneously obtained by fusion of opposite mating type cells. For A24 (*ho/ho*), one isolated haploid meiospore was diploidized via transient expression of the HO endonuclease (Albertin et al. 2009). These strains, called W1, D1, D2, E2, E3, E4 and E5 for *S. cerevisiae* and U1, U2, U3 and U4 for *S. uvarum*, were used as the parental strains for the construction of a complete diallel design (Figure 1).

All strains were grown at 24 °C in YPD medium containing 1 % yeast extract (Difco Laboratories, Detroit, MI), 1 % Bacto peptone (Difco), and 6 % glucose, supplemented or not with 2 % agar. When necessary, antibiotics were added at the following concentrations: 100 µg/mL for G418 (Sigma, l'Isle d'Albeau Chesnes, France), and nourseothricin (Werner bioagent, Germany) and 300 µg/mL for hygromycin B (Sigma, l'Isle d'Albeau Chesnes, France).

CONSTRUCTION OF THE HYBRID DIALLEL DESIGN

In order to produce interspecific hybrids, the eleven diploid parental strains were transformed with a cassette containing the *HO* allele disrupted by a gene resistance to either G418 (*ho::KanR*), hygromycin B (*ho::HygR*) or nourseothricin (*ho::NatR*) as previously described (Albertin et al. 2013). After transformation, monosporic clones were isolated, and the mating-type (*MATa* or *MATalpha*) of antibiotic-resistant clones was determined using testers of well-known mating-type. Strain transformation allowed (i) conversion to heterothallism for the

homothallic strains (all but D2, see Table 1) and (ii) antibiotic resistance allowing easy hybrid selection.

For each hybrid construction, parental strains of opposite mating type were put in contact 2 to 6 hours in YPD medium at room temperature, and then plated on YPD-agar containing the appropriate antibiotics. The 55 possible hybrids from the 11 parental strains, namely 21 *S. cerevisiae* intraspecific hybrids, 6 *S. uvarum* intraspecific hybrids and 28 interspecific hybrids, were obtained. For each cross, a few independent colonies were collected. After recurrent cultures on YPD-agar corresponding to ~ 80 generations, the nuclear chromosomal stability of the hybrids was controlled by pulsed field electrophoresis (CHEF-DRIII, Biorad, CA) as well as homoplasmy (only one parental mitochondrial genome) as detailed in Albertin *et al.* (Albertin et al. 2013).

YEAST STRAIN CHARACTERIZATION

In order to discriminate rapidly the hybrids and parental strains, we used two polymorphic microsatellites specific to *S. cerevisiae* (Sc-YFR038, Sc-YML091) (Richards et al. 2009) and two specific to *S. uvarum* (locus 4 and 9) (Masneuf-Pomarède et al. 2007). These four markers were amplified in a multiplex PCR reaction with the labeled primers (Table S1). The PCR was carried out in a final volume of 8 µL using the following program: 95°C for 5 min for initial denaturation step; 95 °C for 30 s, 55 °C for 90 s and 72 °C for 60 s repeated 35 times; a final elongation step of 30 min at 60 °C. The PCR products were analyzed on an ABI3730 apparatus (Applied Biosystem, Villebon-sur-Yvette, France) by the genotyping facilities of Bordeaux University. Microsatellite lengths were analyzed using the Peak Scanner tool (Applied Biosystem, Villebon-sur-Yvette, France)

ALCOHOLIC FERMENTATION EXPERIMENTS

EXPERIMENTAL DESIGN

The 66 strains (11 parental and 55 hybrids) were grown in three replicates at two temperatures, 26 °C and 18 °C. The 396 fermentations (66 strains x 2 temperatures x 3 replicates) were performed following a randomized experimental design. The design was implemented considering a block as two sets of 27 fermentations (26 fermentations and a control without yeast to check for contamination), one carried out at 26 °C and the other at 18 °C. The distribution of the strains within the blocks was randomized to minimize the residual variance of the estimators of the *Strain* and *Temperature* effects.

GRAPE MUST AND FERMENTATION CONDITIONS

White grape must was obtained from *Sauvignon blanc* grapes, harvested in vineyards in Bordeaux area (2009 vintage). Tartaric acid precipitation was stabilized and turbidity was adjusted to 100 NTU (Nephelometric Turbidity Unit) before storage at – 20°C. Grape juice had a sugar concentration of 189 $g.L^{-1}$, a pH of 3.3 and an assimilable nitrogen content of

242 mg $N.L^{-1}$. The indigenous yeast population was estimated by YPD-plate counting after must thawing and was consistently lower than 20 CFU (Colony-Forming Unit) *per* mL.

Yeast pre-cultures (20 mL) were run in half-diluted must filtered through a 0.45 µm nitrate-cellulose membrane, during 24 h, at 24°C with orbital agitation (150 rpm). Cell concentration was quantified using a flow cytometer (see below) and grape must was inoculated at 10^6 viable cells *per* mL. Fermentations were run in 125 mL glass-reactors, locked to maintain anaerobiosis, with permanent stirring (300 rpm) at 18 °C or 26 °C. Yeast strains implantation in grape must was checked when the stationary phase was reached (40 % of alcoholic fermentation). The DNA of fermenting yeast was extracted using FTA clone saver cards (Whatman, France), and strain identity was controlled by microsatellite analysis.

MULTI-TRAIT PHENOTYPING IN WINEMAKING CONDITIONS

For each alcoholic fermentation, *i.e.* each replicate of each strain-temperature combination, four series of experimental data were measured or estimated over time or at the end of the fermentation: fermentation kinetics parameters, life-history traits, basic enological parameters and aromatic traits.

- *ESTIMATING FERMENTATION KINETICS PARAMETERS*

The amount of CO_2 released was monitored daily by the weight loss of the bioreactors. The fermentations were considered as completed when the CO_2 production rate was lower than 0.05 $g.L^{-1}.h^{-1}$.

The amount of CO_2 released (Y_{it}) for the fermentation i at time t was modeled as:

$$Y_{it} = f(t, t_{0i}, d_i, \alpha_i, b_i) + \varepsilon_{it}$$

with $\{\varepsilon_{it}\}$ i.i.d. $\sim N(0, \sigma_{f,i}^2)$. Function f was a discontinuous function of time t, allowing for a lag-time of duration t_0. The lag-time corresponds to the gradual saturation of the medium by carbon dioxide, implicating the CO_2 released was undetectable before it. After t_0, the release of CO_2 was supposed to follow a Weibull function of parameters (d, α, b) (Figure 2A and Figure S1):

$$\begin{cases} t \le t_0: \ f(t) = 0 \\ t \ge t_0: \ f(t, t_0, d, \alpha, b) = d\,(1 - \exp[-\alpha\,(t - t_0)^b]\,) \end{cases}$$

where d was the total amount of CO_2 released at the end of the fermentation (*t.end*), α was a shape parameter and b was a parameter that gave information on the presence ($b > 1$), or absence ($b < 1$) of an inflection point in the curve (Figure S1). Based on common fermentation knowledge, we applied two additional constraints to the model: $d \le 93$ $g.L^{-1}$ and $b > 1$.

A grid of values was first assigned to t_{0i}. For each t_{0i} value, the parameters d_i, a_i and b_i were estimated from nonlinear least squares, through an iterative procedure by using nls2 R

package (Huet 1996). At the end, the set of four parameters $\left(\hat{t}_{0i}, \hat{d}_i, \hat{\alpha}_i, \hat{b}_i\right)$ minimizing the residual sum of squares between Y_{it} and $f\left(t, \hat{t}_{0i}, \hat{d}_i, \hat{\alpha}_i, \hat{b}_i\right)$ was selected. The homogeneity and independence of the residues was checked by pooling all fermentations and plotting the residues against the fitted values (Figure S2). The standardized residuals ranged between – 3 and + 3 indicating that the model fitted well the data. The structure found for the residuals indicated that the amount of CO_2 computed from the model underestimated or overestimated the amount of CO_2 released, depending on the progress of the fermentation. A better fit could have been obtained at the price of an increase of the number of parameters. Since the number of data points was not large enough, we chose to keep the Weibull model for the CO_2 release after the lag time. Because all strains were treated in the same way, this did not impair the conclusions of the work.

Seven fermentative traits were computed from the model (Figure 2A and 2B):

- *t.lag* (h) = $\hat{t}_0$, the fermentation lag-phase that is the time between inoculation and the beginning of CO_2 release (when the CO_2 production rate was higher than 0.05 $g.L^{-1}.h^{-1}$);

- $t.V_{max}$ (h) was the time to reach the inflexion point, out of the fermentation lag phase;

- *t.45* (h) was the fermentation time at which 45 $g.L^{-1}$ of CO_2 was released, out of the fermentation lag phase;

- *t.75* (h) was the fermentation time at which 75 $g.L^{-1}$ of CO_2 was released, out of the fermentation lag phase;

- *AFtime* (Alcoholic Fermentation time, h) was the time between *t.lag* and the time at which the CO_2 emission rate became less than, or equal to, 0.05 $g.L^{-1}.h^{-1}$;

- V_{max} ($g.L^{-1}.h^{-1}$) was the value of the first derivative of the Weibull function *f*, at $t.V_{max}$ (h), ($f' = \alpha db * (t.V_{max} - t_0)^{b-1} * \exp[-\alpha (t.V_{max} - t_0)^b]$), and corresponded to the maximum CO_2 released rate;

- CO_{2max} ($g.L^{-1}$) = $\hat{d}$, the total amount of CO_2 released at the end of the fermentation.

- *LIFE-HISTORY TRAITS*

The monitoring of population growth, cell size and viability was performed using a cytometer FC500 MPL. Samples were collected regularly and were filtered before flow cytometry analysis (10 µm disposable filters, CellTric, Partec). Samples were diluted with McIlvaine buffer pH 4 (0.1 M citric acid, 0.2 M sodium phosphate dibasic) added with propidium iodide (0.3 % v/v) in order to stain dead cells, and dilution was adapted to reach a flow rate lower than 2500 particules/sec. Fluorescent beads (Cell Counter, Beckman Coulter) were used to normalize the quantification of cellular concentration. Eight life-history traits (Figure 2C) were considered:

Cell growth parameters

Experimental measurements of the logarithm of cell concentration (Z_{it}) for each alcoholic fermentation i at time t, was modeled as: $Z_{it} = g\ (t,\ t_{Ni},\ t_{N\max i},\ r_i,\ m_i,\ I_i,\ C_i) + \varepsilon_{it}$, with $\{\varepsilon_{it}\}$ i.i.d. $\sim$ N $(0,\ \sigma_{g,i}^2)$ and where g was a discontinuous function of time. We assumed that after a lag-time of duration t_N, each cell population grew exponentially and reached its carrying capacity at time $t_{N\max}$. Then, the population size could either stay constant (reduced model R) or change exponentially at a different rate due to mortality (full model F). Under model F, the function g writes:

$$\begin{cases} t \le tN: & g\ (t, tN, tN_{\max}, r, m, I, C) = I \\ tN < t < tN_{\max}: & g\ (t, tN, tN_{\max}, r, m, I, C) = I + r\ (t - tN) \\ t \ge tN_{\max}: & g\ (t, tN, tN_{\max}, r, m, I, C) = I + r\ (tN_{\max} - tN) + C + m\ (t - tN_{\max}) \end{cases}$$

where tN (h) was the lag-time, $tN_{\max}$ (h) was the time to reach the carrying capacity, I (log[cells/mL]) was the initial cell concentration, r (logarithm of the number of cell divisions *per* hour) was the growth rate, m was the growth rate after $tN_{\max}$, and C (log[cells/mL]) was a parameter which accounted for the possible lack of experimental points around $tN_{\max}$.

For each model, a grid of values was first assigned to tN_i (model R) or to the couple $(tN_i,\ tN_{\max i})$ (model F). For each model and each possible values, parameters r_i, m_i, I_i and C_i, were obtained from segmented linear regression using lm and home-written code in R-software. The different models were then compared using the Akaike Information Criterion (AIC) and the best model was chosen. Notice that under model R, $m_i = C_i = 0$ and $t.N_{\max}$ varied between tN and 378 h which corresponded to the latest experimental time point among all realized fermentations. The homogeneity and independence of the residuals were checked by pooling all fermentations and plotting the residuals against the fitted values. Five population dynamics traits were then derived from the model's parameters (Figure 2C):

- $t.N_0$ (h) = $\hat{t}N$, the growth lag-phase (time between inoculation and the beginning of population growth);

- $t.N_{\max}$ (h) = $\hat{t}N_{\max}$, the time at which the carrying capacity K was reached;

- r (logarithm of the number of cell divisions *per* mL *per* hour) was the intrinsic growth rate;

- K (log[cells/mL]) was the carrying capacity computed as: $K = I + r\ (t.N_{\max} - t.lag) + C$.

Maximum CO_2 production rate per *cell*

For each alcoholic fermentation, the maximum CO_2 specific flux, $J_{\max}$ (g.h^{-1}.10^{-8} cell^{-1}), was computed as the maximum value of the ratio $f\left(t, \hat{t}_{0i}, \hat{d}_i, \hat{\alpha}_i, \hat{b}_i\right) \Big/ g\left(t, \hat{t}N_i, \hat{t}N_{\max i}, \hat{r}_i, \hat{m}_i, \hat{I}_i, \hat{C}_i\right)$, the estimated CO_2 production rate divided by the estimated cell concentration.

Cell size and viability traits

Experimental measurements of cell size (W_{it}) for each alcoholic fermentation i at time t were assumed to follow a linear model: $W_{it} = H + w.t + \varepsilon_{it}$, $\{\varepsilon_{it}\}$ i.i.d. $\sim$ N (0, $\sigma_{w,i}^2$) between $t.N_{max}$ and the time at which 93 % of the total amount of CO_2 was released. All the data points outside this time range were discarded for the following reasons: (i) cell size tended to increase before $t.N_{max}$ but we did not have enough experimental data points before tN_{max} to fit such a model; (i) cells tended to flocculate at the end of the fermentation and we observed that the data were no more reliable beyond about 93 % of total amount of CO_2 released. Again, after fitting the model, the homogeneity and independence of the residuals was checked by pooling all fermentations and plotting the residuals against the fitted values.

From the model's parameters, we estimated $Size.t.N_{max}$ (µm), the average cell size at $t.N_{max}$. The same model was used for estimating the percentage of living cells (V_{it}), and two viability traits were computed:

- *Viability*.$t.N_{max}$, the percentage of living cells at $t.N_{max}$

- *Viability.t.75*, the percentage of living cells at *t.75*.

- BASIC ENOLOGICAL PARAMETERS (BEP)

At the end of the fermentation, six Basic Enological Parameters (BEP) were quantified: *Residual.Sugar* ($g.L^{-1}$), *Ethanol* (%vol), *Sugar.Ethanol.Yield* ($g.L^{-1}.\%vol^{-1}$) (ratio between the amount of metabolized sugar and the amount of released ethanol), *Acetic.acid* ($g.L^{-1}$ H_2SO_4), *Total.SO*$_2$ and *Free.SO*$_2$ ($mg.L^{-1}$). *Residual.Sugar* and *Ethanol* were measured by infrared reflectance using an Infra-Analyzer 450 (Technicon, Plaisir, France). For some strains, *Residual.Sugar* was below the threshold of detection. In these cases, instead of inferring the value "0", which is not biologically realistic, we used the value: $(x/1.05) + y$, where x is the lowest value measured in the whole data and y is a value drawn in a uniform distribution $\sim$ U(0, 0.001). *Acetic.acid* was quantified by colorimetry (*A*460) in continuous flux (Sanimat, Montauban, France). *Total.SO*$_2$ and *Free.SO*$_2$ were assayed by Pararosaniline titration [31].

- AROMATIC TRAITS (AT)

The aromatic profile of fermenting yeast was estimated by quantifying 14 aromatic traits (AT). The main volatile compounds were measured at the end of the alcoholic fermentation by GC-MS. For esters, higher alcohols and volatile acids, HSSE HeadSpace Sorptive Extraction followed by GC-MS analysis was used according to Weldegergis (2007). For volatile thiols, a specific extraction was performed according to Tominaga (1998). These analytical methods allowed us to detect up to 22 compounds in the analyzed samples (suppl. Table S2). However only 13 of them were quantified in a sufficient number of samples and were retained after statistical analysis. These compounds were two superior alcohols (*Phenylethanol*, *Hexanol*, $mg.L^{-1}$), seven esters (*Phenylethanol.acetate*, *Isoamyl.acetate*,

Ethyl.propanoate, Ethyl.butanoate, Ethyl.hexanoate, Ethyl.octanoate, Ethyl.decanoate, mg.L–1), three medium chain fatty acids (Hexanoic.acid, Octanoic.acid, Decanoic.acid, mg.L–1) and one volatile thiol (*4-methyl-4-mercaptopentan-2-one*, or *X4MMP*, ng.L^{-1}). For *Ethyl.decanoate* and *Ethyl.octanoate*, which were sometimes below the threshold of detection, we proceeded as described above for *Residual.Sugar*. Finally the *Acetate.Ratio*, ratio between *Phenylethanol.acetate* and *Phenylethanol*, was computed. This parameter represents the acetylation ratio of higher alcohols.

DATA ANALYSIS

- *SINGLE-TRAIT ANALYSES*

For each of the 35 traits collected, the effects of the strain, of the temperature and of the strain-by-temperature interaction, as well as the random block effect, were estimated through the following mixed model of analysis of variance (R program, *lme4* package (Computing, Austria)):

$$Y_{ijk} = m + s_i + temp_j + (s*temp)_{ij} + W_k + E_{ijk}$$

where Y_{ijk} was the value of the trait for strain i (i = 1, ..., 66) at temperature j (j = 1, 2) obtained the week k (k = 1, ..., 22), m was the overall mean, s_i was the fixed strain effect, $temp_j$ was the fixed temperature effect, $(s*temp)_{ij}$ was the interaction effect between temperature and strain and W_k was the random block effect. For each trait, normality of residual distributions and the homogeneity of the variances were checked. Some of them displayed heteroscedasticity, which decreased the power of the ANOVA. This was due to strains with weak fermentation abilities ($t.lag$ > 40 h, $t.V_{max}$ > 20h, CO_{2max} < 88 g.L^{-1} and/or $t.75$ > 110 h). The predicted means $\hat{Y}_{ij} = m + s_i + temp_j + (s*temp)_{ij}$ were computed from the model's parameters, as well as their standard deviations. The decomposition of the total phenotypic variance of each trait into its genetic and environmental components was computed after correction for the random block effects. Multiple non parametric comparisons were carried out using *nparcomp* package of the R program with adjusted *p*-values [33].

- *MULTI-TRAIT ANALYSES*

To get a general overview of the data, a Principal Component Analysis (PCA) was performed on the ANOVA predicted means for each temperature-strain combination (R program, *ade4* package (Dufour 2007)). In addition, a multivariate analysis of variance was realized (MANOVA, R program) with the six combinations "hybrid type – temperature" as factor: "*S. cerevisiae*: 18", "*S. uvarum*: 18", *S. cerevisiae* * *S. uvarum*: 18", "*S. cerevisiae*: 26", "*S. uvarum*: 26" and "*S. cerevisiae* * *S. uvarum*: 26". A Linear Discriminant Analysis (LDA, R program, *ade4* package (Dufour 2007)) was performed to represent the MANOVA results. The parental strains were added as supplementary individuals, for both PCA and LDA.

RESULTS

LARGE-SCALE PHENOTYPING OF A COMPLETE YEAST DIALLEL UNDER WINEMAKING CONDITIONS

Eleven parental strains (seven strains of *S. cerevisiae* and four strains of *S. uvarum*, Table 1) and their 55 intra- and inter-specific hybrids were phenotyped under enological conditions, at two temperatures (26 °C, favorable for *S. cerevisiae*, and 18 °C, favorable for *S. uvarum*), in three replicates (Figure 1 and 2). A total of 396 alcoholic fermentations were performed (66 strains * 2 temperatures * 3 replicates). A few fermentations (26) were discarded due to incomplete or absent implantation of the expected strain. The alcoholic fermentations were characterized in depth through 35 phenotypic traits, leading to almost 13 000 numerical data (35 * 370) for all the fermentations. The traits were classified into four categories (Table 2): (i) Seven fermentation kinetics (FK) parameters: *t.lag*, *t.*V_{max}, *t.45*, *t.75*, *AFtime*, V_{max}, CO_{2max}; (ii) Eight life-history traits (LHT): *t.*N_0, *t.*N_{max}, *r*, *K*, J_{max} (growth traits), *Size.t.*N_{max}, *Viability.t.*N_{max}, *Viability.t.75* (size and viability traits); (iii) Six basic enological parameters (BEP): *Residual.Sugar*, *Ethanol*, *Sugar.Ethanol.Yield, Acetic.acid*, *Total.*SO_2 and *Free.*SO_2; (iv) Fourteen aromatic traits (AT): *Phenylethanol*, *Hexanol*, *Phenylethanol.acetate*, *Isoamyl.acetate*, *Ethyl.propanoate*, *Ethyl.butanoate*, *Ethyl.hexanoate*, *Ethyl.octanoate*, *Ethyl.decanoate*, *Hexanoic.acid*, *Octanoic.acid*, *Decanoic.acid*, *X4MMP (4-methyl-4-mercaptopentan-2-one*) and *Acetate.Ratio*, the acetylation rate of higher alcohols.

THE SOURCES OF PHENOTYPIC VARIATION DIFFER ACCORDING TO TRAIT CATEGORIES

The sources of variation of each phenotypic trait were studied by analyses of variance (ANOVA) to estimate the *Strain*, *Temperature*, and *Strain*Temperature* interaction effects (Table 2). The part of phenotypic variation explained by the model (block effect removed) depended on the trait category, with Fermentation Kinetics parameters (FK) showing the highest R^2 values (0.60 to 0.92) and Aromatic Traits (AT) the smallest (0.09 to 0.66). All the traits but three (*Isoamyl.acetate*, *Ethyl.butanoate* and *Ethyl.octanoate*) displayed a significant *Strain* effect, accounting for 11 to 67% of the variance explained (p-value < 0.05). The temperature had contrasted effects according to the trait category: the ten traits for which temperature explained at least 10% of the model variance were mainly found in the Fermentation Kinetics (FK) and Life-history Traits (LHT) categories, with R^2 values up to 79%: *t.45*, *AFtime*, V_{max}, *t.75* and *t.lag* (FK), *r*, J_{max} and *t.*N_{max} (LHT), *Acetic.acid* (BEP), and *Hexanol* (AT). Finally highly significant *Strain*Temperature* interactions were found for CO_{2max}, *t.lag* and *t.*V_{max} (FK), *t.*N_0, *K*, *Size.t.*N_{max} and *Viability.t.75* (LHT), *Ethanol*, *Residual.Sugar* and *Sugar.Ethanol.Yield* (BEP) and *Acetate ratio* (AT).

Overall, FK traits display *Strain* effects and large *Temperature* effects, and in a lesser extent *Strain*temperature* interactions (except for CO_{2max} with $R^2 = 0.32$), LHT traits have *Strain* and *Strain*temperature* effects but also high *Temperature* effects for *r* and *t.*N_{max}, BEP traits

have both *Strain* and *Strain*temperature* effects with almost no effect of temperature, and finally AT traits have almost exclusively *Strain* effects.

COMPARING FERMENTATION PHENOTYPES BETWEEN *S. CEREVISIAE*, *S. UVARUM* AND INTERSPECIFIC HYBRIDS

For each trait*temperature combination, we compared three means using non-parametric tests ($\alpha = 0.05$): the mean of the *S. cerevisiae* strains (parental and intraspecific hybrids), the mean of the *S. uvarum* strains (parental and intraspecific hybrids) and the mean of the interspecific hybrids. In 42 cases out of 70 (2 temperatures x 35 traits), at least one mean was significantly different from the others (Figures S4 and S5). For 12 traits, a difference was observed at both temperatures, for 5 traits at 18 °C only and for 12 traits at 26 °C only. Some of the variable traits were relevant for winemaking. For instance *Phenylethanol* and its acetate, which have been widely described as being contrasted between *S. cerevisiae* and *S. uvarum* (Antonelli et al. 1999, Masneuf-Pomarède et al. 2010, Gamero et al. 2013), are indeed found very different between the two species at 18 °C as well as at 26 °C (Figure 3A1 and A2). Moreover there was a significant *Species*Temperature* interaction for these compounds (2 % and 6 % of variance explained for *Phenylethanol* and *Phenylethanol.acetate*, respectively): their concentration was significantly lower at 26 °C than at 18 °C in the *S. uvarum* group but neither in the *S. cerevisiae* group nor in the interspecific hybrids. As a consequence the interspecific hybrids are intermediary between parental species at 18 °C and close to the *S. uvarum* group at 26 °C. Another striking difference between groups was the yield of alcoholic fermentation, a key parameter in winemaking industry because strains with high *Sugar.Ethanol.Yield* are required to reduce ethanol content in wine (Aguera et al. 2010, Schmidtke et al. 2012, Tilloy et al. 2014). At 18 °C, the *S. uvarum* group and the interspecific hybrids required respectively 0.56 and 0.35 g/L more sugar than the *S. cerevisiae* for producing 1% vol. of ethanol (Figure 3B). This species discrepancy was highly significant and showed a slight *Species*Temperature* interaction with a reduced difference between species at 26 °C as compared to 18 °C. The production levels of several ethyl-esters, which positively impact wine quality by conferring fruity notes (Sumby et al. 2010), was higher in interspecific hybrids than in either parental species at both temperatures. This effect was illustrated by summing the concentrations of all the ethyl-esters Figure 3C. At 26 °C the interspecific hybrids produced less acetic acid than the parental species, consistent with previous results (Caridi et al. 2002, Masneuf-Pomarède et al. 2010). Finally the production of 4MMP was significantly lower in the *S. cerevisiae* group than in the two other groups, as already reported (Masneuf et al. 2002).

Beyond these particular traits, the inspection of the differences between the three groups for all trait*temperature combinations makes it difficult to draw general conclusions regarding species differences and effects of hybridization (Figures S4 and S5). Therefore we performed a Principal Component Analysis (PCA) in order to get a multi-trait representation of the strains.

HYBRIDIZATION RESHAPES THE MULTI-TRAIT PHENOTYPES AND INCREASES HOMEOSTASIS

It is difficult to capture the consequences of hybridization by analyzing the data variable by variable with a so high number of variables that are more or less correlated. For that reason we performed principal component analyses (PCA) with the entire data file. The first PCA axis (PCA1) accounted for 20 % of the total variance and clearly separated the strains according to the fermentation temperature (Figure 4A1). As expected, the first axis was mainly explained by traits showing a large temperature effect in the ANOVA (p-value < 0.0001) (Figure 4A2). All the FK time traits (*t.lag*, *t.75, t.45*, $t.N_{max}$) had low values at 26 °C, which reduced the alcoholic fermentation time (*AFtime*). These traits were strongly correlated with each other (Figure S3), which explained the major temperature effect seen on axis 1. The first axis was also clearly explained by V_{max} and r, two traits with highly significant temperature effects, and in a lesser extent by K, J_{max}, *Acetic.acid*, *hexanol*, $t.N_0$ and three ethyl esters. The second and the third axes of the PCA (Figure 4A and 4B) accounted for 13 % and 12 % of the total inertia, respectively, and clearly separated the data according to the strain type (*S. cerevisiae*, *S. uvarum* and interspecific hybrids). The cloud of the 28 interspecific hybrids (green points) had roughly an intermediate position between the groups of the parental species. Volatile compounds such as *Phenylethanol* and *Phenylethanol.acetate*, as well as most of ethyl esters (AT traits), largely contributed to discriminate the *S. uvarum* and *S. cerevisiae* groups, which is consistent with their large R^2 for *Strain* effects. Life-history traits such as cell size and viability also contributed to separate the species. *S. uvarum* strains had smaller cell size and a lower viability than *S. cerevisiae* strains. Note the negative correlation between carrying capacity K and cell size (Figure S3), previously reported in various studies (Spor et al. 2008, Albertin et al. 2011), which is here confirmed in a multi-species context. Finally, three basic enological parameters, *Ethanol*, *Residual.Sugar* and *Sugar.Ethanol.Yield*, and one FK trait, CO_{2max}, also discriminated the species. Thus both ANOVAs and PCA showed that genetic and environmental variations did not affect in the same way the different trait categories. Temperature strongly influenced fermentation kinetics and life-history traits, while fermentation byproducts (AT and BEP) were mainly influenced by strain origin.

In order to assess the extent to which the multi-trait phenotype of the hybrids is intermediate between the multi-trait phenotypes of the parents, we performed another PCA including in the data file the *in silico* hybrids assuming additive inheritance for all traits. The first discriminating plan accounted for 38 % of variance (Figure 4C). As expected, whatever the temperature, the intraspecific *in silico* hybrids perfectly overlapped the groups of their respective parental strains, and the interspecific *in silico* hybrids were in an intermediary position between the two species groups. But interestingly, the *in vivo* hybrids did no overlap the *in silico* hybrids, which meant that both intra- and interspecific hybridizations created original multi-trait phenotypes that are not the "average" of their parents. In addition, the shift tended to decrease the temperature effect, since the dispersion of the groups on the first axis

was lower for the *in vivo* hybrids than for the *in silico* hybrids, suggesting partial homeostasis resulting from hybridization.

THE THREE GROUPS OF HYBRIDS ARE NOT SIMILARLY AFFECTED BY TEMPERATURE

To investigate more specifically which combination of traits allowed distinguishing between types of hybrids (intraspecific hybrids of *S. cerevisiae* and *S. uvarum* and interspecific hybrids), a multivariate analysis of variance was performed (MANOVA). Six groups defined by the combination "species-temperature" were used to compute the discriminant function: "*S. cerevisiae*-18", "*S. cerevisiae***S. uvarum*-18", "*S. uvarum*-18", "*S. cerevisiae*-26", "*S. cerevisiae***S. uvarum*-26" and "*S. uvarum*-26". According to the Pillai's criterion, the group effect was highly significant (p-value = 2.2 x 10^{-16}). The robustness of the classification was then checked using a cross-validation: after a phase of training, the *a posteriori* probability of allocating successfully the hybrids to their respective groups was estimated between 68 % and 78 % at 18 °C and between 68 % and 81 % at 26 °C (Table 3). To illustrate the MANOVA results, a Linear Discriminant Analysis (LDA) was performed. The first axis (Discriminant Axis 1, 62 % of the inertia) separated the hybrids according to their growth temperature (Figure 5A). As expected, this axis was mainly explained by the variation of traits showing high temperature effects in the ANOVA and PCA (p-value < 0.001): five FK traits (*t.45, AFtime,* V_{max}*, t.75* and *t.lag*), one BEP trait (*Actetic.acid*), three LHT traits (*r,* $t.N_{max}$ and J_{max}) and two AT traits (*Ethyl.propanoate* and *Hexanol*) (Figure 5C). In addition, we observed that *S. uvarum* intraspecific hybrids were better separated according to their growth temperature than interspecific hybrids and *S. cerevisiae* intraspecific hybrids, illustrating *Strain*Temperature* interaction effects. The second axis (Discriminant Axis 2, 23 % of the inertia) separated *S. cerevisiae* intraspecific hybrids (*S.c*S.c*) from *S. uvarum* intraspecific hybrids (*S.u*S.u*) better at 18 °C than at 26 °C, which again exemplifies *Strain*Temperature* interaction. Interspecific hybrids *S.c*S.u* were intermediate. Accordingly this axis was significantly explained (p-value < 0.001) by traits that all had both *Strain* effects and *Strain*Temperature* interaction effects, with no or negligible *Temperature* effects: $t.V_{max}$ (FK), *Sugar.Ethanol.Yield* and *Ethanol* (BEP), $Size.t.N_{max}$ (LHT) and *Phenylethanol.acetate*, *Phenylethanol Hexanoic.acid* and *Octanoic.acid* (AT) (Figure 5C).

