Hybridization Outcomes Have Strong Genomic and Environmental Contingencies

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ABSTRACT: Extreme F2 phenotypes known as transgressive segregation can cause increased or decreased fitness in hybrids beyond the ranges seen in parental populations. Despite the usefulness of transgression for plant and animal breeding and its potential role in hybrid speciation, the genetic mechanisms and predictors of transgressive segregation remain largely untested. We generated seven hybrid crosses between five widely divergent Saccharomyces yeast species and measured the fitness of the parents and their viable F1 and F2 hybrids in seven stressful environments. We found that on average 16.6% of all replicate F2 hybrids had higher fitness than both parents. Against our predictions, transgression frequency was not a function of parental genetic and phenotypic distances across test environments. Within environments, some relationships were significant, but not in the predicted direction; for example, genetic distance was negatively related to transgression in ethanol and hydrogen peroxide. Significant effects of hybrid cross, test environment, and cross × environment interactions suggest that the amount of transgression produced in a hybrid cross is highly context specific and that outcomes of hybridization differ even among crosses made from the same two parents. If the goal is to reliably predict hybrid fitness and forecast the evolutionary potential of admixed populations, we need more efforts to identify patterns beyond the idiosyncrasies caused by specific genomic or environmental contexts.

Keywords: transgressive segregation, hybridization, fitness, heterosis, yeast, quantitative genetics.

Introduction

Hybridization instantaneously increases the genetic variation contained in populations (Grant and Grant 2019) and can play an important role in adaptive evolution (Stebbins 1959; Barton 2001; Seehausen 2004; Abbott et al. 2013; Arnold 2015). Some hybrids are able to colonize ecological niches that are not accessible to parental genotypes (Rieseberg et al. 2003a; Stelkens et al. 2014), a process that may ultimately result in hybrid speciation, as demonstrated in a broad range of taxa, including fungi (Leducq et al. 2016; Mixão and Gabaldón 2020), plants (Ungerer et al. 1998; Yang et al. 2019), and animals (Nice et al. 2012; Lamichhaney et al. 2018; Meier et al. 2019).

Reticulate evolution events, including hybridization, introgression, and horizontal gene transfer, have also played an important role in the evolutionary diversification of microbial species (Mallet et al. 2016; Shapiro et al. 2016). One of the best-documented cases are the species in the genus of the model microbe Saccharomyces budding yeast. Interspecies hybridization has featured prominently in Saccharomyces’ ancient (Marcet-Houben and Gabaldón 2015; Peris et al. 2017) and recent (Libkind et al. 2011; Peris et al. 2014; Gallone et al. 2019) evolutionary history (reviewed in Boynton and Greig 2014; Gabaldón 2020). Saccharomyces currently includes eight distinct species with origins ~4–20 million years ago (Kellis et al. 2003; Borneman and Pretorius 2015; Shen et al. 2018). Of the eight species, five are known to hybridize in the wild and/or in domestication environments (Boynton and Greig 2014; Peter et al. 2018; Pontes et al. 2019). Although sexual reproduction and especially hybridization are considered rare events in nature (approximately one sexual cycle per 1,000 asexual generations; Gonzalez et al. 2006; Tsai et al. 2008), all species readily mate with each other under laboratory conditions, forming diploid F1 hybrids (Naumov 1996; Greig 2009). While F1 hybrids are viable, they are mostly sterile and produce only a small fraction of healthy gametes during meiosis because the large sequence divergence...
between species impairs crossing over and proper segregation of homologous chromosomes into daughter cells. However, the few gametes that do survive F1 hybrid meiosis can mate and form viable and sexually fertile F2 hybrids, which can be reproductively isolated from their parents through their genomic composition, structural genetic variation, and ecological profile, representing a potential route to hybrid speciation (Leducq et al. 2016; Eberlein et al. 2019).

