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# Heterosis in hybrids within and between yeast species

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## Abstract

The performance of hybrids relative to their parents is an important factor in speciation research. We measured the growth of 46 *Saccharomyces* yeast F1 interspecific and intraspecific hybrids, relative to the growth of each of their parents, in pairwise competition assays. We found that the growth of a hybrid relative to the average of its parents, a measure of mid-parent heterosis, correlated with the difference in parental growth relative to their hybrid, a measure of phenotypic divergence, which is consistent with simple complementation of low fitness alleles in one parent by high fitness alleles in the other. Interspecific hybrids showed stronger heterosis than intraspecific hybrids. To manipulate parental phenotypic divergence independently of genotype, we also measured the competitive growth of a single interspecific hybrid relative to its parents in 12 different environments. In these assays, we not only identified a strong relationship between parental phenotypic divergence and mid-parent heterosis as before, but, more tentatively, a weak relationship between phenotypic divergence and best-parent heterosis, suggesting that complementation of deleterious mutations was not the sole cause of interspecific heterosis. Our results show that mating between different species can be beneficial, and demonstrate that competition assays between parents and offspring are a useful way to study the evolutionary consequences of hybridization.

## Introduction

When individuals from different species or from genetically distinct populations mate, they may produce hybrid offspring (Barton & Hewitt, 1985).

Hybridization can bring alleles together in combinations that have never before been exposed to natural selection, often with unpredictable results. Genetic incompatibilities between independently diverged alleles at different loci might reduce hybrid fertility or viability, restricting gene flow between diverging populations (Orr & Turelli, 2001) through Bateson–Dobzhansky–Muller (hereafter BDM) incompatibilities. But interactions among novel combinations of alleles from different populations or species can also increase aspects of hybrid fitness (Shull, 1948). There is evidence from a variety of taxa including plants (Rieseberg *et al.*, 2003), fish (Nolte & Sheets, 2005), insects (Schwarz *et al.*, 2005) and yeast (Stelkens *et al.*, 2014) that hybrids can colonize new environments which are inaccessible to their parents. Thus, hybridization can increase or decrease fitness, and both promote or prevent speciation (Barton & Hewitt, 1985).

It is difficult to determine experimentally the factors that can enable hybrids to outcompete their parents. Various traits contribute to the single trait called fitness, including traits that affect viability (e.g. vigour, survival, growth rate) and those that affect sexual reproduction (e.g. mating success, fertility, fecundity). Hybridization can simultaneously improve some fitness-determining traits, such as vigour, while diminishing others, such as fertility. Different generations of hybrids may also be affected differently; for example, 'hybrid breakdown' describes a reduction in fitness affecting later, but not earlier, generations of hybrids, due to homozygous recessive allelic incompatibilities (Edmands, 2002; Stelkens *et al.*, 2015). And because

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hybridization can greatly increase phenotypic variance, it is possible for some hybrid individuals to be much fitter than their parents, even when most are much less fit or even inviable. Hybrid effects on fitness may also depend on the local environment; for example, BDM incompatibilities often depend on environmental conditions (Nosil, 2012). Thus, to evaluate the evolutionary potential of a hybrid, it is helpful to sample hybrid fitnesses in multiple environments (Lexer *et al.*, 2003; Rieseberg *et al.*, 2003; Stelkens *et al.*, 2014).

Many of these complexities can be avoided using a simple experimental model system. The facultatively sexual yeasts of the *Saccharomyces sensu stricto* species complex are ideal for experimental studies of hybridization. They are well characterized genetically and phenotypically, they have short generation times, and they are easy to cultivate in large populations under controlled and repeatable conditions (Scannell *et al.*, 2011). All members of the *sensu stricto* complex can mate with each other, forming diploid F1 hybrids (Naumov, 1996). Diploids do not have sexes or mating types, but they can undergo meiosis to produce haploid gametes of two mating types, 'a' and 'alpha', which can fuse to restore diploidy and complete the sexual cycle. Because both haploids and diploids can undergo mitosis, individuals can be isolated and propagated as clones, allowing the effects of hybridization to be studied at all life stages and across many generations. Different genotypes can be genetically marked so that they and their offspring can be distinguished, allowing competitive growth assays in a common environment. These advantages allow different methods that are not possible in traditional plant or animal model systems, and although the results from yeast may not be directly applicable to obligate outcrossing species, they are likely to be relevant to a large number of other sexual microbial eukaryotes.

The most striking and best studied characteristic of diploid F1 hybrids between different *Saccharomyces sensu stricto* species is their greatly reduced sexual fertility: <1% of the gametes they produce are viable (Hunter *et al.*, 1996). BDM incompatibilities contributing to this interspecific F1 hybrid gamete inviability have not been found (Kao *et al.*, 2010). Instead, antirecombination has been shown to be the major cause of yeast F1 hybrid sterility (Hunter *et al.*, 1996). When chromosomes from different parents are sufficiently diverged, they cannot crossover during meiosis and so fail to segregate accurately. The genomes of *Saccharomyces cerevisiae* and *Saccharomyces paradoxus* differ at about 14% of nucleotides, which impairs chromosome crossing-over and meiotic segregation so much that most gametes produced by F1 hybrids lack essential chromosomes and are inviable. Despite the low number of gametes that survive F1 hybrid meiosis, those that do, and that are capable of mating, can form F2 hybrids. Some of these F2 hybrids are both viable and sexually fertile, capable of producing viable gametes themselves, yet reproductively isolated from their parents by their new chromosome compositions, thus demonstrating a potential mechanism of hybrid speciation (Greig *et al.*, 2002).