The discriminant axes 2 and 3, which represents 32 % of the inertia, highlighted that the interspecific hybrids, unlike the intraspecific hybrids, were very close to each other at 18 °C and 26 °C (Figure 5B). In addition to the traits already mentioned, these axes were also significantly explained by two end-products, namely CO_{2max} and *Residual.Sugar* (Figure 5C), which also displayed high *Strain*temperature* interaction effects. This homeostasis of interspecific hybrids regarding temperature could also be illustrated by the projection of the strains (including parental strains) on the LDA axes (Figure 6), which clearly showed that the average behavior of the interspecific population at 18 °C and at 26 °C was similar, while parental and intraspecific hybrid strains had not the same average value at the two temperatures.

DISCUSSION

DIALLEL DESIGN

In this study, a diallel design of 55 newly synthetized hybrids was obtained from 11 parental strains belonging to the two main species involved in grape juice fermentation, *S. cerevisiae* and *S. uvarum*. This kind of genetic design has been widely used in plant and animal breeding to analyze the genetic bases of complex traits and identifying heterotic groups (Crusio et al. 1984, Hallauer A.R. and Miranda Filho J.B. 1988). In yeast, a diallel design has been recently developed by different authors using the collection of yeast strains sequenced by Liti et al. 2009 (Timberlake et al. 2011, Plech et al. 2014, Shapira et al. 2014, Zörgö et al. 2012). Our design included for the first time interspecific hybrids, allowing us to investigate possible synergies between the genomes of *S. cerevisiae* and *S. uvarum*. From 370 controlled fermentation experiments at two temperatures in a natural grape juice (Sauvignon blanc), we measured or estimated through sophisticated mathematical models various fermentation kinetics parameters, life-history traits and a series of metabolites including wine aromatic compounds such as esters and volatile thiols, resulting in about 13 000 data points for 35 phenotypic traits.

INTERSPECIFIC HYBRIDIZATION BETWEEN *S. CEREVISIAE* AND *S. UVARUM* STRAINS PROVIDES A NEW TYPE OF YEAST WITH MANY SUITABLE TRAITS FOR WINEMAKING

Multivariate analysis clearly showed that interspecific hybrids can be separated from *S. cerevisiae* and *S. uvarum* strains (Figure 4B) mostly by aromatic traits and other parameters crucial for enology (AT and BEP groups). Hybridization between *S. cerevisiae* and *S. uvarum* strongly reshapes the production of several secondary metabolites in interspecific hybrids (Figure S3 and S4). This finding was previously reported for glycerol (Caridi et al. 2002), acetic acid (Caridi et al. 2002, Masneuf-Pomarède et al. 2010), volatile thiols (*4MMP*) (Masneuf et al. 2002) and higher alcohols like phenylethanol (Antonelli te al. 1999, Masneuf-Pomarède et al. 2010). Except for glycerol that was not assayed here, these discrepancies were confirmed for a large set of hybrids. At 26 °C, the interspecific hybrids produced less acetic acid than the parental species, which can prove to be useful for wine yeast selection (Vilela-Moura et al. 2008). Interestingly, at the same temperature the 4MMP production was three fold higher in interspecific hybrids and *S. uvarum* group than in *S. cerevisiae* group. This could be explained by the inheritance of *S. uvarum Irc7p* allele encoding a cystathionin β-lyase able to cleave efficiently the cysteinylated precursor of this compound (Roncoroni et al. 2011, Dufour et al. 2013). The production of phenylethanol and its acetate in the interspecific hybrids confirmed to be intermediate between the parental species (Masneuf et al. 2002). The high level of these molecules is a major characteristic of *S. uvarum* species and could be due to the more active shikimate and phenylalanine pathways found in this species (Blein-Nicolas et al. 2013, Lopez-Malo et al. 2013). Interestingly

interspecific hybrids produced lower amount of this compounds than *S. uvarum*. In wine these compounds may mask more subtle fragrance (Masneuf et al. 2002), so their moderate production during alcoholic fermentation is interesting.

Beside these already described features, our data provide new interesting results. First, interspecific hybrids display a much higher production of ethyl esters (2.45 folds) than pure species at both temperatures. The production of ethyl esters can be dependent on two factors. (i) The availability of short and medium chain fatty acid which depends on the must composition, the fermentation temperature (Torija et al. 2003, Beltran et al. 2008) and the fermenting species (Torija et al. 2003); (ii) The esterification of these fatty acids with ethanol is mediated by specific ethyl esterases (Eeb1p, Eht1p, Ymr210p) (Mason et al. 2000, Scherens et al. 2006). The high production of ethyl esters in interspecific hybrids could result from the combination of both factors. Interestingly, *S. uvarum* and *S. cerevisiae* show large differences in lipid metabolism (Lopez-Malo et al. 2013) with a higher level of Medium Chain Fatty Acids (MCFA) production in *S. uvarum* (Torija et al. 2003), likely due to a more active fatty acid pathway (Blein-Nicolas et al. 2013). Conversely a recent gene expression survey on the brewery yeast *S. pastorianus* demonstrated that the allele of the major ethyl esterase *Eeb1p* was much more expressed in *S. cerevisiae* than in *S. eubayanus* (He et al. 2014). This could indirectly suggest that *S. cerevisiae* might produce more esterase than *S. uvarum*, a species closely related to *S. eubayanus* (Libkind et al. 2011). A second interesting result was the higher sugar/ethanol yield found in both interspecific hybrids and *S. uvarum* strains as compared to *S. cerevisiae* strains. To date the natural intraspecific variation among *S. cerevisiae* strains was very low for this trait (Marullo et al. 2006, Albertin et al. 2011, Camarasa et al 2011). Due to the continuous increasing level of ethanol in wines, the sugar/ethanol yield is becoming an important trait for wine yeast selection (Tilloy et al. 2014). Recent works demonstrated that *S. uvarum* and *S. kudriavzevii* species have an sugar/ ethanol yield higher than the one of *S. cerevisiae*, especially at low temperature (Arroyo-Lopez et al. 2009, Masneuf-Pomarède et al. 2010). However these species are susceptible to high ethanol content and elevated temperature and are not adapted to harsh fermentation conditions. Additional investigations with higher sugar concentrations confirm that some of these hybrids can reduce the ethanol content in wine up to 0.4 % without excessive production of acetic acid (Bely et al. 2013).

INTRA- AND INTERSPECIFIC YEAST HYBRIDS HARBOR PHENOTYPIC HOMEOSTASIS

The temperature had a major effect on many variables, particularly on the fermentation kinetics traits. However we showed that both intra- and interspecific hybrids were more robust with respect to temperature than the parental strains. This phenomenon was not detected using variable-by-variable tests (not shown), but emerged only from multivariate analyses, PCA and LDA. This means that, even though a hybrid strain is affected by temperature for various individual traits, it is more stable than its parents in the multidimensional space of the 35 variables.

Homeostasis is particularly interesting from an evolutionary viewpoint. The fitness is typically a multi-trait property, and hybrids with robust fitness may have higher chance to colonize winemaking environments. Alternatively, homeostasis for Basic Enological Parameters, Fermentation Kinetics and Aromatic Traits may have been selected by human for winemaking, allowing the dissemination of strains having quite stable phenotypes over temperature changes. Conscious or unconscious anthropic selection may explain why intra- and inter-specific hybridization is so frequent in yeast. Indeed, numerous natural hybrids were described associated with enology (Lopandic et al. 2007, Belloch et al. 2008, Capello et al. 2010, Borneman et al. 2012), but also with other bioprocesses producing alcoholic beverages (beer, cider, etc.) (Masneuf et al 1998, Libkind et al. 2011, Nguyen et al. 2011). Interestingly, besides homeostasis, interspecific hybrids showed global heterosis for a few characters. Such transgressive phenotypes, associated with global homeostasis, could explain the prevalence of hybridization in natural or domesticated yeast.

ACKNOWLEDGMENTS

This work was supported by the project ANR-08-ALIA-09 HeterosYeast, the regional grants Aquitaine technological transfert “Cytoviable” and “Sagesse”.

REFERENCES

Albertin W., Marullo P., Aigle M., Bourgais A. et al. (2009) Evidence for autotetraploidy associated with reproductive isolation in *Saccharomyces cerevisiae*: towards a new domesticated species. J Evol Biol 22: p.2157-2170.

Albertin W., Marullo P., Aigle M., Dillmann C. et al. (2011) Population size drives industrial *Saccharomyces cerevisiae* alcoholic fermentation and is under genetic control. Appl Environ Microbiol 77: p.2772-2784.

Albertin W., da Silva T., Rigoulet M., Salin B., Masneuf-Pomarede I. et al. (2013) The Mitochondrial Genome Impacts Respiration but Not Fermentation in Interspecific *Saccharomyces* Hybrids. PLoS ONE 8: e75121.

Aguera E, Bes M., Roy A., Camarasa C. and Sablayrolles J.M. (2010) Partial removal of ethanol during fermentation to obtain reduced-alcohol wines. American Journal of Enology and Viticulture 61: p.53-60.

Antonelli A., Castellari L., Zambonelli C. and Carnacini A. (1999) Yeast influence on volatile composition of wines. J Agric Food Chem 47: p.1139-1144.

Antunovics Z., Irinyi L. and Sipiczki M. (2005) Combined application of methods to taxonomic identification of Saccharomyces strains in fermenting botrytized grape must. J Appl Microbiol 98: p.971-979.

Arroyo-Lopez F.N., Orlic S., Querol A. and Barrio E. (2009) Effects of temperature, pH and sugar concentration on the growth parameters of Saccharomyces cerevisiae, S. kudriavzevii and their interspecific hybrid. Int J Food Microbiol 131: p.120-127.

Arroyo-Lopez F.N., Perez-Torrado R., Querol A. and Barrio E (2010) Modulation of the glycerol and ethanol syntheses in the yeast Saccharomyces kudriavzevii differs from that exhibited by Saccharomyces cerevisiae and their hybrid. Food Microbiol 27: p.628-637.

Baack E.J., Whitney K.D. and Rieseberg L.H. (2005) Hybridization and genome size evolution: timing and magnitude of nuclear DNA content increases in *Helianthus* homoploid hybrid species. New Phytologist 167: p.623-630.

Belloch C., Orlic S., Barrio E. and Querol A. (2008) Fermentative stress adaptation of hybrids within the *Saccharomyces* sensu stricto complex. Int J Food Microbiol 122: p.188-195.

Belloch C., Perez-Torrado R., Gonzalez S.S., Perez-Ortin J.E. et al. (2009) Chimeric genomes of natural hybrids of *Saccharomyces cerevisiae* and *Saccharomyces kudriavzevii*. Appl Environ Microbiol 75: p.2534-2544.

Beltran G., Novo M., Guillamon J.M., Mas A. and Rozes N. (2008) Effect of fermentation temperature and culture media on the yeast lipid composition and wine volatile compounds. International Journal of Food Microbiology 121: p.169-177.

Bely M., Renault P., da Silva T., Masneuf-Pomarède I. et al. (2013) Non conventional yeasts and alcohol levels reduction. Bordeaux.

Blein-Nicolas M., Albertin W., Valot B., Marullo P. et al. (2013) Yeast proteome variations reveal different adaptative responses to grape must fermentation. Mol Biol Evol 30: p.1368-1383.

Bon E., Neuveglise C., Casaregola S., Artiguenave F. et al. (2000) Genomic exploration of the hemiascomycetous yeasts: 5. *Saccharomyces bayanus var. uvarum*. FEBS Lett 487: p.37-41.

Borneman A.R., Desany B.A., Riches D., Affourtit J.P. et al. (2012) The genome sequence of the wine yeast VIN7 reveals an allotriploid hybrid genome with *Saccharomyces cerevisiae* and *Saccharomyces kudriavzevii* origins. FEMS Yeast Res 12: p.88-96.

Camarasa C., Sanchez I., Brial P., Bigey F. and Dequin S. (2011) Phenotypic Landscape of *Saccharomyces cerevisiae* during Wine Fermentation: Evidence for Origin-Dependent Metabolic Traits. PLoS ONE 6: e25147.

Caridi A., Cufari A. and Ramondino D. (2002) Winemaking from gaglioppo grapes with hybrid strains of *Saccharomyces*. Folia Microbiologica 47: p.407-408.

Castellari L., Ferruzzi M., Magrini A., Giudici P. et al. (1994) Unbalanced Wine Fermentation by Cryotolerant Vs Non-Cryotolerant *Saccharomyces* Strains. Vitis 33: p.49-52.

Computing. RDCTRRFfS A language and environment for statistical computing. Vienna, Austria.

Coque, M. and Gallais, A. (2008) Genetic variation for N-remobilization and postsilking N-uptake in a set of maize recombinant inbred lines. II. With line *per se* evaluation, comparison with testcross performance. Maydica 53: 29–38.

Crusio W.E., Kerbusch J.M.L. and Abeelen J.H.F. (1984) The replicated diallel cross: A generalized method of analysis. Behavior Genetics 14: p.81-104.

de Barros Lopes M., Bellon J.R., Shirley N.J. and Ganter P.F. (2002) Evidence for multiple interspecific hybridization in *Saccharomyces* sensu stricto species. FEMS Yeast Research 1: p.323-331.

Demuyter C., Lollier M., Legras J.L. and Le Jeune C. (2004) Predominance of *Saccharomyces uvarum* during spontaneous alcoholic fermentation, for three consecutive years, in an Alsatian winery. J Appl Microbiol 97: p.1140-1148.

Dufour SDaAB (2007) The ade4 package: implementing the duality diagram for ecologists. Journal of Statistical Software 22: p.1-20.

Dufour M., Zimmer A., Thibon C. and Marullo P. (2013) Enhancement of volatile thiol release of Saccharomyces cerevisiae strains using molecular breeding. Appl Microbiol Biotechnol 97: p.5893-5905.

Dunn B., Paulish T., Stanbery A., Piotrowski J. et al. (2013) Recurrent Rearrangement during Adaptive Evolution in an Interspecific Yeast Hybrid Suggests a Model for Rapid Introgression. PLoS Genet 9: e1003366..

Erny C., Raoult P., Alais A., Butterlin G. et al. (2012) Ecological success of a group of *Saccharomyces cerevisiae*/*Saccharomyces kudriavzevii* hybrids in the northern european wine-making environment. Appl Environ Microbiol 78: p.3256-3265.

Fischer G., Neuvéglise C., Durrens P., Gaillardin C. and Dujon B (2001) Evolution of Gene Order in the Genomes of Two Related Yeast Species. Genome Research 11: p.2009-2019.

Gamero A., Tronchoni J., Querol A. and Belloch C. (2013) Production of aroma compounds by cryotolerant *Saccharomyces* species and hybrids at low and moderate fermentation temperatures. J Appl Microbiol 114: p.1405-1414.

Gonzalez S.S., Barrio E. and Querol A. (2008) Molecular characterization of new natural hybrids of *Saccharomyces cerevisiae* and *S. kudriavzevii* in brewing. Appl Environ Microbiol 74: p.2314-2320.

Hallauer, A.R. and Miranda, J.B. (1981) Quantitative Genetics in Maize Breeding. Iowa University Press.

He Y., Dong J., Yin H., Chen P. al. (2014) Monitoring of the production of flavour compounds by analysis of the gene transcription involved in higher alcohol and ester formation by the brewer's yeast Saccharomyces pastorianus using a multiplex RT-qPCR assay. Journal of the Institute of Brewing: n/a-n/a.

Jarosz D.F., Taipale M., and Lindquist S. (2010) Protein Homeostasis and the Phenotypic Manifestation of Genetic Diversity: Principles and Mechanisms. Annual Review of Genetics 44: p.189-216.

Leamy L. and Thorpe R.S. (1984) Morphometric studies in inbred and hybrid house mice. Heterosis, homeostasis and heritability of size and shape. Biological Journal of the Linnean Society 22: p.233-241.

Le Jeune C., Lolier M., Demuyter C., Enry C. et al. (2007) Characterization of natural hybrids of Saccharomyces cerevisiae and Saccharomyces bayanus var. uvarum. FEMS Yeast Res 7: p.540-549.

Lerner I.M. (1954) Genetic homeostasis. Edinburgh: Oliver and Boyd.

Libkind D., Hittinger C.T., Valerio E., Goncalves C. et al. (2011) Microbe domestication and the identification of the wild genetic stock of lager-brewing yeast. Proc Natl Acad Sci U S A 108: p.14539-14544.

Liti G., Carter D., Moses A., Warringer J. et al. (2009) Population genomics of domestic and wild yeasts. Nature 458: p.337-341.

Lopandic K., Gangl H., Wallner E., Tscheik G. et al. (2007) Genetically different wine yeasts isolated from Austrian vine-growing regions influence wine aroma differently and contain putative hybrids between *Saccharomyces cerevisiae* and *Saccharomyces kudriavzevii*. FEMS Yeast Res 7: p.953-965.

Lopandic K., Tiefenbrunner W., Gangl H., Mandl K. et al. (2008) Molecular profiling of yeasts isolated during spontaneous fermentations of Austrian wines. FEMS Yeast Res.

Lopes C.A., Barrio E. and Querol A. (2010) Natural hybrids of *S. cerevisiae* x *S. kudriavzevii* share alleles with European wild populations of *Saccharomyces kudriavzevii*. FEMS Yeast Res 10: p;412-421.

Lopez-Malo M., Querol A. and Guillamon J.M. (2013) Metabolomic comparison of Saccharomyces cerevisiae and the cryotolerant species *S. bayanus var. uvarum* and *S. kudriavzevii* during wine fermentation at low temperature. PLoS One 8: e60135.

Mallet J. (2005) Hybridization as an invasion of the genome. Trends Ecol Evol 20: p.229-237.

Marullo P., Bely M., Masneuf-Pomarede I., Pons M. et al. (2006) Breeding strategies for combining fermentative qualities and reducing off-flavor production in a wine yeast model. FEMS Yeast Res 6: p.268-279.

Masneuf I., Hansen J., Groth C., Piskur J. and Dubourdieu D. (1998) New hybrids between *Saccharomyces* sensu stricto yeast species found among wine and cider production strains. Appl Environ Microbiol 64: p.3887-3892.

Masneuf I., Murat M., Naumov G., Tominaga T. and Dubourdieu D. (2002) Hybrids *Saccharomyces cerevisiae x Saccharomyces bayanus var. uvarum* having a high liberating ability of some sulfur varietal aromas of Vitis vinifera Sauvignon blanc wines. Journal international des sciences de la vigne et du vin 36: p.205-212.

Masneuf-Pomarede I., Le Jeune C., Durrens P., Lollier M. al. (2007) Molecular typing of wine yeast strains *Saccharomyces bayanus var. uvarum* using microsatellite markers. Syst Appl Microbiol 30: p.75-82.

Masneuf-Pomarede I., Bely M., Marullo P., Lonvaud-Funel A. and Dubourdieu D. (2010) Reassessment of phenotypic traits for Saccharomyces bayanus var. uvarum wine yeast strains. Int J Food Microbiol 139: p.79-86.

Mason A.B. and Dufour J.P. (2000) Alcohol acetyltransferases and the significance of ester synthesis in yeast. Yeast 16: p.1287-1298.

Meyers L.A. and Bull J.J. (2002) Fighting change with change: adaptive variation in an uncertain world. Trends in Ecology & Evolution 17: p.551-557.

Mino, M. (1980). Hybrid vigor found in some characters of maize seedlings. Jpn J Breed 30: 131–38.

Morales L. and Dujon B. (2012) Evolutionary Role of Interspecies Hybridization and Genetic Exchanges in Yeasts. Microbiology and Molecular Biology Reviews 76: p.721-739.

Naumov G.I. (2000) *Saccharomyces bayanus var. uvarum* comb.nov., a new variety established by genetic analysis. Mikrobiologiia 69: p.410-414.

Naumov G.I., Masneuf I., Naumova E.S., Aigle M. and Dubourdieu D. (2000) Association of *Saccharomyces bayanus var. uvarum* with some French wines: genetic analysis of yeast populations. Res Microbiol 151: p.683-691.

Naumov G.I., Naumova E.S., Antunovics Z. and Sipiczki M. (2002) *Saccharomyces bayanus var. uvarum* in Tokaj wine-making of Slovakia and Hungary. Appl Microbiol Biotechnol 59: p.727-730.

Newman H.H. (1917) On the Production of Monsters by Hybridization. Biological Bulletin 32: p.306-321.

Nguyen H.V., Lepingle A. and Gaillardin C.A. (2000) Molecular typing demonstrates homogeneity of *Saccharomyces uvarum* strains and reveals the existence of hybrids between *S. uvarum* and *S. cerevisiae*, including the *S. bayanus* type strain CBS 380. Syst Appl Microbiol 23: p.71-85.

Nguyen H.V., Legras J.L., Neuveglise C. and Gaillardin C. (2011) Deciphering the hybridisation history leading to the Lager lineage based on the mosaic genomes of *Saccharomyces bayanus* strains NBRC1948 and CBS380. PLoS One 6: e25821.

Peris D., Belloch C., Lopandic K., Alvarez-Perez J.M. et al. (2011) The molecular characterization of new types of *Saccharomyces cerevisiae* x *S. kudriavzevii* hybrid yeasts unveils a high genetic diversity. Yeast.

Peris D., Lopes C.A., Belloch C., Querol A. and Barrio E (2012) Comparative genomics among *Saccharomyces cerevisiae* x *Saccharomyces kudriavzevii* natural hybrid strains isolated from wine and beer reveals different origins. BMC Genomics 13: p.407.

Piotrowski J.S., Nagarajan S., Kroll E., Stanbery A. et al. (2012) Different selective pressures lead to different genomic outcomes as newly-formed hybrid yeasts evolve. BMC Evol Biol 12: p.46.

Plech M., de Visser J.A.G.M. and Korona R. (2014) Heterosis Is Prevalent Among Domesticated but not Wild Strains of *Saccharomyces cerevisiae*. G3: Genes|Genomes|Genetics 4(2): p.315–323.

Rainieri S., Zambonelli C., Tini V., Castellari L. and Giudici P. (1998) The Enological Traits of Thermotolerant *Saccharomyces* Strains. American Journal of Enology and Viticulture 49: p.319-324.

Rainieri S., Zambonelli C., Hallsworth J.E., Pulvirenti A. and Giudici P. (1999) *Saccharomyces uvarum*, a distinct group within *Saccharomyces* sensu stricto. Fems Microbiology Letters 177: p.177-185.

Richards K.D., Goddard M.R. and Gardner R.C. (2009) A database of microsatellite genotypes for *Saccharomyces cerevisiae*. Antonie Van Leeuwenhoek 96: p.355-359.

Roncoroni M., Santiago M., Hooks D.O., Moroney S. et al. (2011) The yeast *IRC7* gene encodes a beta-lyase responsible for production of the varietal thiol 4-mercapto-4-methylpentan-2-one in wine. Food Microbiol 28: p.926-935.

Sarkissian, I.V., Kessinger, M.A. and Harris, W. (1964) Differential rates of development of heterotic and non heterotic young maize seedlings. I. Correlation of differential morphological development with physiological differences in germinating seeds. Proc Natl Acad Sci USA 51: 212–18.

Scherens B., Feller A., Vierendeels F., Messenguy F. and Dubois E. (2006) Identification of direct and indirect targets of the Gln3 and Gat1 activators by transcriptional profiling in response to nitrogen availability in the short and long term. FEMS Yeast Research 6: p.777-791.

Schmidtke L.M., Blackman J.W. and Agboola S.O. (2012) Production Technologies for Reduced Alcoholic Wines. Journal of Food Science 77: R25-R41.

Schnell F.W. and Becker H.C. (1986) Yield and yield stability in a balanced system of widely differing population structures in *Zea mays* L. Plant Breeding 97: p.30-38.

Sebastiani F., Barberio C., Casalone E., Cavalieri D. and Polsinelli M. (2002) Crosses between *Saccharomyces cerevisiae* and *Saccharomyces bayanus* generate fertile hybrids. Res Microbiol 153: p.53-58.

Shapira R., Levy T., Shaked S., Fridman E. and David L. (2014) Extensive heterosis in growth of yeast hybrids is explained by a combination of genetic models. Heredity (Edinb).

Sipiczki M. (2008) Interspecies hybridization and recombination in *Saccharomyces* wine yeasts. FEMS Yeast Res 8: p.996-1007.

Solieri L., Antunez O., Perez-Ortin J.E., Barrio E. and Giudici P (2008) Mitochondrial inheritance and fermentative : oxidative balance in hybrids between *Saccharomyces cerevisiae* and *Saccharomyces uvarum*. Yeast 25: p.485-500.

Spor A., Wang S., Dillmann C., de Vienne D. and Sicard D. (2008) "Ant" and "Grasshopper" Life-History Strategies in *Saccharomyces cerevisiae*. PLoS ONE 3: e1579.

Sprague, G.F. (1983) Heterosis in maize: theory and practice. In: R. Frankel (ed). Heterosis. Reappraisal of Theory and Practice. Springer-Verlag: 47–70.

Sumby K.M., Grbin P.R. and Jiranek V. (2010) Microbial modulation of aromatic esters in wine: current knowledge and future prospects. Food Chemistry 121: p.1-16.

Tilloy V., Ortiz J.A. and Dequin S. (2014) Reducing ethanol and improving glycerol yield by adaptive evolution of Saccharomyces cerevisiae wine yeast under hyperosmotic conditions. Appl Environ Microbiol.

Timberlake W.E., Frizzell M.A., Richards K.D. and Gardner R.C. (2011) A new yeast genetic resource for analysis and breeding. Yeast 28: p.63-80.

Tominaga T., Furrer A., Henry R. and Dubourdieu D. (1998) Identification of new volatile thiols in aroma of Vitis vinifera L. var. Sauvignon blanc wines. Flavour Fragr J 13: p.159-162.

Torija M.J., Beltran G., Novo M., Poblet M. et al. (2003) Effects of fermentation temperature and *Saccharomyces* species on the cell fatty acid composition and presence of volatile compounds in wine. International Journal of Food Microbiology 85: p.127-136.

Torriani S., Zapparoli G. and Suzzi G. (1999) Genetic and phenotypic diversity of *Saccharomyces* sensu stricto strains isolated from Amarone wine. Antonie Van Leeuwenhoek 75: p.207-215.

Tosi E., Azzolini M., Guzzo F. and Zapparoli G. (2009) Evidence of different fermentation behaviours of two indigenous strains of *Saccharomyces cerevisiae* and *Saccharomyces uvarum* isolated from Amarone wine. Journal of Applied Microbiology 107: p.210-218.

Tronchoni J., Gamero A., Arroyo-Lopez F.N., Barrio E. and Querol A (2009) Differences in the glucose and fructose consumption profiles in diverse Saccharomyces wine species and their hybrids during grape juice fermentation. Int J Food Microbiol 134: p.237-243.

Vilela-Moura A., Schuller D., Mendes-Faia A. and Corte-Real M. (2008) Reduction of volatile acidity of wines by selected yeast strains. Appl Microbiol Biotechnol 80: p.881-890.

Waddington C.H. (1942) The canalization of development and the inheritance of acquired characters. Nature 150: p.563-565.

Welcker, C., Thé, C., Andréau, B., de Léon, C., Parentoni, S.N., Bernal, J., Félicité, J., Zonkeng, C., Salazar, F., Narro, L., Charcosset, A. and Horst, W.J. (2005) Heterosis and combining ability for maize adaptation to tropical acid soils: implications for future breeding strategies. Crop Sci 45: 2405–13.

Weldegergis B.T., Tredoux A.G.J. and Crouch A.M. (2007) Application of a Headspace Sorptive Extraction Method for the Analysis of Volatile Components in South African Wines. J Agric Food Chem 55: p.8696-8702.

Zaidi, P.H., Selvan, P.M., Sultana, R., Srivastava, A., Singh, A.K., Srinivasan, G., Singh, R.P. and Singh, P.P. (2007) Association between line *per se* and hybrid performance under excessive soil moisture stress in tropical maize (*Zea mays L.*). Field Crops Res 101: 117–26.

Zörgö E., Gjuvsland A., Cubillos F.A., Louis A.J. et al. (2012) Life History Shapes Trait Heredity by Accumulation of Loss-of-Function Alleles in Yeast. Mol Biol Evol 29(7): p.1781-9.

FIGURES

Figure 1. General scheme of the experimental approach. Fully homozygous diploid strains were used as parental strains. W1, D1, D2, E2, E3, E4 and E5 are *S. cerevisiae* strains. U1, U2, U3 and U4 are *S. uvarum* strains.

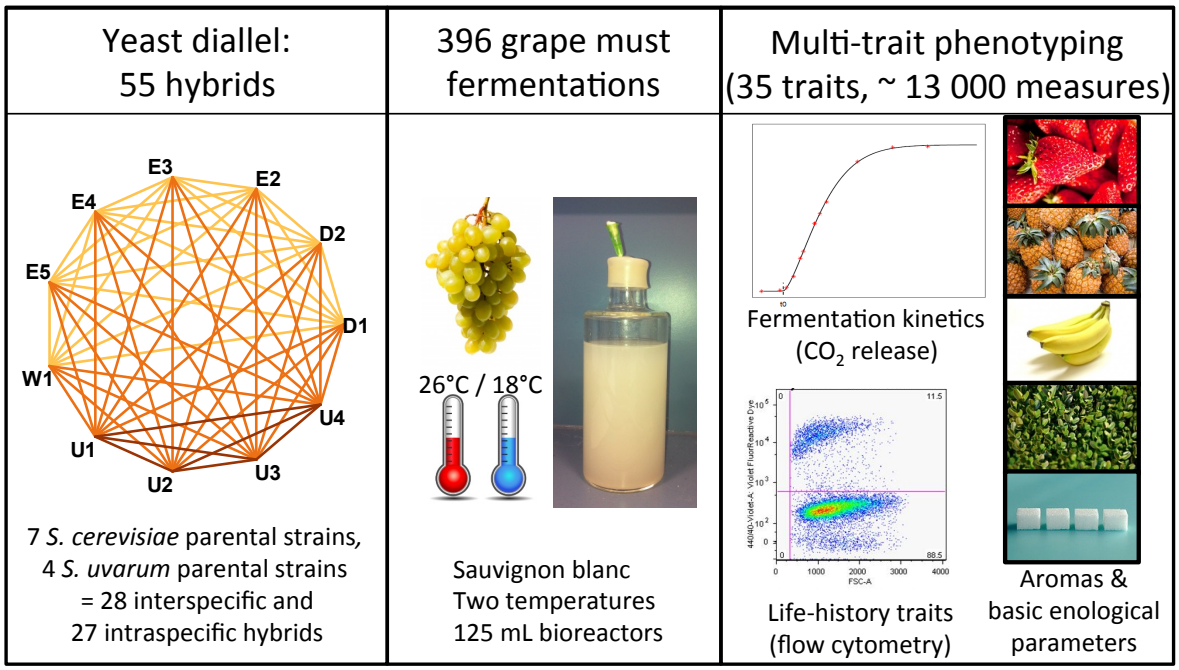


Figure 2 Fermentation kinetics and population dynamics parameters during alcoholic fermentation.