Extreme F2 and later-generation hybrid yeast phenotypes, known as transgressive segregants, have been shown to outcompete their parents in stressful environments (Greig et al. 2002; Stelkens et al. 2014; Zhang et al. 2019) and are frequently exploited to improve biotechnological processes such as wine and beer fermentation (Krogerus et al. 2017; Langdon et al. 2019). The genetic mechanisms underlying transgression are varied and not yet fully understood, but most evidence points to epistasis and complementary gene action playing important roles (DeVicente and Tanksley 1993; Rieseberg et al. 2003b; Koide et al. 2019). Complementary gene action is the additive action of alleles at quantitative trait loci that are fixed for opposite trait signs in parental species and add up to extreme trait values in F1 hybrids. Transgression due to epistasis and complementation can, once established, persist indefinitely in hybrid populations because fit recombinant homozygous genotypes can breed true, as opposed to overdominance-based heterosis, which is consecutively lost with every generation (Fitzpatrick and Shaffer 2007). Thus, transgression can have lasting effects on hybrid fitness and the evolutionary outcomes of hybridization.

Because of its largely additive and epistatic nature, theory predicts that transgression should increase with parental genetic divergence and decrease with parental phenotypic differences (Rieseberg et al. 1999). It is, however, notoriously difficult to assess the relative impact of these predictors of transgression independently, with the same set of experimental crosses, because they often covary; more genetically divergent lineages are often also phenotypically different. Covariance between genetic and phenotypic divergence can potentially mask or override their opposing effects on transgression. Here, in the hope of providing a sounder testing ground for theoretical predictions and to explicitly parse the effects of genetic and phenotypic distance on transgression, we used a set of seven interspecific hybrid yeast crosses in which parental genetic and multivariate phenotypic distances were not correlated.

We made interspecies F1 and F2 hybrid crosses between five yeast species: *S. cerevisiae*, *S. paradoxus*, *S. mikatae*, *S. kudriavzevii*, and *S. uvarum*. Most of these hybrids have been found in natural or fermentation environments before (except for Sk × Sm and Sk × Sp). These species vary in relatedness from 13% to 21% nucleotide divergence based on whole-genome alignments (fig. 1), capturing the entire range of genetic distances in this species group. Their ecological niches and geographic distributions vary widely. Baker’s yeast (*S. cerevisiae*) has a global, human-associated distribution; it is used in the food, biofuel, and medical industries and is typically found in warm, nutrient-rich environments, like grapes and other fruits. *Saccharomyces paradoxus* is mainly found in the Northern Hemisphere, and *S. uvarum* mostly occurs in the Southern Hemisphere.

![Figure 1](image-url)  
*Figure 1:* Phylogenetic relationships of parental *Saccharomyces* species. The tree was built using orthogroup inferences from whole-genome assemblies. The support values are the proportion of times that the bipartition was found in each of the individual species tree estimates. Branch lengths represent the average number of substitutions per site across the sampled gene families. *Kluyveromyces lactis* and *Torulaspora delbrueckii* were used as out-groups but are not shown. Interspecific hybrids generated from parental strain crosses are shown.
Saccharomyces mikatae and S. kudriavzevii have narrow geographic distributions mostly confined to East Asia. Saccharomyces paradoxus, S. mikatae, S. kudriavzevii, and S. uvarum occur in colder, less nutrient-rich habitats, like tree bark and forest soils.

We measured the fitness of parents and their viable F1 and F2 hybrids under seven stressful conditions and tested whether the frequency of hybrid segregants with higher or lower fitness than the parents (transgressive segregation) was a function of parental genetic and phenotypic distance. We also tested whether transgression was environment dependent and whether any interactions among phenotype, genotype, and environment explained variation in hybrid stress response. As a prerequisite to being used in this experiment, this set of parental strains had pairwise phenotypic distances (measured in the experimental environments we used) that were not correlated to pairwise genetic distances.

Material and Methods

Parental Strains and F1 Hybrids

We made seven interspecific hybrid crosses from five members of the Saccharomyces species group (fig. 1; table 1). Note that crosses were not all phylogenetically independent, as S. kudriavzevii and S. cerevisiae were used repeatedly. Parental strains (gifts from C. T. Hittinger and D. Greig) used for mating were previously engineered to be stable, isogenic heterothallic haploids with homothallic switching endonuclease (HO) deletion using drug resistance markers (HygMX, KanMX, or NatMX). We generated parental diploid strains by mating haploid derivatives with markers (HygMX, KanMX, or NatMX). We generated parental diploid strains by mating haploid derivatives with opposite mating types, generating homozygotes (except at the mating-type [MAT] locus). To make F1 hybrid crosses, haploid parents with opposite mating types were grown clonally in liquid YEPD overnight, and frozen for later use.