Although much work has concentrated on the reduced sexual fertility of interspecies *Saccharomyces* F1 hybrids, there has been relatively little work on the competitive ability of the F1 hybrids themselves. This is surprising, because the first challenge a new F1 yeast hybrid faces is not its sexual fertility, but its viability and ability to compete under asexual growth. Interspecies F1 hybrid vigour has not been systematically quantified by competitive asexual growth assays against parent species, as far as we know. As most yeast reproduction is by asexual diploid mitosis, the competitive growth of F1 hybrids is likely to determine the success of further generations when hybrids compete for the same resource as their parents: in principle, high F1 asexual competitiveness could completely compensate for their low sexual fertility, or conversely, low F1 asexual competitiveness might greatly strengthen the barrier already established between species. Thus, the ability of F1 hybrids to compete against their parents is evolutionarily important. Furthermore, F1 hybrids are ideal for studying the net contribution of all genetic effects of hybridization at all loci: a single diploid genotype captures the entire range of genetic differences between its two parents. This contrasts to F2 hybrids in which parental differences between loci and within loci are reduced by recombination and segregation respectively, as a result of the preceding sexual cycle. F2 hybrids derived from the same two parent species can vary genetically, containing any combination or proportion of parental alleles, and therefore being more or less affected by hybridization. This presents a sampling problem for researchers studying speciation, particularly with interspecific yeast crosses, where many F2 hybrid individuals have zero viability. For these practical and evolutionary reasons, we set out to measure the factors that affect the competitive ability of F1 hybrids relative to their parents.

When genetically diverged parents mate, their F1 hybrid offspring inherit a complete set of alleles from both parents and might therefore be expected to be phenotypically intermediate. However, parental phenotypes often interact nonadditively, producing hybrid trait values that are different from the average of the parental trait values, and which can even fall outside the range of parental values. For many crosses, these nonadditive genetic interactions may reduce viability enough that the F1 hybrids are rendered completely inviable, preventing traits from being quantified. But fitness-determining traits can also be enhanced by the high heterozygosity of hybrids relative to their parents: this is known as hybrid vigour or heterosis (Shull, 1948). In this article, we will use the term positive heterosis (or sometimes just heterosis) to refer to an

increase in fitness of a F1 hybrid due to heterozygosity and the term negative heterosis when F1 hybrid fitness is diminished due to heterozygosity.

It should be possible to predict the strength and sign of heterosis in F1 hybrids from the characteristics of their parents, so many experimental studies have measured the effect of evolutionary divergence between parents on F1 hybrid traits (for review see Edmands, 2002). One would expect that there should be an optimum level of divergence, between the occurrences of inbreeding depression and outbreeding depression, at which positive heterosis for fitness is maximized, so natural selection should act on mating systems to achieve this intermediate level of outcrossing (Waser, 1993). However, although some researchers do find such a humped-shaped relationship between parental evolutionary divergence and hybrid traits (Moll *et al.*, 1965), others find only positive relationships (Xiao *et al.*, 1996; Gonzáles *et al.*, 2007), negative relationships (McClelland & Naish, 2007; Pekkala *et al.*, 2012) or no relationship at all (Hung *et al.*, 2012). One problem with such experiments is determining which traits to measure. Yeast has an advantage over other study systems in that experimental strains can be made homozygous and propagated as pure clones, so parents and hybrids can be grown simultaneously in a common environment to determine their direct competitive ability in standardized and repeatable – albeit artificial and highly simplified – conditions. This method is commonly used in experimental evolution studies with asexual microbes to determine relative fitness (Lenski, 1991). Relative fitness is the evolutionary important measure: this is what natural selection acts to improve and in competition in batch cultures allows several fitness-associated traits – such as faster maximal growth rate, shorter lag phase, higher carrying capacity or better survival in stationary phase (Vasi *et al.*, 1994) – to be incorporated in a single evolutionary-relevant measurement, albeit one that excludes sexual parts of the life cycle. Studying heterosis in yeast can also help address another practical problem, in that the best measure of evolutionary distance between parents is not obvious: geographic distance, difference in local environments, general phenotypic divergence in multiple traits and general genetic divergence in DNA sequences or markers have all been used (for review see Edmands, 2002). The ability of yeast to grow clonally allows the same genotypes to be tested and retested in different ways, potentially allowing one measure of parental divergence to be manipulated independently of another. For example, genetic distance can be fixed and phenotypic distance varied by retesting the same genotypes in different environments in which their phenotypic differences vary.

Here, we used F1 hybrids between wild *S. paradoxus* parents differing by up to 4% in nucleotide divergence and between *S. paradoxus* and *S. cerevisiae* parents differing by up to 14%. These crosses represent much greater genetic divergence than the intraspecific *S. cerevisiae* hybrids used in previous yeast studies on heterosis, which were <1% divergent according to SNP data (Zörgö *et al.*, 2012; Plech *et al.*, 2014; Shapira *et al.*, 2014). Rather than measuring growth rates in isolation, we determined the growth of these hybrids relative to their parents in direct competition. We determined the relationship between heterosis and both genetic divergence (genome sequence divergence) and phenotypic divergence (the difference in competitive growth) of the parents. Then, to determine the relationship between heterosis and phenotypic divergence independently from genetic divergence, we retested the competitive growth of a single interspecific hybrid relative to its parents under different environmental conditions, to manipulate parental phenotypic divergence.