A. Fermentation kinetics: CO_2 released was expressed in $g.L^{-1}$. *t.lag* (h) is the time between inoculation and the beginning of CO_2 released. *t.45* (h) and *t.75* (h) are respectively the fermentation times at which 45 $g.L^{-1}$ and 75 $g.L^{-1}$ of CO_2 are released minus *t.lag*. *AFtime* (h) is the time necessary to ferment all the sugar in the medium minus *t.lag*, and CO_{2max} ($g.L^{-1}$) is the total amount of CO_2 released at the end of the fermentation.

B. CO_2 production rate $g.L^{-1}.h^{-1}$). V_{max} ($g.L^{-1}.h^{-1}$) is the maximum CO_2 production rate; $t.V_{max}$ (h) is the fermentation time at which V_{max} is reached.

C. Cell growth: the carrying capacity *K* was expressed in $cell.mL^{-1}$. $t.N_0$ (h) and $t.N_{max}$ (h) are respectively the time to reach the initial growth point and the carrying capacity. Viability was measured at $t.N_{max}$ and *t.75*.

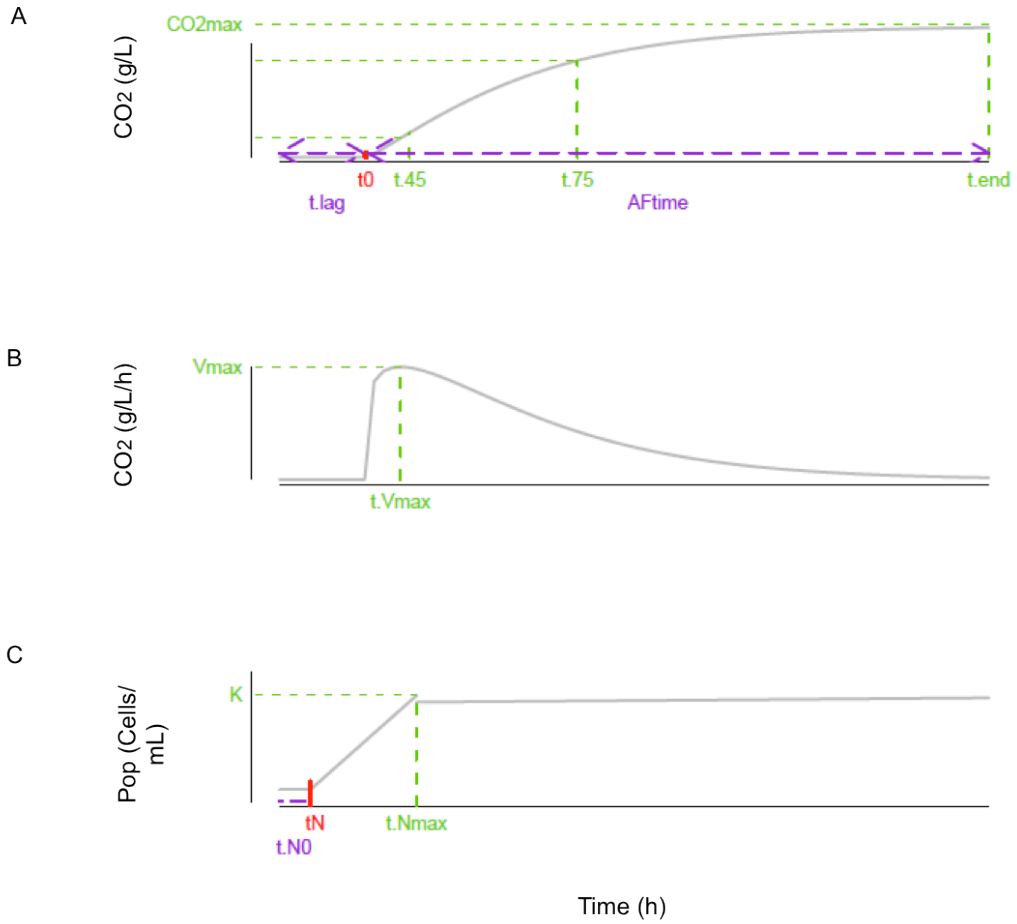


Figure 3. Species effect for quantitative traits of enological interest. *Phenylethanol.acetate* (A1), *Phenylethanol* (A2), *Sugar.Ethanol.Yield* (B) and sum of ethyl esters (C) concentrations in the *S. cerevisiae* (Sc, in red), *S. uvarum* (Su, in blue) and interspecific hybrid (H, in green) strains at 18 °C (dots) and 26 °C (triangles). Statistical differences between groups were tested using a multiple non-parametric test with corrected *p* values. Different letters indicate groups showing significant differences ($p < 0.01$). Capital and lower cases were used for 18° and 26°C, respectively.

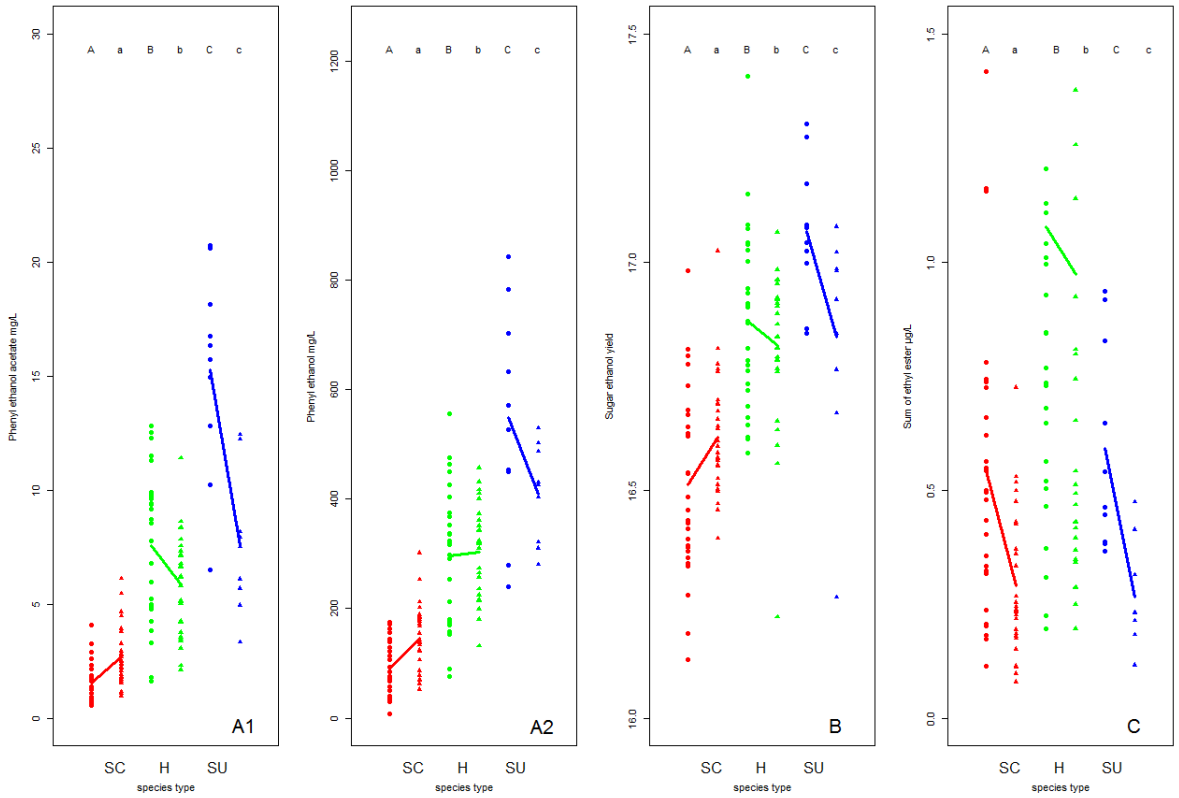
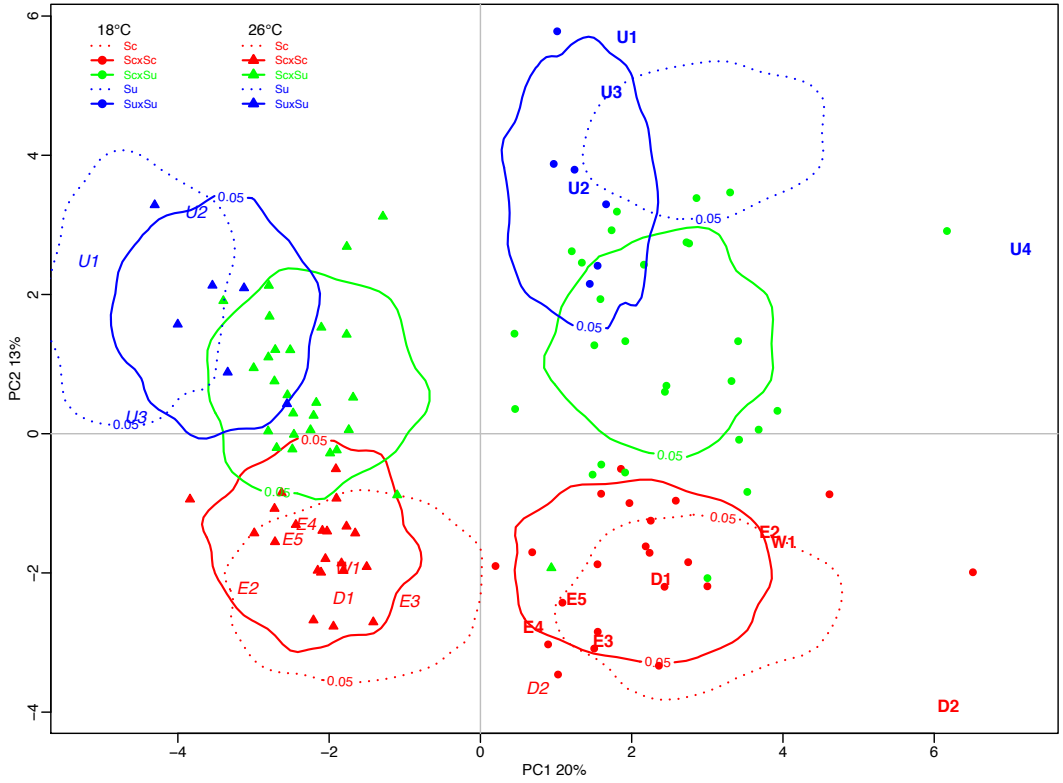
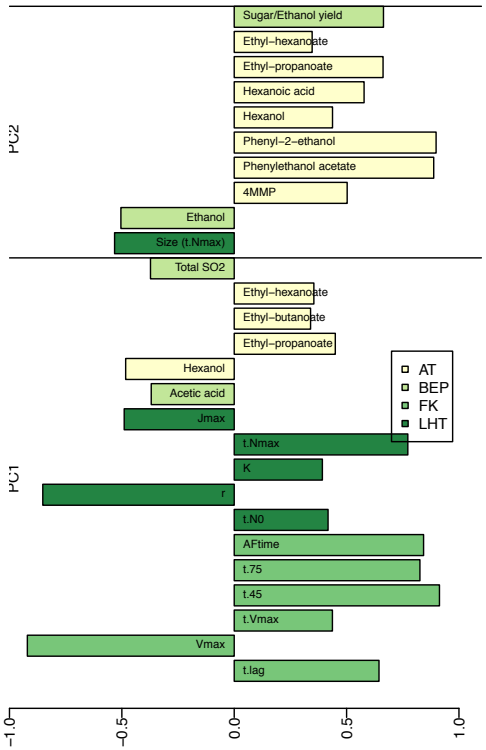


Figure 4. Principal Components Analysis (PCA) made from the 35 variables listed Table 2. Each point represents one of the 55 hybrid strains, and the names of the parental strains are noted in italics (26 °C) or in right (18 °C) characters. A: axes 1 and 2 (33 % of the total inertia). B: axes 2 and 3 (25 % of the total inertia). C: correlation of the variables to discriminant axes DA1, DA2 and DA3. Only variables showing a significant correlation (p-value < 0.0001) are shown. The four-color palette corresponds to the four variable categories (FK: Fermentation Kinetics, LHT: Life-history Trait, BEP: Basic Enological Parameters, AT: Aromatic Traits).

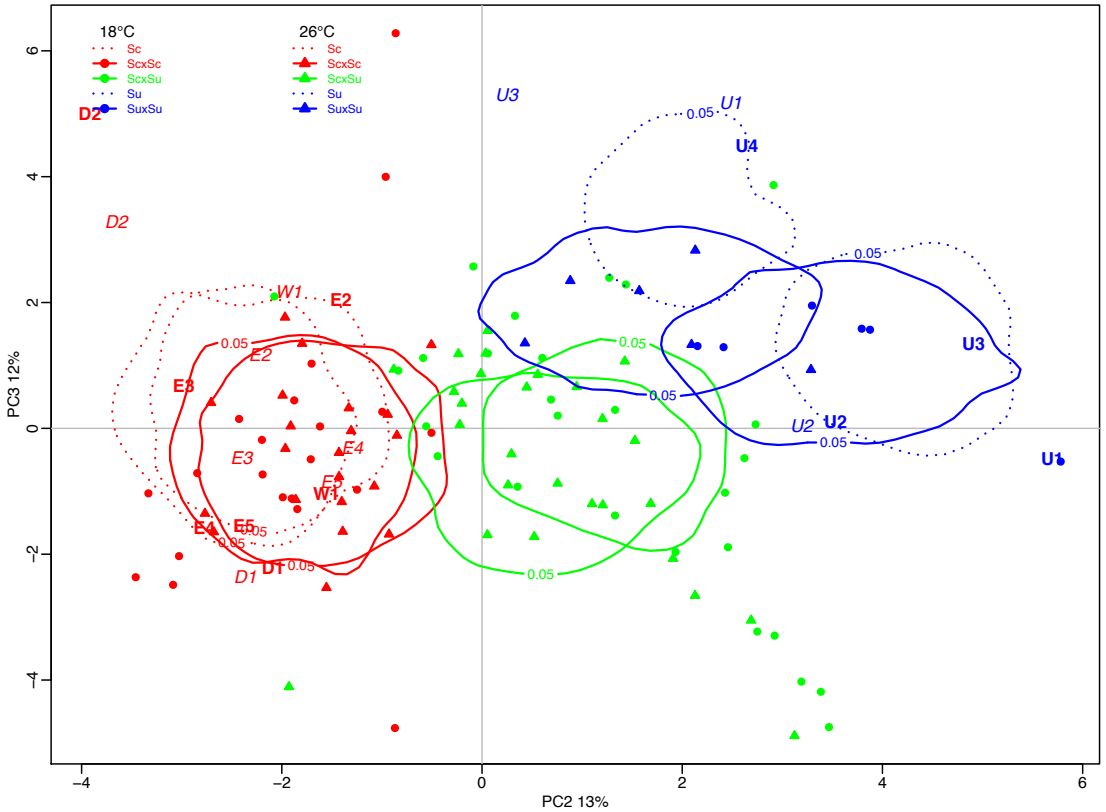
A1



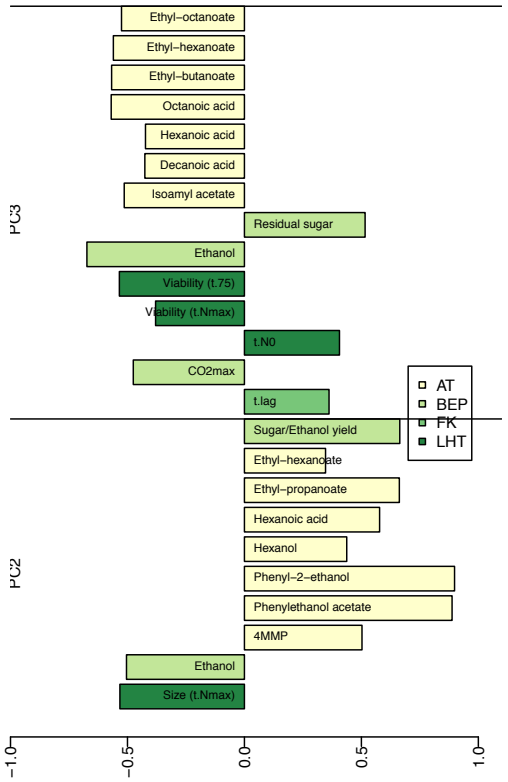
A2



B1


18°C
Sc
ScxSc
ScxSu
Su
SuxSu
26°C
Sc
ScxSc
ScxSu
Su
SuxSu
PC3 12%
PC2 13%
U1
U2
U3
U4
D1
D2
E1
E2
E3
E4
E5
W1
0.05

B2


Ethyl−octanoate
Ethyl−hexanoate
Ethyl−butanoate
Octanoic acid
Hexanoic acid
Decanoic acid
Isoamyl acetate
Residual sugar
Ethanol
Viability (t.75)
Viability (t.Nmax)
t.N0
CO2max
t.lag
Sugar/Ethanol yield
Ethyl−hexanoate
Ethyl−propanoate
Hexanoic acid
Hexanol
Phenyl−2−ethanol
Phenylethanol acetate
4MMP
Ethanol
Size (t.Nmax)
PC3
PC2
AT
BEP
FK
LHT

C

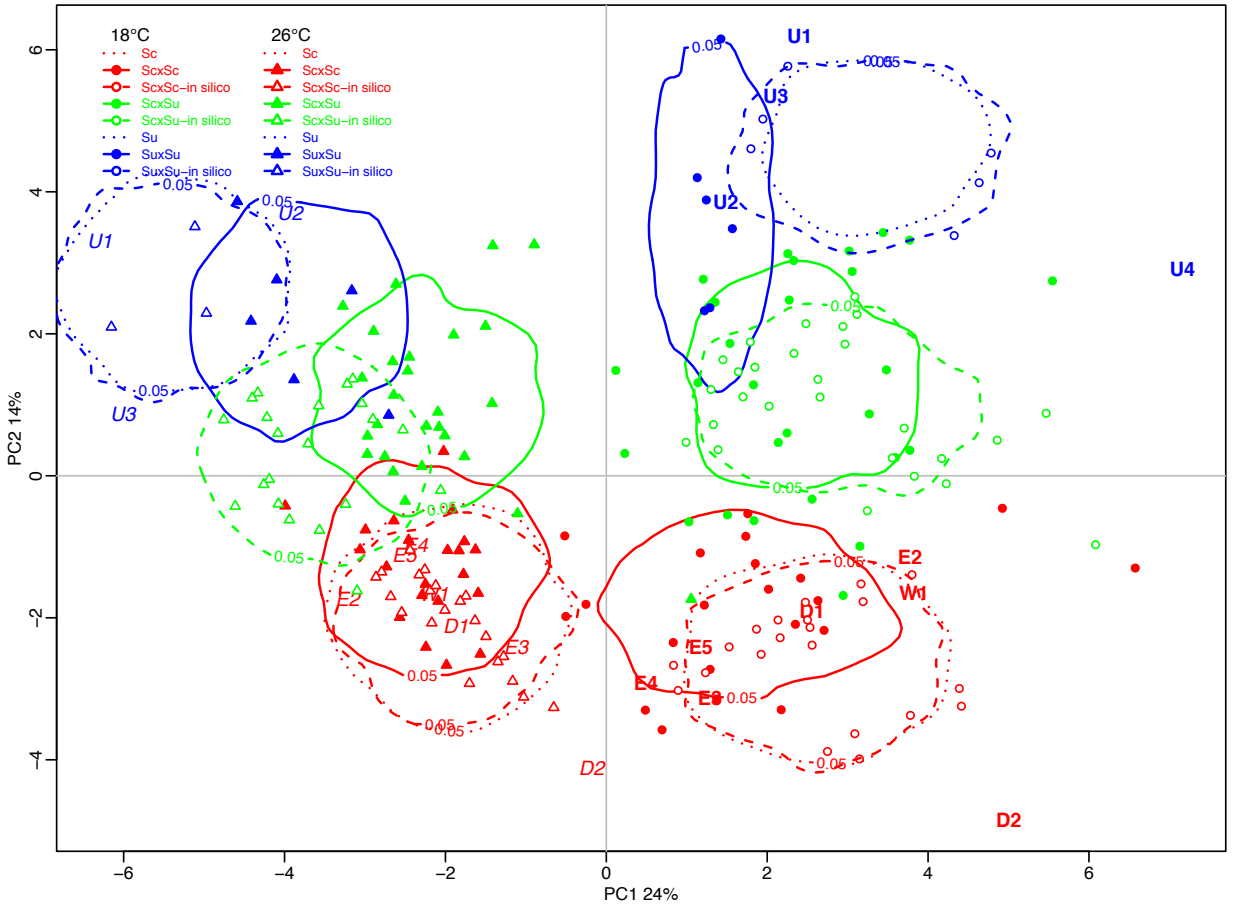

18°C
26°C
Sc
ScxSc
ScxSc–in silico
ScxSu
ScxSu–in silico
Su
SuxSu
SuxSu–in silico
U1
U2
U3
U4
E1
E2
E3
E4
E5
D1
D2
W1
0.05
PC1 24%
PC2 14%

Figure 5. Linear Discriminant Analysis (LDA) for six groups of hybrids: "*S. cerevisiae*-18", "*S. cerevisiae*S. uvarum*-18", "*S. uvarum*-18", "*S. cerevisiae*-26", "*S. cerevisiae*S. uvarum*-26" and "*S. uvarum*-26". The names of the parental strains are written in right (26 °C) or in italics (18 °C) characters. The confidence surfaces of the groups (95 %) are indicated by dotted (18 °C) or solid (26 °C) contour lines.

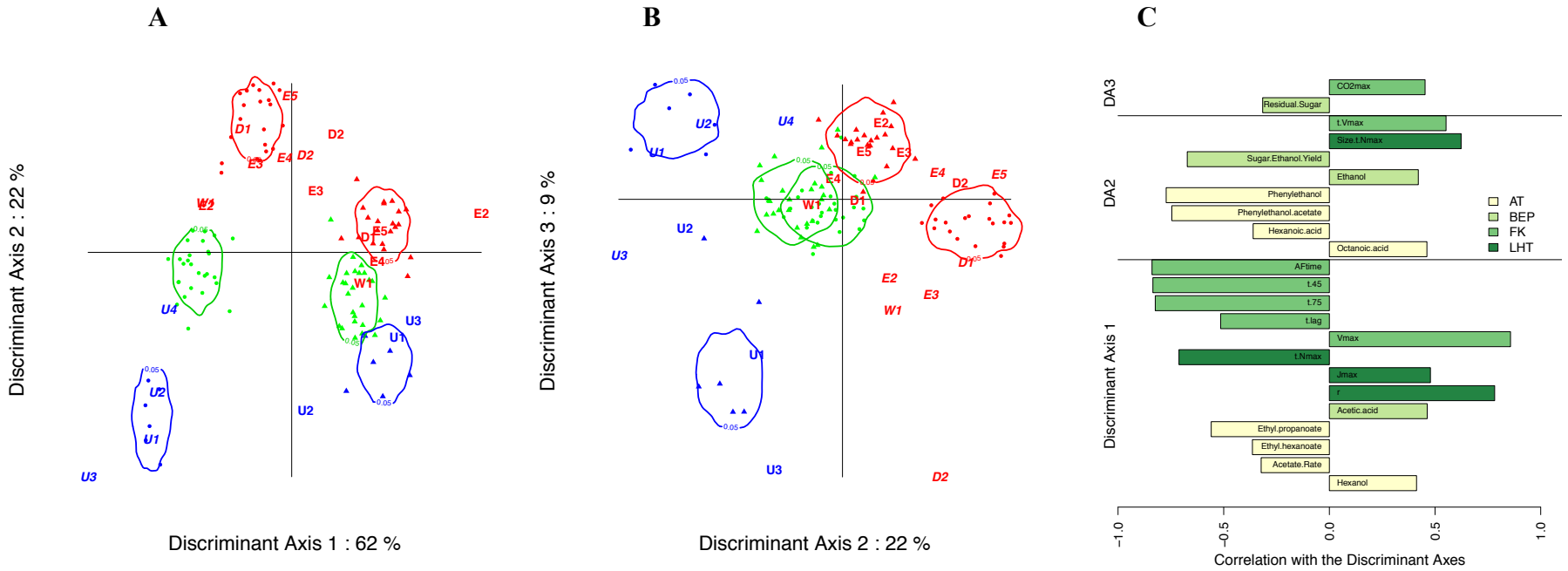
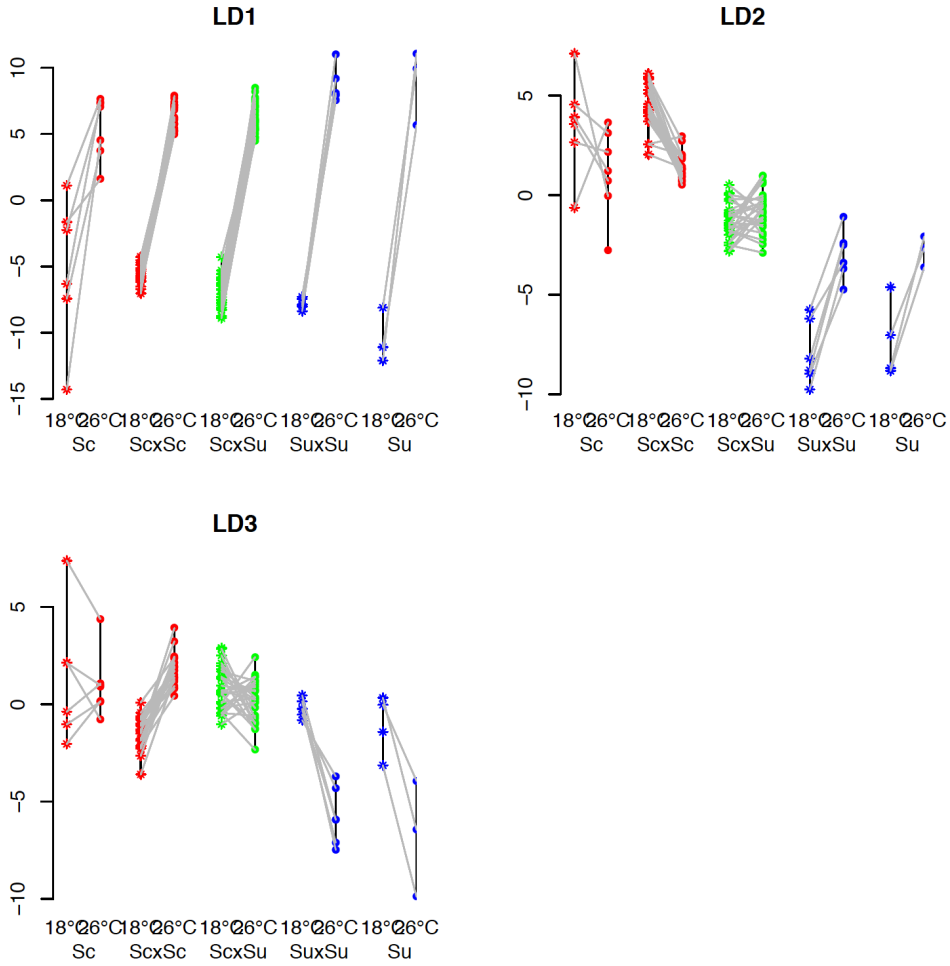


Figure 6. Projection of the strains (including parental strains) on the LDA axes at 18 °C (stars) and 26 °C (dots). Color codes as in figure 4.



TABLES

Table 1 - Yeast strains used in this study.

Strain	Genotype	Ploidy	Collection/supplier	Origin	Reference
Original strains					
YSP128	*HO/HO* (*S. cerevisiae*)	diploid	SGRP	Forest Oak exudate, Pennsylvania, USA	Liti et al., 2009
Alcotec 24	*ho/ho* (*S. cerevisiae*)	diploid	Hambleton Bard	Distillery, UK	Albertin et al 2011
CLIB-294	*HO/HO* (*S. cerevisiae*)	diploid	CIRM-Levures	Distillery, Cognac, France	Albertin et al 2011
VL1	*HO/HO* (*S. cerevisiae*)	diploid	Laffort Œnologie	Enology, Bordeaux, France	Marullo et al 2006
F10	*HO/HO* (*S. cerevisiae*)	diploid	Laffort Œnologie	Enology, Bordeaux, France	Marullo et al 2009
VL3c	*HO/HO* (*S. cerevisiae*)	diploid	Laffort Œnologie	Enology, Bordeaux, France	Marullo et al, 2004
BO213	*HO/HO* (*S. cerevisiae*)	diploid	Laffort Œnologie	Enology, Bordeaux, France	Marullo et al 2006
PM12	*HO/HO* (*S. uvarum*)	diploid	ISVV	Grape must fermentation, Jurançon, France	Masneuf et al, 2007
PJP3	*HO/HO* (*S. uvarum*)	diploid	ISVV	Grape must fermentation, Sancerre, France	Masneuf et al, 2007
Br6.2	*HO/HO* (*S. uvarum*)	diploid	*ADRIA Normandie*	Cider fermentation, Normandie, France	Albertin et al 2013
RC4-15	*HO/HO* (*S. uvarum*)	diploid	*ISVV*	Grape must fermentation, Alsace, France	Masneuf et al, 2007
Homozygous diploid parental strains					
W1	Derived from YSP128, HO/HO	diploid	ISVV		Blein et al.
D2	Derived from Alcotec24, ho/ho	diploid	ISVV		Albertin et al 2011
D1	Derived from CLIB-294, HO/HO	diploid	ISVV		Albertin et al 2011
E3	Derived from VL1, HO/HO	diploid	ISVV		Albertin et al 2011
E4	Derived from F10, HO/HO	diploid	ISVV		Albertin et al 2011
E5	Derived from VL3c, HO/HO	diploid	ISVV		Blein et al.
E2	Derived from BO213, HO/HO	diploid	ISVV		Marullo et al 2009
U1	Derived from PM12, HO/HO	diploid	ISVV		Blein et al.
U2	Derived from PJP3, HO/HO	diploid	ISVV		Blein et al.
U3	Derived from Br6.2, HO/HO	diploid	ISVV		Blein et al.

