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The effect of hybrid transgression on environmental tolerance in experimental yeast crosses

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Abstract

Evidence is rapidly accumulating that hybridization generates adaptive variation. Transgressive segregation in hybrids could promote the colonization of new environments. Here, we use an assay to select hybrid genotypes that can proliferate in environmental conditions beyond the conditions tolerated by their parents, and we directly compete them against parental genotypes in habitats across environmental clines. We made 45 different hybrid swarms by crossing yeast strains (both *Saccharomyces cerevisiae* and *S. paradoxus*) with different genetic and phenotypic divergence. We compared the ability of hybrids and parents to colonize seven types of increasingly extreme environmental clines, representing both natural and novel challenges (mimicking pollution events). We found that a significant majority of hybrids had greater environmental ranges compared to the average of both their parents' ranges (mid-parent transgression), but only a minority of hybrids had ranges exceeding their best parent (best-parent transgression). Transgression was affected by the specific strains involved in the cross and by the test environment. Genetic and phenotypic crossing distance predicted the extent of transgression in only two of the seven environments. We isolated a set of potentially transgressive hybrids selected at the extreme ends of the clines and found that many could directly outcompete their parents across whole clines and were between 1.5- and 3-fold fitter on average. *Saccharomyces* yeast is a good model for quantitative and replicable experimental speciation studies, which may be useful in a world where hybridization is becoming increasingly common due to the relocation of plants and animals by humans.

Introduction

Evidence is accumulating rapidly from all parts of the tree of life that hybridization between species or highly diverged populations can generate adaptive variation. Despite increasing interest in hybrid speciation (see issues in Heredity 110(2) 2013 and Journal of Evolutionary Biology 26(2) 2013), current knowledge mainly comes from *post hoc* analysis of existing natural hybrid species (e.g. Keller *et al.*, 2013; Trier *et al.*, 2014). There are only a few studies of experimental hybrid speciation, that is those in which hybrid speciation, or processes leading to it, is induced experimentally (Greig *et al.*, 2002; Lexer *et al.*, 2003; Johnston *et al.*, 2004; Rosenthal *et al.*, 2005; Johansen-Morris & Latta, 2006).

Hybrids often have trait values that lie between those of their parents. They usually become outcompeted by the parent species because their phenotypic intermediacy leaves them poorly adapted to both ancestral habitats (Vamosi *et al.*, 2000; Gow *et al.*, 2007; Svedin *et al.*, 2008). However, intermediacy is not the only possible outcome of hybridization. Hybrids can express trait values that fall outside the range of both parent species, which is known as transgression (Slatkin & Lande, 1994; Rieseberg *et al.*, 1999). Transgression has been

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described in many different taxa, ranging from microbes to vertebrates (e.g. Albertson & Kocher, 2005; Marullo *et al.*, 2006; Stelkens *et al.*, 2009; Parsons *et al.*, 2011; Pritchard *et al.*, 2013; Latour *et al.*, 2014). If given the right ecological opportunity, these extreme phenotypes can provide hybrids with higher fitness and a selective advantage over their parents.

The potential benefits of hybridization are well known in agriculture, where transgressive crop plants are breeding targets because they produce more seed or fruit than their parents (Kuczyńska *et al.*, 2007; Knox *et al.*, 2012; Shivaprasad *et al.*, 2012). But transgression of hybrids has only recently caught the attention of evolutionary biologists as a potential mechanism for speciation by natural selection (Rieseberg *et al.*, 1999; Seehausen, 2004; Arnold, 2006; Mallet, 2007; Dittrich-Reed & Fitzpatrick, 2012). The best-studied natural hybrid system showing transgression is in sunflowers: the wild hybrid species *Helianthus paradoxus* grows better on salty soils than either of its parents (Welch & Rieseberg, 2002; Lexer *et al.*, 2003). Quantitative genetics research has shown that transgressive segregation in hybrids beyond the F1 generation is often caused either by epistasis or by the additive effects of alleles of opposite signs at QTLs that sum to extreme trait values in the hybrid genome (reviewed in Rieseberg *et al.*, 1999; Stelkens & Seehausen, 2009). In the F1, transgressive phenotypes are caused by heterosis. Previous data from laboratory-bred fish hybrids (Stelkens *et al.*, 2009) and a meta-analysis of transgressive traits in hybrid plants and animals (Stelkens & Seehausen, 2009) suggest that the amount of transgression in a given cross can, to an extent, be predicted by the genetic distance of the parental species.

The presence of a transgressive phenotype in a hybrid is only evolutionarily significant if it provides a fitness advantage (Arnold & Martin, 2010). Thus, to determine whether transgressive can promote hybrid speciation, it is necessary to demonstrate that hybrids can proliferate in habitats beyond the extremes of the parental ranges or that they can outcompete parental genotypes within those ranges. To do this, we used *Saccharomyces* yeast, which has several key advantages over traditional models for speciation and is ideally suited for high-throughput testing of strains, replicates and environments. *Saccharomyces* is a rapidly growing, genetically tractable model microbe that can reproduce sexually – a prerequisite when testing for the effects of genetic exchange between lineages (e.g. Greig *et al.*, 2002). As a model eukaryote for molecular genetics, large sets of genomic and phenotypic data exist for many *Saccharomyces* strains, and transgressive phenotypes have been described repeatedly (e.g. Marullo *et al.*, 2006; Liti *et al.*, 2009; Cubillos *et al.*, 2011).

We crossed *Saccharomyces* yeast strains along genetic and phenotypic distance axes, creating both intraspecific and interspecific F1 hybrids, and we induced sex in these to create F2 'hybrid swarms' potentially containing a mixture of F1 hybrids, the gametes they produce and the resulting F2 hybrids, but not backcrosses to parents. We tested (i) whether F1 hybrids or F2 hybrid swarms could proliferate in habitats beyond the parental ranges (i.e. were positively transgressive), (ii) whether transgression was affected by phenotypic or genotypic crossing distance between parents and (iii) whether positively transgressive hybrids could directly outcompete their parents in a shared environment. We tested the ability of parents and hybrids to grow along seven distinct environmental clines representing both natural challenges and novel environments, which would, for instance, mimic anthropogenic pollution events. We then tested the fitness of selected transgressive hybrids relative to their parents by direct competition.