## Materials and methods

### Strains and hybrid crosses

We used 32 homozygous strains of *S. paradoxus* and *S. cerevisiae* from the National Collection of Yeast Cultures (NCYC, http://www.ncyc.co.uk/) to produce 46 F1 hybrids: 28 intraspecific hybrids between *S. paradoxus* and *S. paradoxus* and 18 interspecific hybrids between *S. paradoxus* and *S. cerevisiae* (Stelkens *et al.*, 2014). Strains and crosses were selected to maximize the ranges of genetic and phenotypic divergence within a manageable set of hybrids (Stelkens *et al.*, 2014). Strains are available on request (see Table S1). Parental strains came from around the world. Most of the *S. paradoxus* strains were collected from oak trees, but *S. cerevisiae* strains came from diverse habitats with high ecological diversity such as soil, trees, diseased human tissue, faeces, insects, fruit, beer and wine (Liti *et al.*, 2009; see Table S1). F1 hybrid strains were made by mixing equal volumes of the haploid parental strains of opposite mating types, mating overnight on YEPD agar (1% yeast extract, 2% peptone, 2% glucose, 2% agar), streaking onto new YEPD plates and replica-plating the resulting single colonies onto KAC agar (1% potassium acetate, 0.1% yeast extract, 0.05% glucose, 2% agar) to induce sporulation. After 48 h incubation, we identified the colonies that had sporulated (and were therefore founded by mated diploids) using a microscope and selecting the corresponding colony from YEPD plate. These pure diploid F1 hybrids were stored frozen at −80 °C 20% glycerol stock for later use. The parental haploid strains used to make F1 hybrids strains were genetically marked with one of the two dominant homozygous alleles conferring resistance to the antibiotics G418 and hygromycin: the resulting F1 hybrid was resistant to both antibiotics (*ho::HYGMX/ho::HYGMX, ura3::KANMX/ura3::KANMX*), whereas the

parental diploid strains were homozygous for the wild-type alleles (*HO/HO, URA3/URA3*) and thus sensitive to the antibiotics. Gene transformation was carried out by following methods in Gietz & Woods (2002).

### Measuring heterosis using competitive growth assays

We measured the competitive growth of every diploid hybrid relative to both of its diploid parents using replicated assays in 5 mL liquid YEPD (1% yeast extract, 2% peptone, 2% glucose) shaken cultures at 30 °C. Each assay tested a hybrid strain against one of its parents. The hybrid and both its parental strains were grown in isolation for 24 h before mixing the hybrid with each parent separately in equal volumes. A 50 $\mu$L sample of the mixture was used to inoculate 5 mL of fresh sterile medium, and the initial (t0) cell number of the hybrid and parental strains was estimated by taking a 100 $\mu$L sample, serially diluting it and plating it to solid YEPD agar before incubating it for 2 days to yield ~200 colonies. The proportion of hybrid colonies was determined by replica-plating to YEPD agar, supplemented with 400 mg of the antibiotic G418 in every litre of medium (0.04% final concentration of G418). Multiplying by the dilution factor allowed the initial number of the hybrid and parent cells in the culture to be determined. Meanwhile, the freshly inoculated medium was incubated for 1 day before a second 100 $\mu$L sample was removed, and the final (t1) number of each cell type was determined by serial dilution and replica-plating as before. The competitive growth of the hybrid relative to its parent was determined by the ratio of their Malthusian growth parameters (Lenski, 1991). Each assay was replicated independently three times using the same strains but different primary cultures, and then, the mean of these three competitive growth measurements was taken and log-transformed. Every hybrid was tested against both of its parents, producing two log-transformed hybrid competitive growth values, one relative to each parent. The higher value is a measure of the performance of a hybrid relative to its less competitive parent, whereas the lower values represent its performance against the more competitive parent, which we therefore use as our measure of best-parent heterosis. Thus, the average of the two hybrid competitive growth values is our measure of mid-parent heterosis. Heterosis values below zero mean the parent (s) outperform the hybrid, whereas heterosis values higher than zero mean the hybrid outperforms its parent(s). The absolute difference between the two values represents the difference between the competitive growths of the two parents relative to their hybrid and is therefore a measure of phenotypic divergence for competitive growth against a common competitor (the hybrid). Genetic divergence between the parents in each cross was calculated using SNP data (personal communication with Gianni Liti), by dividing the number of bases that differed between species by the total number of aligned bases.

To quantify any systematic effect on competitive growth due to the genetic markers used to distinguish hybrids (*ho::HYGMX/ho::HYGMX, ura3::KANMX/ura3::KANMX*) from their parents (*HO/HO, URA3/URA3*), we competed each parental diploid against a marked (*ho::HYGMX/ho::HYGMX, ura3::KANMX/ura3::KANMX*) version of the same parental strain, under the same conditions used for the competitive growth assays between parents and offspring described above.

### Effect of environment on heterosis

To determine the effect of phenotypic divergence independently from the genetic divergence of the parent strains, we measured heterosis in a single interspecific hybrid under different environmental conditions. To facilitate future investigation into the molecular mechanisms of heterosis, we chose two genetically tractable laboratory strains as parents: s288c (*S. cerevisiae*) and N17 (*S. paradoxus*). We again used genetic markers to identify competing strains in our growth assays. The parents were marked with dominant drug resistance cassettes conferring resistance to G418 and to hygromycin as a heterozygote in the same locus, *ura3* (i.e. *ura3::KANMX/ura3::HYGMX*). The hybrid was simply homozygous for a *ura3* deletion (thus *ura3/ura3*) and sensitive to the two drugs. Gene transformation was carried out by following methods in Gietz & Woods (2002). Mid-parent heterosis and best-parent heterosis were measured as before using competitive growth assays replicated three times, except that instead of conducting the assays in YEPD medium at 30 °C, we used 12 different media. Assays were all conducted in shaken liquid minimum medium with added uracil (MIN+URA: 0.67% yeast nitrogen base without amino acids, 2% glucose, 0.003% uracil) with the following supplements: caffeine (10%, 30 °C), zinc sulphate (10%, 30 °C), citric acid (10%, 30 °C), acetylsalicylic acid or aspirin (10%, 30 °C), sodium chloride (10%, 30 °C), peroxide (10%, 30 °C), nipagin (10%, 30 °C), ethanol (1%, 30 °C), lithium acetate (1%, 30 °C), dimethyl sulphoxide or DMSO (1%, 30 °C) as well as at 15 and 30 °C with no supplement.