U4	Derived from RC4-15, HO/HO	diploid	ISVV		this work
Monosporic clones used for crosses					
D1-HYG-1A	*ho::hygR, MATa*	haploid	ISVV		this work
D1-HYG-4C	*ho::hygR, MATalpha*	haploid	ISVV		this work
D2-3A-HYG	*ho::hygR, MATalpha*	haploid	ISVV		Albertin et al 2013
E2-KAN-4A	*ho::kanR, MATalpha*	haploid	ISVV		this work
E2-KAN-4D	*ho::kanR, MATa*	haploid	ISVV		this work
E3-NAT-1C	*ho::natR, MATa*	haploid	ISVV		this work
E3-NAT-2C	*ho::natR, MATalpha*	haploid	ISVV		this work
E3-KAN-1B	*ho::kanR, MATa*	haploid	ISVV		this work
E4-NAT-3A	*ho::natR, MATalpha*	haploid	ISVV		this work
E4-NAT-3B	*ho::natR, MATa*	haploid	ISVV		this work
E5-KAN-1C	*ho::kanR, MATa*	haploid	ISVV		this work
E5-HYG-5B	*ho::hygR, MATalpha*	haploid	ISVV		this work
E5-HYG-5D	*ho::hygR, MATa*	haploid	ISVV		this work
W1-NAT-1B	*ho::natR, MATa*	haploid	ISVV		Albertin et al 2013
W1-NAT-1C	*ho::natR, MATalpha*	haploid	ISVV		this work
U1-KAN-4A	*Suho::kanR, MATalpha*	haploid	ISVV		this work
U1-KAN-5D	*Suho::kanR, MATa*	haploid	ISVV		this work
U2-KAN-2A	*Suho::kanR, MATa*	haploid	ISVV		this work
U2-KAN-3B	*Suho::kanR, MATalpha*	haploid	ISVV		Albertin et al 2013
U3-KAN-3A	*Suho::kanR, MATa*	haploid	ISVV		Albertin et al 2013
U3-KAN-3B	*Suho::kanR, MATalpha*	haploid	ISVV		this work
U4-KAN-2C	*Suho::kanR, MATa*	haploid	ISVV		this work
U4-KAN-2B	*Suho::kanR, MATalpha*	haploid	ISVV		this work
Hybrids of the diallel design					
DD12	D1-HYG-1A * D2-3A-HYG	diploid	ISVV		this work

DE12	D1-HYG-1A * E2-KAN-4A	diploid	ISVV		this work
DE13	D1-HYG-1A * E3-NAT-2C	diploid	ISVV		this work
DE14	D1-HYG-1A * E4-NAT-3A	diploid	ISVV		this work
DE15	D1-HYG-4C * E5-KAN-1C	diploid	ISVV		this work
DE22	D2-3A-HYG * E2-KAN-4D	diploid	ISVV		this work
DE23	D2-3A-HYG * E3-NAT-1C	diploid	ISVV		this work
DE24	D2-3A-HYG * E4-NAT-3B	diploid	ISVV		this work
DE25	D2-3A-HYG * E5-KAN-1C	diploid	ISVV		this work
DU11	D1-HYG-1A * U1-KAN-4A	diploid	ISVV		this work
DU12	D1-HYG-1A * U2-KAN-3B	diploid	ISVV		this work
DU13	D1-HYG-1A * U3-KAN-3B	diploid	ISVV		this work
DU14	D1-HYG-1A * U4-KAN-2B	diploid	ISVV		this work
DU21	D2-3A-HYG * U1-KAN-5D	diploid	ISVV		this work
DU22	D2-3A-HYG * U2-KAN-2A	diploid	ISVV		this work
DU23	D2-3A-HYG * U3-KAN-3A	diploid	ISVV		this work
DU24	D2-3A-HYG * U4-KAN-2C	diploid	ISVV		this work
DW11	D1-HYG-1A * W1-NAT-1C	diploid	ISVV		this work
DW21	D2-3A-HYG * W1-NAT-1B	diploid	ISVV		this work
EE23	E2-KAN-4A * E3-NAT-1C	diploid	ISVV		this work
EE24	E2-KAN-4D * E4-NAT-3A	diploid	ISVV		this work
EE25	E2-KAN-4A * E5-HYG-5D	diploid	ISVV		this work
EE34	E3-KAN-1B * E4-NAT-3A	diploid	ISVV		this work
EE35	E3-NAT-2C * E5-KAN-1C	diploid	ISVV		this work
EE45	E4-NAT-3A * E5-KAN-1C	diploid	ISVV		this work
EU21	E2-KAN-4A * U1-KAN-5D	diploid	ISVV		this work
EU22	E2-KAN-4A * U2-KAN-2A	diploid	ISVV		this work
EU23	E2-KAN-4A * U3-KAN-3A	diploid	ISVV		this work

EU24	E2-KAN-4A * U4-KAN-2C	diploid	ISVV		this work
EU31	E3-NAT-1C * U1-KAN-4A	diploid	ISVV		this work
EU32	E3-NAT-1C * U2-KAN-3B	diploid	ISVV		this work
EU33	E3-NAT-1C * U3-KAN-3B	diploid	ISVV		this work
EU34	E3-NAT-1C * U4-KAN-2B	diploid	ISVV		this work
EU41	E4-NAT-3B * U1-KAN-4A	diploid	ISVV		this work
EU42	E4-NAT-3B * U2-KAN-3B	diploid	ISVV		this work
EU43	E4-NAT-3B * U3-KAN-3B	diploid	ISVV		this work
EU44	E4-NAT-3B * U4-KAN-2B	diploid	ISVV		this work
EU51	E5-HYG-5D * U1-KAN-4A	diploid	ISVV		this work
EU52	E5-HYG-5D * U2-KAN-3B	diploid	ISVV		this work
EU53	E5-HYG-5D * U3-KAN-3B	diploid	ISVV		this work
EU54	E5-HYG-5D * U4-KAN-2B	diploid	ISVV		this work
EW21	E2-KAN-4A * W1-NAT-1B	diploid	ISVV		this work
EW31	E3-KAN-1B * W1-NAT-1C	diploid	ISVV		this work
EW41	E4-NAT-3A * W1-NAT-1B	diploid	ISVV		this work
EW51	E5-HYG-5B * W1-NAT-1B	diploid	ISVV		this work
UU12	U1-KAN-4A * U2-KAN-2A	diploid	ISVV		this work
UU13	U1-KAN-4A * U3-KAN-3A	diploid	ISVV		this work
UU14	U1-KAN-4A * U4-KAN-2C	diploid	ISVV		this work
UU23	U2-KAN-3B * U3-KAN-3A	diploid	ISVV		this work
UU24	U2-KAN-2A * U4-KAN-2B	diploid	ISVV		this work
UU34	U3-KAN-3B * U4-KAN-2C	diploid	ISVV		this work
WU11	W1-NAT-1B * U1-KAN-4A	diploid	ISVV		this work
WU12	W1-NAT-1B * U2-KAN-3B	diploid	ISVV		this work
WU13	W1-NAT-1B * U3-KAN-3B	diploid	ISVV		this work
WU14	W1-NAT-1B * U4-KAN-2B	diploid	ISVV		this work

Table 2 - Results of the ANOVAs for 35 variables representative of fermentation and life-history traits in yeast. R^2, proportion of variance explained by the model (block effect removed). SS, sum of squares. Temp, temperature. Resid, residual. S*T, strain*temperature interaction. *p*-val, *p*-value.

Trait	Trait category	Strain number	Mean	Unit	R^2	Block effect	SS Strain	SS Temp	SS S*T	SS Resid.	*p*-val Strain	*p*-val Temp	*p*-val S*T
t.lag	FK	63	19.70	h	0.80	*	0.54	0.23	0.11	0.13	0.00000	0.00000	0.00000
$t.V_{max}$	FK	63	8.47	h	0.60	*	0.53	0.06	0.14	0.26	0.00000	0.00000	0.00019
t.45	FK	63	31.53	h	0.91	*	0.14	0.75	0.04	0.06	0.00000	0.00000	0.00000
t.75	FK	63	71.04	h	0.92	*	0.21	0.68	0.06	0.05	0.00000	0.00000	0.00000
AFtime	FK	63	142.39	h	0.91	*	0.20	0.69	0.05	0.06	0.00000	0.00000	0.00000
V_{max}	FK	63	1.80	g/(L*h)	0.92	ns	0.11	0.79	0.05	0.05	0.00000	0.00000	0.00000
CO_{2max}	FK	63	90.43	g/L	0.65	*	0.45	0.00	0.32	0.23	0.00000	0.07273	0.00000
$t.N_0$	LHT	63	4.17	h	0.63	*	0.60	0.02	0.15	0.23	0.00000	0.00028	0.00010
$t.N_{max}$	LHT	63	28.75	h	0.40	*	0.19	0.32	0.11	0.38	0.02071	0.00000	0.61236
R	LHT	63	0.15	log(cells/mL)/h	0.55	*	0.19	0.44	0.08	0.29	0.00040	0.00000	0.66772
K	LHT	63	162163078.27	cell/ml	0.36	*	0.32	0.04	0.24	0.40	0.00000	0.00001	0.00025
J_{max}	LHT	63	0.0047	$g/(L*10^8*cell)$	0.40	*	0.36	0.16	0.11	0.38	0.00000	0.00000	0.57488
$Size.t.N_{max}$	LHT	61	6.13	µm	0.49	*	0.44	0.00	0.26	0.29	0.00000	0.33193	0.00003
$Viability.t.N_{max}$	LHT	62	90.98	%	0.33	*	0.43	0.00	0.19	0.38	0.00000	0.19832	0.18694
Viability.t.75	LHT	62	78.20	%	0.64	*	0.50	0.04	0.25	0.20	0.00000	0.00000	0.00000
Residual.Sugar	BEP	63	1.13	g/L	0.71	*	0.45	0.00	0.35	0.19	0.00000	0.02983	0.00000
Ethanol	BEP	63	11.13	%vol	0.68	*	0.55	0.00	0.24	0.21	0.00000	0.66762	0.00000
Sugar.Ethanol.Yield	BEP	63	16.73	g/L/%	0.50	*	0.50	0.00	0.17	0.32	0.00000	0.76264	0.00041
Acetic.acid	BEP	63	0.13	g/L	0.38	*	0.33	0.10	0.18	0.40	0.00000	0.00000	0.02117
$Total.SO_2$	BEP	63	172.50	mg/L	0.18	*	0.30	0.02	0.16	0.52	0.00071	0.00923	0.46632
$Free.SO_2$	BEP	63	67.95	mg/L	0.25	*	0.31	0.00	0.22	0.47	0.00009	0.56268	0.04659

Table 2 (Continued)

Trait	Trait category	Strain number	Mean	Unit	R^2	Block effect	SS Strain	SS Temp	SS S*T	SS Resid.	*p*-val Strain	*p*-val Temp	*p*-val S*T
Phenylethanol	AT	63	191.60	mg/L	0.66	*	0.64	0.02	0.11	0.22	0.00000	0.00000	0.00124
Hexanol	AT	63	1.32	mg/L	0.29	*	0.33	0.11	0.10	0.46	0.00000	0.00000	0.91234
Phenylethanol.acetate	AT	63	3.86	mg/L	0.66	*	0.67	0.00	0.11	0.22	0.00000	0.34696	0.00212
Isoamyl.acetate	AT	63	0.94	mg/L	0.09	*	0.23	0.00	0.18	0.59	0.05512	0.79194	0.54684
Ethyl.propanoate	AT	63	0.07	mg/L	0.41	*	0.40	0.06	0.16	0.38	0.00000	0.00000	0.03700
Ethyl.butanoate	AT	63	0.05	mg/L	0.11	*	0.20	0.05	0.17	0.58	0.20289	0.00002	0.63904
Ethyl.hexanoate	AT	63	0.11	mg/L	0.19	*	0.27	0.08	0.12	0.53	0.00087	0.00000	0.88627
Ethyl.octanoate	AT	63	0.06	mg/L	0.11	*	0.23	0.04	0.15	0.58	0.06347	0.00013	0.83217
Ethyl.decanoate	AT	63	0.07	mg/L	0.13	*	0.31	0.00	0.13	0.56	0.00042	0.94066	0.89555
Hexanoic.acid	AT	63	11.16	mg/L	0.20	*	0.33	0.01	0.14	0.52	0.00002	0.02999	0.62936
Octanoic.acid	AT	63	2.30	mg/L	0.17	*	0.26	0.06	0.14	0.54	0.00518	0.00000	0.87192
Decanoic.acid	AT	63	0.99	mg/L	0.13	*	0.23	0.04	0.17	0.57	0.03997	0.00015	0.45642
X4MMP	AT	63	9.28	ng/L	0.44	*	0.51	0.01	0.11	0.36	0.00000	0.00766	0.34848
Acetate.Ratio	AT	63	0.03	-	0.22	*	0.21	0.01	0.27	0.51	0.03548	0.01387	0.00044

Table 3. Multivariate analysis of variance. *A posteriori* probability of allocating successfully the hybrids to their respective groups.

	ScxSc-18	**ScxSu-18**	**SuxSu-18**	**ScxSc-26**	**ScxSu-26**	**SuxSu-26**
ScxSc-18	0.78	0.12	0.03	0.04	0.02	0.02
ScxSu-18	0.12	0.73	0.08	0.02	0.05	0.01
SuxSu-18	0	0.28	0.68	0.01	0.01	0.02
ScxSc-26	0.02	0.01	0	0.81	0.16	0.01
ScxSu-26	0.03	0.04	0.01	0.18	0.68	0.07
SuxSu-26	0.01	0.01	0	0.02	0.26	0.69

Figure S1. Sensitivity of the Weibull model to the variation of parameters *d* (A), *b* (B) and *α* (C).

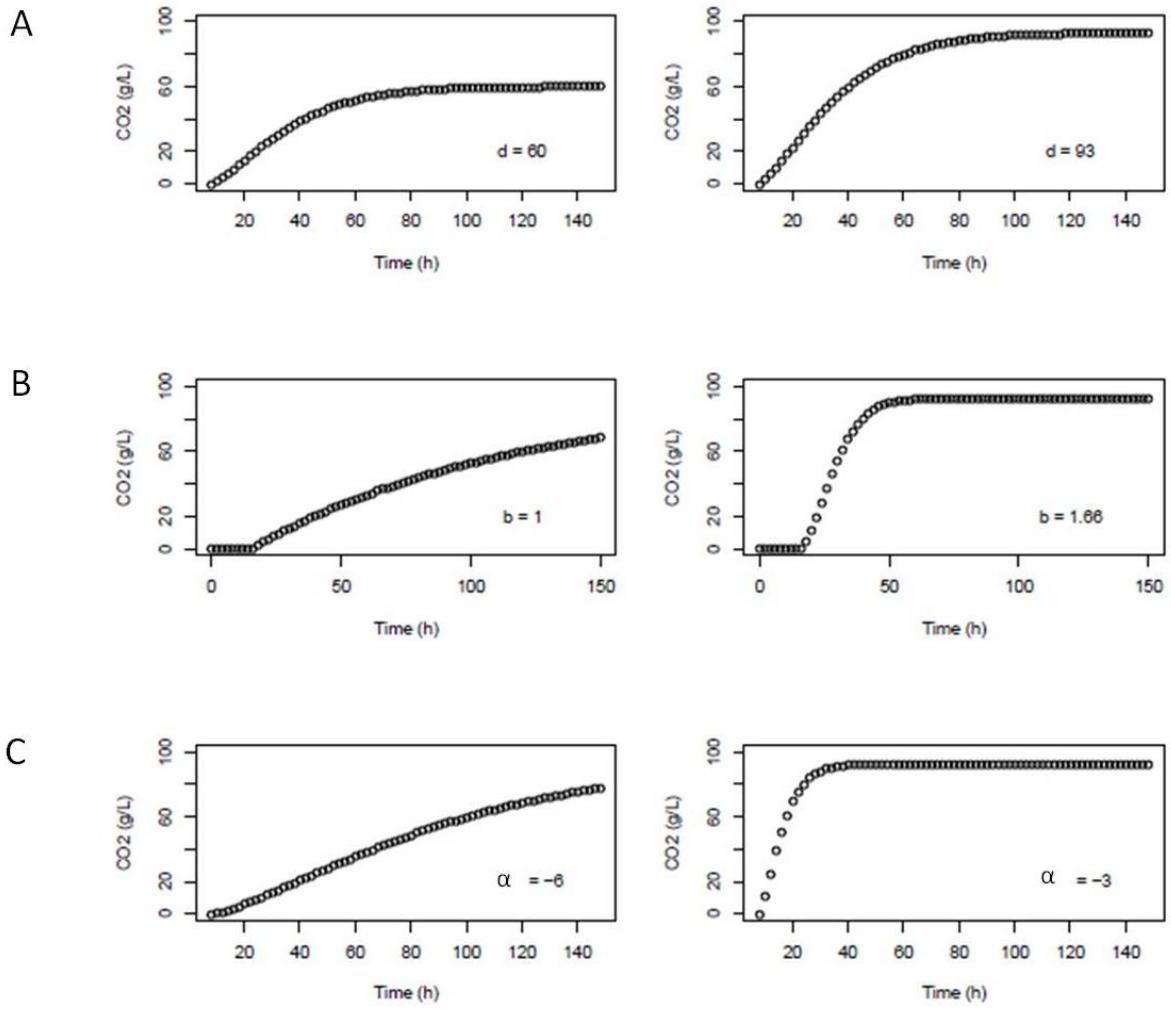


Figure S2. Fitting the data of CO_2 release with the Weibull model. Plot of the standardized residues against the CO_2 values estimated from the model. Data from all the fermentations were pooled.

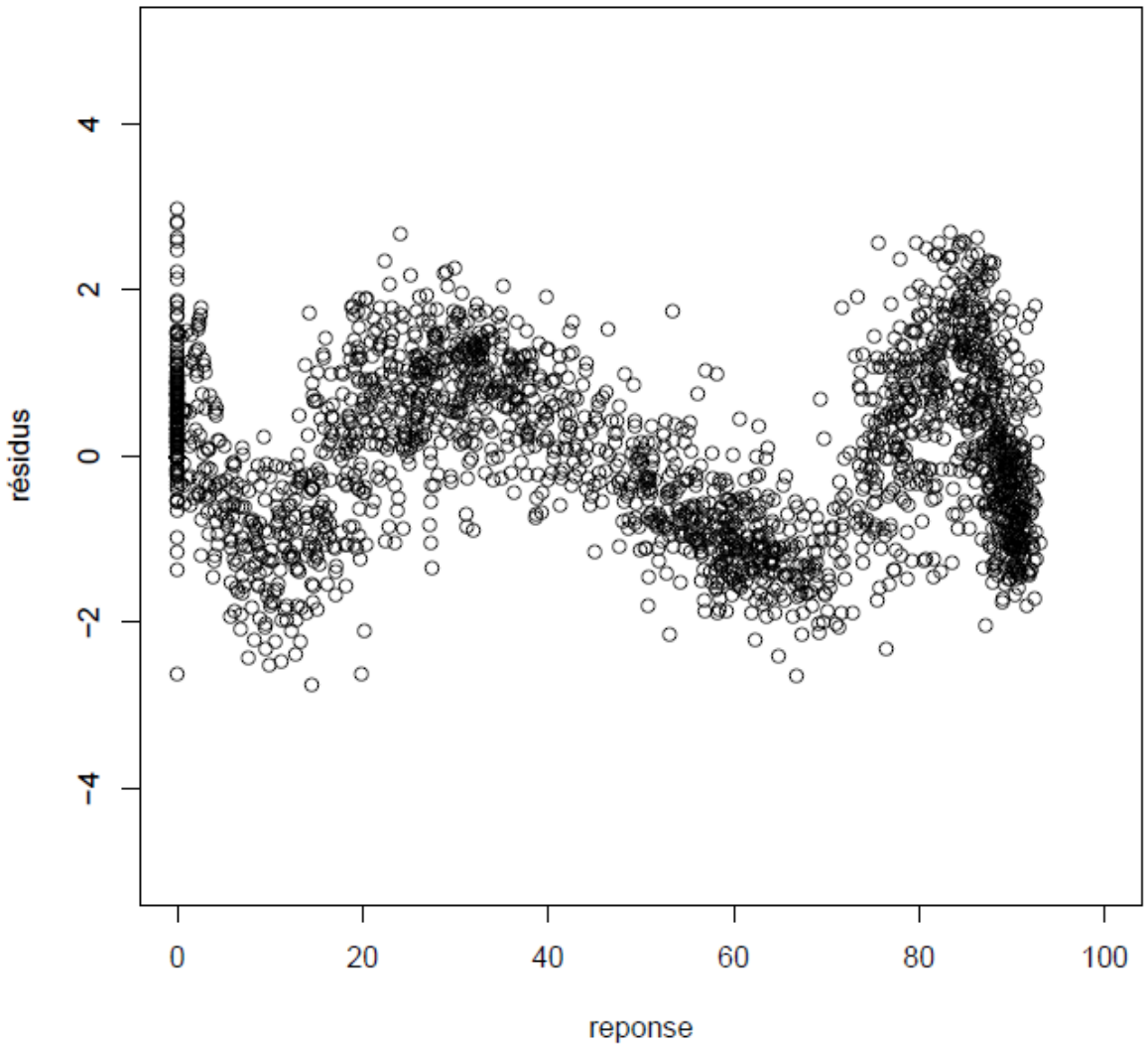
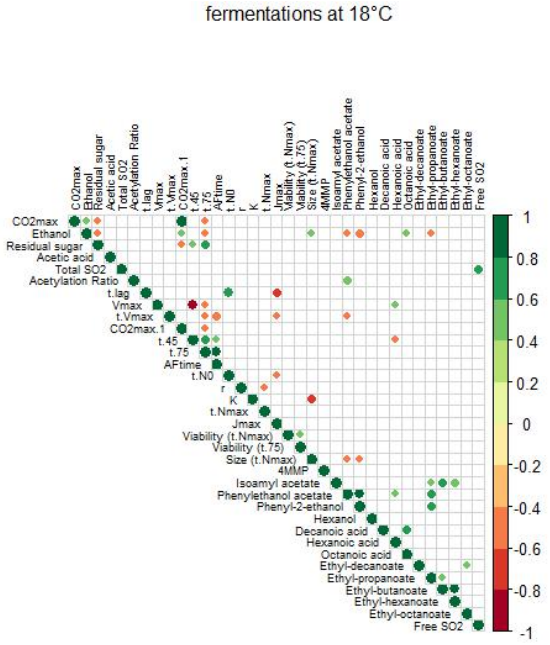


Figure S3 Correlation between the 35 fermentation traits analyzed at 18 °C (A) and 26 °C (B). Only parameters showing a significant correlation (p-value < 0.05 after Benjamini-Hochberg adjustment) were represented by a dot. Green and red tones correspond to positive and negative correlation, respectively.

A



B

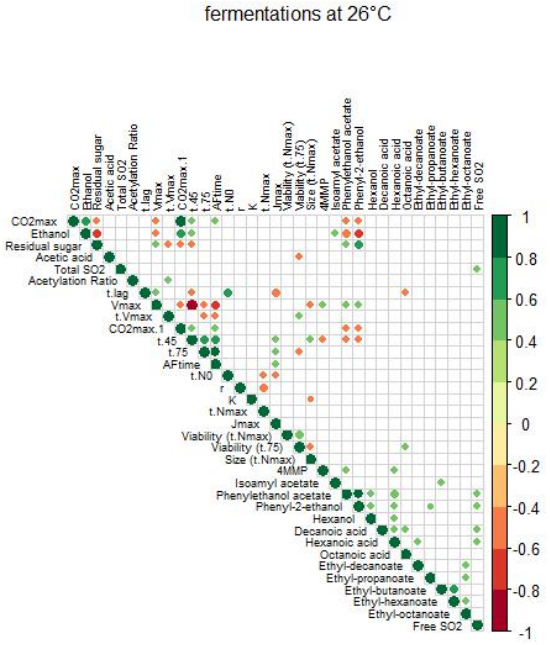


Figure S4. Traits with a significant species effect at 18 °C

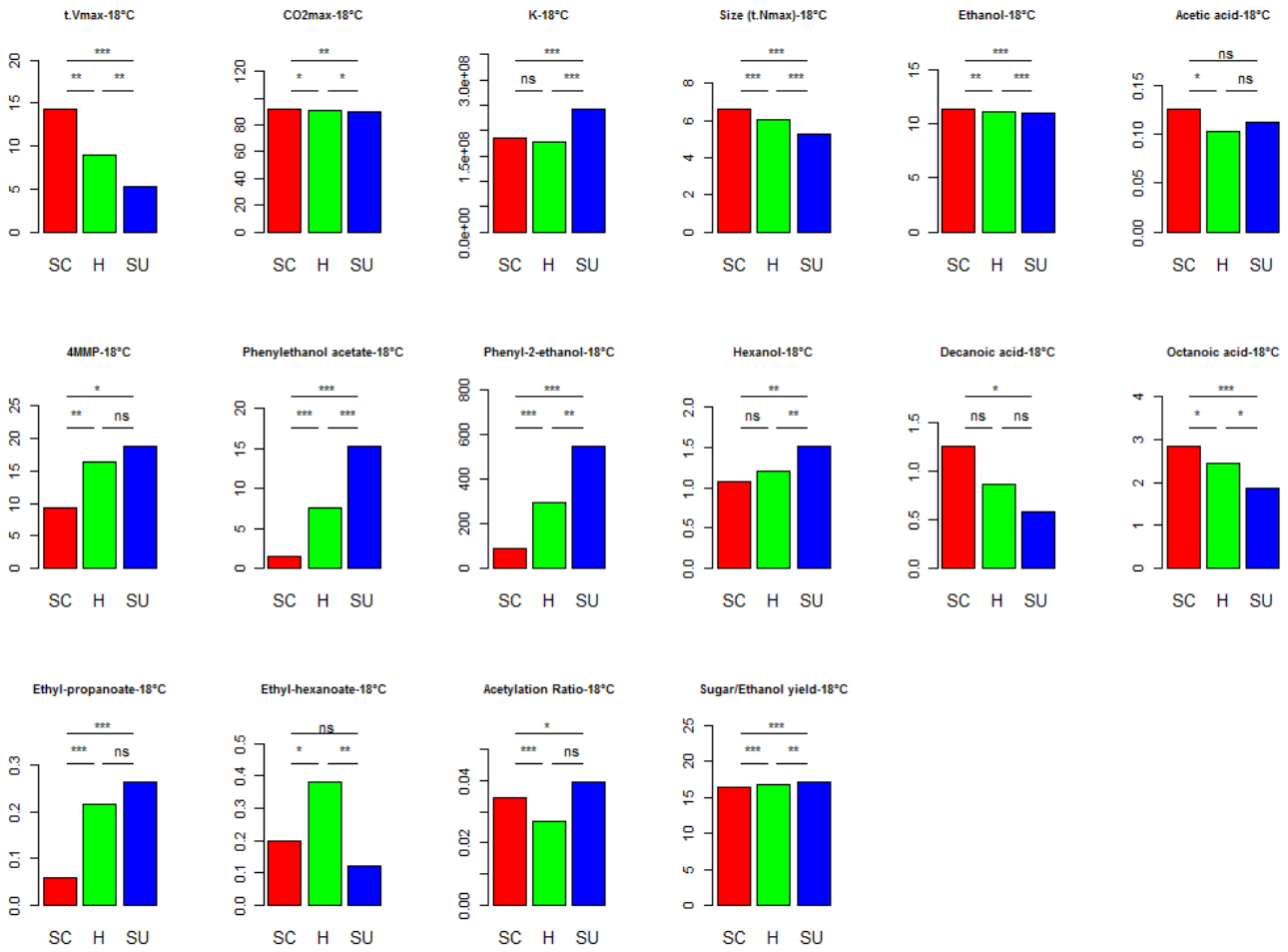


Figure S5. Traits with a significant species effect at 26 °C.

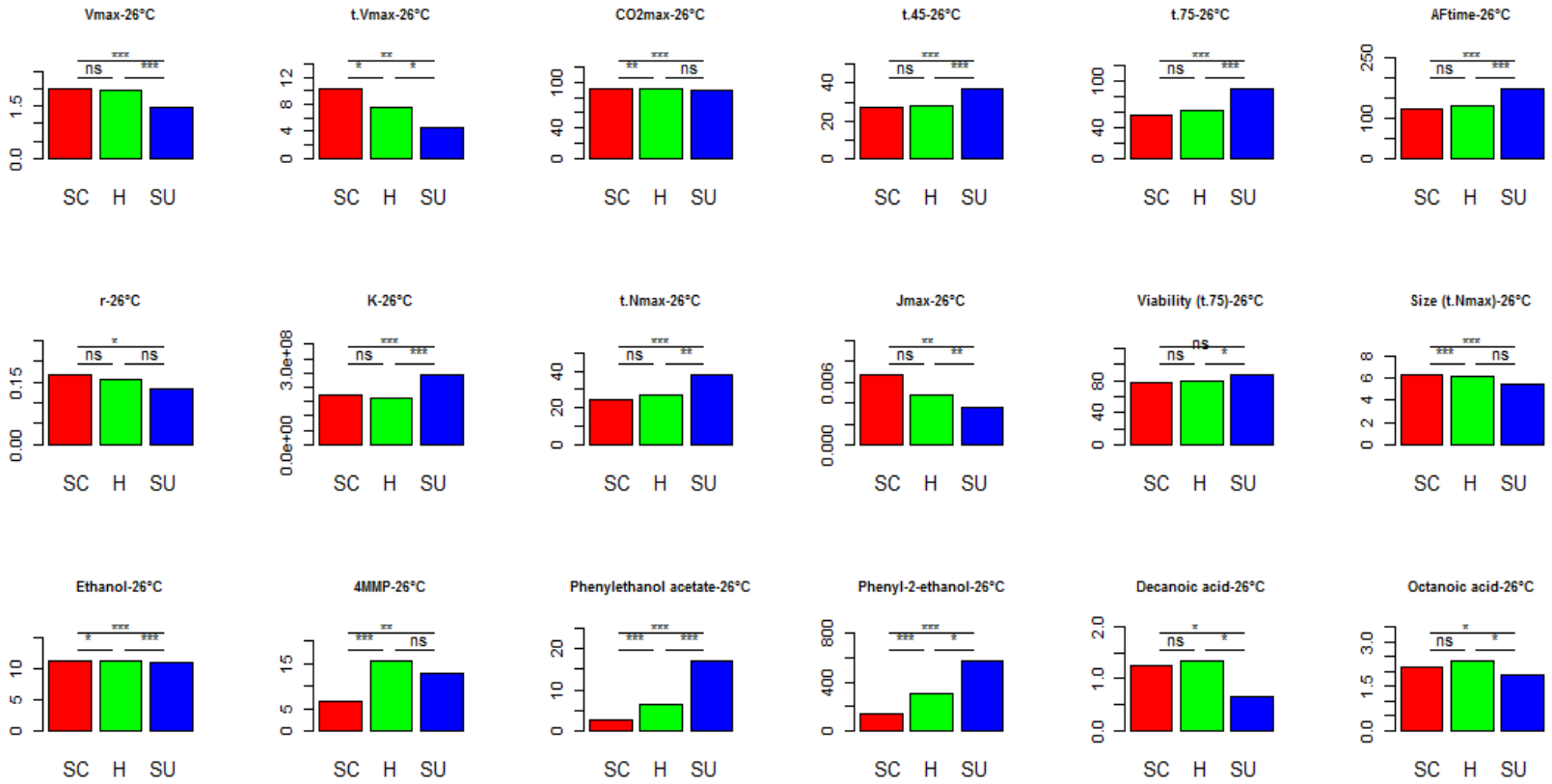


Figure S5. Traits with a significant species effect at 26 °C (continued).