We found that hybridization significantly increased ecological range compared to the range of the mid-parent, but not compared to the range of the best parent. We found that selected hybrid genotypes could directly outcompete their parents within the same habitat, under a large range of environmental conditions. Genetic and phenotypic crossing distance predicted the extent of transgression in two of seven environments.

Materials and methods

Strains and crosses

We used 31 strains of *S. cerevisiae* or *S. paradoxus* from the National Collection of Yeast Cultures (http://www.ncyc.co.uk/). Strains were originally collected from a wide diversity of habitats (soil, trees, faeces, insects, fruit, clinical, beer, wine) from across the world (Europe, UK, Siberia, Russia, Japan, Malaysia, Australia, West Africa, North and South America, Hawaii). Matching parental strains from such vast variety of geographical and ecological sources to make pairs with a large range of genetic and phenotypic distances (Liti *et al.*, 2009) produced a total of 45 representative F1 hybrid strains, some with parents that were different species (*S. paradoxus* × *S. cerevisiae*) and some with parents that were different isolates of the same species (*S. paradoxus* × *S. paradoxus*). Unlike others (Zörgö *et al.*, 2012; Plech *et al.*, 2014), we did not include laboratory strains such as S288c to limit effects of domestication (i.e. heterotic release in F1 hybrids due to complementation of deleterious mutations). Parental strains were isogenic heterothallic (*HO*-deleted) haploid versions of the original wild-type homothallic strains (Cubillos *et al.*, 2009). They each carried two antibiotic markers (*ho::HygMX* and *ura3::KanMX*). Genome-wide SNP data (obtained from Liti *et al.*, 2009) were used to calculate genetic distances between pairs of parental strains (number of different base pairs/total number of aligned base pairs). The total number of aligned base

pairs per cross ranged from 27 760 to 5 763 735 bp. The number of SNPs per cross ranged from 28 to 790 401 bp. *Saccharomyces* genomes contain about 12 million base pairs in total. The hybrids had parents with between 0.06% and 14% SNP sequence divergence. Natural *S. paradoxus* populations show strong genetic structure; strains isolated from geographically distant places are highly diverged. Because strains have only been sampled from a few different geographic locations, the genetic distances in our crosses are discontinuous, forming four clusters: crosses between strains isolated from the same part of a continent are closely related, those from distant parts of the same continent are more diverged, those from different continents are more diverged still, and crosses between *S. paradoxus* and *S. cerevisiae* are most diverged. A list of all 45 hybrid crosses and their parents, with NCYC accession numbers, genetic distances and phenotypic distances (calculations below), can be found in Table S1. The 45 hybrid crosses were not entirely independent genetic entities because some parental strains were used in more than one cross (Table S1).

Hybridization protocol

Strains were grown from frozen samples and incubated at 30 °C in 10 mL YEPD (1% yeast extract, 2% peptone, 2% dextrose) in a shaking incubator for 24 h. Diploid F1 hybrids were made by mixing equal volumes of two haploid parental strains of different mating types and incubating the mixture on YEPD plates (with the addition of 2.5% agar) overnight (culture 1). To purify the resulting diploid F1 hybrids, culture 1 was streaked to new YEPD plates (culture 2), grown for 48 h, and the resulting colonies, each derived from a single cell, were replica plated to KAC agar plates (2% potassium acetate, 2% agar) and incubated for 48 h at 25 °C to induce sporulation, which was verified microscopically. Because diploids can sporulate but haploids cannot, a sporulating colony could be identified as a pure F1 hybrid and not a parent haploid. A colony of each pure F1 hybrid was spread on to a new YEPD plate (culture 3), grown for 48 h, replica plated to KAC and incubated as before to induce sporulation (meiosis) and obtain a large sample of F1 spores (gametes). F1 spores were washed off the KAC plates with 10 mL liquid YPD and glass beads, and the resulting suspension of F1 spores was propagated for 24 h (culture 4), to allow germination and mating. Culture 4 thus contained a 'hybrid swarm' of mated F2 hybrids, unmated F2 gametes produced by F1 hybrid meiosis and F1 hybrid cells that had not undergone meiosis. We acknowledge that normally the term hybrid swarm often refers to a population also containing backcrosses to one or both parental types, which are not present in our F2 hybrid swarms. We have adopted the term here because we think it conveys the genetic and phenotypic variation of this experimental group. A sample of each F2 hybrid swarm (culture 4) was frozen for later use.

Nonhybrid diploid parents were obtained using the same protocol as for hybrids. We crossed isogenic haploid strains of different mating types and purified the resulting nonhybrid diploid colonies in the same way that pure F1 hybrids were obtained. A pure diploid colony of each parent was then spread, sporulated and washed off in YEPD liquid, and the spores were allowed to germinate and mate as before. Each parental culture (culture 5) thus contained a mixture of homozygous diploids and some unmated but isogenic haploid cells. The ploidy status of parent and hybrid cultures is therefore comparable, which is important as ploidy can be a key determinant of trait variation in yeast (Zörgö *et al.*, 2013).

Measurement of environmental range

We tested pure F1 hybrids (culture 3), F2 hybrid swarms (culture 4) and their parents (culture 5) for their ability to grow on environmental clines of increasing concentrations of seven different substances. Four of these substances (ethanol, acetic acid, glucose and hydrogen peroxide) represent naturally occurring stresses (fruit rots, plant surfaces). The remaining three (lithium acetate, sodium chloride and cycloheximide) are novel challenges not encountered naturally by any of the parental strains and may be interpreted to mimic novel anthropogenic interference such as those associated with chronic contamination by pollutants. Clines were made in 96-well flat-bottomed culture plates. Each cline occupied a column of the plate, with the lowest concentration of the substance (diluted with growth medium MIN + URA) in the bottom well, and increasing in concentration in eight steps so that the top well contained a sufficiently high concentration of the substance to inhibit the growth of the parent strains (see all concentrations in Table S2). The assay was designed to identify hybrids that could proliferate in conditions that are not tolerated by their parents (transgression).

Cultures were grown from frozen stocks overnight in 10 mL minimal medium plus uracil (MIN + URA, 0.67% yeast nitrogen base without amino acids, 2% glucose, 2% agar, 0.003% uracil) at 30 °C. Each cline received a F1 population, an F2 hybrid swarm or a parent culture inoculated into each well. Allocation of strains to columns and plates was made randomly. Every strain was tested in three replicate clines in each of the seven substances. Plates were incubated for 48 h at 30 °C.