To test for any systematic effect on growth of the genetic markers used to identify competing strains, we ran control assays in which each drug-resistant diploid (*ura3::KANMX/ura3::HYGMX*) s288c and N17 parent was competed against an isogenic drug-sensitive diploid containing only a homozygous *ura3* deletion (*ura3/ura3*) diploids. Assays were conducted as described above in the different supplemented media (not including temperature this time) and replicated three times independently, using the same strains but different primary cultures.

### Statistical analysis

All statistical analyses were performed in R (R version 3.0.2, packages: 'lawstat' version 2.4.1, 'lme4' version 1.17 and 'nlme' version 3.1-120). Individual statistical tests are listed in the Results. All hybrid relative competitive growth measures were log-transformed to produce measures of positive or negative heterosis for competitive growth. We tested the intraspecies and interspecies competitive growth for normality (Shapiro–Wilk test: mid-parent heterosis: $W = 0.953$, $P = 0.063$, Best-parent heterosis: $W = 0.984$, $P = 0.781$) and homogeneity of variances (Levene's test: mid-parent heterosis: $F_{1,44} = 0.041$, $P = 0.840$, best-parent heterosis: $F_{1,44} = 0.001$, $P = 0.971$) to ensure the correct use of parametric tests.

## Results

Colony counts from all competitive growth assays are provided as Tables S1 and S2. Note that although some authors use the word heterosis only to refer to cases of hybrid outperforming parents, our measure of heterosis can be negative (see Methods), which thus describes parent outcompeting their hybrids.

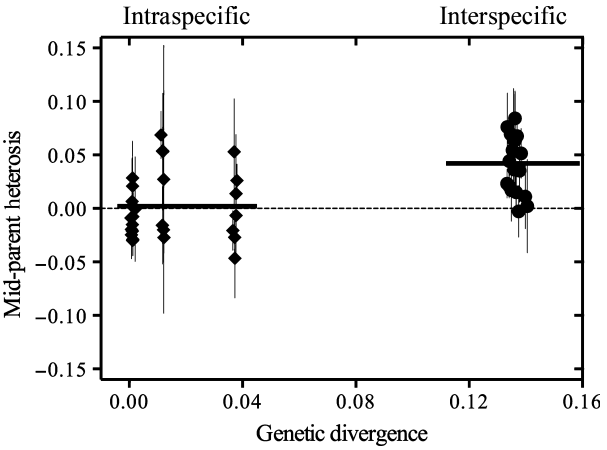


**Fig. 1** Mid-parent heterosis in intraspecific and interspecific hybrids. Horizontal lines indicate the average mid-parent heterosis for intraspecific (mean = 0.004) and interspecific hybrids (mean = 0.045). Points with error bars indicate the means and standard deviations, respectively, of the replicates measures of mid-parent heterosis (see Methods). Diamonds indicate intraspecific hybrids, which are crosses between *Saccharomyces paradoxus* strains, and circles indicate interspecific hybrids, which are crosses between *Saccharomyces cerevisiae* and *S. paradoxus*.

### Heterosis and genetic divergence in different crosses

Genetic divergence ranged from 0.06% to 14% (Table S1), but because of the global population structure of *S. paradoxus*, divergence clustered into four categories (Fig. 1): hybrids between *S. paradoxus* parents from within the same continent (i.e. within Europe, Asia or America, resulting in <1% sequence divergence) with similar competitive growth than the parental average (group mean = −0.001%), hybrids between *S. paradoxus* parents from adjacent continents (i.e. between Europe and Asia, resulting in 1–2% sequence divergence) with similar competitive growth to the parental average (group mean = 0.012%), hybrids between *S. paradoxus* parents from continents isolated by oceans (i.e. crosses between America and Europe and between America and Asia, resulting in 3–4% sequence divergence) also with similar competitive growth to the parental average (group mean = −0.001%) and finally interspecific hybrids between *S. paradoxus* and *S. cerevisiae* (13–14% sequence divergence) with higher competitive growth than the parental average (group mean = 4.3%).

Overall, there was a significant increase in mid-parent heterosis for relative competitive growth with increasing genetic divergence ($F_{44} = 2127$, $P < 0.001$, Fig. 1), but the relationship was driven entirely by the interspecific hybrids, which as a group showed strong and significant positive mid-parent heterosis with hybrids on average growing 4.3% better than the average parent (one-sample *t*-test: $t_{18} = 7.142$, $P < 0.001$). The intraspecific hybrids grew on average 0.2% better than their parents, but not significantly (one-sample *t*-test: $t_{26} = 0.628$, $P = 0.536$). Interspecific hybrids had significantly higher mid-parent heterosis for competitive growth than intraspecific hybrids (two-sample *t*-test: $t_{44} = 4.547$, $P < 0.001$). There was no significant relationship between genetic divergence and heterosis within intraspecific hybrids as a group ($F_{1,25} = 0.108$, $P = 0.746$), nor within interspecific hybrids as a group ($F_{1,17} = 2.883$, $P = 0.108$).