F2 Hybrid Crosses

F1 hybrids were grown in liquid YEPD overnight. Cells were pelleted by centrifugation and washed two times with distilled water. Cells were transferred to a 500-mL Erlenmeyer flask containing 50 mL of liquid KAC, which was shaken at room temperature for 7 days to induce meiosis (see the schematic of the experimental procedure in fig. 2). After microscopic verification of sporulation, we centrifuged and washed cells, resuspended them in 50 mL of liquid YEPD, and distributed them to 2-mL Eppendorf tubes. Tubes were incubated at 30°C with shaking (300 rpm) for 30 min. Then cells were pelleted, and vegetative F1 cells were eliminated by adding 1 mL of 1% NaOH solution onto YEPD plates (in triplicate) and found to be on average 16.3 ± 6.1 cells. The remaining populations, now containing only F1 hybrid genotypes, were frozen for later use.

Stressful Environments

We supplemented liquid synthetic complete (SC) medium (2% glucose, 0.67% bacto-yeast nitrogen base without amino acids, 0.00079% Formedium complete supplement mixture [CSM] powder) with a range of concentrations of seven toxic substances: caffeine (C8H10N4O2), citric acid (C6H8O7), zinc sulfate (ZnSO4), salicylic acid (C7H6O3), ethanol (C2H6O), hydrogen peroxide (H2O2), and dimethyl sulfoxide (CH3SO2).

Table 1: Parental Saccharomyces strains with geographic and ecological origin

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain ID</th>
<th>Origin</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. cerevisiae</td>
<td>Y55</td>
<td>Europe, laboratory strain</td>
<td>Liti et al. 2009</td>
</tr>
<tr>
<td>S. paradoxus</td>
<td>Q89.8</td>
<td>United Kingdom, oak</td>
<td>Liti et al. 2009</td>
</tr>
<tr>
<td>S. mikatae</td>
<td>IFO1815</td>
<td>Japan, soil</td>
<td>Scannell et al. 2011</td>
</tr>
<tr>
<td>S. kudriavzevii</td>
<td>FM1109 (ZP 591)</td>
<td>Portugal, oak</td>
<td>Hittinger et al. 2010</td>
</tr>
<tr>
<td>S. uvarum</td>
<td>CBS7001</td>
<td>Spain, insect</td>
<td>Scannell et al. 2011</td>
</tr>
</tbody>
</table>

Note: References provide more background information on each strain.
sulfoxide (DMSO; C₂H₆OS). We created environmental gradients on 96-well plates, so that the top row (along the X-axis of the plate) contained the lowest concentration and every row below increased in concentration until the bottom row contained the highest concentration lethal for all strains (details are in table S1; tables S1, S2 are available online). Every well contained 170 µL volume.

Fitness Assays

We measured the growth of parental diploids, F₁ hybrids, and F₂ hybrids in seven environmental gradients, each with eight levels of increasing concentration of a substance (fig. 2; table S1). For this, parents and F₁ hybrids (which were clonal) were grown in SC medium overnight. F₂ populations were taken directly from frozen samples and resuspended in SC medium to avoid preselection and thus loss of genetic diversity in SC overnight. Every well on a 96-well plate (plate A) was inoculated with 10 µL of yeast culture. After inoculation, plates were incubated at 30°C for 48 h. Then, 1 µL of culture from each well was transferred to the same position on a fresh 96-well plate (plate B) containing identical concentrations of the same substance. Another transfer to a fresh plate (plate C) was done 48 h later. Immediately following