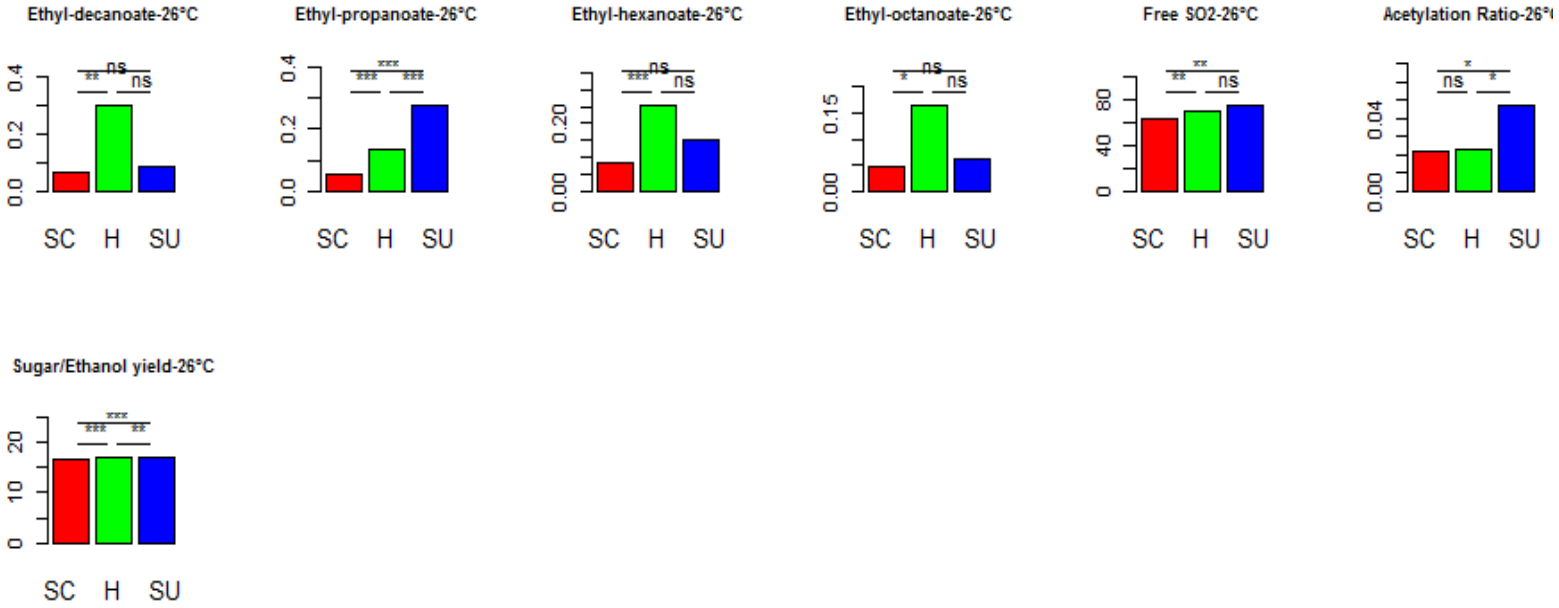


Table S1. Microsatellite primers used to discriminate hybrids and parental strains.

Name	Fluorescence label	Locus	Sequence	Final concentration (µM)	Reference
pC5fw	Fam	*YFR038*	gtgtcttgacacaatagcaatggccttca	0.3	[29]
pC5rev			gcaagcgactagaacaacaatcaca	0.3	
p91fw	Ned	*YML091*	gtgtctaagcctcttcaagcatgac	1.0	
p91rev			ctgtctggacaattttgccacctta	1.0	
p703	Fam	Locus 4	ggacactagagttcgtctcg	0.3	
p704			gccaccactatcagttcg	0.3	[30]
p705	Ned	Locus 9	cacggcaatcagcacattt	1.0	
p706			tgaagtttcatcatcggcaa	1.0	

Table S2. The 22 aromatic compounds detected in the samples

Class	Unit	Compound	Retained
esters	mg/L	*Hexyl acetate*	*no*
esters	mg/L	*Isoamyl acetate*	*yes*
esters	mg/L	*2-Phenylethanol-acetate*	*yes*
esters	mg/L	*Ethyl 2-Methylpropanoate [mC3C2]*	*no*
esters	mg/L	*Ethyl butanoate [C4C2]*	*yes*
esters	mg/L	*Ethyl 2-Methylbutanoate [mC4C2]*	*no*
esters	mg/L	*Ethyl hexanoate [HC6C2]*	*yes*
esters	mg/L	*Ethyl propanoate [C6C2]*	*yes*
esters	mg/L	*Ethyl octanoate [C8C2]*	*yes*
esters	mg/L	*Ethyl decanoate [C10C2]*	*yes*
fatty acid	mg/L	*Butyric acid*	*no*
fatty acid	mg/L	*Isobutyric acid*	*no*
fatty acid	mg/L	*Propionic acid*	*no*
fatty acid	mg/L	*Isovaleric acid*	*no*
fatty acid	mg/L	*Hexanoic acid*	*yes*
fatty acid	mg/L	*Octanoic acid*	*yes*
fatty acid	mg/L	*Decanoic acid*	*yes*
higher alcohols	mg/L	*Hexanol*	*yes*
higher alcohols	mg/L	*2-Phenylethanol*	*yes*
volatile thiols	ng/L	*3-mercapto-hexan-1-ol*	*no*
volatile thiols	ng/L	*4-methyl-4-mercaptopentan-2-one*	*yes*
volatile thiols	ng/L	*3-mercapto-hexyl acetate*	*no*

CONCLUSION – PERSPECTIVES

Ma thèse avait pour ambition d'explorer le phénomène d'hétérosis chez deux espèces de levure d'importance agro-économique majeure, *S. cerevisiae* et *S. uvarum,* dans des conditions proches de celles de l'œnologie. Pour la première fois, des hybrides interspécifiques ont été inclus dans un dispositif diallèle complet. Un autre aspect original de notre projet résidait dans l'approche intégrative choisie, qui combinait l'étude de phénotypes à différents niveaux : métabolique, protéomique, cellulaire et populationnel. Un panel de 66 souches (55 hybrides et leurs 11 parents) a été analysé pour 35 caractères à deux températures et avec trois répétitions. Au total 396 fermentations alcooliques ont été réalisées, dont 370 exploitables, produisant près de 13 000 *data points*.

Certains caractères ont été directement mesurés, comme les métabolites importants pour l'œnologie (éthanol, sucre résiduel, acide acétique, dioxyde de soufre). D'autres caractères sont des paramètres estimés à partir de modèles d'ajustement des données, comme les caractères décrivant la cinétique fermentaire et la dynamique des populations. Les modèles élaborés pour l'estimation de ces paramètres sont originaux et devraient être utilisables pour l'analyse d'autres expériences de croissance microbiennes.

LES INTERACTIONS NUCLEO-CYTOPLASMIQUES N'INFLUENCENT PAS LA VARIATION DES CARACTERES ETUDIES EN CONDITIONS FERMENTAIRES

Le premier chapitre de ma thèse décrit un travail effectué en collaboration dont le but était de savoir si le génome mitochondrial était susceptible d'influencer les caractères mesurés chez les hybrides interspécifiques *S. cerevisiae* * *S. uvarum*, comme cela a été décrit chez des végétaux et des animaux (ex. Burgess et al. 2004, Rand et al. 2006). Des levures hybrides isogéniques pour l'ADN nucléaire, mais possédant soit le patrimoine mitochondrial de *S. cerevisiae*, soit celui de *S. uvarum*, ont été comparées en régime respiratoire et en régime fermentaire. En conditions respiratoires, des différences significatives ont été observées entre hybrides isogéniques selon leur cytoplasme. Ce résultat n'était pas inattendu, étant donné les connaissances déjà acquises sur l'effet du remplacement d'un génome mitochondrial de *S. cerevisiae* par celui d'une autre espèce (Spirek et al. 2000, Sulo et al. 2003). Dans les conditions fermentaires utilisées dans cette thèse, l'origine du cytoplasme n'avait par contre aucune influence détectable sur les phénotypes mesurés (cinétique fermentaire et paramètres

œnologiques). Ces résultats sont conformes à ceux de Rosenfeld et al. (2002) qui montraient que, dans des conditions de culture semblables aux nôtres, les traces d'oxygène dissous dans le milieu ne sont pas consommées par la mitochondrie mais sont utilisées pour la biosynthèse de stérols et pour les systèmes dépendant du NADPH localisés dans les membranes microsomiques. Ainsi dans les conditions expérimentales du projet, la part génétique de la variation phénotypique observée ne peut donc être qu'imputable au polymorphisme des gènes nucléaires.

LES DIFFERENTS TYPES DE CARACTERES NE SONT PAS INFLUENCES DE LA MEME MANIERE PAR LA TEMPERATURE ET LES INTERACTIONS SOUCHE * TEMPERATURE

Les analyses de variance ont révélé que si presque tous les caractères sont génétiquement variables (effet souche significatif pour 33 caractères sur 35), en revanche les effets température et souche * température dépendent des catégories de variable. Les caractères qui mesurent la cinétique de la fermentation, ainsi que le taux de croissance et le flux de CO_2 maximum, sont très sensibles à la température, tandis que la production de métabolites importants pour l'œnologie est peu ou pas affectée. En revanche parmi ces derniers, les caractères œnologiques de base (BEP) sont fortement sujets aux effets d'interaction souche * environnement, de même que le rapport phenyléthanol acetate/phénylethanol, ainsi que plusieurs caractères de fermentation et d'histoire de vie, comme le CO_2 maximum, la capacité biotique, la viabilité et la taille des cellules. En d'autres termes, pour ces caractères-là, le classement des souches dépend de la température.

L'HYBRIDATION INTERSPECIFIQUE *S. CEREVISIAE* * *S. UVARUM* CREE UN NOUVEAU TYPE DE LEVURE AVEC DE MEILLEURES APTITUDES POUR LA PRODUCTION DE VIN

En raison du réchauffement climatique, les moûts de raisin contiennent depuis quelques décennies des concentrations toujours croissantes en sucre, faisant augmenter le degré d'alcool des vins. La sélection de souches de levures ayant un rendement sucre/éthanol plus élevé devient un objectif pertinent pour l'œnologie (Tilloy et al. 2014). Des études récentes ont montré que *S. uvarum* et *S. kudriavzevii* avaient un rendement plus élevé que celui de *S. cerevisiae* (Masneuf-Pomarède et al. 2010, Arroyo-Lopez et al. 2013). Toutefois ces deux espèces ne sont pas adaptées à des conditions de fermentations difficiles (température élevée et concentration en éthanol élevée). Mon travail a montré que les hybrides interspécifiques *S. cerevisiae* * *S. uvarum*, par ailleurs moins sensibles à la température, avaient un rendement sucre/éthanol plus élevé que celui des souches de *S. cerevisiae*, et semblable à celui des souches de *S. uvarum*. Ce résultat est en accord avec ceux de Bely et al. (2013) qui ont démontré que ces mêmes hybrides *S. cerevisiae* * *S. uvarum* pouvaient réduire la concentration en éthanol du vin jusqu'à 0.4 % sans produire plus d'acide acétique.

Un autre avantage des hybrides interspécifiques concerne les arômes du vin. Les éthyl-esters, qui confèrent des notes fruitées aux vins, sont plus concentrés chez les hybrides

interspécifiques que chez les espèces parentales, ce qui pourrait être intéressant pour l'œnologie.

Les souches de levures hybrides cultivees en conditions fermentaires presentent de la robustesse face aux perturbations environnementales

Que la température ait un effet marqué sur différentes variables décrivant la cinétique de fermentation (vitesses, taux de croissance, flux de CO_2) était évidemment attendu. Mais comme l'a montré l'ACP incluant les hybrides *in silico* construits en supposant l'additivité pour tous les caractères, cet effet est un peu moins fort chez les hybrides intra- et interspécifiques que chez les parents. Par ailleurs la LDA a révélé que pour une série de variables présentant à la fois un effet souche et un effet souche*température (CO_{2max}, rendement sucre/éthanol, production d'éthanol, de sucre résiduel et de divers métabolites), les hybrides interspécifiques étaient plus robustes vis-à-vis de la température que les hybrides *S. cerevisiae * S. cerevisiae*, qui eux-mêmes le sont plus que les hybrides *S. uvarum * S. uvarum*. Cette homéostasie, ainsi que les transgressions observées pour certains caractères, pourrait expliquer le succès de certains hybrides interspécifiques dans les milieux naturels ou anthropiques.

Perspectives

Beaucoup d'analyses restent à faire pour exploiter entièrement les données de cette thèse. En particulier l'étude de l'hétérosis pour chacun des croisements aux deux températures n'a pas été faite. Ceci permettra d'identifier les croisements les plus hétérotiques pour chaque caractère, d'estimer les aptitudes générales et spécifiques à la combinaison des souches parentales, et de vérifier le potentiel des hybrides interspécifiques suggéré par mes résultats pour l'œnologie.

Un aspect plus fondamental sera de relier les différents niveaux phénotypiques, en intégrant le niveau protéomique déjà analysé en détail. Blein-Nicolas et al. (article soumis en annexe) ont étudié l'hérédité de 1396 protéines dans ce même diallèle. Ils ont montré que la proportion de protéines hétérotiques dépendait fortement de l'hybride considéré et de la température. Globalement, pour les hybrides intraspécifiques, cette proportion est plus élevée lorsque la température de culture n'est pas optimale, et ces hybrides présentent un fort hétérosis positif. Par ailleurs, leur protéome se distingue clairement de celui des souches intraspécifiques par un groupe de protéines présentant de l'hétérosis meilleur-parent. L'hybridation interspécifique crée un phénotype protéomique « exotique » pour certaines catégories de protéines (codées par des gènes essentiels, impliquées dans le métabolisme des protéines, etc.). Il sera intéressant de relier la fonction de ces protéines avec les différents caractères mesurés dans ma thèse pour aborder la recherche des bases moléculaires de leur hérédité.

RÉFÉRENCES BIBLIOGRAPHIQUES

Albertin W. and Marullo P. (2012) Polyploidy in fungi: evolution after whole-genome duplication. Proc Biol Sci 279(1738): p.2497–509.

Albertin W., da Silva T., Rigoulet M. and Salin B. (2013) The Mitochondrial Genome Impacts Respiration but not Fermentation in Interspecific *Saccharomyces* Hybrids. PLoS One, 8(9): e75121.

Almeida P., Goncalves C., Teixeira S., Libkind D. et al. (2014) A Gondwanan Imprint on the Global Diversity and Domestication of the Wine and Cider Yeast *Saccharomyces uvarum*. Nature communications in press.

Arroyo-Lopez F.N., Orlic S., Querol A. and Barrio E. (2009) Effects of temperature, pH and sugar concentration on the growth parameters of *Saccharomyces cerevisiae*, *S. kudriavzevii* and their interspecific hybrid. Int J Food Microbiol 131: p.120–127.

Barnett J. A., Payne R.W. and Yarrow D. (2000) Yeasts: Characteristics and Identification. 3e ed Cambridge University Press.

Belloch C., Orlic S., Barrio E. and Querol A. (2008) Fermentative stress adaptation of hybrids within the Saccharomyces sensu stricto complex. Int. J. Food Microbiol. 122: p.188–195.

Bely M., Renault P., da Silva T., Masneuf-Pomarède I. et al. (2013) Non conventional yeasts and alcohol levels reduction. Bordeaux.

Berlan J.P. (2002) Ordre et désordres dans l'économie monde. Paris : PUF.

Birchker J.A., Auger D.L and Riddle R.C. (2003). In Search of the Molecular Basis of Heterosis. The Plant Cell. 15: p.2236–2239.

Blank L.M., Lehmbeck F. and Sauer U. (2005) Metabolic-flux and network analysis in fourteen hemiascomycetous yeasts. FEMS Yeast Research 5: p.545–558.

Bruce A.B. (1910) The mendelian theory of heredity and the augmentation of vigor. Science 32: p.627–628.

Buerkle C. A., Morris R. J., Asmussen M. A. and Rieseberg L. H. (2000) The likelihood of homoploid hybrid speciation. Heredity 84(July 1999): p.441–451.

Burgess K.S. and Husband B.C. (2004) Maternal and paternal contributions to the fitness of hybrids between red and white mulberry (Morus, Moraceae). American Journal of Botany 91: 1802–1808. 30.

Castellari L., Ferruzzi M., Magrini G., Giudici P. et al. (1994). Unbalanced wine fermentation by cryotolerant vs. non-cryotolerant *Saccharomyces* strains. Vitis 33: p.49–52.

Chen Z.J. (2010) Molecular mechanisms of polyploidy and hybrid vigor. Trends in Plant Science 15(2): p.57–71. doi:10.1016/j.tplants.2009.12.003.

Crabtree H.G. (1929) Observations on the carbohydrate metabolism of tumours. Biochem J 23: p.536–545.

Crow J.F. (1948) Alternative hypotheses of hybrid vigor. Genetics 33: p.477–487.

Darwin C. (1876) The effects of cross and self fertilization in the vegetable Kingdom. John Murray, London (Ed. française : Des effets de la fécondation croisée et de la fécondation directe dans le règne végétal. Trad. De E. Heckel, C. Reinwald et al. 1877).

Davenport B.B. (1908) Degeneration, albinism and inbreeding. Science 28: p.454–455.

Dettman J.R., Sirjusingh C., Kohn L.M. et al. (2007). Incipient speciation by divergent adaptation and antagonistic epistasis in yeast. NATURE 447: p.585.

De Wit H.C.D. and Baudière A. (1993) Histoire du développement de la biologie. Volume 2. Presses Polytechniques Universitaires Romandes.

Dooner H.K., Robbins T.P., Jorgensen R.A. (1991) Genetic and developmental control of anthocyanin biosynthesis. Annu. Rev. Genet. 25: p.173–199.

Dufour M., Zimmer A., Thibon C. and Marullo P. (2013) Enhancement of volatile thiol release of Saccharomyces cerevisiae strains using molecular breeding. Appl Microbiol Biotechnol 97(13): p.5893–905.

Dujon B. (1996) The yeast genome project: what did we learn? Trends in Genetics 12(7): p.263.

Dujon B. (2010). Yeast evolutionary genomics. Nature Publishing Group, 11(7): p.512–524. doi:10.1038/nrg2811

Dunn B., Paulish T., Stanbery A., Piotrowski J., Koniges G. et al. (2013) Recurrent Rearrangement during Adaptive Evolution in an Interspecific Yeast Hybrid Suggests a Model for Rapid Introgression. PLoS Genet 9(3): p.e1003366.

Erny C., Raoult P., Alais A., Butterlin G. et al. (2012) Ecological success of a group of *Saccharomyces cerevisiae*/*Saccharomyces kudriavzevii* hybrids in the northern european wine-making environment. Appl Environ Microbiol 78(9): p.3256–65.

Fay J.C and Benavides J.A. (2005) Evidence for domesticated and wild populations of *Saccharomyces cerevisiae*, PLoS Genet. 1 66–71.

Fiechter A. and Seghezzi W. (1992) Regulation o glucose metabolism in growing yeast cells. Journal of Biotechnology 27: p.27–45.

Flint-garcia S.A., Buckler E.S., Tiffin P., Ersoz E. and Springer, N.M. (2009) Heterosis Is Prevalent for Multiple Traits in Diverse Maize Germplasm. PLoS One 4.

Gallais A. (2009) Hétérosis et variétés hybrides en amélioration des plantes. Quae, Versailles.

Giudici P., Caggia C., Pulvirenti A. and Rainieri S. (1998) Karyotyping of *Saccharomyces* strains with different temperature profiles. J Appl Microbiol 84: p.811–819.

Giudici P., Solieri L., Pulvirenti A.M. and Cassanelli S. (2005) Strategies and perspectives for genetic improvement of wine yeasts. Appl. Microbiol. Biotechnol. 66(6): p.622–8.

Goff S.A. (2010) Tansley review A unifying theory for general multigenic heterosis: energy efficiency, protein metabolism, and implications for molecular breeding. New Phytologist.

Goffeau A., Barrell B.G., Bussey H., Davis R.W. et al. (1996) Life with 6000 genes. Science 274 : p.546–567.

Goldman and Hallauer (1997) CIMMYT Book of Abstracts. The Genetics and Exploitations of Heterosis in Crops; An International Symposium. Mexico. D,F. Mexico. p.4–p.350.

Groszmann M., Greaves I. K., Albertyn Z. I., Scofield G. N. et al. (2011) Changes in 24-nt siRNA levels in Arabidopsis hybrids suggest an epigenetic contribution to hybrid vigor. PNAS 108: p.2617–2622.

Ha M., Lu J., Tian L., Ramachandran V. et al. (2009). Small RNAs serve as a genetic buffer against genomic shock in Arabidopsis interspecific hybrids and allopolyploids. PNAS 106(42): p.17835–17840.

Hagman A., Säll T., Compagno C. and Piskur J. (2013) Yeast "Make-Accumulate-Consume" Life Strategy Evolved as a Multi-Step Process That Predates the Whole Genome Duplication. Plos One 8(7): e68734.

Hall J.G. and Wills C. (1987) Conditional overdominance at an alcohol desydrogenase locus in yeast. Genetics 117: p.421–7.

Hittinger C.T. (2013) Saccharomyces diversity and evolution: a budding model genus. Trends in Genetics 29(5): p.309–317.

Howell K.S., Swiegers J.H., Elsey G.M, Siebert T.E. et al. (2004) Variation in 4-mercapto-4-methyl-pentan-2-one release by *Saccharomyces cerevisiae* commercial wine strains. FEMS Microbiol Lett 240(2): p.125–9.

Hull F.H. (1946) Overdominance and corn breeding where hybrid seed is not feasible. Journal of the Society of Agronomy 38: p.1100–1103.

Jiranek V., Langridge P. and Henschke P.A. (1995) Regulation of hydrogen sulfide liberation in wine-producing *Saccharomyces cerevisiae* strains by assimilable nitrogen. Appl Environ Microbiol 61(2): p.461–7.

Jiranek V., Langridge P. and Henschke P.A. (1996) Determination of sulphite reductase activity and its response to assimilable nitrogen status in a commercial *Saccharomyces cerevisiae* wine yeast. J Appl Bacteriol 81: p.329–336.

Jones D.F. (1917) Dominance of linked factors as a means of accounting for heterosis. PNAS 3: p.310–312.

Jones D.F. (1945) Heterosis resulting from degenerative changes. Genetics 30: p.527–542.

Jones D.F. (1952) Plasmagenes and chromogenes in heterosis. Heterosis, 2,-2345 J.W. GowErx, Ed., Ames, Iowa.

Kappeli O. (1986) Regulation of carbon metabolism in Saccharomyces cerevisiae and related yeasts. Advanced Microbial Physiology 28: p.181–209.

Keeble F. and Pellew C. (1910) The mode of inheritance of stature and of time of flowering in peas (*Pisum sativum*). J. Genet 1: p.47–56.

Kishimoto M. and Goto S. (1995) Growth temperatures and electrophoretic karyotyping as tools for practical discrimination of *Saccharomyces bayanus* and *Saccharomyces cerevisiae*. Journal of General and Applied Microbiology A. 41(3): p.239–247.

Legras J.L., Merdinoglu D., Cornuet J.M. and KARST F. (2007) Bread, beer and wine: *Saccharomyces cerevisiae* diversity reflects human history. Molecular Ecology 16: p.2091–2102. doi: 10.1111/j.1365-294X.2007.03266.x.

Le Jeune C., Lollier M., Demuyter C., Erny C. et al. (2007) Characterization of natural hybrids of *Saccharomyces cerevisiae* and *Saccharomyces bayanus* var. *uvarum*. FEMS Yeast Research 7: p.540–549. doi: 10.1111/j.1567-1364.2007.00207.x.

Libkind D., Todd C., Valério E., Gonçalves C. et al. (2011) Microbe domestication and the identi fi cation of the wild genetic stock of lager-brewing yeast. PNAS, 108(35): p.14539–14544. doi: 10.1073/pnas.1105430108.

Lintz F.A. (1963) Théories de l'hétérosis et relations karyocytoplasmiques. Acta biotheor., Ser. A., 16, -126 (in Plant eByed Abstr., 34, 5) 0.77.

Liti G., Carter D.M., Moses A.M., Warringer J. et al. (2009) Population genomics of domestic and wild yeasts. Nature 458(7236): p.337–341.

Magwene P.M., Kay Ö., Granek J.A., Reininga J.M. et al. (2010) Trade-offs shape genome evolution in *Saccharomyces cerevisiae*. PNAS, 108(5): p.1987–1992. doi: 10.1073/pnas.1012544108.

Mallet J. (2005) Hybridization as an invasion of the genome. Trends in Ecology and Evolution, 20(5): p.229–237. doi: 10.1016/j.tree.2005.02.010.

Marchal A., Marullo P. Moine V. and Dubourdieu D. (2011) Influence of yeast macromolecules on sweetness in dry wines: role of the saccharomyces cerevisiae protein Hsp12. J Agric Food Chem 59(5): p.2004–10.

Marullo P., Bely M., Masneuf-Pomarède I., Pons D. et al. (2006) Breeding strategies for combining fermentative qualities and reducing off-flavor production in a wine yeast model. FEMS Yeast Res. 6(2): p.268–79.

Marullo P., Yvert G., Bely M., Aigle M. and Dubourdieu D. (2007) Efficient use o DNA molecular markers to construct industrial yeast strains. 8th internatinal symposium of enology. Bordeaux : Vigne et Vin Publications internationales éditeurs.

Masneuf I., Murat M.L., Namov G.I., Tomnaga T. and Dubourdieu D. (2002) Hybrids *Saccharomyces cerevisiae* x *Saccharomyces bayanus var. uvarum* having a high liberating ability of some sulfur varietal aromas of Vitis vinifera Sauvignon blanc wines. Journal international des sciences de la vigne et du vin 36(4): p.205–212.

Masneuf-Pomarede I., Bely M., Marullo P., Lonvaud-Funel A. and Dubourdieu D. (2010) Reassessment of phenotypic traits for *Saccharomyces bayanus var. uvarum* wine yeast strains. Int J Food Microbiol 139: p.79–86.

Mayr E. (1996) What is a species, and what is not? Philosophy of Science 63: p.262-277.

Morales L. and Dujon B. (2012) Evolutionary Role of Interspecies Hybridization and Genetic Exchanges in Yeasts. Microbiology and Molecular Biology Reviews 76(4): p.721-739.

Naumov G.I. (1996) Genetic identification of biological species in the*Saccharomyces* sensu stricto complex. Journal of Industrial Microbiology 17(3-4): p.295–302.

Ng D.W., Lu J. and Chen Z.J. (2012) Big roles for small RNAs in polyploidy, hybrid vigor, and hybrid incompatibility. Curr. Opin. Plant Biol. 15: p.154–161.

Noble A.C. and Bursick G.F. (1984) The contribution of glycerol to perceived viscosity and sweetness in white wine. American Journal of Enlogy and Viticulture 35: p.110–112.

Novo M., Bigey F., Beyne E., Galeote V. et al. (2009) Eukaryote-to-eukaryote gene transfer events revealed by the genome sequence of the wine yeast *Saccharomyces cerevisiae* EC1118. Proc Natl Acad Sci U S A 106(38): p.16333-8.

Peyrot des Gachons C., Tominaga T. and Dubourdieu D. (2000) Measuring the aromatic potential of Vitis vinifera L. Cv. Sauvignon blanc grappe by assaying S-cysteine conjugates, precursors of the volatile thiols responsible for their varietal aroma. J Agric Food Chem 48: p.3387-91.

Plech M., de Visser J.A.G.M. and Korona R. (2014) Heterosis Is Prevalent Among Domesticated but not Wild Strains of *Saccharomyces cerevisiae*. G3: Genes|Genomes|Genetics 4(2): p.315–323.

Pronk J.T., Yde Steensma H. and Van Dijken J.P. (1996) Pyruvate metabolism in *Saccharomyces cerevisiae*. Yeast 12(16): p.1607-33.

Rand D.M., Fry A., and Sheldahl L. (2006) Nuclear-mitochondrial epistasis and drosophila aging: introgression of Drosophila simulans mtDNA modifies longevity in D. melanogaster nuclear backgrounds. Genetics 172: p.329–341.

Ribéreau-Gayon P., Dubourdieu D., Donèche B. and Lonvaud A. (2004) Traité d'oenologie Tome 1 5e éd. Microbiologie Du Vin. Vinifications.

Rood S.B., Buzell R.I., Mander L.N., Pearce D. and Pharis R.P. (1988) Gibberellins - A phytohormonal basis for heterosis in maize. Science 251(4870): p. 1216–1218.

Rosenfeld E., Beauvoit B., Rigoulet M. and Salmon J.M. (2002) Non-respiratory oxygen consumption pathways in anaerobically-grown *Saccharomyces cerevisiae*: evidence and partial characterization. Yeast 19: p.1299–1321.

Saerens S.M., Verstrepen K.J., Van Laere S.D.M, Voet A.R.D. et al. (2006) The *Saccharomyces cerevisiae* EHT1 and EEB1 genes encode novel enzymes with medium-chain fatty acid ethyl ester synthesis and hydrolysis capacity. J Biol Chem 281(7): p.4446–56.

Saerens S.M., Thevelein J. and Delvaux F. (2008) Ethyl ester production during brewery fermentation: a review. *Cerevisia* 33.

Schacherer J., Shapiro J.A., Ruderfer D.M. and Kruglyak L. (2009) Comprehensive polymorphism survey elucidates population structure of *Saccharomyces cerevisiae*. Nature 458(7236): p.342–5.

Schull G. H. (1908) The composition of a field of maize. Am Breeders, Assoc Rep. 4: p.296–301.

Shapira R., Levy T., Shaked S., Fridman E. and David L. (2014) Extensive heterosis in growth of yeast hybrids is explained by a combination of genetic models. Heredity (Edinb) p.1–11.

Sharma S. and Tauro P. (1986) Control of ethanol production by yeast:Role of pyruvate decarboxylase and alcohol desydrgenase. Biotechnology Letters 8: p.735–738.

Shen H., He H., Li J., Chen W. et al. (2012). Genome-Wide Analysis of DNA Methylation and Gene Expression Changes in Two Arabidopsis Ecotypes and Their Reciprocal Hybrids. The Plant Cell, 24(March): p875–892. doi: 10.1105/tpc.111.094870.

Sicard D. and Legras J. (2011) Bread, beer and wine: Yeast domestication in the *Saccharomyces* sensu stricto complex. Comptes Rendus Biol. doi: 10.1016/j.crvi.2010.12.016.

Sinha H., Nicholson B.P., Steinmetz L.M. and McCusker J.H. (2006) Complex genetic interactions in a quantitative trait locus. PLoS Genet 2(2): e13.

Spirek M., Horvath A., Piskur J. and Sulo P. (2000) Functional co-operation between the nuclei of *Saccharomyces cerevisiae* and mitochondria from other yeast species. Curr Genet 38: 202–207.

Srivastava H.K. (1981) Intergenomic interaction, heterosis and improvement of crop yield. Advances in Agronomy 34: p.117–195.

Steensels J., Snoek T., Meersman E., Nicolino M.P. et al. (2014) Improving industrial yeast strains: exploiting natural and artificial diversity. FEMS Microbiol Rev.

Steinmetz L.M., Sinha H., Richards D.R., Spiegelman J.I. et al. (2002) Dissecting the architecture of a quantitative trait locus in yeast. Nature 416(6878): p.326–30.

Subileau M., Schneider R., Salmon J.M. and Degryse E. (2008) New insights on 3-mercaptohexanol (3MH) biogenesis in Sauvignon Blanc wines: Cys-3MH and (E)-hexen-2-al are not the major precursors. J Agric Food Chem 56(19): p.9230-5.

Sulo P., Spirek M., Soltesova A., Marinoni G. and Piskur J. (2003) The efficiency of functional mitochondrial replacement in *Saccharomyces* species has directional character. FEMS Yeast Res 4: p.97–104.

Sumby K.M., Grbin P.R. and Jiranek V. (2010) Microbial modulation of aromatic esters in wine: current knowledge and future prospects. Food Chemistry 121(1): p.1-16.

Thibon C., Marullo P., Claisse O., Cullin C. et al. (2008) Nitrogen catabolic repression controls the release of volatile thiols by *Saccharomyces cerevisiae* during wine fermentation. FEMS Yeast Res 8(7): p.1076-86.

Tilloy V., Ortiz J.A. and Dequin S. (2014) Reducing ethanol and improving glycerol yield by adaptive evolution of *Saccharomyces cerevisiae* wine yeast under hyperosmotic conditions. Appl Environ Microbiol.

Timberlake W.E., Frizzell M.A., Richards K.D. and Gardner R.C. (2010) A new yeast genetic resource for analysis and breeding. Yeast 28(1): p.63-80.