Best-parent heterosis for competitive growth also increased significantly with genetic distance ($F_{1,44} = 10.49$, $P = 0.002$), but, as for mid-parent heterosis, the relation was driven by the higher best-parent heterosis of the interspecific hybrid group compared to the intraspecific group (Fig. S1). Interspecific hybrids had significantly higher best-parent heterosis for competitive growth than intraspecific hybrids (two-sample *t*-test: $t_{44} = 3.307$, $P = 0.002$), but there was no significant relationship between genetic divergence and best-parent heterosis within either of the two subgroups (Fig. S1: intraspecific hybrids: $F_{1,26} = 0.003$, $P = 0.954$; interspecific hybrids: $F_{1,18} = 0.397$, $P = 0.535$). Interspecific hybrids grew on average 0.5% better than their best parent but not significantly (one-sample *t*-test: $t_{18} = 0.812$, $P = 0.427$). Intraspecific hybrids grew on average 2% worse than their best parents, a significant difference (one-sample *t*-test: $t_{26} = 4$, $P < 0.001$).

### Heterosis and phenotypic divergence in different crosses

Mid-parent heterosis significantly increased with phenotypic divergence of the parents (i.e. the absolute difference between the competitive growth of the two parents relative to their hybrid $F_{1,44} = 25.73$, $P < 0.001$ – Fig. 2). Unlike the general relationship between genetic divergence and heterosis discussed above, this relationship did not appear to be driven by any outlying group of strains; however, we note that phenotypic divergence was positively correlated with genetic divergence (Fig. S2: $F_{1,44} = 8.535$, $P = 0.006$). There was no significant relationship between best-parent heterosis and phenotypic divergence ($F_{1,44} = 0.235$, $P = 0.630$).

### Effect of genetic marker in different crosses

The genetic markers (*ho::HYGMX/ho::HYGMX, ura3::KANMX/ura3::KANMX*) used to identify hybrids from their competing parent strains had a significant cost on competitive growth when tested in the 32 parent genetic backgrounds (one-sample *t*-test: $t_{30} = 2.065$, $P = 0.047$). On average, unmarked parents grew 2.07% (SD = ±0.026%) better than the marked versions of the same strains. In the competitions between hybrids and their parents, the hybrids were marked, so the cost of the marker might cause a systematic underestimation of the strength of positive heterosis. To account for this, we adjusted all log-transformed hybrid relative competitive growth rate values by adding the average log-transformed growth advantage of unmarked parents relative to unmarked parents (Fig. S3, Table S3). This adjustment made some of our results more significant. As before the adjustment, the interspecific hybrid had significant mid-parent heterosis (one-sample *t*-test: $t_{18} = 10.08$, $P < 0.001$), but their best-parent heterosis was now also significant after the adjustment (one-sample *t*-test: $t_{18} = 4.246$, $P < 0.001$). As before, intraspecific hybrids show no best-parent heterosis (one-sample *t*-test: $t_{26} = 0.135$, $P = 0.894$), but they now show significant mid-parent heterosis (one-sample *t*-test: $t_{26} = 3.574$, $P = 0.001$). As before, mid-parent heterosis significantly increased with phenotypic divergence across the entire set of crosses ($F_{1,44} = 25.73$, $P < 0.001$), and best-parent heterosis remained unrelated to phenotypic divergence ($F_{1,44} = 0.235$, $P = 0.630$). As before, interspecific hybrids had significantly higher heterosis for competitive growth than intraspecific hybrids, both for mid-parent heterosis (two-sample *t*-test: $t_{44} = 4.681$, $P < 0.001$) and for best-parent heterosis (two-sample *t*-test: $t_{44} = 3.307$, $P = 0.002$). Thus, although some differences became significant that were previous not significant, the adjustment did not change the pattern of the effect or our interpretation. We therefore present and discuss the more conservative, unadjusted heterosis values in the main body of the manuscript, but provide the adjusted values as Table S3.

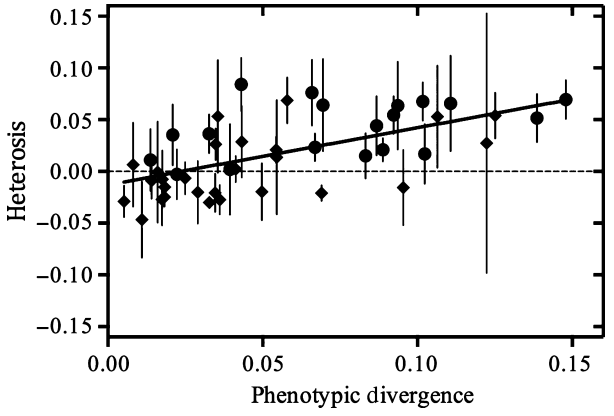


**Fig. 2** The relationship between mid-parent heterosis for competitive growth and phenotypic divergence. Solid line indicates a significant positive correlation ($r_{44} = 0.607$, $P < 0.001$). Points and error bars as for Fig. 1.

### Heterosis in different environments

To investigate the effect of phenotypic divergence independently of genetic divergence, we tested the competitive growth of an interspecific hybrid relative to its parents in different environments. The interspecific hybrid (s288c x N17) we tested grew on average 13% better than the average of its parents across 12 different environments (Fig. 3, Tables S2 and S4), and it grew significantly better than at least one of its parent in all environments (one-sample *t*-test corrected for multiple comparisons using the Holm–Sidak method: see Table S4 for statistics). In ten of the twelve environments (all except for aspirin and zinc sulphate), the competitive growth of the interspecific hybrid was higher against the *S. paradoxus* parent than against *S. cerevisiae* parent. Phenotypic distance correlated with both mid-parent heterosis ($F_{1,10} = 150.4$, $P < 0.001$, Fig. 4a) and best-parent heterosis ($F_{1,10} = 5.684$, $P = 0.038$, Fig. 4b) across all environments.