Figure 2: Schematic of experimental procedure designed to identify transgressive interspecific yeast hybrids, proliferating along a stress gradient that is not tolerated by their parents. Serial transfers into fresh medium are common practice and help to exclude dead cells, which can otherwise confound optical density (OD) measurements. Total population growth was calculated as the difference in OD between the start and end of the 48-h growth period on plate C. Darker-shaded yeast cells signify F₂ hybrids carrying beneficial genetic variation, allowing them to thrive under stressful conditions. SC = synthetic complete; YEPD = 1% yeast extract, 2% peptone, 2% dextrose.
this last transfer (time point \(t_t\)), we measured the optical density (OD; at 600 nm) of every well with a microplate reader (Tecan, Sunrise). OD scales proportionally with cell number and is a common high-throughput measure of microbial population growth and Malthusian fitness in liquid medium. After incubation at 30°C for 48 h, we took a second OD measure (time point \(t_2\)). We calculated population growth as the difference in OD between \(t_0\) and \(t_1\) in each well. We collected data from six replicates for each parental and F1 hybrid strain and from 12 replicates for F2 hybrids in each of the seven environmental gradients. Note that in the case of F2 hybrids, every well well contained different genotypes (the result of chance meiotic segregation and recombination between parental genomes).

**Genetic and Phenotypic Distance**

Genetic distances between parental strains were calculated using estimated DNA-DNA hybridization values generated using the Genome-to-Genome Distance Calculator 2.1 with formula 2 (Meier-Kolthoff et al. 2013). The distances are estimated by locally aligning each genome pair used in our hybrid crosses using BLAST+ (Camacho et al. 2009), producing high-scoring segment pairs. Previously published genome assemblies for each *Saccharomyces* species were used for genetic distance estimation (Scannell et al. 2011; Yue et al. 2017; Bendixsen et al. 2021). Notably, because of the lack of an assembled genome for *S. paradoxus* strain Q89.8, a closely related *S. paradoxus* strain (CBS432) was used. Similarly, for *S. kudriavzevii* strain FM1109, the strain ZP 591 (from which FM1109 was originally derived) was used (Sampaio and Gonçalves 2008). To understand the phyloge netic relationships of the parental *Saccharomyces* species, we built a consensus species tree using orthogroup inference. Orthologs were identified from the same previously assembled and annotated *Saccharomyces* genomes (Scannell et al. 2011; Yue et al. 2017; Bendixsen et al. 2021). *Kluyveromyces lactis* and *Torulaspora delbrueckii* were used as out-groups in the phylogenetic analysis. We used OrthoFinder version 2.5.2 (Emms and Kelly 2015; Emms and Kelly 2019) and aligned all orthologous protein-coding genes identified in the five *Saccharomyces* species. In total, 5,304 orthologs were identified, and gene trees were constructed for each group. The consensus species tree was inferred using STAG (Emms and Kelly 2018) and rooted using STRIDE (Emms and Kelly 2017).

To obtain pairwise phenotypic distances between parental strains, we used the growth measurements (OD) of the parents measured along the seven environmental gradients as explained above and entered these multivariate data into a multidimensional scaling (MDS) analysis (using the magrittr, dplyr, ggpubr, and ggplot2 packages in R). Because we measured growth in seven environmental gradients, each with eight levels of increasing concentrations of a different substance, we first calculated pairwise OD differences between all parental strains per environment and per concentration to create an input distance matrix. We then extracted and plotted the first two dimensions and used paired Euclidean distances between strain means as pairwise phenotypic distances. The first two dimensions together explained large amounts of variance (from 92.3% to 97.6%) in the individual environment analyses and thus captured the most important variation in this data set for our downstream analysis of phenotypic distance.

**Heterosis in F1 Hybrids**

The molecular underpinnings of heterosis are manifold. They can be classified into genetic effects of overdominance, where variation in the parent populations is maintained by balancing selection and results in heterozygote advantage in the hybrid, and dominance effects, which is the genome-wide masking of recessive mutations that are mildly deleterious in the homozygous parents, giving heterozygous hybrids fitness advantages. There is evidence that digenic or higher-order epistasis can also play a significant role in causing heterosis in F1 hybrids (Yu et al. 1997; Shapira et al. 2014; Shapira and David 2016).