After 48 h, we measured the optical density of every well with a microplate reader (Infinite M200 Pro, Tecan). Plates were also scanned on a flatbed scanner (Color LaserJet CM2320fxi MFP, Hewlett-Packard) for visual inspection and troubleshooting (e.g. dried-up

wells). We normalized the optical density readings within each combination of cross and cline type so that the highest and lowest readings were 100% and 0%, respectively, and summed the number of contiguous wells (going from low to high concentration of each substance), which had readings of 50% or higher. We added 0.5 to this sum if the normalized optical density in the next extreme concentration was between 25% and 50%, to account for wells with low but noticeable growth. Measurements were averaged across three replicates per strain and used as response variable 'environmental range' in downstream analyses.

Although this method of measuring environmental range produced only discrete data (i.e. the number of wells across a cline that a culture could grow in), it is more useful for our purposes than using the raw optical density measurements. The strains used in this experiment differ in cell size, shape and tendency to form clumps, causing differences in optical density measurements that do not reflect differences in cell number. Further, we were not so much interested in comparing absolute cell numbers, which are expected to vary greatly across ecological ranges, as we were in comparing the extents of the ranges themselves. This captures the phenomenon we are most interested in: the ability of hybrids to colonize environments that are inaccessible to their parents. We compared the optical densities of all wells across the cline to determine only whether the population inoculated into a particular well was growing there or not. Our method only gives a measure of the ecological range, not of fitness, neither of the population nor of the individuals within a population (see Relative fitness analysis below).

Phenotype analysis

To obtain phenotypic distances between parental strains, parental growth range data from all environments were entered into principal component analysis (PCA) and pairwise distances were extracted from the rotated, centred, scaled data (Table S1). Our phenotypic distances matched the distances calculated from previously published multivariate phenotypes in Warringer *et al.* (2011) ($R^2 = 0.42$, $F_{44} = 30.87$, $P < 0.001$), which were estimated from over 600 traits per strain. The close match between their and our data indicates that, despite measuring growth ranges in only seven environments, we have successfully explored multivariate phenotype.

To measure the extent of transgression, for each cross, we subtracted the average parental range (i.e. the mid-parent range) from the F1 hybrid range and from the F2 hybrid swarm range, to give measures of F1 hybrid and F2 hybrid swarm 'transgression', respectively. Each transgression assay was replicated three times. Hybrid ranges were also compared to the parent with the largest environmental range (rather than comparing to mid-parent ranges). Sign tests were used to determine whether the environmental ranges of hybrids were larger or smaller than their mid-parent ranges. Hybrid ranges were also compared to the parent with the largest environmental range (rather than comparing to mid-parent ranges). A two-way ANOVA was used containing 'environment' (7 levels), 'cross' (45 levels) and interaction between environment and cross, to test for their effects on transgression in F1 hybrids and F2 hybrid swarms. *Post hoc* Tukey's tests showed which environments differed in their extent of transgression. We used regression analysis to test whether genetic or phenotypic crossing distance predicted transgression in different environments. Distance variables were Box-Cox-transformed prior to analysis. Genetic crossing distance had a tenuous distribution which is due to the limited geographic locations that have been used to sample the highly structured global *S. paradoxus* population, as well as the high genetic divergence between *S. paradoxus* and *S. cerevisiae*. We therefore also performed analyses on the genetic distance separated into four categories ('intraspecific close', 'intraspecific intermediate', 'intraspecific distant' and 'interspecific'), or into two categories ('intraspecific' or 'interspecific'), using one-way ANOVAS on transgression in F1 hybrids and F2 hybrid swarms. To test for differences between the mean ranges of F2 hybrid swarms and pure F1 hybrids, we used regression analysis and paired t-tests across and within environments. All analyses were performed in JMP11 (SAS).

Relative fitness analysis

We tested whether transgressive hybrid strains could outcompete their parental strains in direct competition. We tested individuals isolated from the five most transgressive F2 hybrid swarms (Table S3) from each of three environments (ethanol, lithium acetate and cycloheximide). The entire content from the well with the highest substance concentration that supported growth was streaked on YEPD and allowed to form colonies. Then a single hybrid genotype was chosen at random and purified. These 15 strains were stored as frozen stocks.

We competed each hybrid, which was hygromycin resistant (*ho::HygMX*), with the nonresistant isogenic diploid version of its best parent from NCYC, using the drug resistance phenotype to distinguish them. A list of hybrid and parental strains used in this assay can be found in Table S3, with parental NCYC accession numbers. For each fitness assay, the hybrid and the nonresistant version of its parent were first grown up separately for 48 h in the same medium used for the fitness assay (5 mL MIN + URA supplemented with the appropriate concentration of one of the three substances). Tubes were kept at 30 °C in a shaking incubator. Equal volumes of the two cultures were then

mixed, and 50 μL was used to inoculate 5 mL of fresh medium of the same type (time point t_0). A sample of this initial culture was serially diluted, plated on YEPD to yield single colonies, and then replica plated to YEPD supplemented with 300 mg/L hygromycin, which only allows the hybrid colonies to grow. All samples were plated out in duplicates. The colonies growing on YEPD plates were counted and multiplied by the dilution factor to determine the initial total number of cells in the liquid culture, and the colonies growing on YEPD supplemented with hygromycin were counted to determine how many of these were hybrid colonies. The mixed liquid culture was grown for 48 h. Then, a second sample was taken (time point t_1), diluted, plated to yield single colonies and counted as before to determine the final number of hybrid and nonhybrid cells in the culture. The fitness of the hybrid relative to its parent was calculated by dividing the Malthusian growth parameter of the hybrid strain (the natural log of the dilution rate and the proportion of hygromycin-resistant CFU at t_1 over t_0) by the Malthusian growth parameter of the parental strain (Lenski *et al.*, 1991).