### Effect of genetic marker in different environments

The marker (*ura3::KANMX/ura3::HYGMX*) used to identify the parent strains in the experiment in different environments increased competitive growth by an average of 1.21% relative to the marker carried by the hybrids (*ura3/ura3*), when both markers were tested in the parental genetic back grounds in all environments (Table S3). Thus, the benefit of the parental marker might cause an underestimation of heterosis. To adjust for this, we added the log-transformed measured growth advantage of the parental marker, for each

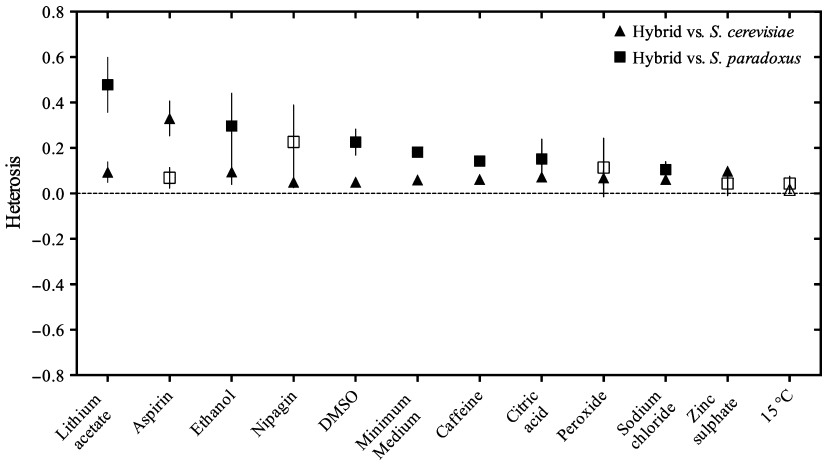


**Fig. 3** Heterosis for competitive growth of a single interspecific cross in twelve different environments. Triangles show average heterosis relative to the *Saccharomyces cerevisiae* parent; squares show average heterosis relative to *Saccharomyces paradoxus* parent. Open shapes indicate heterosis not significant after correction for multiple testing (Table S4; see Results). Error bars indicate standard deviation of the mean of the replicate measurements.

parent in each environment except low temperature, to the log-transformed competitive growth of the hybrids relative to each parent in each environment except low temperature (Table S3). The adjustment generally increased our estimates of heterosis, but did not change our interpretation of the results. As before, the interspecific hybrid grew significantly better than at least one of its parents for all the environments tested (one-sample *t*-test corrected for multiple comparisons using the Holm–Sidak method: see Table S4 for statistics, Fig. S4). Adjusting for the measured marker effect did not affect the relationship between phenotypic distance and mid-parent heterosis, which stayed significantly positive ($F_{1,9} = 75.50$, $P < 0.001$), but it made the correlation between phenotypic distance and best-parent heterosis not significant ($F_{1,9}<0.001$, $P = 0.976$). Because the unadjusted results provide a more conservative measure of heterosis, we present and discuss the unadjusted results here, but we provide the adjusted results as supporting data (Table S3).

## Discussion

Here, we find that hybrids between *S. cerevisiae* and the wild species *S. paradoxus* can grow on average 4.3% better than their parents in direct competition. In contrast, crosses between genetically diverged *S. paradoxus* strains had much less strong heterosis. We show that the strength of heterosis is best predicted by the difference in the competitive growth rates of parents relative to their common hybrid, both when different strains are tested in the same environment, and when the same strains are tested in different environments.

Recent studies of intraspecific *S. cerevisiae* x *S. cerevisiae* crosses have attributed positive heterosis to complementation of deleterious alleles that have accumulated in this species as the result of its domestication by humans (Zörgö *et al.*, 2012; Plech *et al.*, 2014). Cellular functions that are maintained in the wild may be lost in simplified winery or brewery habitats. Two features of yeast domestication might exacerbate this process: drift due to reduced effective population size and disruptive selection in different environments allowing fixation of loss-of-function mutations in different metabolic pathways. Zörgö *et al.* (2012) crossed nine genetically diverged *S. cerevisiae* strains in all pairwise combinations and grew the F1 hybrids asexually under various environmental conditions. Mid-parent heterosis was prevalent and was correlated with poor parental growth, consistent with the simple complementation of loss-of-function mutations that reduce growth in the experimental environment. A follow-up study with larger sample of parental strains confirmed that heterosis was indeed much more likely when parents originated from domesticated, rather than natural environments (Plech *et al.*, 2014).

Could the presence of deleterious mutations in *S. cerevisiae* due to domestication explain the general heterosis we observe when it is crossed to wild *S. paradoxus* strains that lack such mutations? In our experiments, *S. paradoxus* x *S. paradoxus* crosses have much lower heterosis than our *S. cerevisiae* x *S. paradoxus* crosses (Fig. 1), consistent with the wild species having fewer deleterious mutations (or less deleterious mutations). We also found that the larger the difference in parental competitive growth, the stronger the mid-parent heterosis was, both in the full set of crosses tested in a single environment (Fig. 2) and in a single *S. cerevisiae* x *S. paradoxus* cross tested in multiple environments (Fig. 4a). Simple complementation of recessive deleterious mutations in one parent, such as a domesticated *S. cerevisiae* strain, by functional alleles in another, such as a wild *S. paradoxus* strain, would be expected to give exactly this pattern of autocorrelation. To visualize this, imagine that *S. cerevisiae* strains carrying recessive deleterious mutations with different effect sizes (and therefore with different low fitnesses) are crossed to *S. paradoxus* strains lacking such deleterious