Heterosis was estimated for each cross in each environment as in Shapira et al. (2014). In brief, mode of inheritance was determined by calculating the degree of dominance \((d/a)\) using the midparent value \(m\) (mean growth of parent 1 + mean growth of parent 2)/2, additive genetic variation \(a\) (mean growth of the better parent — m), and dominance variation \(d\) (mean growth of F1 hybrid — m). Dominance was grouped into seven categories: codominance \((d/a = 0)\), partial dominance of fitter parent \((0 < d/a < 1)\), partial dominance of less fit parent \((-1 < d/a < 0)\), complete dominance of fitter parent \((d/a = 1)\), complete dominance of less fit parent \((d/a = -1)\), overdominance of F1, \((d/a > 1)\), and underdominance of F1, \((d/a < 1)\). Only F1 hybrids with overdominant growth profiles were considered heterotic.

**Transgressive Segregation Frequency in F2 Hybrids**

We counted positively transgressive segregants as those F2 hybrids with fitness exceeding the midparent value by two standard deviations (+2 SDs) and negatively transgressive segregants as F2 hybrids with fitness less than two standard deviations below midparent value (−2 SDs; DeVicente and Tanksley 1993; Diaz et al. 2014). In both cases, only environments and concentrations with sufficient growth were considered. In a given environment and concentration, if either of the parental species or the F1 hybrid had an OD greater than 0.1, the well was included in the analysis,
resulting in 2,937 hybrid measurements. For each concentration level, wells showing transgressive growth were divided by the total number of replicate wells (i.e., wells containing the same concentration of a substance). For a given cross and environment, transgression frequency was calculated by averaging all concentration levels with at least one OD more than 0.1.

We used linear regression to test whether genetic and phenotypic parental distance predicted variation in transgression frequency across environments and crosses. To explore whether positive transgression of the F1 hybrid populations can be explained by cross type, test environment, or concentration level of stress, we used a generalized linear mixed model (family = binomial). The binary response variable was whether an F2 hybrid was positively transgressive (0: F2 < midparent + 2 SDs; 1: F2 > midparent + 2 SDs). Hybrid cross, environment, concentration level, their two-way interactions, and the combined three-way interaction were examined as fixed factors. Replicate was used as a random factor. Analyses were done using the nlme and lme4 packages in R version 3.6.1 (R Core Team 2019). To identify the model with the best fit, we applied model reduction to the full model. Replicates of F1 and F2 hybrids also clustered together in multidimensional space and did not overlap with the parents or with each other (fig. S2).

Pairwise phenotypic and genetic distances (p-distances from whole-genome alignments) of the parental strains were not correlated (fig. 5); that is, more genetically divergent lineages were not more likely to also be phenotypically different in the experimental environments (R² = 0.005, P = .88). This is important, as it allowed us to independently test these variables for their effect on hybrid transgression.

**F₁ Hybrid Heterosis**

We considered F₁ hybrid performance as heterotic (overdominant) if d1/a > 1 (for details, see “Material and Methods”). Overall, across all crosses and environmental conditions, heterosis was very common (40% of cases). The amount of heterosis varied among crosses (F₁,m = 2.97, P = .016), ranging from 16% (in Sc × Sk) to 55% (in Su × Sc). Among environments, heterosis also varied but not significantly (F₁,Sk = 2.11, P = .072), ranging from 21% in ethanol to 52% in salicylic acid and zinc sulfate (fig. S3).

Partial dominance of the fitter parent was rather common, being present in a total of 24% of cases across all crosses and conditions. Complete dominance of the fitter parent was found in 13% of all cases, and complete dominance of the less fit parent was found in 8% of cases. Partial dominance of the less fit parent was found in 9% of all cases. Underdominance was found in 5%, and codominance was found in 0.3%. Heterosis was not predicted by genetic (R² = 0.04, P = .66) or phenotypic (R² = 0.1, P = .65) distance between parents (fig. S4).