We wanted to test the ability of each transgressive hybrid to compete against its fittest parent under conditions ranging from those that might be expected to favour the hybrid to those that might be expected to favour the parent. We therefore ran fitness assays for every hybrid in five increasing concentrations of ethanol, lithium acetate or cycloheximide (Table S3). The highest concentration corresponded to the well with the highest concentration in the previous transgression assay that still permitted hybrid growth; the lowest concentration contained none of the substances at all. Fitness assays were replicated three times for every combination of strains in every concentration. One-sample t-tests, calculated on means per strain, were used to determine whether hybrid fitness was larger or smaller than their parents' fitness across concentrations. Because we wanted to know whether hybrid strains differ in fitness, and to test for effects of increasingly toxic conditions on relative hybrid fitness, a model was fitted with hybrid 'strain', 'concentration' and their interaction ('concentration x strain') for each substance separately. An outlying data point from lithium acetate was excluded prior to analysis (identified as outlier using Cook's D).

Results

Hybrid transgression

We were interested in the ability of hybrids to colonize niches that were unavailable to their parents. We therefore looked at the differences between the environmental ranges of 45 hybrids and the mean ranges of each hybrid's two parents (mid-parent range). Overall, across strains, environments and replicates, transgression was weak but positive. F1 transgression was 0.1 wells of a possible 8-well cline (SEM 0.05 wells) on average, and F2 hybrid swarm transgression was 0.07 wells (SEM 0.05 wells) on average. Of 945 combinations of crosses, environments and replicates, F1 transgression was positive in 449 cases, negative in 354 cases and zero in 142 cases. Thus, transgression was significantly positive overall (two-tailed sign test; $P < 0.001$ from binomial distribution generated by the null hypothesis that positive and negative transgressions are equally likely). For F2 hybrid swarms, there were 457 cases of positive transgression, 345 cases of negative transgression and 143 cases of zero transgression, which is also significantly positive (two-tailed sign test as before, $P < 0.001$). We also looked at the differences between the environmental ranges of each hybrid and its parent with the largest range (best-parent transgression), rather than the mean range of both parents (mid-parent transgression). We found that overall best-parent transgression tended to be negative for both F1 hybrids ($P < 0.001$, two-tailed sign test) and F2 hybrid swarms ($P < 0.001$, two-tailed sign test). Taken together, these data show that hybrids tended to have ranges above the mid-parent but below the best parent. All further analysis was performed on mid-parent transgression estimates.

Two-factor ANOVAs showed that cross, environment and their interaction had significant effects on transgression in both F1 hybrids (cross: $F_{44,630} = 2.30$, $P < 0.001$; environment: $F_{6,630} = 4.01$; $P < 0.001$; interaction: $F_{264,630} = 1.8$, $P < 0.001$) and F2 hybrid swarms (cross: $F_{44,630} = 2.36$, $P < 0.001$; environment: $F_{6,630} = 4.45$; $P < 0.001$; interaction: $F_{264,630} = 1.97$, $P < 0.001$). *Post hoc* Tukey's tests indicated that transgression in ethanol, glucose and hydrogen peroxide was significantly higher than transgression in lithium acetate in F1 hybrids, but transgression in acetic acid, cycloheximide and sodium chloride was not different from any other environment. Similarly, in F2 hybrid swarms, transgression in ethanol, glucose and hydrogen peroxide was significantly higher than transgression in sodium chloride, but transgression in acetic acid, cycloheximide and lithium acetate was not different from any other environment (Fig. 1).

Transgression as a function of genetic and phenotypic crossing distance

To further investigate the effect of cross on transgression, we tested the relationship between parental genetic distance and phenotypic distances on the average of the three replicate measurements of hybrid transgression for each cross. Overall, there was no significant relationship between genetic distance and transgression for either F1 hybrids ($R^2 = 0.0$, $F_{1,313} = 1.05$, $P = 0.31$) or F2 hybrid swarms ($R^2 = 0.0$, $F_{1,313} = 0.8$, $P = 0.37$). Phenotypic distance predicted an overall increase in transgression in F1 hybrids ($R^2 = 0.02$,