Tominaga T., Furrer A., Henry R. and Dubourdieu D. (1998) Identification of new volatile thiols in aroma of Vitis vinifera L. var. Sauvignon blanc wines. Flavour Fragr J 13: p.159-162.

Tosi E., Azzolini M., Guzzo F. and Zapparoli G. (2009) Evidence of different fermentation behaviours of two indigenous strains of *Saccharomyces cerevisiae* and *Saccharomyces uvarum* isolated from Amarone wine. Journal of Applied Microbiology 107: p.210–218. doi: 10.1111/j.1365-2672.2009.04196.x.

Urk H., Bruinenberg P.M., Veenhuis M., Scheffers W.A. and Van Dijken J.P. (1989) Respiratory capacities of mitochondria of *Saccharomyces cerevisiae* CBS 8066 and *Candida utilis* CBS 621 grown under glucose limitation. Antonie Van Leeuwenhoel 56 : p.211-220.

Veitia R. A. and Vaiman D. (2011) Exploring the mechanistic bases of heterosis from the perspective of macromolecular complexes. FASEB. doi: 10.1096/fj.10-170639.

Wang Q.M., Liu W.Q., Liti G., Wang S.A. and Bai F.Y. (2012) Surprisingly diverged populations of *Saccharomyces cerevisiae* in natural environments remote from human activity. Molecular Ecology 21: p.5404–5417. doi: 10.1111/j.1365-294X.2012.05732.x.

Weusthuis R.A., Visser W., Pronk J.T., Scheffers W.A. and Van Dijken J.P. (1994) Effects of oxygen limitation on sugar metabolism in yeasts: a continuous-culture study of the Kluyver effect. Microbiology 140:703–715.

Zörgö E., Gjuvsland A., Cubillos F.A., Louis A.J. et al. (2012) Life History Shapes Trait Heredity by Accumulation of Loss-of-Function Alleles in Yeast. Mol Biol Evol 29(7): p.1781–9.

ANNEXE

Heterosis for protein abundance in yeast affects primarily highly regulated and evolutionary constrained proteins.

Heterosis for protein abundance in yeast primarily affects highly regulated and evolutionary constrained proteins

Mélisande Blein-Nicolas[1], Warren Albertin[2,3], Telma da Silva[1,4], Benoît Valot[5], Thierry Balliau[1], Isabelle Masneuf-Pomarède[3,6], Marina Bely[3], Philippe Marullo[3,7], Delphine Sicard[8,9], Christine Dillmann[8], Dominique de Vienne[8], Michel Zivy[10]*

1 INRA, PAPPSO, UMR 0320/UMR 8120 Génétique Végétale, F-91190, Gif-sur-Yvette, France

2 CNRS, UMR 0320/UMR 8120, Génétique Végétale, F-91190 Gif-sur-Yvette, France

3 Université Bordeaux, Unité de Recherche Œnologie, EA 4577, ISVV, 210 chemin de Leysotte, 33140 Villenave d'Ornon, France

4 Ariana Pharmaceuticals, 28 rue du Docteur Finlay, 75015 Paris

5 CNRS, Université de Franche-Comté, UMR 6249 Chrono-Environnement, F-25000, Besançon, France

6 Bordeaux Sciences Agro, Gradignan, France

7 BIOLAFFORT, F-33034 Bordeaux, France

8 Univ Paris-Sud, UMR 0320/UMR 8120 Génétique Végétale, F-91190, Gif-sur-Yvette, France

9 INRA, UMR1083, 2 Place Viala, F-34060 Montpellier, France

10 CNRS, PAPPSO, UMR 0320/UMR 8120 Génétique Végétale, F-91190, Gif-sur-Yvette, France

* Corresponding author: Michel Zivy

Email: zivy@moulon.inra.fr

Address: PAPPSO, Plateforme d'Analyse Protéomique de Paris Sud-Ouest, UMR de Génétique Végétale, INRA - Univ Paris Sud - CNRS, Ferme du Moulon, F-91190 Gif-sur-Yvette, France

Fax : +33 1 69 33 23 40

ABSTRACT

Background

Heterosis is a universal phenomenon, which is of immense socio-economic value for agriculture. Its genetic and molecular bases have been studied for more than 100 years, but still remain elusive. To study the molecular manifestations of this intriguing issue, we analyzed the inheritance of 1396 proteins in 55 inter- and intra-specific hybrids obtained from *Saccharomyces cerevisiae* and *S. uvarum* and grown in grape juice at two temperatures.

Results

The proportion of heterotic proteins was highly variable depending on the parental strain and on the temperature considered. In intra-specific hybrids, this proportion was higher at non-optimal temperature. Inter-specific hybrids displayed a strong bias toward positive heterosis. Their proteome clearly differed from that of the other strains by a group of proteins showing best-parent heterosis. These proteins exhibited several features suggesting that their abundances are under evolutionary constraint: they presented little quantitative variations between parents and between temperatures, were highly abundant and were enriched in proteins encoded by essential genes and in proteins involved in protein metabolism. More generally, the proportion of hybrids in which a protein was heterotic was correlated to the number of putative transcription factors of the encoding gene. This correlation was particularly strong when proteins were grouped by functional category.

Conclusions

Together, these results highlight mechanisms of heterosis for protein abundance that may be related to selection pressures acting on the regulators controlling protein abundances.

KEYWORDS

heterosis, Saccharomyces, quantitative proteomics

BACKGROUND

Non-additive inheritance, in which the hybrid phenotype is non-intermediary between the parental phenotypes, is commonly observed in all species. For monogenic traits, the departure from additivity is called dominance [1], or over-dominance if the hybrid phenotype is outside the range defined by homozygous phenotypes [2]. Regarding polygenic traits, gene effects producing non-additive inheritance are more complex and are collectively referred to as heterosis.

Heterosis has fascinated scientists and breeders for more than 100 years for its effects on traits such as growth rate, biomass, size, yield or fertility [3, 4]. It is exploited since the 1930s in plant breeding to produce hybrids of high agronomic value [5]. In this context, heterosis has proven to efficiently accelerate the process of selection for various crops (reviewed in [6]). Heterosis is opposite to inbreeding depression, which is supposed to be predominantly caused by the homozygosity of deleterious recessive alleles [7]. Heterosis provides a heterozygote advantage by buffering against these alleles and confers genetic plasticity to adapt to environmental changes [8].

Given the importance of heterosis for agriculture and because it is an intriguing phenomenon, many studies have focused on the understanding of its genetic and molecular bases [8–19]. Three non-exclusive hypotheses based on genetic effects are classically put forward to explain heterosis. First, the dominance hypothesis attributes heterosis to complementation: in the hybrid, the different recessive deleterious alleles are masked by dominant superior alleles [20, 21]. Second, the over-dominance hypothesis assumes that heterosis results from the inherent superiority of the heterozygote over its homozygous parents [2, 22]. Third, the epistasis hypothesis proposes that heterosis is due to favourable intergenic interactions created in the hybrid [23, 24]. Scientists have long sought for a unifying theory to account for heterosis, but it is now commonly admitted that this phenomenon likely arises from the combination of several mechanisms, the effects of which vary according to the trait, the cross or the species [17, 19].

In spite of the complex and versatile nature of heterosis, general trends have emerged when

compiling the results achieved so far across different studies. For example, heterosis is of greatest magnitude for highly complex traits such as yield [17, 25]; it is largest in allogamous than in autogamous species [25]; it requires genetic divergence between parents, and inter-specific crosses generally produce higher levels of heterosis [15, 18, 26]; positive heterosis is much more common than its negative counterpart [14].

Heterosis has long been studied for macroscopic traits, but it can occur at all biological levels. For instance, heterosis was reported for molecular traits such as transcript abundance [27–29], protein abundance [30–33], enzyme activity [34–36], metabolite abundance [37] or metabolic flux [38]. To address the issue of the molecular manifestations of heterosis, we analyzed heterosis for the abundances of a large number of proteins in intra- and inter-specific hybrids of yeast. Proteins are particularly relevant because they play key roles in all the cellular functions. In addition, protein abundances are polygenic molecular traits [39] that can be measured at high-throughput by quantitative proteomics [40, 41].

Although yeast is a model species amenable to large laboratory experiments, it has not been frequently used to study heterosis [42–47]. Given that yeast is also an organism of industrial interest for wine-making, we used two species well adapted to oenological conditions, namely *Saccharomyces cerevisiae* and *S. uvarum* Beijerinck. Hybrids with exceptional performances were reported in *S. cerevisiae* [43, 44, 48, 49]. In addition, inter-specific hybrids between *S. cerevisiae* and *S. uvarum* seem to have important biotechnological potentials for wine-making [49–52]. Our experimental design included 11 parental strains of *S. cerevisiae* and *S. uvarum* and their 55 intra- and inter-specific hybrids, which were grown at two temperatures to take into account adaptation differences between parental species (18°C and 26°C optimal for *S. uvarum* and *S. cerevisiae*, respectively [53–55]). We specifically addressed the following questions: what is the extent of heterosis for protein abundance? Do inter-specific crosses produce more heterosis for protein abundance than intra-specific crosses? Are there specific features of the proteins exhibiting

heterosis?

RESULTS

Protein quantification by LC-MS/MS

A total of 396 alcoholic fermentations (66 strains x 2 temperatures x 3 replicates) were performed, of which 34 were discarded due to the poor fermenting abilities of some strains (Additional file 1, Table S1). Yeast samples taken from the 362 successful fermentations were analyzed by shotgun label-free quantitative proteomics. Peptides were quantified by integrating precursor ion peak areas. The quantification measurements obtained for each peptide as well as detailed information on all the peptides and all the proteins identified in all LC-MS/MS runs were deposited on-line using PROTICdb database [56–58] at the following URL: http://moulon.inra.fr/protic/heterosyeast2 (username: heterosyeast and password: yeast. These data will be made freely available after publication).

In total, 1514 proteins were quantified in at least one strain x temperature combination. Of them, 1396 proteins were quantified both in a hybrid and its parents at the same temperature (Additional file 2). These 1396 proteins were assigned to 16 functional categories following the MIPS Functional Catalogue Database [59] (Additional File 3, Figure S1, Additional File 1, Table S2). Metabolism was the most represented category, with 534 proteins (31.1% coverage; Additional File 3, Figure S1).

Representation of protein abundances as heatmap showed that the strain x temperature combinations were separated in three main clusters corresponding globally to *S. uvarum* strains (cluster A), inter-specific hybrids (cluster B) and *S. cerevisiae* strains (cluster C; Figure 1). Inter-specific hybrids differed from all the other strains by a cluster of proteins that were globally more abundant than in the other strains (cluster II). *S. uvarum* strains and *S.cerevisiae* strains differed by two clusters of proteins: cluster I containing proteins that were more abundant in *S. cerevisiae* and cluster III containing proteins that were more abundant in *S. uvarum*. Except for a

particular group containing the parental strain D2 and all its descendants (including inter-specific hybrids; cluster D), the strains x temperature combinations within the clusters A, B and C were grouped by temperature.

Protein inheritance patterns

To analyze the inheritance of protein abundances at a given temperature, we considered the triplets (formed by one hybrid and its parents) where at least two successful fermentations were obtained for each member. This was the case for 53 triplets at 18°C and for 44 triplets at 26°C (Additional File 1, Table S1). For each protein x hybrid x temperature combination, we computed the deviation from additivity (*d*) as the difference between hybrid and mid-parental abundances. A protein was considered as heterotic whenever *d* was significantly different from zero (Wald test adjusted $P < 0.05$, Additional File 2). A total of 97 360 protein x hybrid x temperature combinations were examined. For 65.2% (63 469) of them, no significant abundance variation was detected neither between a hybrid and its parent, nor between parents (invariant proteins). The remaining 33 891 protein x hybrid x temperature combinations were classified depending on their inheritance pattern (Figure 2; Additional File 2; Additional File 1, Table S3): 66.8% (22 634) displayed additivity; 11.7% (3965) displayed negative or positive mid-parent heterosis (MPH), meaning that the protein abundance in the hybrid was within the parental range; 11.0% (3746) displayed best-parent or worst-parent heterosis (BPH and WPH, respectively), meaning that the protein abundance in the hybrid fell outside the parental range; 10.5% (3546) corresponded to cases of unresolved heterosis because statistical tests did not allow us to distinguish between mid-parent and best/worst-parent heterosis.

The proportion of heterotic proteins per hybrid x temperature combination (invariant proteins omitted) was highly variable, ranging from 8.4 to 61.2% with a median at 31.4% (Table 1). The parent, the temperature and the parent x temperature interaction were significantly involved in the

variations of this proportion (Figure 3A). Globally, hybrids having at least one *S. cerevisiae* strain as parent showed more heterotic proteins at 18°C than at 26°C (Figures 3B and C). On the contrary, hybrids having a *S. uvarum* strain as parent showed slightly more heterotic proteins at 26°C than at 18°C in intra-specific crosses while this effect was not visible in inter-specific crosses (Figure 3B and C).

Inter-specific hybrids exhibit specific characteristics regarding protein abundance inheritance

We further analyzed heterosis for protein abundance in inter- *versus* intra-specific hybrids. By examining the distribution of relative additivity deviation (computed as *d/m*, where *m* is the parental mean), we showed that *d/m* was globally higher in inter- than in intra-specific hybrids (Figure 4A). In addition, the proportion of heterotic proteins with positive *d* values was, on average, much higher in inter- than intra-specific hybrids (78.8%, 52.3% and 42.6% in inter-specific, *S. cerevisiae* and *S. uvarum* hybrids, respectively; Figure 4B). This indicates a strong bias toward positive heterosis in inter-specific hybrids.

We next looked whether the temperature affected protein inheritance similarly in inter-specific hybrids compared to intra-specific hybrids. For the majority of the protein x hybrid combinations (82.5%), the protein was heterotic at only one temperature. For the remaining 17.5%, four scenarios were possible depending on the sign of *d* at the two temperatures: positive at both 18°C and 26°C (+/+), negative at both 18°C and 26°C (–/–), positive at 18°C and negative at 26°C (+/–), negative at 18°C and positive at 26°C (–/+). Globally, inter-specific hybrids presented an excess of +/+ scenarios (451 over 656, χ^2 $P = 7.0\text{x}10^{-12}$; Figure 4C). This result holds true for nearly all inter-specific hybrids (Additional File 3, Figure S2A). Regarding intra-specific hybrids, *S. cerevisiae* hybrids presented an excess of +/– scenarios (252 over 719, χ^2 $P = 1.2\text{x}10^{-8}$; Additional File 3, Figure S3A), while *S. uvarum* hybrids presented an excess of –/+ scenarios (6 over 79, χ^2 $P = 1.2\text{x}10^{-8}$; Figure 3B). However, this result largely depended on the hybrid

considered (Additional File 3, Figure S2B).

Altogether, these results show that inter-specific hybrids exhibit specific characteristics, which are the same whatever the environmental and genetic context.

The remodeling of the proteome of inter-specific hybrids predominantly affects particular categories of proteins

Principal component analysis (PCA) based on the protein abundances estimated in all the strain x temperature combinations was performed in order to visualize the effects of the strains and of the temperature on the proteome (Figure 5). The first axis (PC1, 15.4% of the total variance) separated the parental and hybrid strains of *S. cerevisiae* from those of *S. uvarum*, with inter-specific hybrids located between the two species. Interestingly, within each type of hybrid (*S. cerevisiae*, *S. uvarum* and inter-specific), PC1 also separated hybrid x temperature combinations according to the temperature. Especially, *S. uvarum* strains moved along PC1 toward *S. cerevisiae* when temperature changed from 18°C to 26°C, and reciprocally *S. cerevisiae* strains moved along PC1 toward *S. uvarum* when temperature changed from 26°C to 18°C. This result shows that, when a species is grown at non-optimal temperature, its proteome tends to resemble that of the other species for which the temperature is optimal.

The second axis (PC2, 13.0% of the total variance) separated inter-specific hybrids from the other strains. PC2 contributed nearly as much as PC1 to the total variance, indicating that inter-specific hybridization has extensively remodeled the proteome. To characterize the proteins involved in the differentiation of inter-specific hybrids, we analyzed the proteins significantly correlated to PC2 (adjusted $P < 0.01$) with $|r| > 0.5$ (set H, 103 proteins; Additional File 1, Table S4). For all of them but one, r was positive, which indicates that these proteins were globally more abundant in inter-specific hybrids than in the other strains. This is in agreement with what observed on Figure 1. The proteins with $r > 0$ (set H+) contributed poorly to PC1, indicating that they

presented low abundance variations between *S. cerevisiae* and *S. uvarum* and between temperatures. Compared to the proteins that were not correlated to PC2 (set NH, 259 proteins; Additional File 1, Table S4), these proteins exhibited other specific characteristics. First, they were significantly enriched in heterotic proteins in inter-specific hybrids (+ 8.8%) but not in intra-specific hybrids (Figure 6A). Second, they were more abundant than other proteins (average abundance in parental strains: $2.2x10^7$ in NH *vs* $3.2x10^7$ in H+; Figure 6B). Third, they were significantly enriched in proteins encoded by essential genes, *i.e.* genes that are required for viability of *S. cerevisiae* under standard laboratory conditions [60, 61] (+ 161%; Figure 6C). Fourth, they were slightly enriched in proteins involved in protein metabolism (protein synthesis and protein fate; + 49.0%; Figure 6D).

Altogether, these results show that inter-specific hybridization caused BPH for a defined portion of the proteome that contains proteins characterized by the stability of their abundances toward genetic and environmental changes, by their high abundances and by their importance for the cell viability.

Heterosis for protein abundance is partly related to the complexity of transcriptional regulation

To determine the extent to which the factors controlling protein abundances could be involved in protein heterosis, we focused on the transcription factors (TFs) possibly involved in the regulation of the genes encoding the proteins quantified in our study. A total of 162 TFs sharing a consensus DNA-binding sequence were retrieved from the Yeastract database (www.yeastract.com; [62–65]). On average, the genes encoding proteins that were heterotic in at least one hybrid x temperature combination were putative targets of a higher number of TFs than the genes encoding non-heterotic proteins (27.7 vs 21.4; Mann-Whitney test $P = 1.96x10^{-15}$; Figure 7A). In addition, a significant correlation was found between the number of putative TFs of a gene and the proportion of hybrids x temperature combinations in which the encoded protein was heterotic ($r = 0.18$,

$P < 2.2x10^{-16}$, Additional File 3, Figure S4). This correlation was found both in intra- and in inter-specific crosses and at the two temperatures. Given that the proteins in H+ were more frequently heterotic in inter-specific hybrids than proteins in NH, we wondered whether they presented a higher number of putative TFs. This was not the case since the average number of TFs putatively binding to a gene was 25.4 and 27.8 for H+ and NH, respectively.

The number of putative TFs of a gene depended significantly on the functional category of the gene (generalized linear model, ANOVA $P < 2.2x10^{-16}$). As a consequence, the frequency at which a protein was heterotic was also dependent on its functional category. For example, the genes involved in metabolism, energy and cell rescue, defense and virulence had, on average, more putative TFs and their proteins were more frequently heterotic than those involved in cell differentiation (Figure 7B). Note that the protein synthesis category appeared as an outlier, containing proteins that were heterotic in a high proportion of hybrids but not presenting a very high number of putative TFs.

Altogether, these results indicate that the complexity of transcriptional regulation is involved in heterosis for protein abundance, which would also explain why some functional categories are more prone to heterosis than others. However, this is not sufficient to explain the heterosis observed in specific groups of proteins, such as H+ and protein synthesis.

DISCUSSION

We used label-free quantitative proteomics in yeast to perform a large scale study of heterosis for protein abundance. We successfully handled proteomic data obtained from several hundred of samples, which shows that quantitative proteomics is suitable to study large experimental designs and opens the way for its use in quantitative genetics. In agreement with previous results [66], we confirmed that the proteomes of *S. cerevisiae* and *S. uvarum* are highly differentiated. Interestingly, this differentiation is maximal when the strains grow in their respective optimal temperature, 18°C for *S. uvarum* and 26°C for *S. cerevisiae.* The *S. cerevisiae* proteome is closer to the *S. uvarum* proteome at 18°C, while the *S. uvarum* proteome is closer to the *S. cerevisiae* proteome at 26°C. These results are consistent with the adaptation of these two species to specific temperatures [53–55].

Heterosis for protein abundance is subject to genotype x environment interactions

Heterotic proteins were detected in every hybrid x temperature combinations analyzed. This is consistent with previous results showing that heterosis for gene expression and protein abundance is a common occurrence, regardless the species or genotypes considered (reviewed in [8]). The proportion of heterotic proteins varied from 8.4 to 61.2% depending on the hybrid x temperature combination considered. Comparatively, Khan *et al.* [67] found 85.9% of heterotic proteins (342 out of 398) in one *S. cerevisiae* x *S. uvarum* cross. However, these authors used an arbitrary threshold without statistical test to decide on the inheritance of the proteins, which may explain the discrepancy with our results. In any case, our study is much more representative of both the proteome and the genetic diversity of *S. cerevisiae* and *S. uvarum* since we examined 1396 proteins quantified in 55 crosses and at two temperatures. This allowed us to show that there were genotype x environment interactions for heterosis since the temperature did not affect protein inheritance

similarly in the different types of hybrid examined. Indeed, the proportion of heterotic proteins was higher at 18°C for *S. cerevisiae* and inter-specific hybrids and at 26°C for *S. uvarum* hybrids. Note that in the case of intra-specific hybrids, these temperatures were non-optimal, suggesting that there may be a relationship between the proportion of heterotic proteins and stressful growth conditions. In addition, the sign of *d* was little affected by temperature in inter-specific hybrids, which was not the case in intra-specific hybrids.

Heterosis in inter-specific hybrids fits well with the concave genotype-phenotype relationship

For metabolic fluxes and traits proportional to fluxes, dominance of the 'high' over the 'low' alleles [68], and hence positive heterosis [38, 69], are the consequence of the concave relationship between enzyme parameters and the flux through a metabolic system. Beyond the metabolic control theory, there are many examples of such a non-linear genotype-phenotype relationship at various levels of cell organization, from transcription to integrated phenotypes [70–74].

This is consistent with the observation that positive heterosis has been reported to be much more common than its negative counterpart, and fits well with our results for inter-specific hybrids. For these hybrids, BPH was more particularly related to a special group of proteins (set H+) whose abundances seemed to be under evolutionary constraint: (i) the proteins of this set were highly abundant. Previous observations have shown that highly expressed proteins evolve slowly [75–77]; (ii) the proteins of this set exhibited little abundance variations between temperatures and between *S. cerevisiae* and *S. uvarum*, yet two distantly related species [78]; (iii) this set was enriched in proteins encoded by essential genes, which are thought to be under strong purifying selection since they are highly conserved across large evolutionary distances in yeasts and mammals [79, 80]; (iv) this set was enriched in proteins involved in protein metabolism, among which proteins of ribosomes and proteasome that are structurally and functionally conserved [81, 82]. Observation of inter-specific heterosis for these proteins possibly reveals that the two species are genetically

contrasted and complementary at the loci controlling protein abundances (PQL, protein quantitative loci [39]), so that hybridization results in relaxation of abundance constraints.

By contrast, and unexpectedly, no bias toward positive heterosis was found in intra-specific hybrids, suggesting that the assumption of the dominance of 'high' over 'low' alleles may not prevail in intra-specific hybrids. We have no explanation for this discrepancy between intra- and inter-specific hybrids.

Heterosis for protein abundance is globally related to the complexity of transcriptional regulation

Our results show that the number of putative TFs of a gene is a proper predictor of heterosis for the abundance of the encoded protein. Regulation of transcription is complex, involving a combination of several TFs individually acting as activator and/or repressor [83]. Previous studies have shown that genetic polymorphism in cis and trans regulators can influence the inheritance pattern of gene expression level, polymorphism of trans regulators being preferentially associated to heterotic patterns [46, 84–86]. If the number of polymorphic TFs increases with the number of TFs, the relationship between the number of putative TFs of a gene and the frequency at which the encoded protein is heterotic is consistent. Conceptually, this is similar to what is observed for agronomic traits in plants, the genetic complexity of which is related to heterosis [17, 25].

The number of putative TFs of a gene depended significantly on the functional category of the gene, explaining why some functional categories were more prone to heterosis than others. Among the functional categories containing genes putatively regulated by a high number of TFs and showing frequently heterotic proteins, we found energy, metabolism and cell rescue, defense and virulence. This result is consistent with many studies in plants that show these categories to be involved in heterosis for gene expression (reviewed in [16, 18]). In addition, these categories are generally involved in response to environmental changes [87] and were therefore expected to be

highly regulated.

The proteins in H+ and the proteins involved in protein synthesis appeared as outliers regarding the relationship between the number of putative TFs of a gene and heterosis for protein abundance, presenting frequencies of heterosis higher than expected based on the number of putative TFs of their encoding genes. These two groups of proteins were partly redundant, since H+ was enriched for proteins involved in protein metabolism. To explain the peculiar behavior of these proteins, we assume that factors other than TFs are involved in heterosis for protein abundance as, for example, post-translational modifications, that were recently shown to be related to the variations of phenotypic traits [88].

CONCLUSIONS

We identified a special group of proteins that presented BPH in inter-specific hybrids and were characterized by several features suggesting that their abundances are under evolutionary constraint. These results highlight mechanisms of heterosis for protein abundance that may be related to the selection pressures acting on the regulators controlling protein abundances. In agreement with a role of these regulators in heterosis, we also showed that the complexity of transcriptional regulation, estimated through the number of putative TFs of a gene, is a general factor of heterosis for protein abundance, which supports the relationship between heterosis and trait complexity. Other factors are also probably involved in heterosis for protein abundance, such as post-translational modifications. The symmetry observed in intra-specific hybrids between positive and negative heterosis was unexpected in the light of previous results and of predictions from modeling of heterosis, which would require further investigation. Taken together, our results show the interest of high-throughput technologies to provide a more comprehensive view of complex biological phenomena such as heterosis.

METHODS

Yeast strains

Four diploid *S. uvarum* strains, seven diploid *S. cerevisiae* strains and their 55 hybrids produced from a diallel design [89] were analyzed in this study. Parental strains were derived from strains isolated from different geographical locations and from either natural or food-processing origins (Additional File 1, Table S5). The genetic variability of parental strains was assessed by amplification and sequence alignment of six genes (*ACC1*, *ALA1*, *ADP1*, *GLN4*, *VSP13* and *RPN2*), as previously described [66].

Alcoholic fermentation in grape must

All the 66 strains (11 parents and 55 hybrids) were grown in white grape must obtained from Sauvignon grapes harvested in vineyards in Bordeaux area (2009 vintage). Tartaric acid precipitation was stabilized and turbidity was adjusted to 100 NTU (Nephelometric Turbidity Unit) before storage at −20°C. The sugar concentration was 189 $g.l^{-1}$, the nitrogen content was 242 $mg.l^{-1}$ and the pH was 3.3. The indigenous yeast population, estimated by YPD-plate (Yeast extract Peptone Dextrose) counting after must thawing, was less than 20 CFU (Colony-Forming Unit) *per* ml. Pre-cultures of each strain were run in half-diluted must filtered through a 0.45 µm nitrate-cellulose membrane (24°C, 150 RPM (Rounds Per Minute) during 24h, after what one million cells per ml were sampled and added to a final volume of 125 ml of Sauvignon must. Then, fermentations were run into 125 ml glass-reactors at two different temperatures (18°C and 26°C, 300 RPM) and repeated three times independently. In total, 396 alcoholic fermentations were performed (66 strains x 2 temperatures x 3 replicates) following a randomized experimental design. Of them, 31 failed due to the poor fermenting abilities of some strains (Additional File 1, Table S1). The amount of CO_2 released was regularly determined by measurement of glass-reactor weight loss.

375

376 **Protein extraction and digestion**

377 Samples were harvested at 40% of CO_2 release to perform proteomic analyses. At this time, all
378 strains had reached their maximum population size and performed alcoholic fermentation without
379 growing. Only strain x temperature combinations with at least two successful fermentations were
380 kept for further mass-spectrometry analysis (Additional File 1, Table S1). Five ml of fermentative
381 media were sampled and centrifuged (5 min, 2750 g). The pellets were rinsed two times with 5 ml
382 of water, frozen in liquid nitrogen and stored at -80°C until protein extraction. Total protein extracts
383 were isolated via acetone precipitation as described in Blein-Nicolas et al. (2013). Dried protein
384 pellets were solubilized in 300 µl of a solution containing 6M of urea, 2M of thiurea, 10 mM of
385 dithiothreitol (DTT), 30mM of TrisHCl pH 8.8 and 0,1% of Zwitterionic Acid Labile Surfactant
386 (ZALS, Proteabio) and centrifugated for 10 mn at 14000 rpm. Protein concentration was determined
387 using PlusOne 2-D Quant Kit (GE Healthcare) and adjusted to 4 $\mu g.\mu l^{-1}$. After a 10-times dilution in
388 50 mM of ammonium bicarbonate, proteins were reduced 1 hour in 100mM DTT, alkylated 1 hour
389 in 40mM iodoacetamide and digested overnight at 37°C with 1/50 (w/w) trypsin (Promega).
390 Digestion was stopped by adding 0.4% of TFA (trifluoroacetic acid). Peptides were purified on solid
391 phase extraction using polymeric C18 column (Phenomenex) with a washing solution containing
392 0.06% acetic acid and 3% acetonitrile (ACN). After elution with 0.06% acetic acid and 70% ACN,
393 peptides were speedvac-dried and suspended in 2% ACN and 0.08% TFA.

394

395 **LC-MS/MS analysis**

396 LC-MS/MS analyses were performed using a NanoLC-Ultra System (nano2DUltra, Eksigent)
397 connected to a Q-Exactive mass spectrometer (Thermo Electron). A 700 ng of protein digest were
398 loaded onto a PepMap C18 precolumn (0.3 × 5 mm, 100 Å, 5 µm; Nanoseparation) at 7.5 $\mu l.min^{-1}$
399 and desalted with 0.1% formic acid and 2% ACN. After 3 min, the precolumn was connected to a

PepMap C18 nanocolumn (0.075 × 150 mm, 100 Å, 3 µm). Buffers were 0.1% formic acid in water (A) and 0.1% formic acid and 100% ACN (B). Peptides were separated using a linear gradient from 5 to 35% buffer B for 40 min at 300 nl.min^{-1}. One run took 60 min, including the regeneration step at 100% buffer B and the equilibration step at 100% buffer A.

Ionization was performed with a 1.3-kV spray voltage applied to an uncoated capillary probe (10 µm tip inner diameter; New Objective). Peptide ions were analyzed using Xcalibur 2.2 (Thermo Electron) with the following data-dependent acquisition steps: (1) MS scan (mass-to-charge ratio (m/z) 400 to 1400, 70 000 resolution, profile mode), (2) MS/MS (17 500 resolution, collision energy = 30%, profile mode). Step 2 was repeated for the eight major ions detected in step 1. Dynamic exclusion was set to 40 s. Xcalibur raw datafiles were transformed to mzXML open source format using msconvert software in the ProteoWizard 3.0.3706 package [90]. During conversion, MS and MS/MS data were centroided.

MS data availability

The raw MS output files were deposited online using PROTICdb database [56–58] at the following URL: http://moulon.inra.fr/protic/heterosyeast. They are currently available with the following username: heterosyeast and password: yeast. They will be made freely available after publication.