**F₂ Transgression Frequency**

Positive transgression frequency was 16.6% across all environments, concentrations, and crosses. Negative transgression frequency was only 6.1%. All further calculations are based on positive transgression. Transgression frequency differed among crosses and ranged between 21.4% (in Su × Sk) and 7.5% (in Sm × Sk). It also varied strongly between environments, ranging from 39.0% in salicylic acid to only 4.3% in ethanol. Transgression frequency was a function of increasing concentrations in salicylic acid (R² = 0.84, P = .001). In ethanol, transgression frequency decreased significantly with increasing concentration.
Figure 3: Optical density (OD; a measure of population growth and Malthusian fitness) of five parental Saccharomyces species and seven F1 and F2 hybrid crosses, measured along seven stress gradients with increasing concentration (M or %). Parent 1 refers to the species listed first, and parent 2 is the species listed second in the cross in each row (e.g., S. cerevisiae is the black line, S. kudriavzevii the gray line in the Sc × Sk cross). Data points represent independent replicates. Lines are LOESS (locally weighted smoothing) regression lines with shaded confidence intervals.
The other five environments showed no significant linear correlation with concentration level. Overall, transgression frequency was not a function of parental genetic distance ($R^2 = 0.49, P = .05$) or of parental phenotypic distance ($R^2 = 0.03, P = .24$). However, when analyzed separately by environment, transgression frequency was inversely correlated with genetic distance in ethanol ($R^2 = 0.96, P < .001$) and hydrogen peroxide ($R^2 = 0.85, P = .003$; note that this is the opposite of what theory predicts; fig. 6A). These relationships remained significant when excluding the cross with most transgressive hybrids (Sc × Sp; ethanol: $R^2 = 0.79, P = .017$; hydrogen peroxide: $R^2 = 0.73, P = .009$).
H_2O_2; R^2 = 0.72, P = .034). Phenotypic distance predicted a decrease in transgression frequency in salicylic acid only (fig. 6B), in agreement with theoretical predictions. No other significant correlations were found.

A generalized linear mixed effect model showed that all main effects (cross, environment, and concentration level), as well as their two-way interaction terms, explained significant amounts of variation in the number of transgressive F_2 hybrid populations (table S2). The number of positive transgressive segregants ranged across hybrid crosses from 106 (Su × Sk) to 25 (Sm × Sk), with an average of ∼70 per environment (fig. 7). Environmental impact was strong with ∼54% (262) of the total number of transgressive segregants found within a single environment (salicylic acid). Transgressive segregants were most frequent in the lowest concentration level (108) and least frequent at intermediate level 3 (27). Visualizing the number of positive transgressive segregants in pairwise interactions between hybrid cross, environment, and concentration level revealed the complex nature of these interactions (fig. 7). Although salicylic acid was the test environment with the most transgressive segregants, the segregants were not evenly distributed among hybrid crosses. In fact, two hybrid crosses had very few (Sc × Sm) or no (Sm × Sk) transgressive segregants. Similarly, transgressive segregants in salicylic acid were not evenly distributed among concentration levels but increased with increasing concentration. Other environments showed the highest levels of transgressive segregants at lower concentration levels (caffeine, ethanol, H_2O_2). Some hybrid crosses showed a uniform distribution of transgressive segregants across concentration levels (Sp × Sk, Su × Sk), while others were bimodal (Sc × Sp). Still others showed transgression primarily either at low (Sc × Sm) or at high (Sc × Sk) concentration levels. Overall, these analyses and visualizations show that positive transgression is a result of complex genetic and environmental interactions.

**Discussion**

There is evidence from theoretical and experimental work—for example, in sunflowers (Lexer et al. 2003), fish (Nolte and Sheets 2005; Parsons et al. 2011), and fungal pathogens (Shahid et al. 2008)—that transgressive segregation in hybrids can cause increased rates of adaptation and major ecological shifts (Rieseberg et al. 2003a; Kagawa and Takimoto 2018). This can potentially promote hybrid speciation, especially if reproductive isolation between hybrids and parents prevents backcrosses, which may evolve more easily in distant species crosses (Comeault and Matute 2018). However, there are little data on how frequently transgression occurs in hybrids and on how often transgression serves to promote or diminish hybrid fitness, and few attempts have been made to reliably predict hybridization outcomes, especially in nondomesticated crosses (Taylor and McPhail 2000; Gompert et al. 2017; Mandeville et al. 2019). Here, we used a set of seven interspecific yeast crosses in which parental genetic and phenotypic distances were not correlated (fig. 5), to test whether transgression frequency in F_2 hybrids was predictable from parental attributes, the experimental environment, or both.