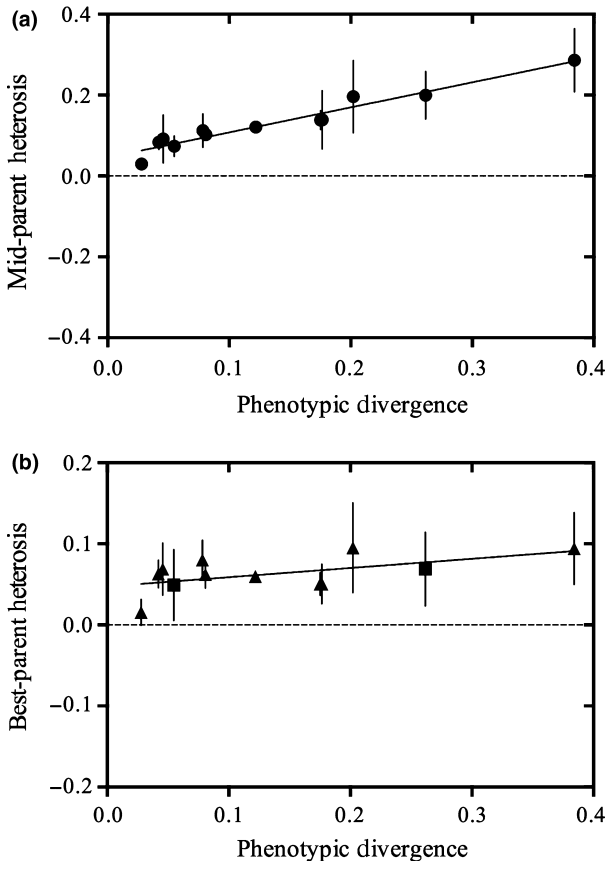


**Fig. 4** The relationship between parental phenotypic divergence and heterosis of a single interspecific hybrid in twelve different environments. (a) Mid-parent heterosis. Circles with error bars indicate means and standard deviations, respectively, of the replicate measures of mid-parent heterosis (see Methods). Solid line indicates a significant positive correlation ($r_{10} = 0.968$, $P < 0.001$). (b) Best-parent heterosis. Points with error bars indicate means and standard deviations, respectively, of the replicates measures of best-parent heterosis (see Methods). Triangles indicate that the best parent was *Saccharomyces cerevisiae* parent, and squares indicate that the best parent was *Saccharomyces paradoxus*. Solid line indicates a significant positive correlation ($r_{10} = 0.566$, $P = 0.038$).

mutations (and therefore of approximately equal, high fitness). Under this simple complementation model, all recessive defects will be complemented so all hybrids will have approximately equal high fitness, but those hybrids showing the strongest mid-parent heterosis will be those whose parents have the largest fitness difference and thus the lowest mid-parent fitness. Zörgö *et al.* (2012) found such a relationship between the difference in growth between *S. cerevisiae* parents and the mid-parent heterosis of their resulting intraspecific hybrids and also interpreted it as simple complementation of domestication defects in one parent by wild-type alleles in another.

However, several aspects of our data make this model of simple complementation of defective *S. cerevisiae* alleles by functional *S. paradoxus* alleles questionable. Plech *et al.* (2014) found that intraspecific heterosis was more prevalent when *S. cerevisiae* parents had been isolated from human-made habitats rather than wild habitats. But we did not find significant higher heterosis in the 13 interspecific crosses made with *S. cerevisiae* strains from human habitats, than in the six interspecific crosses made with *S. cerevisiae* isolated from natural habitats (crosses with *S. cerevisiae* strains from human habitats grew only 1.2% better, two-sample *t*-test: $t_{17} = 0.935$, $P = 0.363$), but we note that this test has little power, especially given that the domestication history of a strain cannot reliably be inferred from the habitat it was isolated from. A clearer prediction of the simple complementation model is that if interspecific heterosis was due to growth defects in *S. cerevisiae*, then *S. cerevisiae* parents should grow less well than *S. paradoxus* parents in competition with their shared hybrids. But in general, the opposite was true: for 13 out of 19 hybrids (not a significant majority, one-way two-tailed chi-squared test: $\chi^2_1 = 1.746$, $P = 0.186$) and for 10 out of 12 environments (a significant majority, one-way two-tailed chi-square test: $\chi^2_1 = 3$, $P = 0.042$), the *S. cerevisiae* parent actually grew better than the *S. paradoxus* parent, relative to their common hybrid. Finally, and perhaps, most importantly, simple complementation of defective *S. cerevisiae* alleles by functional *S. paradoxus* alleles is expected to produce only mid-parent heterosis, in which the hybrid grows at best as well as the functional *S. paradoxus* parent, not best-parent heterosis in which it grows better. Best-parent heterosis can occur when two parents carrying defects at different loci are crossed (Zörgö *et al.*, 2012; Plech *et al.*, 2014). For example when a strain with loss-of-function mutation in one of the genes in the galactose utilization pathway was crossed to a strain with a loss-of-function mutation in another gene of the same pathway, function was restored because the defects were recessive and the intraspecies cross grew better on galactose than either of its parents (Zörgö *et al.*, 2012). However, we see evidence for best-parent heterosis in our interspecies hybrids, both in multiple crosses after the marker effect is corrected for (Fig. S3 and Table S3) and in the single hybrid we studied, which could outcompete both parents in many different environments (Fig. 3). Although recessive deleterious mutations might be fixed in *S. cerevisiae* strains because of relaxed selection due to domestication, we would not expect such mutations in *S. paradoxus*, which is undomesticated, so we would not expect best-parent heterosis, nor would we expect it to correlate with phenotypic divergence (Fig. 4b). Thus, our results suggest that mechanisms in addition to complementation of recessive deleterious alleles, such as overdominance, might also contribute to best-parent heterosis of interspecies yeast hybrids,

although we note that our sample size is too small to be conclusive.