Protein identification

Protein identification was performed using the custom database described in [66], in which proteins of *S. cerevisiae* and of *S. uvarum* encoded by orthologous genes were attributed unique labels. A contaminant database containing the sequences of standard contaminants and the sequences of 16 proteins of *Vitis vinifera* previously identified in extracts of yeast grown in grape juice was also interrogated. The decoy database comprised the reverse protein sequences of the

custom database. Database search was performed with X!Tandem (version 2011.12.01.1; http://www.thegpm.org/TANDEM/) with the following settings. Enzymatic cleavage was declared as a trypsin digestion with one possible misscleavage. Carboxyamidomethylation of cysteine residues and oxidation of methionine residues were set to static and possible modifications, respectively. Precursor mass precision was set to 10 ppm. Fragment mass tolerance was 0.02 Th. A refinement search was added with the same settings, except that protein N-ter acetylations were also searched. Only peptides with a E-value smaller than 0.05 were reported.

Identified proteins were filtered and sorted by using X!TandemPipeline (version 3.3.0, http://pappso.inra.fr/bioinfo/xtandempipeline/). Criteria used for protein identification were (i) at least two different peptides identified with an E-value smaller than 0.05, (ii*)* a protein E-value (product of unique peptide E-values) smaller than 10^{-4}. These criteria led to a false discovery rate estimated by using the decoy database of 0.12% and 1.15% for peptide and protein identification, respectively.

Peptide quantification and processing intensity data

Peptides were quantified based on extracted ion chromatograms using MassChroQ software version 1.2.2 [91], with the parameters given in Additional File 4. Due to progressive fouling of the quadrupole, sensitivity losses were observed over time, leading to a global decrease of measured intensities, particularly for hydrophobic peptides. To take these sensitivity losses into account, samples were classified according to their running order and divided into five blocks representing homogeneous global intensities. For each peptide, the block effect was retrieved and substracted from intensity measures by using an analysis of variance (ANOVA). Then, normalization was performed to take into account possible global quantitative variations between LC-MS runs. For each LC-MS run, the ratio of all peptide values to their value in the chosen reference LC-MS run was computed. Normalization was performed by dividing peptide values by the median value of

peptide ratios.

Raw data (containing intensity measures of 25 060 peptides) were then filtered to remove (i) dubious peptides for which standard deviation of retention time was superior to 60 s, (ii) peptide x strain x temperature combinations quantified in only one replicate, (iii) peptides shared by several proteins, representing less than 5% of all the quantified peptides. To avoid bias on the estimation of total protein abundances in hybrids, we removed parent-specific peptides by using peptides presenting presence/absence variation among parental strains as a proxy. However, parent-specific peptides were confounded with species-specific peptides, which represented nearly 65% of the valid peptides. To exploit as far as possible the data available for intra-species crosses, we thus split the dataset into three subsets: one contained *S. cerevisiae* triplets (hybrid and its parents), another contained *S. uvarum* triplets and the last one contained inter-specific triplets. Parent-specific peptides were removed separately in the three subsets. To finish, in order to estimate the peptide effect properly, peptides quantified in less than four strains x temperature combinations in a given subset of data were removed.

Detection of protein abundance changes

Protein abundances were estimated independently in the three subsets of data by using the following mixed effect model:

$$\log(I_{istr}) = \theta_{kst} + D_i + B_r + C_{str} + \epsilon_{istr}$$

where I_{istr} is the normalized intensity value for peptide *i* in strain *s*, temperature *t* and replicate *r,*

θ_{kst} is the natural logarithm of the abundance of protein *k* in strain *s* and temperature *t,*

$B_r \sim N(0, \sigma_B^2)$ is an error due to the biological variation of replicate *r,*

$C_{str} \sim N(0, \sigma_C^2)$ is an error due to the technical variation of sample *str,*

$D_i \sim N(0, \sigma_D^2)$ is an error due to the LC-MS response of peptide *i,*

475 $\epsilon_{istr} \sim N(0, \sigma^2_\epsilon)$ is the residual error.

476 Estimation of the parameters of the model was performed as described in [92]. Protein abundance
477 changes were detected by multiple test procedure across four different contrasts: (i) hybrid–mean of
478 parents, (ii) hybrid–parent$_1$, (iii) hybrid–parent$_2$, (iv) parent$_1$–parent$_2$. Since several couples of
479 strains x temperature combinations and several proteins were tested, p-values were adjusted for
480 multiple testing by a Benjamini-Hochberg procedure [93]. Of note, the statistical power was
481 reduced in the subset of data containing inter-specific hybrids compared to the two other subsets
482 since intensity data were more drastically filtered (on average, there were 6.2 peptides per protein in
483 the subset containing inter-specific hybrids against 8.9 and 8.2 in the subsets containing
484 *S. cerevisiae* hybrids and *S. uvarum* hybrids, respectively).
485
486 **Data analysis**
487 Protein abundances estimated in different subsets of data were not directly comparable. To
488 overcome this drawback, the subset of data containing inter-specific hybrids (further named *B* for
489 between) was taken as a reference and the following linear regression was performed for each
490 protein in the subsets of data containing intra-specific hybrids (referred to as *W* for within):
491 $\theta^W_{pt} = a + b\,\theta^B_{pt} + \epsilon_{pt}$
492 where θ^W_{pt} and θ^B_{pt} are the abundances estimated in parental strain *p* at temperature *t* in the
493 subsets of data *W* and *B*, respectively
494 *a* and *b* are the parameters of intercept and slope, respectively
495 ϵ_{pt} is the residual error
496 The median of the coefficient of determination R^2 was 0.83 indicating that the protein abundances
497 estimated separately in different subsets of data were globally well correlated. For proteins with *b*
498 significantly different from 0 (adjusted $P < 0.05$), estimators of *a* and *b* were used to correct the
499 abundances estimated for intra-specific hybrids:

$$\omega_{ht}^{W} = (\theta_{ht}^{W} - \hat{a}) / \hat{b}$$

where θ_{ht}^{W} is the abundance estimated in hybrid *h* at temperature *t* in the subset *W*. Then, protein abundances in the subset *B* were gathered with the ω_{ht}^{W} computed in the subset *W*.

A total of 615 proteins quantified in more than 122 strains x temperature combinations were kept for data representation as heatmap and principal component analysis (PCA). Missing data were imputed from a uniform distribution with minimum = 0 and maximum = 10^7 under the hypothesis that they corresponded to low abundance values.

All data analyses and graphical representations were performed using R version 3.0.2 [94].

LIST OF ABBREVIATIONS

ACN: Acetonitrile; ANOVA: Analysis of variance; BPH: Best-parent heterosis; CFU: Colony-forming unit; DTT: Dithiothreitol; LC-MS/MS: Liquid chromatography-Tandem mass spectrometry; MPH: Mid-parent heterosis; NTU: Nephelometric turbidity unit; PCA: Principal component analysis; PQL: Protein quantitative locus; RPM: Round per minute; TF: Transcription factor; TFA: Trifluoroacetic acid; WPH: Worst-parent heterosis; YPD: Yeast extract peptone dextrose; ZALS: Zwitterionic Acid Labile Surfactant.

COMPETING INTERESTS

The authors declare no competing interests.

AUTHORS' CONTRIBUTIONS

IMP, MB, PM, DS, CD, DdV and MZ designed research; WA and TdS performed the fermentations and the strain phenotyping; MBN, BV and TB performed the proteomics experiments; MBN and WA analyzed the results; MBN wrote the manuscript. All authors discussed the results and commented the manuscript. All authors read and approved the final manuscript.

ADDITIONAL FILES

Additional file 1 (ods file): Table S1 to S5. Table S1: Number of successful fermentations obtained for the 55 hybrids and their 11 parents at 18°C and 26°. Table S2: Functional classification of the quantified proteins. Table S3: Decision rules used to determine the inheritance pattern of a protein. Table S4: Correlation to PC2 of the proteins used to built the PCA shown in Figure 5. Table S5. Origin of parental strains.

Additional file 2 (txt file): Quantification measurement, pvalues of the statistical tests and

inheritance pattern for each protein x hybrid x temperature combination.

Additional file 3 (pdf file): Figures S1 to S4. Figure S1: Functionnal classification of the 1396 proteins quantified in both a hybrid and its parents in either of the two temperatures. Figure S2: Distributions of the proteins exhibiting heterosis at the two growth temperatures in four scenarios depending on the sign of additivity deviation. Figure S3: Relationships between additivity deviation at 18°C and additvity deviation at 26°C for the proteins exhibiting heterosis at the two growth temperatures in *S. cerevisiae* hybrids and *S. uvarum* hybrids. Figure S4: Relationship between the number of putative transcription factors of a gene and the proportion of hybrid x temperature combinations in which the encoding protein is heterotic.

Additional file 4 (txt file): parameters used for the peptide quantification with MassChroQ.

ACKNOWLEDGMENTS

This work was supported by the Agence Nationale de la Recherche (ANR-08-ALIA-09 HeterosYeast).

549 **REFERENCES**

1. Mendel G: **Versuche Über Pflanzenhybriden.** *Verh Naturforsch Ver Brünn* 1865, **4:**3–47.

2. Hull FH: **Overdominance and corn breeding where hybrid seed is not feasible.** *J Am Soc Agron* 1946, **38:** 1100-1103.

3. East EM: **Inbreeding in corn**. In *Report on the Connecticut Agricultural Experimental Station 1907-1908* . Connecticut Agricultural Experimental Station; 1908:419–428.

4. Shull GH: **The composition of a filed of maize**. *Am Breed Assoc* 1908, **4:**296–301.

5. Crow JF: **90 years ago: the beginning of hybrid maize**. *Genetics* 1998, **148:**923–928.

6. Duvick DN: **Heterosis: feeding people and protecting natural resources.** In *The genetics and exploitation of heterosis in crops*. Madison: American Society of Agronomy, Crop Science Society of America; 1999:19-29.

7. Charlesworth D, Willis JH: **The genetics of inbreeding depression.** *Nat Rev Genet* 2009, **10:**783–96.

8. Lippman ZB, Zamir D: **Heterosis: revisiting the magic.** *Trends Genet* 2007, **23:**60–66.

9. Sprague GF: **Heterosis in maize: theory and practice.** In *Heterosis. Reappraisal of theory and practice.* Edited by Frankel PDR. Berlin Heidelberg New York: Springer; 1983:47–70.

10. Tsaftaris SA: **Molecular aspects of heterosis in plants.** *Physiol Plant* 1995, **94:**362–370.

11. Tsaftaris AS, Kafka M: **Mechanisms of heterosis in crop plants.** *J Crop Prod* 1997, **1:**95–111.

12. Birchler JA, Auger DL, Riddle NC: **In search of the molecular basis of heterosis.** *Plant Cell* 2003, **15:**2236–2239.

13. Hochholdinger F, Hoecker N: **Towards the molecular basis of heterosis.** *Trends Plant Sci* 2007, **12:**427–432.

14. Birchler JA, Yao H, Chudalayandi S, Vaiman D, Veitia RA: **Heterosis.** *Plant Cell* 2010, **22:**2105–2112.

15. Chen ZJ: **Molecular mechanisms of polyploidy and hybrid vigor.** *Trends Plant Sci* 2010, **15:**57–71.

16. Baranwal VK, Mikkilineni V, Zehr UB, Tyagi AK, Kapoor S: **Heterosis: emerging ideas about hybrid vigour.** *J Exp Bot* 2012, **63:**6309–6314.

17. Kaeppler S: **Heterosis: many genes, many mechanisms—end the search for an undiscovered unifying theory.** *ISRN Bot*, **2012:**1–12.

18. Chen ZJ: **Genomic and epigenetic insights into the molecular bases of heterosis.** *Nat Rev Genet* 2013, **14:**471–482.

19. Schnable PS, Springer NM: **Progress toward understanding heterosis in crop plants.** *Annu Rev Plant Biol* 2013, **64:**71–88.

20. Davenport CB: **Degeneration, albinism and inbreeding.** *Science* 1908, **28:**454–455.

21. Bruce AB: **The mendelian theory of heredity and the augmentation of vigor.** *Science* 1910, **32:**627–628.

22. Crow JF: **Alternative hypotheses of hybrid vigor.** *Genetics* 1948, **33:**477–487.

23. Richey FD: **Mock-dominance and hybrid vigor.** *Science* 1942, **96:**280-281.

24. Powers L: **An expansion of Jones's theory for the explanation of heterosis.** *Am Nat* 1944, **78:**275–280.

25. Becker HC: *Pflanzenzüchtung.* Stuttgart: UTB Verlag Eugen Ulmer*;* 1993.

26. East EM: **Heterosis.** *Genetics* 1936, **21:**375–397.

27. Kollipara KP, Saab IN, Wych RD, Lauer MJ, Singletary GW: **Expression profiling of reciprocal maize hybrids divergent for cold germination and desiccation tolerance.** *Plant Physiol* 2002, **129:**974–992.

28. Sun Q: **Differential gene expression patterns in leaves between hybrids and their parental inbreds are correlated with heterosis in a wheat diallel cross.** *Plant Sci* 2004, **166:**651–657.

29. Hoecker N, Keller B, Muthreich N, Chollet D, Descombes P, Piepho H-P, Hochholdinger F: **Comparison of maize (*Zea mays* L.) F1-hybrid and parental inbred line primary root transcriptomes suggests organ-specific patterns of nonadditive gene expression and conserved expression trends.** *Genetics* 2008, **179:**1275–1283.

30. Leonardi A, Damerval C, de Vienne D: **Inheritance of protein amounts: comparison of two-dimensional electrophoresis patterns of leaf sheaths of two maize lines (*Zea mays* L.) and their hybrids.** *Genet Res* 1987, **50:**1–5.

31. Song X, Ni Z, Yao Y, Xie C, Li Z, Wu H, Zhang Y, Sun Q: **Wheat (*Triticum aestivum* L.) root proteome and differentially expressed root proteins between hybrid and parents.** *Proteomics* 2007, **7:**3538–3557.

32. Marcon C, Lamkemeyer T, Malik WA, Ungrue D, Piepho H-P, Hochholdinger F: **Heterosis-associated proteome analyses of maize (Zea mays L.) seminal roots by quantitative label-free LC-MS.** *J Proteomics* 2013, **93:**295–302.

33. Mohayeji M, Capriotti AL, Cavaliere C, Piovesana S, Samperi R, Stampachiacchiere S, Toorchi M, Lagana A: **Heterosis profile of sunflower leaves: a label free proteomics approach.** *J Proteomics* 2014, **99:**101–110.

34. Warner RL, Hageman RH, Dudley JW, Lambert RJ: **Inheritance of nitrate reductase activity in *Zea mays* L**. *Proc Natl Acad Sci U S A* 1969, **62:**785–792.

35. Nagy A, Bokany A, Bacs B, Doman NG, Faludi-Daniel A: **Carboxylating enzymes in leaves of two lines of *Sorghum vulgare* cv. frumentaceum and their first generation hybrids.** *Photosynthetica* 1972, **6:**7–12.

36. Causse M, Rocher J-P, Pelleschi S, Barrière Y, de Vienne D, Prioul J-L: **Sucrose phosphate**

synthase: an enzyme with heterotic activity correlated with maize growth. *Crop Sci* 1995, **35:**995-1001.

37. Lisec J, Römisch-Margl L, Nikoloski Z, Piepho H, Giavalisco P, Selbig J, Gierl A, Willmitzer L: **Corn hybrids display lower metabolite variability and complex metabolite inheritance patterns.** *Plant J* 2011, **68:**326–336.

38. Fiévet JB, Dillmann C, de Vienne D: **Systemic properties of metabolic networks lead to an epistasis-based model for heterosis.** *Theor Appl Genet* 2010, **120:**463–73.

39. Damerval C, Maurice A, Josse JM, de Vienne D: **Quantitative trait loci underlying gene product variation: a novel perspective for analyzing regulation of genome expression.** *Genetics* 1994, **137:**289–301.

40. Cox J, Mann M: **Quantitative, high-resolution proteomics for data-driven systems biology.** *Annu Rev Biochem* 2011, **80:**273–299.

41. Bantscheff M, Lemeer S, Savitski MM, Kuster B: **Quantitative mass spectrometry in proteomics: critical review update from 2007 to the present.** *Anal Bioanal Chem* 2012, **404:**939–965.

42. Lindegren CC, Braham JE, Calle JDD: **Heterosis in *Saccharomyces*.** *Nature* 1953, **172:**800–802.

43. Steinmetz LM, Sinha H, Richards DR, Spiegelman JI, Oefner PJ, McCusker JH, Davis RW: **Dissecting the architecture of a quantitative trait locus in yeast.** *Nature* 2002, **416:**326–30.

44. Timberlake WE, Frizzell MA, Richards KD, Gardner RC: **A new yeast genetic resource for analysis and breeding.** *Yeast* 2011, **28:**63–80.

45. Zörgö E, Gjuvsland A, Cubillos FA, Louis EJ, Liti G, Blomberg A, Omholt SW, Warringer J: **Life history shapes trait heredity by promoting accumulation of loss-of-function alleles in yeast.** *Mol Biol Evol* 2012, **29:**1781-1789 .

46. Schaefke B, Emerson JJ, Wang T-Y, Lu M-YJ, Hsieh L-C, Li W-H: **Inheritance of gene expression level and selective constraints on trans- and cis-regulatory changes in yeast.** *Mol Biol Evol* 2013, **30:**2121–2133.

47. Plech M, de Visser JAGM, Korona R: **Heterosis is prevalent among domesticated but not wild strains of *Saccharomyces cerevisiae*.** *G3* 2014, **4:**315–323.

48. Romano P, Soli MG, Suzzi G, Grazia L, Zambonelli C: **Improvement of a wine *Saccharomyces cerevisiae* strain by a breeding program.** *Appl Environ Microbiol* 1985, **50:**1064–1067.

49. Jolly NP, Janse BJH, Rooyen TJV, Louw JH: **Hybridization and typing of yeasts used in sparkling wine fermentations.** *Am J Enol Vitic* 1993, **44:**217–226.

50. Coloretti F, Zambonelli C, Tini V: **Characterization of flocculent *Saccharomyces* interspecific hybrids for the production of sparkling wines.** *Food Microbiol* 2006, **23:**672–676.

51. Zambonelli C, Passarelli P, Rainieri S, Bertolini L, Giudici P, Castellari L: **Technological**

properties and temperature response of interspecific *Saccharomyces* hybrids. *J Sci Food Agric* 1997, **74:**7–12.

52. Masneuf I, Murat M, Naumov G, Tominaga T, Dubourdieu D: **Hybrids *Saccharomyces cerevisiae* x *Saccharomyces bayanus* var. *uvarum* having a high liberating ability of some sulfur varietal aromas of *Vitis vinifera* Sauvignon blanc wines.** *J Int Sci Vigne Vin* 2002, **36:**205-212.

53. Kishimoto M, Goto S: **Growth temperatures and electrophoretic karyotyping as tools for practical discrimination of *Saccharomyces bayanus* and *Saccharomyces cerevisiae*.** *J Gen Appl Microbiol* 1995, **41:**239–247.

54. Naumov G: **Genetic identification of biological species in the *Saccharomyces sensu stricto* complex.** *J Ind Microbiol Biotechnol* 1996, **17:**295–302.

55. Belloch C, Orlic S, Barrio E, Querol A: **Fermentative stress adaptation of hybrids within the *Saccharomyces sensu stricto* complex.** *Int J Food Microbiol* 2008, **122:**188–195.

56. Ferry-Dumazet H, Houel G, Montalent P, Moreau L, Langella O, Negroni L, Vincent D, Lalanne C, de Daruvar A, Plomion C, Zivy M, Joets J: **PROTICdb: a web-based application to store, track, query, and compare plant proteome data.** *Proteomics* 2005, **5:**2069–2081.

57. Langella O, Zivy M, Joets J: **The PROTICdb database for 2-DE proteomics.** *Methods Mol Biol* 2007, **355:**279–303.

58. Langella O, Valot B, Jacob D, Balliau T, Flores R, Hoogland C, Joets J, Zivy M: **Management and dissemination of MS proteomic data with PROTICdb: example of a quantitative comparison between methods of protein extraction.** *Proteomics* 2013, **13:**1457–1466.

59. Ruepp A, Zollner A, Maier D, Albermann K, Hani J, Mokrejs M, Tetko I, Güldener U, Mannhaupt G, Münsterkötter M, Mewes HW: **The FunCat, a functional annotation scheme for systematic classification of proteins from whole genomes.** *Nucleic Acids Res* 2004, **32:**5539–5545.

60. Giaever G, Chu AM, Ni L, Connelly C, Riles L, Véronneau S, Dow S, Lucau-Danila A, Anderson K, André B, Arkin AP, Astromoff A, Bakkoury ME, Bangham R, Benito R, Brachat S, Campanaro S, Curtiss M, Davis K, Deutschbauer A, Entian K-D, Flaherty P, Foury F, Garfinkel DJ, Gerstein M, Gotte D, Güldener U, Hegemann JH, Hempel S, Herman Z, *et al.*: **Functional profiling of the *Saccharomyces cerevisiae* genome.** *Nature* 2002, **418:**387–391.

61. Deutschbauer AM, Davis RW: **Quantitative trait loci mapped to single-nucleotide resolution in yeast.** *Nat Genet* 2005, **37:**1333–1340.

62. Teixeira MC, Monteiro P, Jain P, Tenreiro S, Fernandes AR, Mira NP, Alenquer M, Freitas AT, Oliveira AL, Sá-Correia I: **The YEASTRACT database: a tool for the analysis of transcription regulatory associations in *Saccharomyces cerevisiae*.** *Nucleic Acids Res* 2006, **34:**D446–D451.

63. Monteiro PT, Mendes ND, Teixeira MC, d'Orey S, Tenreiro S, Mira NP, Pais H, Francisco AP, Carvalho AM, Lourenço AB, Sá-Correia I, Oliveira AL, Freitas AT:
YEASTRACT-DISCOVERER: new tools to improve the analysis of transcriptional regulatory associations in *Saccharomyces cerevisiae*. *Nucleic Acids Res* 2008, **36:**D132–D136.

64. Abdulrehman D, Monteiro PT, Teixeira MC, Mira NP, Lourenço AB, dos Santos SC, Cabrito TR, Francisco AP, Madeira SC, Aires RS, Oliveira AL, Sá-Correia I, Freitas AT: **YEASTRACT: providing a programmatic access to curated transcriptional regulatory associations in *Saccharomyces cerevisiae* through a web services interface.** *Nucleic Acids Res* 2011, **39:**D136–D140.

65. Teixeira MC, Monteiro PT, Guerreiro JF, Gonçalves JP, Mira NP, Santos SC dos, Cabrito TR, Palma M, Costa C, Francisco AP, Madeira SC, Oliveira AL, Freitas AT, Sá-Correia I: **The YEASTRACT database: an upgraded information system for the analysis of gene and genomic transcription regulation in *Saccharomyces cerevisiae*.** *Nucleic Acids Res* 2014, **42:**D161-D166.

66. Blein-Nicolas M, Albertin W, Valot B, Marullo P, Sicard D, Giraud C, Huet S, Bourgais A, Dillmann C, de Vienne D, Zivy M: **Yeast proteome variations reveal different adaptive responses to grape must fermentation.** *Mol Biol Evol* 2013, **30:**1368–1383.

67. Khan Z, Bloom JS, Amini S, Singh M, Perlman DH, Caudy AA, Kruglyak L: **Quantitative measurement of allele-specific protein expression in a diploid yeast hybrid by LC-MS.** *Mol Syst Biol* 2012, **8:**602.

68. Kacser H, Burns JA: **The molecular basis of dominance.** *Genetics* 1981, **97:**639–666.

69. De Vienne D, Bost B, Fiévet J, Zivy M, Dillmann C: **Genetic variability of proteome expression and metabolic control.** *Plant Physiol Biochem* 2001, **39:**271–283.

70. Mazat J-P, Rossignol R, Malgat M, Rocher C, Faustin B, Letellier T: **What do mitochondrial diseases teach us about normal mitochondrial functions... that we already knew: threshold expression of mitochondrial defects.** *Biochim Biophys Acta* 2001, **1504:**20–30.

71. Rossignol R, Faustin B, Rocher C, Malgat M, Mazat J-P, Letellier T: **Mitochondrial threshold effects.** *Biochem J* 2003, **370:**751–762.

72. Gout J-F, Duret L, Kahn D: **Differential retention of metabolic genes following whole-genome duplication.** *Mol Biol Evol* 2009, **26:**1067–1072.

73. MacLean RC: **Predicting epistasis: an experimental test of metabolic control theory with bacterial transcription and translation.** *J Evol Biol* 2010, **23:**488–493.

74. Giorgetti L, Siggers T, Tiana G, Caprara G, Notarbartolo S, Corona T, Pasparakis M, Milani P, Bulyk ML, Natoli G: **Noncooperative interactions between transcription factors and clustered DNA binding sites enable graded transcriptional responses to environmental inputs.** *Mol Cell* 2010, **37:**418–428.

75. Pál C, Papp B, Hurst LD: **Highly expressed genes in yeast evolve slowly.** *Genetics* 2001, **158:**927–931.

76. Drummond DA, Bloom JD, Adami C, Wilke CO, Arnold FH: **Why highly expressed proteins evolve slowly.** *Proc Natl Acad Sci U S A* 2005, **102:**14338–14343.

77. Drummond DA, Wilke CO: **Mistranslation-induced protein misfolding as a dominant constraint on coding-sequence evolution.** *Cell* 2008, **134:**341–352.

78. Kellis M, Patterson N, Endrizzi M, Birren B, Lander ES: **Sequencing and comparison of yeast species to identify genes and regulatory elements.** *Nature* 2003, **423:**241–254.

79. Kim D-U, Hayles J, Kim D, Wood V, Park H-O, Won M, Yoo H-S, Duhig T, Nam M, Palmer G, Han S, Jeffery L, Baek S-T, Lee H, Shim YS, Lee M, Kim L, Heo K-S, Noh EJ, Lee A-R, Jang Y-J, Chung K-S, Choi S-J, Park J-Y, Park Y, Kim HM, Park S-K, Park H-J, Kang E-J, Kim HB, *et al.*: **Analysis of a genome-wide set of gene deletions in the fission yeast *Schizosaccharomyces pombe*.** *Nat Biotechnol* 2010, **28:**617–623.

80. Georgi B, Voight BF, Bućan M: **From mouse to human: evolutionary genomics analysis of human orthologs of essential genes.** *PLoS Genet* 2013, **9:**e1003484.

81. Wool IG, Chan Y-L, Glück A: **Structure and evolution of mammalian ribosomal proteins.** *Biochem Cell Biol* 1995, **73:**933–947.

82. Fu H, Doelling JH, Rubin DM, Vierstra RD: **Structural and functional analysis of the six regulatory particle triple-A ATPase subunits from the *Arabidopsis* 26S proteasome.** *Plant J* 1999, **18:**529–539.

83. Hahn S, Young ET: **Transcriptional regulation in *Saccharomyces cerevisiae*: transcription factor regulation and function, mechanisms of initiation, and roles of activators and coactivators.** *Genetics* 2011, **189:**705–736.

84. Lemos B, Araripe LO, Fontanillas P, Hartl DL: **Dominance and the evolutionary accumulation of cis- and trans-effects on gene expression.** *Proc Natl Acad Sci U S A* 2008, **105:**14471–14476.

85. McManus CJ, Coolon JD, Duff MO, Eipper-Mains J, Graveley BR, Wittkopp PJ: **Regulatory divergence in *Drosophila* revealed by mRNA-seq.** *Genome Res* 2010, **20:**816–825.

86. Tirosh I, Reikhav S, Levy AA, Barkai N: **A yeast hybrid provides insight into the evolution of gene expression regulation.** *Science* 2009, **324:**659–662.

87. Gasch AP, Spellman PT, Kao CM, Carmel-Harel O, Eisen MB, Storz G, Botstein D, Brown PO: **Genomic expression programs in the response of yeast cells to environmental changes.** *Mol Biol Cell* 2000, **11:**4241–4257.

88. Albertin W, Marullo P, Bely M, Aigle M, Bourgais A, Langella O, Balliau T, Chevret D, Valot B, da Silva T, Dillmann C, de Vienne D, Sicard D: **Linking post-translational modifications and variation of phenotypic traits.** *Mol Cell Proteomics* 2013, **12:**720–735.

89. Albertin W, da Silva T, Rigoulet M, Salin B, Masneuf-Pomarede I, de Vienne D, Sicard D, Bely M, Marullo P: **The mitochondrial genome impacts respiration but not fermentation in interspecific *Saccharomyces* hybrids.** *PLoS ONE* 2013, **8:**e75121.

90. Kessner D, Chambers M, Burke R, Agus D, Mallick P: **ProteoWizard: open source software for rapid proteomics tools development.** *Bioinformatics* 2008, **24:**2534–2536.

91. Valot B, Langella O, Nano E, Zivy M: **MassChroQ : a versatile tool for mass spectrometry quantification.** *Proteomics* 2011, **11:**3572–3577.

92. Blein-Nicolas M, Xu H, de Vienne D, Giraud C, Huet S, Zivy M: **Including shared peptides**

for estimating protein abundances: a significant improvement for quantitative proteomics. *Proteomics* 2012, **12:**2797–2801.

93. Benjamini Y, Hochberg Y: **Controlling the false discovery rate: a practical and powerful approach to multiple testing.** *J R Stat Soc Ser B Methodol* 1995, **57:**289–300.

94. R core team: *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing; 2013.

FIGURE LEGENDS

Figure 1. Heatmap representation of the abundances of 615 proteins. Each line corresponds to a protein and each column to a strain x temperature combination. Abundance values are indicated by the color-key bar: low abundances are in blue and high abundances in red. Letters on the top indicate clusters of strain x temperature combinations presenting similar proteomes. Roman numerals on the left indicate clusters of proteins exhibiting similar abundance patterns. Membership of a protein to the set H+ (see results and Figure 6) is shown in brown on the right. The type of strain and the growth temperature is indicated in brown at the bottom.

Figure 2. Inheritance pattern of the proteins exhibiting abundance variation between hybrid and parental strains.

Figure 3. Relationships between the proportion of heterotic proteins, the parental strains and the temperature. A. Results of ANOVA performed on the following generalized linear model $H_{ijk} = \mu + P_j + T_k + \epsilon_{ijk}$ where H_{ijk} is the proportion of heterotic proteins in hybrid *i* obtained from parent *j* at temperature *k*, P_j is the genotype of parent *j* and T_k is the temperature in growth condition *k*. * $5.10^{-2} > P \geq 5.10^{-3}$; ** $5.10^{-3} > P \geq 5.10^{-4}$; *** $5.10^{-4} > P$. B. Distribution of the proportion of heterotic proteins according to parental strain and temperature. C. Distributions of the proportion of heterotic proteins among *S. cerevisiae* hybrids, inter-specific hybrids and *S. uvarum* hybrids at the two temperatures.

Figure 4. Characteristics of inter-specific hybrids *versus* intra-specific hybrids. A. Distributions of the medians of absolute values of relative additivity deviation. B. Distribution of the proportions of positive heterosis. C. Relationships between additivity deviation at 18°C and additivity deviation

at 26°C for the proteins exhibiting heterosis at the two temperatures in inter-specific hybrids. The same representation for intra-specific hybrids is shown in Additional file 3, Figure S3.

Figure 5. Principal component analysis based on 615 protein abundances. Parental strains are written in upright (18°C) or italics (26°C) characters. Plain and dotted lines represent the limits that contain 99.9 percent of the distribution of the PC1 and PC2 coordinates of strain x temperature combinations for each group. They were obtained by simulating the kernel densities from group's means and variances supposing bivariate normal distributions and using the R package MASS.