We found that on average 16.6% of all replicate F_2 hybrid populations were transgressive with higher fitness than both parents and that 6.1% of F_2 hybrid populations were transgressive with negative fitness. Transgression frequency was predicted neither by genetic nor by phenotypic parental distances when averaging across all seven

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**Figure 5:** Pairwise genetic (genome-wide p-distances) and phenotypic distances (Euclidean distances from multidimensional scaling of growth data) between parental strains used for making hybrid crosses are not correlated.
test environments. Within environments, we observed only three (of 14) significant relationships with crossing distance, and they were not always in the predicted direction (fig. 6; the negative correlations with genetic distance in ethanol and hydrogen peroxide were unexpected; the negative correlation with phenotypic distance in salicylic acid was expected). Some of the test environments contained substances that yeast might encounter naturally (e.g., ethanol, hydrogen peroxide), and others are more likely novel or entirely artificial (e.g., caffeine, DMSO). Our data do not allow for any conclusions on whether hybridization outcomes are more predictable in more natural environments. It is, however, likely that the genetic architecture of some traits tested here contains genes with strong dominance effects while other traits may be largely controlled by epistasis; others are affected by both mechanisms and may therefore show no clear pattern. The number of transgressive F2 hybrids in our experiment varied significantly among environ-ments, cross backgrounds, and their interactions (fig. 7), suggesting that the outcome of hybridization events is highly context specific with strong genomic and environmental contingencies even when hybrid crosses were made from the same two parents. Transgression has been previously described to vary among phenotypic traits (Albertson and Kocher 2005; Thompson 2020) and experimental environments (Stelkens et al. 2014; Zhang et al. 2019).

Efforts to predict hybrid transgression from genetic and phenotypic parental characteristics have yielded mixed results. Crosses between domesticated crop (e.g., rice, tomatoes, and soy bean; DeVicente and Tanksley 1993; Mansur et al. 1993; Koide et al. 2019) and sunflower species (Kim and Rieseberg 1999) have generated more transgressive F2 offspring in more phenotypically similar parents, in line with theory. Genetic distance has reliably predicted transgression in some hybrid crops (Koide et al. 2019), but not always (Vega and Frey 1980; Cox and Frey 1985). A large

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**Figure 6:** Transgression frequency (%) as a function of genetic (A) and phenotypic (B) parental divergence in seven test environments. Lines show linear regression fit. Shaded areas are 75% confidence intervals. DMSO = dimethyl sulfoxide.
A meta-analysis of nondomesticated plants found a strong positive correlation between transgression and genetic distance, but not the predicted negative relationship with phenotypic distance (Stelkens and Seehausen 2009). Similarly, in interspecific hybrids of African cichlid fish, transgression in body shape increased with genetic distance, but parental phenotypic similarity was not related to hybrid transgression (Stelkens et al. 2009). Finally, neither genetic nor phenotypic parental crossing distance was strongly related to transgression in intra- and interspecific hybrids of two yeast species (Stelkens et al. 2014) that were also included in this study. In summary, no consistent pattern has emerged in the literature, including our study, which would reliably predict transgressive segregation.

The evolutionary distances (5–20 million years) between the species we used may lie outside the range where...
any pattern between sequence divergence and transgression is detectable. These species have accumulated 14%–21% nucleotide differences genome-wide, and hybrid meiosis typically results in less than 1% viable F1 hybrid offspring because of antirecombination and chromosomal missegregation (Hunter et al. 1996; Liti et al. 2006; Kao et al. 2010; Hou et al. 2014; Rogers et al. 2018). Although we have bypassed this constraint using large population sizes and asexual reproduction after F1 meiosis, high F2 hybrid mortality may have caused lower frequencies of transgression than expected and obscured the predicted effects of parental divergence on transgression overall. We are not aware of another study covering similarly large distances to measure hybrid transgression. Another confounding factor is that interspecific yeast hybrids frequently contain aneuploidies (reviewed in Gilchrist and Stelkens 2019). This is often detrimental for the cell, but some aneuploidies have been shown to facilitate adaptation to environmentally stressful conditions (Selmecki et al. 2009; Yona et al. 2012; Chang et al. 2013; Hose et al. 2015; Kaya et al. 2020). While remaining untested here, aneuploidy has likely contributed to both positive and negative transgression in this study.