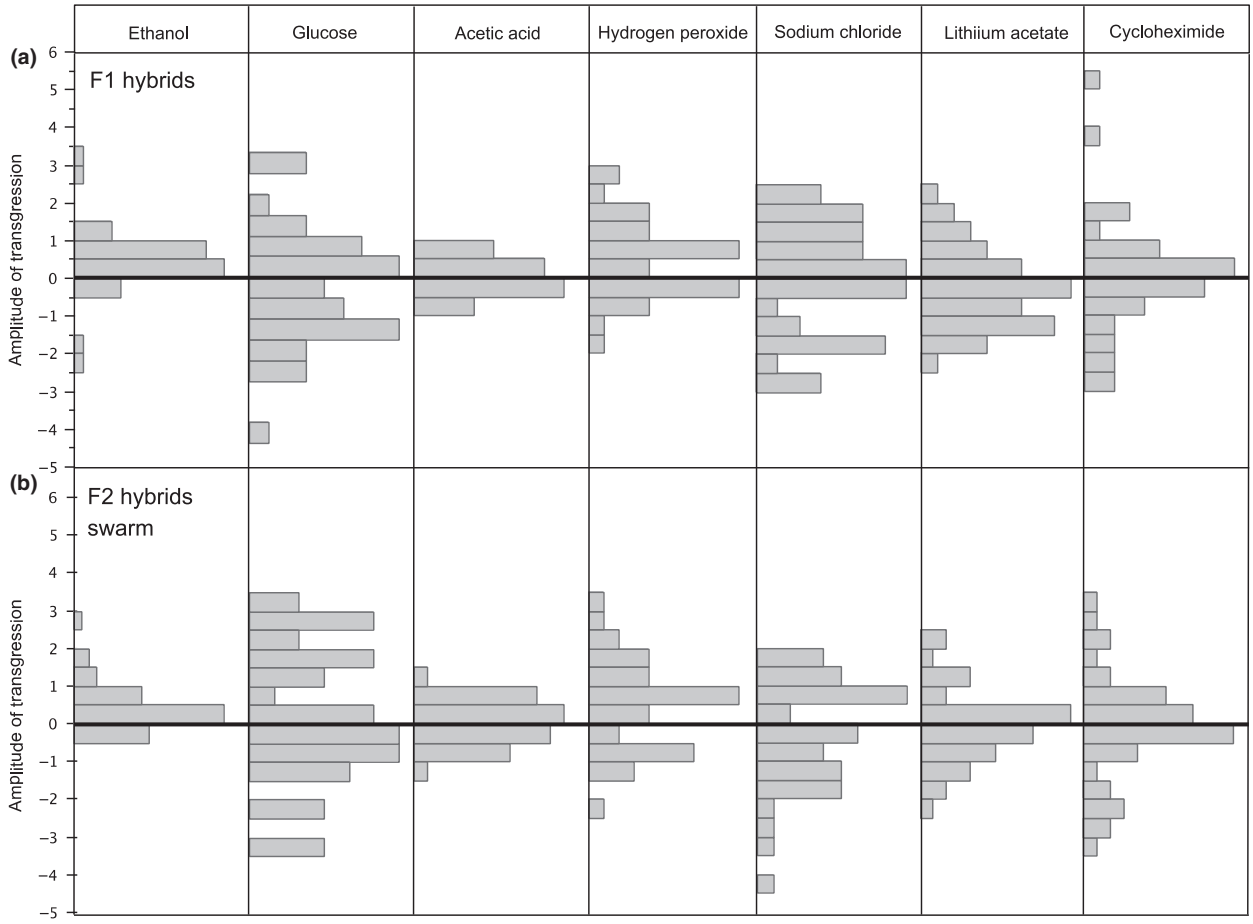

Fig. 1 Amplitude of transgression. Distribution and amplitude of transgression in (a) F1 hybrids and (b) F2 hybrid swarms in each environment. Swarms potentially contained a mix of F1 and F2 hybrids, but no backcrosses. Phenotypes above zero are transgressive (above the bold horizontal line).

$F_{1,313} = 4.85$, $P = 0.028$), but there was no significant relationship in F2 hybrid swarms ($R^2 = 0.0$, $F_{1,313} = 1.65$, $P = 0.19$). Consistent with the significant interaction between cross and environment above, we found that genetic distance predicted a significant increase of transgression in F1 hybrids in lithium acetate ($R^2 = 0.11$, $F_{1,43} = 5.14$, $P = 0.028$), but not in any other environment (Fig. 2a). In hybrid swarms, genetic distance predicted an increase in transgression in ethanol ($R^2 = 0.11$, $F_{1,43} = 5.18$, $P = 0.028$) and hydrogen peroxide ($R^2 = 0.19$, $F_{1,43} = 10.34$, $P = 0.003$), but not in any other environment (Fig. 2b). Phenotypic distance predicted a significant increase in transgression in hydrogen peroxide in both F1 hybrids ($R^2 = 0.22$, $F_{1,43} = 12.32$, $P = 0.001$; Fig. S1a) and F2 hybrid swarms ($R^2 = 0.32$, $F_{1,43} = 20.26$, $P < 0.001$; Fig. S1b). Because genetic distance showed a rather discontinuous distribution, we also tested whether the four cross classes 'intraspecific close', 'intraspecific intermediate', 'intraspecific distant' and 'interspecific' differed from another in the amount of transgression. We found significant differences between these classes in both F1 hybrids ($F_{3,311} = 2.82$, $P = 0.039$; Fig. 3a) and F2 hybrid swarms ($F_{3,311} = 2.88$, $P = 0.036$; Fig. 3b). In both cases, the 'intraspecific distant' group, that is crosses made between geographically distant *S. paradoxus* parents, contained significantly more transgression than another group. Classifying crosses into intraspecific and interspecific did not yield significant differences (F1 hybrids: $F_{1,313} = 0.0$, $P = 0.96$; F2 hybrid swarms: $F_{1,313} = 0.12$, $P = 0.73$).

F1 strains and F2 hybrid swarms show similar transgression

Across all environments, there were no significant differences between the mean ranges of F2 hybrid swarms and pure F1 hybrids. We only found significant differences in lithium acetate, with F2 hybrid swarms significantly exceeding the range of F1 hybrids ($t_{1,44} = 1.44$,

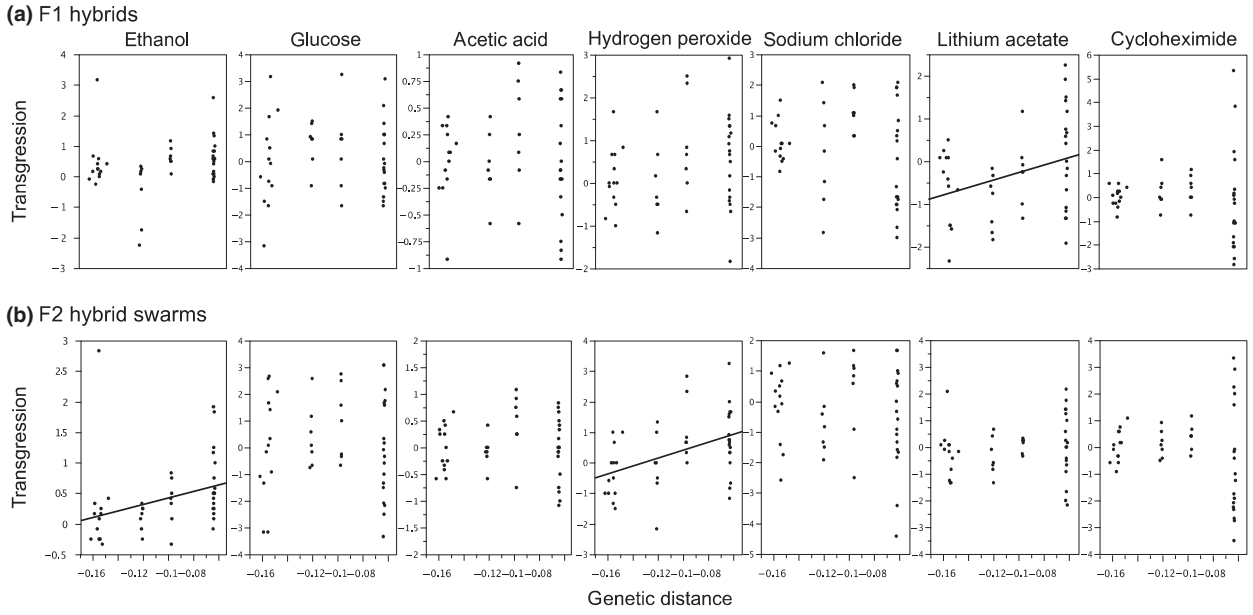

Fig. 2 Hybrid transgression as a function of genetic distance between parents. Panel (a) shows transgression in F1 hybrids; panel (b) shows transgression in F2 hybrid swarms in seven environments. All genetic distance data shown are Box-Cox-transformed. Solid line indicates significant relationship.