A mechanism that can explain the presence of fixed recessive deleterious mutations in both species also presents a caveat that applies to all yeast heterosis studies to date, as far as we know. The parental diploids we used were monosporic isolates, which were originally derived from single haploids that were allowed to divide mitotically, switch mating type, and mate with their identical haploid clone mates to produce perfectly homozygous diploids (Liti *et al.*, 2009). This is a standard practice to produce pure genetic backgrounds that can be sequenced and studied without the complications of segregating genetic variation (Liti *et al.*, 2009). However, there is evidence that natural strains can be highly heterozygous (Magwene *et al.*, 2011), so deriving monosporic isolates would homozygose any recessive deleterious mutations that were previously masked reducing the monosporic strains' fitness relative to their heterozygous parents. Crosses among different monosporic strains would then restore fitness by complementation, giving the illusion of heterosis, even though the resulting F1 hybrids would not necessarily be any fitter than their heterozygous grandparents. It is not easy to eliminate this potential artefact, because most strains available in collections have been treated in this way. Further, natural strains of *Saccharomyces* are usually isolated by enrichment culture, in which an environmental sample (typically a piece of oak bark) is placed into rich liquid growth medium and incubated, before cells from the resulting mixed culture are isolated and their species identified. If oak bark samples usually contain *Saccharomyces* haploid spores rather than vegetative diploid cells, then the rapid germination and growth conditions provided by enrichment culture might promote mating type switching and homozygosis of recessive deleterious mutations, rather than mating with other spores to produce heterozygotes, as might occur under natural conditions. A challenge for yeast biologists studying evolution is therefore to identify a natural source of vegetatively growing *Saccharomyces* from which samples could be taken directly, which without enrichment culturing.

The positive relationship between parental phenotypic divergence and the strength of heterosis, as well as the general heterosis we find in interspecies hybrids, suggests that mating between species might be advantageous. However, any benefit of interspecies hybrids have under mitosis would have to outweigh the cost they suffer under meiosis: 99% of the gametes produced by F1 hybrids are inviable (Hunter *et al.*, 1996), so only if mitotic divisions greatly outnumber meiotic divisions could their increased vigour compensate for their decreased fertility. This might be possible: an estimated based on population genetic suggests that 1000 mitotic divisions occur for every meiosis in wild oak-associated *S. paradoxus* (Tsai *et al.*, 2008), and a F1 hybrid cell with a growth advantage of 4.3% over a cell of its parent species would need only 175 mitotic generations before its population was over 100 times larger (i.e. large enough to compensate for the ~99% spores that die from F1 hybrid meiosis). Indeed, yeast hybrids are well known, especially in wine and beer industry, environments, where, perhaps, meiosis is not required. Best known is *S. pastorianus* the hybrid used to produce low temperature fermented larger beer, which benefits from a combination of the ethanol resistance of its *S. cerevisiae* parent and the cold tolerance of its *S. eubayanus* parent (Vaughan & Martini, 1987; Libkind *et al.*, 2011), but many other hybrids of *S. cerevisiae*, *S. kudriavzevii*, *S. uvarum* and *S. eubayanus* have been found in wine and cider too (Lopandic *et al.*, 2007; Sipiczki, 2008). Genomic methods are now identifying an increasing number of hybrids between *S. cerevisiae* and *S. paradoxus* outside fermentation environments and examples of introgression of *S. cerevisiae* genes into majority wild *S. paradoxus* genomes (Liti *et al.*, 2006) and *vice versa* (Muller & McCusker, 2009), indicating that many sexual cycles occurred since hybridization and suggesting that be benefits of yeast hybridization can indeed sometimes outweigh their fertility costs.

There is increasing awareness in the role that hybridization has played in the evolution of a wide range of species (see the special issue of *Journal of Evolutionary Biology*, 26(2) 2013; Seehausen, 2004; Mallet, 2007; Schumer *et al.*, 2014), not least on our own (Sankararaman *et al.*, 2014). The importance of that role depends very much on the ability of the hybrid to compete against nonhybrids, and yeast offers a useful way to assess the factors contributing to the relative fitness of hybrids.

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## Conflict of interests

The authors declare no conflict of interests.

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## Supporting information

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

**Figure S1** Best-parent heterosis in intra-specific and interspecific hybrids.

**Figure S2** Relationship between genetic divergence and phenotypic divergence.

**Figure S3** Heterosis in intra-specific and inter-specific hybrids adjusted for marker effect.

**Figure S4** Heterosis for competitive growth of a single inter-specific cross in twelve different environments, adjusted for marker effect.

**Table S1** Description of the strains and crosses, raw data for the competition between hybrids and its parents, phenotypic divergence, mid-parent heterosis and best-parent heterosis data.

**Table S2** Description of the environments, raw data for the competition in different environments between inter-specific hybrid and its parents, phenotypic divergence, mid-parent heterosis and best-parent heterosis data.

**Table S3** Raw data for the competition between marked and unmarked strains for different crosses and different environment competition, adjusted phenotypic divergence, mid-parent heterosis and best-parent heterosis for markers' effect.

**Table S4** Multiple comparisons table for the competition between inter-specific hybrid and its parents in different environments, and adjusted multiple comparisons tables for markers' effect.

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