So far, we have discussed our results on the frequency of transgression in F1 hybrids. What about patterns of heterosis in F1 hybrids? F1 hybrid heterosis is widely exploited in plant and animal breeding (Bircher et al. 2003; Schnable and Springer 2013). It is more often observed in crosses of inbred domesticated lines than in outbred wild populations because of the complementation of deleterious or loss-of-function alleles, which become more frequent with domestication (Rieseberg et al. 2003b; Zörgö et al. 2012; Plech et al. 2014). The five parental strains in our experiment are wild isolates and presumably not impacted by domestication. Curiously, heterosis was rampant, with 40% of all F1 populations showing increased fitness compared with the parents, with some variation between crosses. Heterosis is likely caused by a combination of genetic models, including dominance complementation of recessive alleles, overdominance, and epistatic interactions, previously shown to cause increased growth performance in intraspecific yeast hybrids (Shapira et al. 2014). Typically, heterosis increases from small to moderate parental genetic divergence (Cheres et al. 2000; Bernardes et al. 2017; but see Hung et al. 2012; Shapira et al. 2014) and declines rapidly at large distances (Moll et al. 1965; Rieseberg et al. 1999). A recent meta-analysis (Wei and Zhang 2018) confirmed this hump-shaped distribution of heterosis using intraspecific S. cerevisiae crosses (data are mainly from Zörgö et al. 2012; Plech et al. 2014). We found no relationship between heterosis and genetic or phenotypic parental distance across or within any of the environments we tested. As is the case for transgression in the F1 hybrids, we think this lack of relationship is likely due to the extreme sequence divergence of our crosses, located far outside the range of previously tested parental distances. We note, however, that F1 heterosis is not impacted by hybrid mortality here, as this affects only the F2 hybrid generation in yeast. Unlike animal F1 hybrids, which are much more impacted by changes in the function of morphogens during development with often lethal effects, interspecific yeast F1 hybrids are fully viable (yet mostly sterile; Greig 2009).

A number of theoretical models have recently explored the multigenic dynamics of Fisher’s geometric model, accommodating different types of hybrids (ranging from crosses between closely related inbred lines to almost isolated species) and different architectures of hybrid fitness with a variety of mutational effect sizes and underlying genetic structures (Fraïsse et al. 2014; Simon et al. 2018; Dagilis et al. 2019; Yamaguchi and Otto 2020). However, these models simulate the average fitness of a hybrid cross (not a measure of transgression frequency) as a function of the parents’ maladaptation (not necessarily a measure of parental sequence divergence) and can thus provide only limited insight here.

To conclude, hybrids with novel phenotypes, generated through the strategic breeding of parents with specific genetic and phenotypic distances, are an attractive means for agricultural breeding programs and food production. This may become a particularly relevant application under extreme environmental conditions, for example, when transgressive segregation mechanisms better equip hybrid plants and animals to survive droughts or flooding. Improving the forecasting of hybridization outcomes would also be useful to assess conservation risks, for instance, when invasive species interbreed with natives. However, our study demonstrates that reliably predicting hybrid fitness and transgression is not straightforward, especially not across large evolutionary distances and under changing environmental conditions (Gompert et al. 2017). In natural populations with standing genetic variation, transgressive segregation in hybrid offspring may be further obscured by large-effect pleiotropic alleles and compensatory mutations in other traits that are fixed during the adaptive divergence of the parents (Thompson 2020). To understand whether there are any generalizable patterns in hybrid fitness beyond the idiosyncrasies caused by the specific genomic background of the cross, we need more research on the targets of selection in hybrid genomes, the genetic architecture of these traits, and their interactions with the environment.

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Data used in the linear mixed model analysis are available in the Dryad Digital Repository (https://doi.org/10.5061/dryad.66t1gk21; Brice et al. 2021).

**Statement of Authorship**

R.S. conceived the idea for the study; C.B. and R.S. developed the methods and experimental design; C.B. collected the data; Z.Z., D.B., and R.S. analyzed and visualized the data; R.S., C.B., Z.Z., and D.B. wrote the article; and R.B. and D.B. acquired the funding.

**Data and Code Availability**

Data used in the linear mixed model analysis are available in the Dryad Digital Repository (https://doi.org/10.5061/dryad.66t1gk21; Brice et al. 2021).

**Literature Cited**


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