$P = 0.039$). The extent of transgression found in F1 hybrids and F2 swarms was correlated ($R^2 = 0.58$, $F_{1,313} = 425.55$, $P < 0.001$).

Relative fitness of hybrids

We isolated individual hybrid genotypes from the most extreme wells colonized by F2 hybrid swarms of the five most transgressive crosses in three types of environmental clines (ethanol, lithium acetate and cycloheximide). We measured their fitness relative to their parents in a range of conditions representing the whole cline. Tested across all environments, strains and concentrations' relative hybrid fitness was significantly higher than 1 (i.e. higher than the parents' fitness; two-tailed t-test: $t_{62} = 4.8$, $P < 0.001$). Looking at environments separately, hybrid fitness was significantly higher than 1 in ethanol ($t_{13} = 4.75$, $P < 0.001$; Fig. 4a) and in lithium acetate ($t_{23} = 2.97$, $P = 0.007$; Fig. 4b), but not in cycloheximide ($t_{24} = 2.71$, $P = 0.037$; Fig. 4c). Only in lithium acetate, hybrid fitness significantly increased with increasing concentration ($F_{1,14} = 8.7$, $P = 0.005$). In cycloheximide, but not in the other two substances, there were significant differences among hybrid strains ($F_{4,15} = 7.55$, $P < 0.001$) and the relationship between hybrid relative fitness and concentration was strain specific ($F_{4,15} = 4.59$, $P = 0.003$, Fig. S2). The

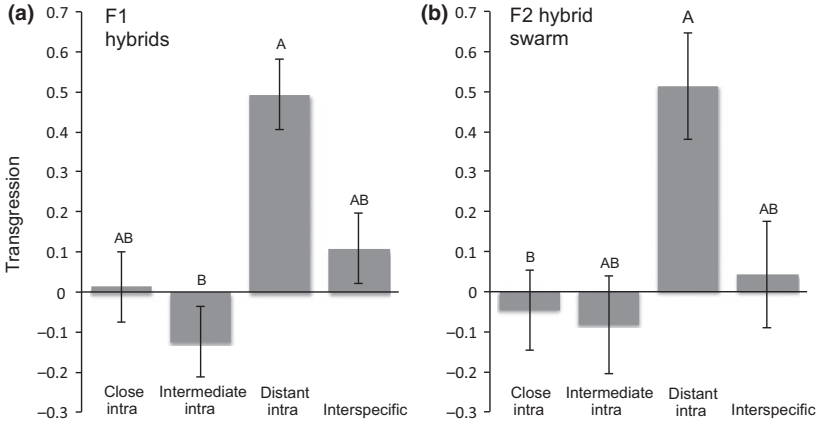

Fig. 3 Hybrid transgression in four genetic distance classes. The classes 'intra close', 'intra intermediate' and 'intra distant' contain crosses made from *S. paradoxus* parents with increasing genetic divergence. The class 'interspecific' contains *S. paradoxus* × *S. cerevisiae* crosses. Panel (a) shows transgression in F1 hybrids; panel (b) shows transgression in F2 hybrid swarms. Error bars show standard errors of the mean. Bars marked with different letters are significantly different.

interaction term was not significant in any other environment.

In ethanol, relative fitness across strains was 2.9 times higher than parent fitness, ranging from 1.12 to 5.6 across concentrations (Fig. 4a). In lithium acetate, hybrid fitness was 2.7 times higher ranging from 0.5 to 13.7 (Fig. 4b). In cycloheximide, hybrid fitness was 1.5 times higher than that of the best parent, ranging from 0.2 to 5 (Fig. 4c).

Discussion

Positive transgressive segregation and heterosis are understudied sources of evolutionary novelty. If transgression affects key ecological traits, hybrid populations may invade habitats not available to either parent and undergo ecological divergence (Buerkle *et al.*, 2000). This process has been suggested to lead to speciation (Rieseberg *et al.*, 2003; Abbott *et al.*, 2013). The potential for hybridization to expand ecological range is especially interesting, because it might allow hybrids to colonize a niche that is inaccessible to their parent species, reducing competition and backcrossing, and thereby promoting speciation. We measured hybrid transgression for ecological range across many genotypes and environments. However, we note that an increased range does not necessarily imply a higher fitness: for example, a 'specialist' may have very high competitive fitness in a narrow range and be able to exclude a generalist that has lower fitness across a much broader range. We therefore tested the competitive fitness of a set of transgressive hybrids not only in the extreme end of the range they were isolated from, but also across the whole range of conditions.

Transgression in extreme environments

In our study of transgression in F1 and F2 hybrid swarms, hybridization slightly increased ecological range compared to the mid-parent. A small, but significant, majority of F1 hybrid populations and F2 hybrid swarms contained genotypes that could colonize environments preventing the growth of their mid-parent. Hybrids did not generally have larger ranges than their best parent. This result is consistent with the proportion of yeast F1 hybrids showing 'best-parent heterosis', reported previously (about 30% in Zörgö *et al.*, 2012; Shapira *et al.*, 2014).