Figure 6. Characteristics of the proteins in the set H+. The proteins correlated with $r > 0.5$ to the second axis of the PCA shown in Figure 5 (set H+) were compared to those that were not correlated (set NH).

. $6.10^{-2} > P \geq 5.10^{-2}$; * $5.10^{-2} > P \geq 5.10^{-3}$; ** $5.10^{-3} > P \geq 5.10^{-4}$; *** $5.10^{-4} > P$

Figure 7. Relationships between the frequency of heterosis and the number of putative TFs of a gene. A. Distributions of the number of putative TFs of a gene for proteins that were heterotic in at least one hybrid x temperature combination (blue) and proteins never observed as heterotic (orange). B. Relationship between the proportion of strain x temperature combinations in which proteins are heterotic and the number of putative TFs of the encoding genes for data organized by functional category.

595 **TABLES**

596 **Table 1. Counting of quantified proteins, invariant proteins and heterotic proteins in each**

597 **hybrid x temperature combination.**

	At 18°C			At 26°C			At both 18 and 26°C		
Hybrid	**Total nb of proteins**	**% of invariant proteins (a)**	**% of heterotic proteins (b)**	**Total nb of proteins**	**% of invariant proteins (a)**	**% of heterotic proteins (b)**	**Total nb of proteins**	**% of invariant proteins (a)**	**% of heterotic proteins (b)**
DD12	1189	64.5	29.6	1210	62.8	41.1	1174	48.5	10.1
DE12	1217	68.4	39.5	1178	65.9	30.6	1164	51.6	11.4
DE13	1212	85.2	23.5	1184	80.8	33.9	1174	72.1	0.9
DE14	1222	81.8	17.9	1210	77.0	37.8	1202	68.3	2.1
DE15	1190	73.6	25.8	1221	72.3	37.0	1182	59.8	7.8
DE22	NA	NA	NA	1127	65.7	30.2	NA	NA	NA
DE23	1178	56.9	51.4	1208	70.1	30.5	1163	47.7	9.5
DE24	1187	63.5	48.0	1216	62.7	17.0	1177	47.6	5.7
DE25	1187	60.7	37.9	1209	58.6	43.1	1170	43.1	10.4
DW11	1214	69.9	20.2	1216	71.2	36.9	1198	57.0	7.2
DW21	1175	64.2	31.4	1212	61.5	46.3	1162	47.3	12.4
EE23	1203	71.0	39.8	1178	69.9	29.1	1156	54.2	4.2
EE24	1211	69.9	46.8	1168	65.8	20.1	1153	51.4	2.9
EE25	1205	59.6	61.2	1176	70.3	25.8	1157	47.2	7.4
EE34	1207	83.8	41.5	1213	77.4	25.9	1193	70.7	6.9
EE35	1179	73.8	46.3	1215	80.1	38.0	1169	63.8	8.7
EE45	1223	76.6	36.7	1219	68.1	33.7	1209	59.5	7.8
EW21	1200	61.7	55.0	NA	NA	NA	NA	NA	NA
EW31	1197	70.7	23.1	891	67.0	33.3	884	54.2	5.2
EW41	1211	67.2	28.5	1214	60.1	25.0	1194	47.1	6.3
EW51	1215	69.4	44.9	1217	66.0	21.7	1200	50.8	4.7
DU11	860	60.3	35.5	870	56.4	24.0	841	42.7	6.2
DU12	883	58.9	25.9	869	59.4	34.3	844	42.5	9.5
DU13	886	52.8	38.0	878	58.4	20.5	857	41.1	7.1
DU14	820	58.8	49.7	NA	NA	NA	NA	NA	NA
DU21	813	59.0	30.9	837	59.1	21.3	790	43.4	5.1
DU22	821	56.3	40.4	851	54.2	39.7	795	39.0	11.1
DU23	804	55.0	24.0	854	55.9	23.3	794	39.7	6.1
DU24	779	61.6	34.8	NA	NA	NA	NA	NA	NA
EU21	833	61.3	32.9	806	57.7	15.5	778	42.4	4.5
EU22	827	54.5	39.9	807	58.9	27.1	772	38.0	3.8

EU23	836	52.3	46.4	813	59.7	20.7	778	39.2	5.9
EU24	781	71.3	19.6	NA	NA	NA	NA	NA	NA
EU31	841	61.1	37.3	865	63.7	31.2	822	49.0	8.8
EU32	833	61.1	27.8	874	66.2	44.7	812	49.9	9.3
EU33	841	64.4	21.7	856	62.1	42.9	812	48.8	7.0
EU34	795	64.3	25.4	NA	NA	NA	NA	NA	NA
EU41	877	58.3	37.4	820	57.3	16.9	811	41.3	5.0
EU42	878	56.0	44.0	834	65.9	24.6	822	45.3	8.9
EU43	880	56.5	32.9	868	56.0	22.8	845	41.4	6.9
EU44	822	57.5	35.5	NA	NA	NA	NA	NA	NA
EU51	870	60.3	46.7	843	66.0	8.4	826	48.9	0.9
EU52	871	53.5	44.2	874	64.6	21.7	844	43.1	5.4
EU53	871	50.4	36.1	869	60.8	25.2	844	39.1	6.4
EU54	816	65.0	36.4	NA	NA	NA	NA	NA	NA
WU11	804	63.8	32.3	803	58.5	28.5	783	44.6	4.8
WU12	803	60.6	33.9	803	59.9	28.9	780	43.3	6.1
WU13	809	56.4	39.9	800	54.5	28.3	782	42.7	13.2
UU12	1050	77.1	23.3	1038	67.3	27.1	1031	59.6	3.8
UU13	1052	72.3	27.1	1044	66.1	39.5	1036	56.9	8.9
UU14	1047	72.0	49.8	NA	NA	NA	NA	NA	NA
UU23	1038	62.3	30.7	1036	72.2	30.6	1023	51.9	4.7
UU24	1053	76.3	28.4	NA	NA	NA	NA	NA	NA
UU34	1050	66.6	24.2	NA	NA	NA	NA	NA	NA

(a) proteins whose abundance did not vary neither between a hybrid and its parent nor between parents
(b) invariant proteins omitted

598

37

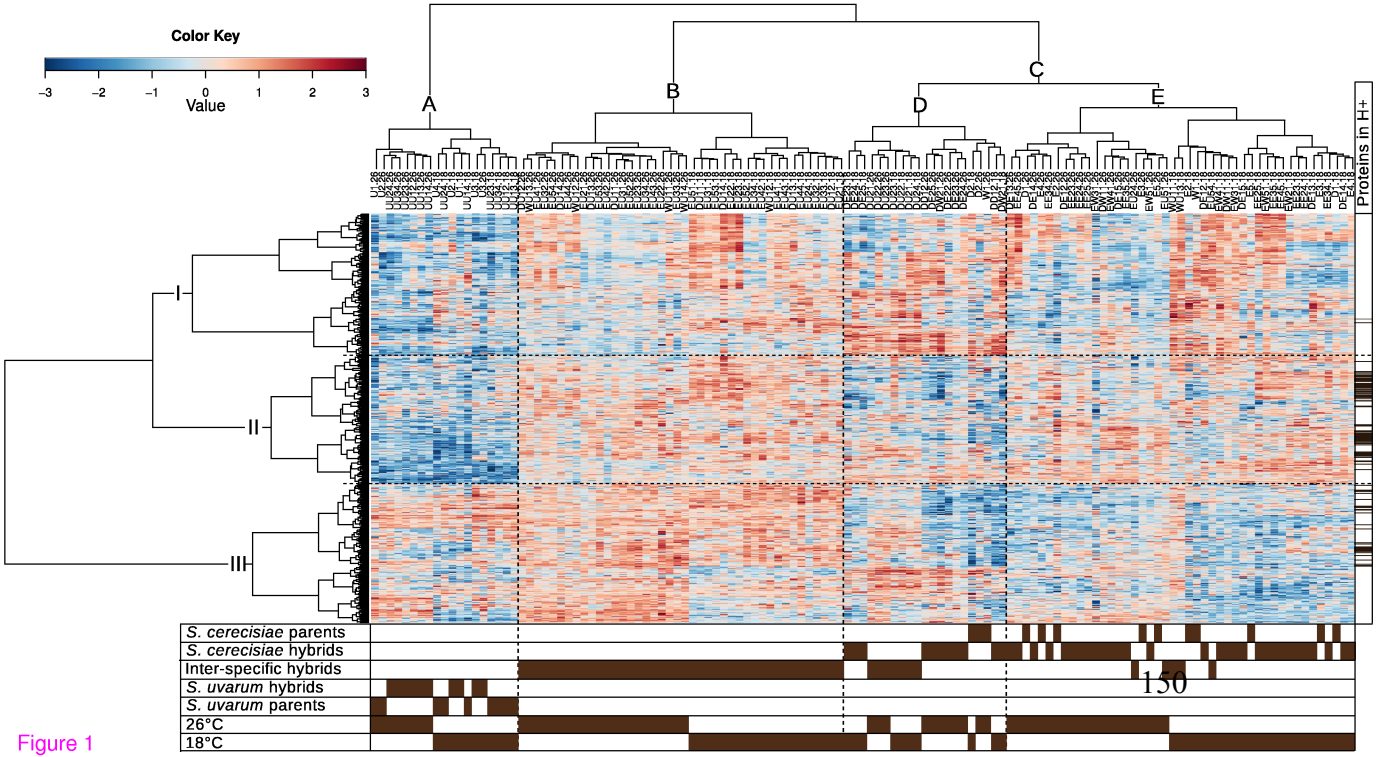

Color Key
−3
−2
−1
0
1
2
3
Value
A
B
C
D
E
Proteins in H+
I
II
III
S. cerecisiae parents
S. cerecisiae hybrids
Inter-specific hybrids
S. uvarum hybrids
S. uvarum parents
26°C
18°C
150

Figure 1

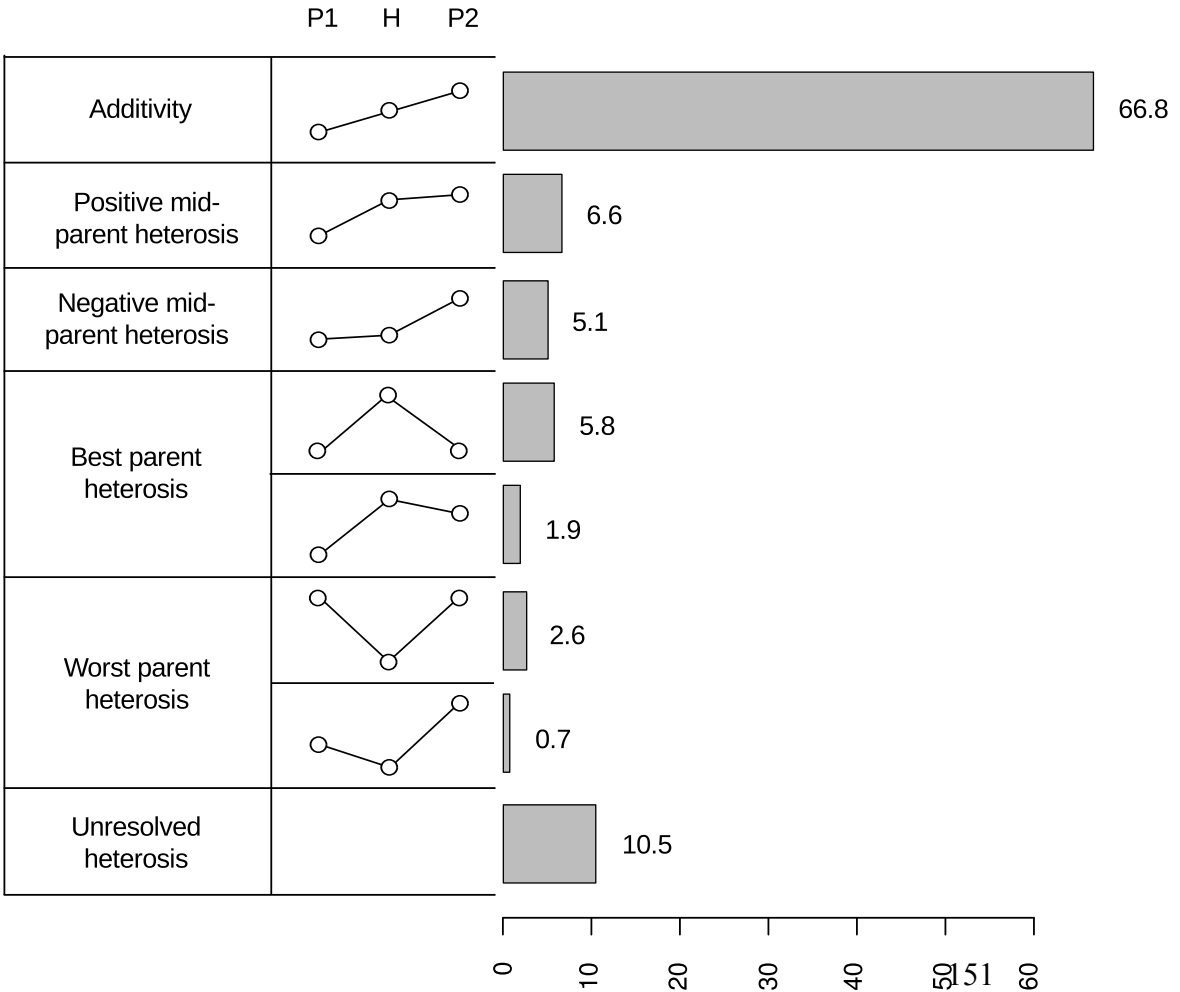

P1
H
P2
Additivity
Positive mid-parent heterosis
Negative mid-parent heterosis
Best parent heterosis
Worst parent heterosis
Unresolved heterosis
66.8
6.6
5.1
5.8
1.9
2.6
0.7
10.5
0
10
20
30
40
50
51
60
% of protein x hybrid x temperature combinations

Figure 2

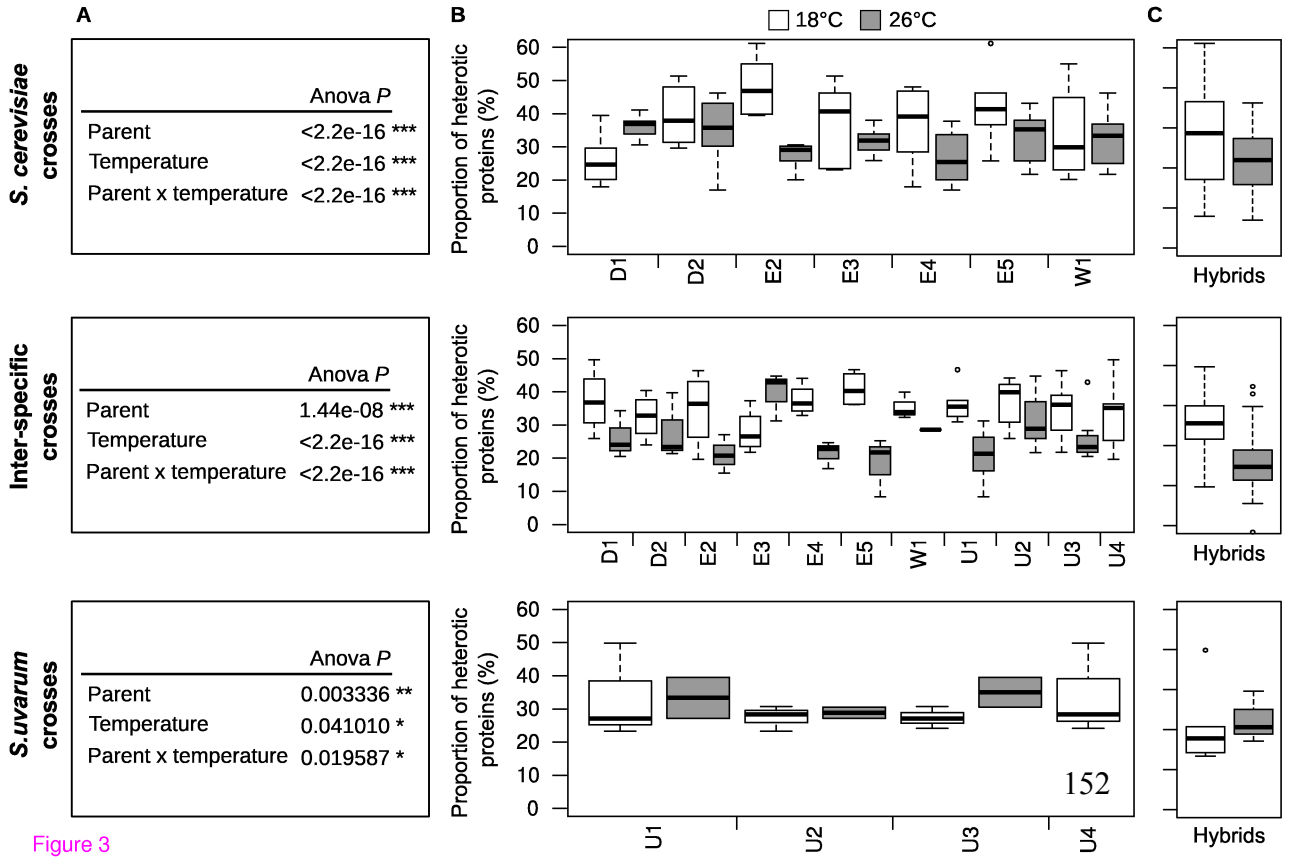

A
B
C
18°C
26°C
S. cerevisiae crosses
Anova P
Parent <2.2e-16 ***
Temperature <2.2e-16 ***
Parent x temperature <2.2e-16 ***
Inter-specific crosses
Anova P
Parent 1.44e-08 ***
Temperature <2.2e-16 ***
Parent x temperature <2.2e-16 ***
S.uvarum crosses
Anova P
Parent 0.003336 **
Temperature 0.041010 *
Parent x temperature 0.019587 *
Proportion of heterotic proteins (%)
D1
D2
E2
E3
E4
E5
W1
U1
U2
U3
U4
Hybrids
152

Figure 3

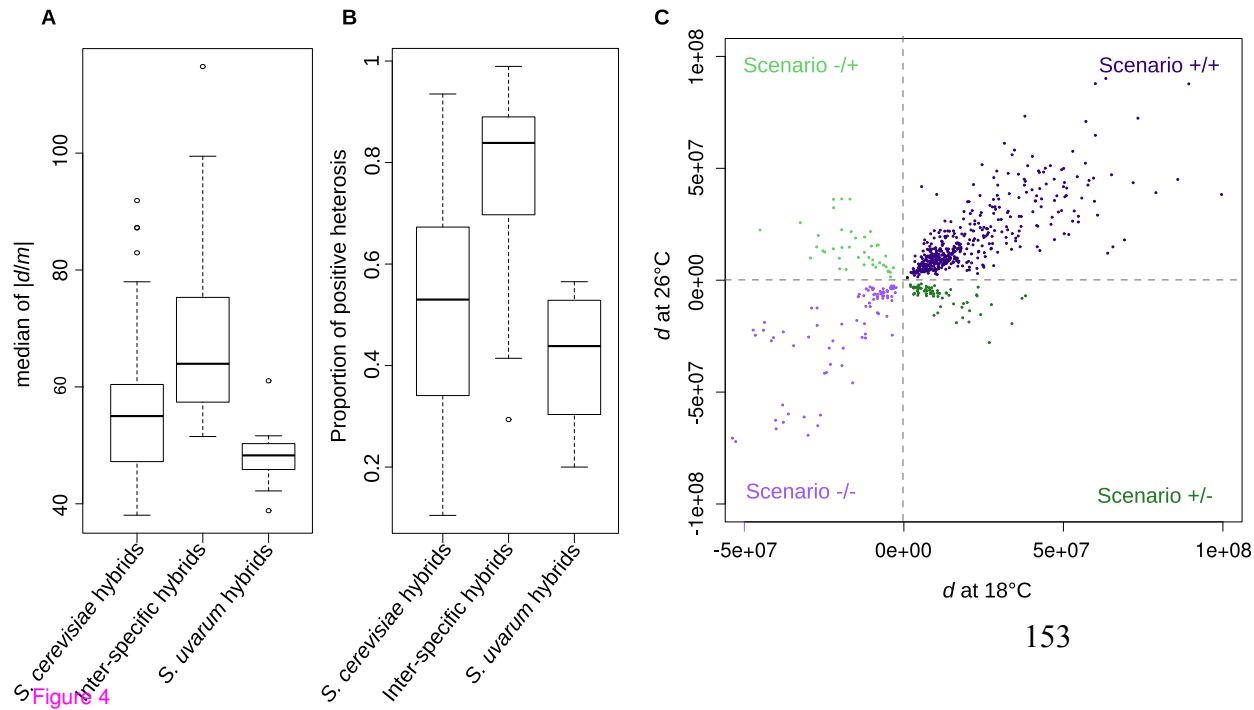

A
median of |d/m|
40
60
80
100
S. cerevisiae hybrids
Inter-specific hybrids
S. uvarum hybrids
B
Proportion of positive heterosis
0.2
0.4
0.6
0.8
1
S. cerevisiae hybrids
Inter-specific hybrids
S. uvarum hybrids
C
Scenario -/+
Scenario +/+
Scenario -/-
Scenario +/-
d at 26°C
-1e+08
-5e+07
0e+00
5e+07
1e+08
-5e+07
0e+00
5e+07
1e+08
d at 18°C

Figure 4

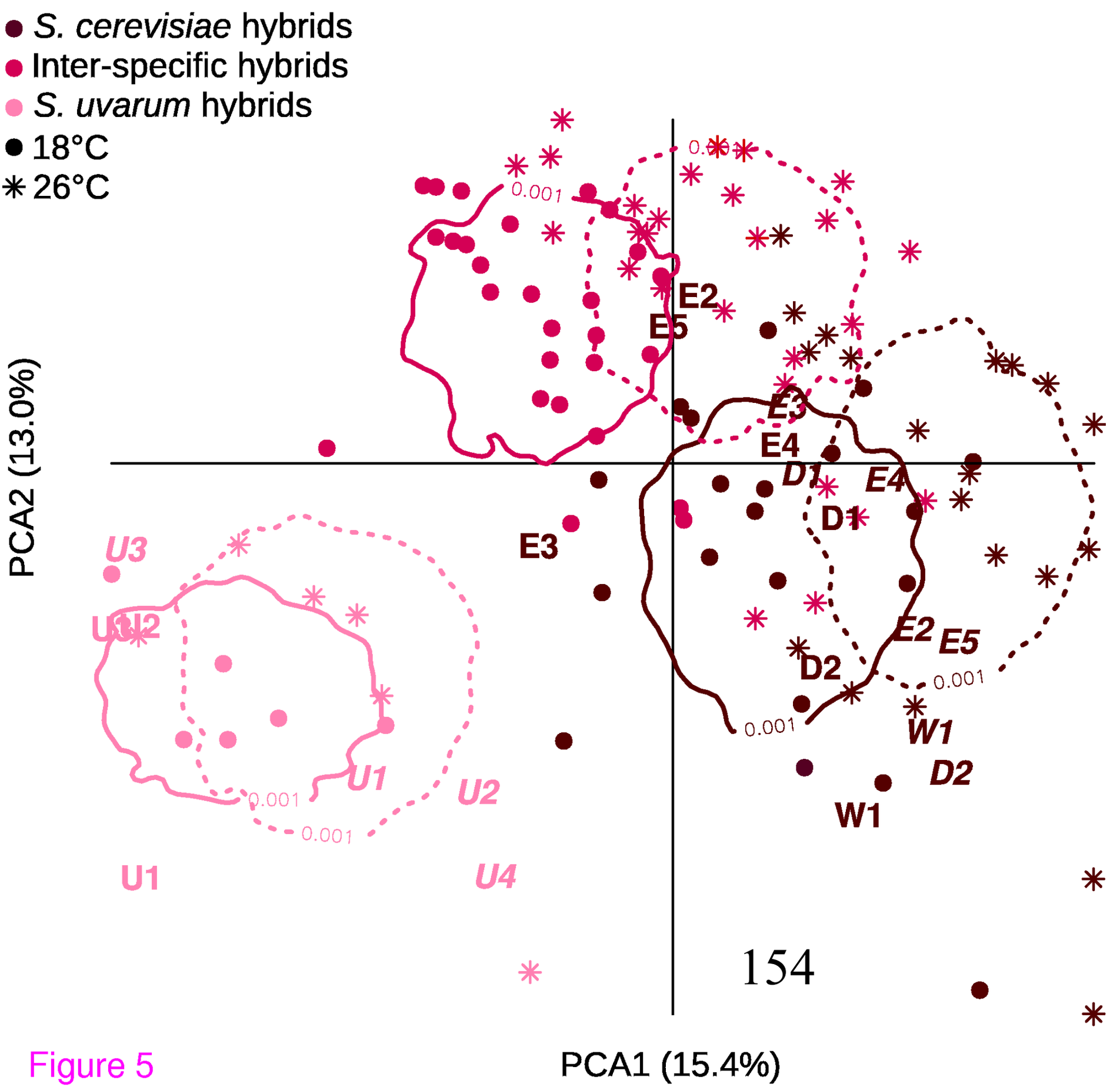
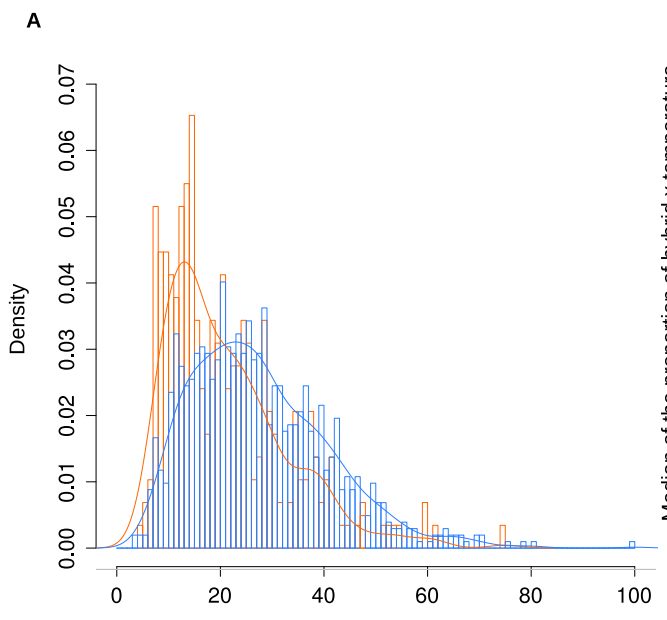

S. cerevisiae hybrids
Inter-specific hybrids
S. uvarum hybrids
18°C
26°C
PCA2 (13.0%)
PCA1 (15.4%)
0.001
E2
E5
E3
E4
D1
D2
W1
U1
U2
U3
U4
154

Figure 5

Protein set NH
Protein set H+
A
% of heterotic proteins in inter-specific hybrids intra-specific hybrids
20 40 60 80
*
B
Mean abundance in parental strains
3e+07 6e+07
***
C
% of proteins encoded by essential genes
10 30 50
***
D
% of proteins involved in protein metabolism
20 40
.
155

Figure 6


A
Density
0.00
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0
20
40
60
80
100
Number of TFs

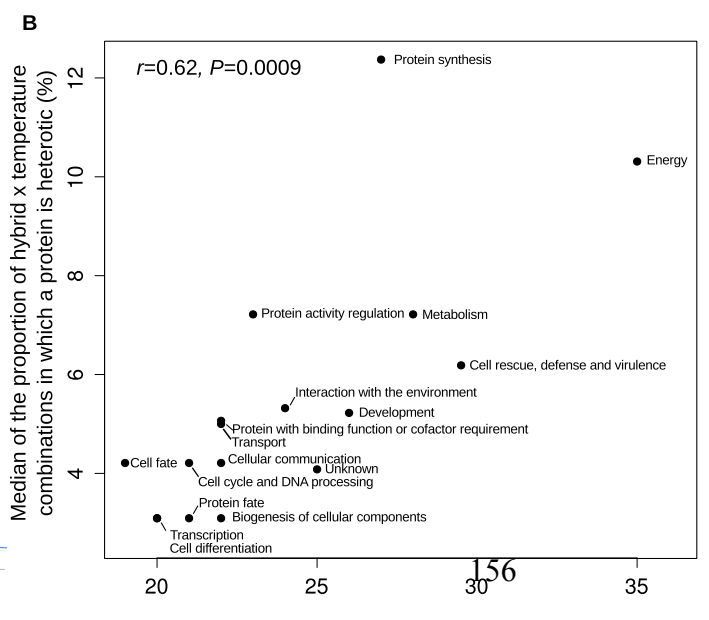

B
r=0.62, P=0.0009
Median of the proportion of hybrid x temperature combinations in which a protein is heterotic (%)
4
6
8
10
12
20
25
30
35
Median of the number of TFs
Protein synthesis
Energy
Protein activity regulation
Metabolism
Cell rescue, defense and virulence
Interaction with the environment
Development
Protein with binding function or cofactor requirement
Transport
Cellular communication
Cell fate
Unknown
Cell cycle and DNA processing
Protein fate
Biogenesis of cellular components
Transcription
Cell differentiation

Figure 7

Additional files provided with this submission:

Additional file 1: Additional_file1.ods, 214K
http://genomebiology.com/imedia/2123523854128839/supp1.ods
Additional file 2: Additional_file2.txt.zip, 10489K
http://genomebiology.com/imedia/7157696841288399/supp2.zip
Additional file 3: Additional_file3.pdf, 636K
http://genomebiology.com/imedia/6981819651288399/supp3.pdf
Additional file 4: Additional_file4.txt, 2K
http://genomebiology.com/imedia/9938730321288549/supp4.txt

RESUME

Malgré son potentiel, l'hétérosis a rarement été étudié, et encore moins exploité, chez les levures, espèces d'intérêt biotechnologique majeur. Ce travail avait pour objectif d'explorer ce phénomène chez deux espèces de levure, *Saccharomyces cerevisiae* et *S. uvarum,* dans des conditions proches de celles de l'œnologie. Pour la première fois des hybrides interspécifiques ont été inclus dans un dispositif diallèle complet. Un autre aspect original de ce travail résidait dans l'approche intégrative choisie, qui combinait l'étude de phénotypes aux niveaux métabolique, cellulaire et populationnel. Un panel de 66 souches (55 hybrides et leurs 11 parents) a été analysé pour 35 caractères à deux températures et avec trois réplicats, soit au total 396 fermentations alcooliques. Ces données nombreuses et complexes nous ont conduits non seulement à utiliser, mais aussi à développer divers outils statistiques et de modélisation originaux pour l'interprétation des données. Après avoir vérifié que les interactions nucléo-cytoplasmiques n'influençaient pas la variation des caractères étudiés, nous avons tout d'abord montré que les sources de variation (effet souche, effet température et interactions souche*température) différaient selon les types de caractères. Nous avons ensuite comparé globalement les trois groupes d'hybrides : intraspécifiques *S. cerevisiae***S. cerevisiae*, intraspécifiques *S. uvarum***S. uvarum* et interspécifiques *S. cerevisiae***S. uvarum*, et avons observé que l'hybridation interspécifique pouvait engendrer des phénotypes présentant de meilleures aptitudes œnologiques et une homéostasie supérieure à celle des hybrides intraspécifiques. Ce dernier résultat pourrait expliquer que l'hybridation interspécifique soit si fréquente chez les levures naturelles et domestiquées.