Although overall hybrid transgression was weak, the transgressive genotypes we sampled were generally very successful. On average, across clines, hybrids were between 1.5- and three-fold fitter than their parents. Remarkably, even though they were selected in extreme conditions, hybrids did not generally decrease in relative fitness in less extreme conditions, except perhaps in one example (lithium acetate, Fig. 4 and Fig. S2). Instead, hybrids were usually fitter than their parents across the entire environmental range. The reason for this may be that the parents were poorly adapted even to the most benign environments and that hybridization allowed rapid adaptation not only to high concentrations of the toxic substances, but also to the common environmental conditions shared across the clines. By testing the fitness of hybrids in direct

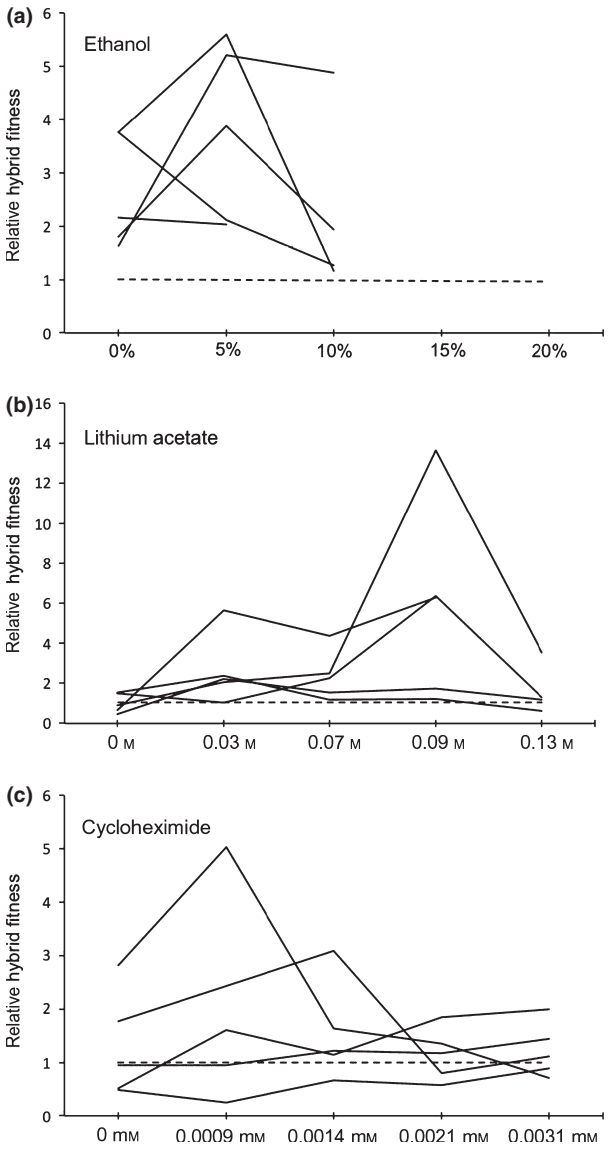

Fig. 4 Relative hybrid fitness assays along three environmental clines. Results of competition experiments in (a) ethanol, (b) lithium acetate and (c) cycloheximide. Lines indicate relative fitness of hybrid strains tested against their best parent. Dashed line denotes equal fitness of hybrid and parents. Every line is a different hybrid strain. Data points are the mean of three technical replicates. X-axes show gradients of concentrations used to simulate environmental clines. 15% and 20% of ethanol proved too strong and no cells survived.

competition with their parents, we can demonstrate the 'realized' adaptive value of transgressive hybrids, within the laboratory setting, as opposed to describing a mere 'hopeful monster' with extreme phenotypic traits (Mallet, 2007; Dittrich-Reed & Fitzpatrick, 2012).

Even though most hybrids did not exceed the ranges of their best parents, hybridization should always be favoured in environments that are lethal to both parent species: even a small chance of survival is better than none. This is especially true when the formation of a hybrid lineage relies on being distinct from the parents in a single trait only – a mechanism known as hybrid trait speciation (Jiggins *et al.*, 2008; The Heliconius Genome Consortium, 2012) Indeed, hybrid species are often found to be ecologically divergent from their parents, and hybrids commonly occupy extreme environments. Examples from natural populations include adaptations to high elevation in butterflies (Gompert *et al.*, 2006; Kunte *et al.*, 2011; Nice *et al.*, 2012), tolerance to extreme drought and soil salinity in *Helianthus* sunflowers (Rieseberg *et al.*, 2003), and tolerance to warmer water temperature in sculpins (Nolte *et al.*, 2005; Czypionka *et al.*, 2012). Besides the colonization of environments that can be interpreted as extreme extensions of parental habitats, divergence of hybrids can also result from adaptation to new challenges brought about by human interference and pollution. Hybrids may express transgressive trait values in response to many substances, including both natural environments relevant to yeast ecology and environments that are newly created through anthropogenic interferences and pollution. Examples for adaptation to such novel challenges are copper tolerance in *Mimulus* (Macnair, 1989; Wu *et al.*, 2008), zinc and cadmium tolerance in *Arabidopsis* (Roux *et al.*, 2011), adaptation to eutrophication in *Daphnia* (Brede *et al.*, 2009) and industrial melanism in the peppered moth (van't Hof *et al.*, 2011).

Environment and genotype affect hybrid transgression

In our experiment, genetic crossing distance was related to the extent of hybrid transgression in two of seven environments (ethanol and hydrogen peroxide). Phenotypic distance only predicted an increase in transgression in one environment. Although a positive relationship between parental divergence and transgressive hybrid phenotypes has been described before in other organisms (Stelkens & Seehausen, 2009; Stelkens *et al.*, 2009), our data extend this finding by the fact that we measured transgression along multiple environmental clines, upon exposure to ecological selection.

In most environments, however, parental crossing distance was not a good predictor of the degree of transgression. A possible explanation for the absence of an effect is that population divergence in yeast may be caused by drift rather than by selection (Dujon, 2010; Zörgö *et al.*, 2012). If this is the case, divergence would be less likely to have been accompanied by directed purging of alleles of opposite signs, and, as a result, the likelihood for complementary gene action in yeast would be equal at any stage of divergence.

A possible explanation for the substance-specific effects observed in our experiment is trait architecture. Whether stress tolerance is determined by few or many genes controls the outcome of segregation variance in hybrids. The more genes are involved, the more likely it is that alleles with opposing signs are present, giving more opportunity for allelic complementation and epistasis (DeVicente & Tanksley, 1993; Rieseberg *et al.*, 1999; Stelkens & Seehausen, 2009). We are currently developing methods using next-generation sequencing to understand the genetic architecture of transgressive traits in *Saccharomyces*.

When we classified crosses into four genetic distance groups, we found significant differences in the amount of transgression (Fig. 3). Interestingly, the group containing the most genetically distant within-species crosses showed the most transgression, that is, the largest environmental ranges. This might reflect potential benefits of outcrossing between divergent genomes that do not yet have to pay the costs of genetic incompatibilities affecting crosses between species.

The genetic basis of hybrid transgression

Yeast usually inbreed by self-fertilization (Ruderfer *et al.*, 2006). Mating between less related individuals could increase fitness in F1 hybrids either by masking deleterious alleles in F1 hybrids, resulting in heterosis, or by positive epistasis between newly combined sets of alleles. Fitness may also decline because of negative epistatic interactions (Dobzhansky, 1937; Müller, 1942; Coyne & Orr, 2004). Fitness can be further affected in F2 hybrids by the breaking up of co-adapted gene complexes (Lynch, 1991) or the production of new beneficial combinations, and by increased offspring aneuploidy due to chromosome mis-segregation during F1 hybrid meiosis (Hunter *et al.*, 1996). Overall, we expected variance in F2 offspring fitness to increase with increasing parental distance, but mean fitness to decrease because of hybrid incompatibilities, including aneuploidy. 99% of all offspring produced from crosses between *S. paradoxus* and *S. cerevisiae* are completely inviable (Hunter *et al.*, 1996), and many offspring produced from diverged crosses within *S. paradoxus* (Greig *et al.*, 2003) or within *S. cerevisiae* (Hou *et al.*, 2014) are similarly affected. In addition to reducing the overall proportion of viable F2 hybrids, the higher rates of aneuploidy expected in more distant crosses can increase genetic variation beyond that which can be achieved by epistasis and dominance effects alone, by also increasing variability in gene dosage. We expected

the highest performing hybrids in our experiment to be aneuploids, whose extra chromosomes increase expression of important phenotypes. Aneuploidy has been associated with stress resistance in yeast and other fungi (Selmecki *et al.*, 2009; Pavelka *et al.*, 2010; Kwon-Chung & Chang, 2012), and polyploidy has been shown to fuel adaptive diversification in yeast (Lidzbarsky *et al.*, 2012).

Our transgression assay had the potential to capture the entire segregational variance generated in the F2 hybrid swarms. We tested whether an F2 hybrid swarm, regardless of its mean fitness, contained transgressive hybrids that could colonize an environment inaccessible to their parents. The relative proportions of F2 hybrids, unmated gametes produced by F1 hybrids and F1 diploids that did not enter meiosis are likely to vary among F2 hybrid swarms from different crosses. Indeed, we observed that F1 hybrid sporulation efficiency was negatively correlated to genetic crossing distance ($R^2 = 0.16$, $F_{1,43} = 8.1$, $P = 0.007$). While a difference in the proportions of these cell types is likely to affect the mean fitness of an F2 hybrid swarm, proportional differences are unlikely to affect the range that a swarm can colonize, because the presence of only a single resistant individual (whether an F1 or F2 hybrid) is required to colonize a toxic well at the extreme end of the cline. The fitness of individuals within an F2 hybrid swarm may also be affected by nonadditive ecological interactions between them. If, for instance, the higher genetic diversity in F2 hybrid swarms from more distant parents resulted in a more positive balance of ecological interactions (productivity; see Cardinale *et al.*, 2011), then this swarm could colonize more extreme wells, even if the fittest genotypes within the swarm could not do so alone.

The ability of yeast to grow clonally, allowing rare high fitness individuals to rapidly produce large populations despite the expected low mean fitness of F2 hybrids, represents an obvious difference to most sexually reproducing organisms. In principle, only a single cell needs to be able to grow clonally in a more extreme well than mid-parent well for transgression to be detected in our assay. However, the high frequency of transgression also found in sexual flowering plants (Rieseberg *et al.*, 1999; Johansen-Morris & Latta, 2006; Stelkens & Seehausen, 2009; Anton *et al.*, 2013) and vertebrates (Stelkens *et al.*, 2009; Parsons *et al.*, 2011) indicates that independent mating events should regularly produce transgressive phenotypes available as mating partners to establish true-breeding hybrid populations with large adaptive potential. After all, transgressive hybrid genotypes may have enough of a selective advantage to quickly increase in numbers, despite the initial hurdle of hybrid breakdown.

Given the multiple potential causes of genetic variation than can be generated in F2 hybrid swarms, and the advantages this should have for adaptation, it is remarkable that the overall extent of transgression was so low. Even if this variation resulted in most variants being unfit or inviable (negative transgression), the transgression assay was designed so that a single positively transgressive genotype should be detected. It is therefore especially surprising that F2 swarms did not do much better than F1 hybrids, which did not contain such genetic variation. The strong correlation between the transgression of pure F1 hybrids and the transgression of F2 hybrid swarms suggests that latter is due to the presence of F1 hybrids within the swarms. This shows that the variation in the F2 hybrid swarms that is produced by meiosis in F1s and subsequent syngamy cannot produce more transgression than simple F1 heterosis alone. We suggest therefore that F1 heterosis, which results from the complementation of deleterious alleles in heterozygotes, is the major factor contributing to hybrid transgression in our study. Two recent studies have tested for heterosis in yeast hybrids measured in different environments using F1 hybrids (Plech *et al.*, 2014) and backcrosses (Shapira *et al.*, 2014). Plech *et al.* found a positive relationship between parental sequence divergence and heterosis (transgressive stress resistance), but heterosis only increased in crosses between domesticated strains, not between wild strains. Shapira *et al.* did not find any such relationship, even though they used wild strains only.

Conclusions

Our transgression assay was designed to select hybrid genotypes from swarms that could grow beyond the environmental range of their clonal parents. Surprisingly, we found that such hybrids only modestly increased environmental range, but that they could nevertheless outcompete their parents in direct competition. This suggests that hybrid speciation is most likely to occur at the edges of species ranges. *Saccharomyces* yeast, a group with extensive natural hybridization (Liti *et al.*, 2005; Muller & McCusker, 2009), allows quantitative and replicable speciation experiments, which is important in a world where hybridization is becoming increasingly common due to the relocation of plants and animals by humans.

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Conflict of interest

The authors declare no conflict of interest.

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Supporting information

Additional Supporting Information may be found in the online version of this article:
Table S1 List of crosses.
Table S2 Concentrations of substances used in transgression assays.
Table S3 List of hybrid and parental strains used in fitness assay.
Figure S1 Hybrid transgression as a function of phenotypic distance between parents.
Figure S2 Relative hybrid fitness.

Data deposited at Dryad: doi:10.5061/dryad.83qh4

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