The long-term evolutionary potential of four yeast species and their hybrids in extreme temperature conditions

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Abstract

Accelerating climate change and extreme temperature events urge us to better understand the potential of populations to tolerate and adapt to thermal challenges. Interspecific hybridization can facilitate adaptation to novel or extreme environments. However, predicting the long-term fitness effects of hybridization remains a major challenge in evolutionary and conservation biology. Experimental evolution with microbes provides a powerful tool for tracking adaptive processes across hundreds of generations in real time. Here, we investigated thermal adaptation dynamics of four species of budding yeast (*Saccharomyces*) and their interspecific F2 hybrids, for 200 generations under extremely cold (5°C) and warm (31°C) conditions. We found significant variation in the evolutionary potential of species and hybrids. Cold-tolerant species showed larger fitness increases in warm temperature, whereas warm-tolerant species showed larger fitness gains in cold temperature. By far the largest fitness improvements occurred in hybrids, with some populations nearly quadrupling in fitness in the cold environment over the course of experimental evolution. Some hybrids exceeded both their parents in thermal adaptive potential. Reciprocal transplanting of evolved populations from the endpoint of evolution into opposite temperatures revealed that hybrids generally have greater resilience than their parents when challenged with sudden temperature shifts. Our results highlight that hybridization alters the fitness outcomes of long-term adaptation to extreme environments and may render populations more resilient to sudden environmental change, presenting both opportunities and challenges for conservation and sustainable agriculture.

Keywords: climate change, experimental evolution, fitness, hybridization, *Saccharomyces,* thermal adaptation

Lay summary

Understanding how populations adapt to extreme temperatures is crucial in times of accelerating climate change. Hybridization and genetic exchange between species can help populations adapt to new or extreme environments, but can also have negative effects on fitness. Predicting the long-term effects of hybridization remains challenging for evolutionary biologists. We usually do not know how hybrid offspring respond to future environmental change. Experimental evolution with microbes is a powerful tool to provide insight here, because we can observe fitness changes in real-time, across many generations. We tracked the fitness of six species of budding yeast *Saccharomyces* and their hybrids, over 200 generations in extreme cold (5°C) and warm (31°C) conditions. We found that species and hybrids have significantly different abilities to adapt. The largest fitness improvements were observed in hybrid populations, particularly in the cold, where some nearly quadrupled in fitness and exceeded both their parents. When exposing evolved populations to a sudden shift into opposite temperatures, hybrids were more resilient than their parent species. Even after 200 generations of evolution under opposite temperature conditions, hybrids still showed a similar performance to their ancestors. Our results suggest that hybridization can enhance the ability of populations to adapt to extreme environments, offering potential benefits for conservation and agriculture.

Introduction

Human actions in the Anthropocene have led to global temperature increases and more frequent extreme climatic events (Perkins-Kirkpatrick & Gibson, 2017), destabilizing ecosystems and driving declines in biodiversity (Hoegh-Guldberg et al., 2018). This has

intensified the need to understand and forecast the evolutionary responses of populations to temperature changes. However, most experimental studies on the effects of climate change rely on short-term exposure of organisms to higher temperatures, often measured within a single generation only. These brief exposures do not reflect the long-term changes occurring in populations over evolutionary time.

Hybridization, the exchange of genetic material between divergent populations and species, can facilitate adaptation, increase diversity, and rescue populations from extinction because it instantly introduces new genetic variation throughout the genome (Abbott et al., 2013; Grant & Grant, 2019; Seehausen, 2004; Stelkens et al., 2014a). Hybridization rates may rise as a consequence of accelerating climate change and globalization (Chunco, 2014; King et al., 2015; McFarlane & Pemberton, 2019), posing both risks and opportunities for adaptive evolution and conservation (Brauer et al., 2023). However, our knowledge about the long-term fitness consequences of hybridization is limited. This is in part because the focus has traditionally been on long-lived species, often with small population sizes, that are of conservation concern (e.g. birds and mammals). Making hybrid crosses in these systems and using hybrids in experiments spanning multiple generations is often difficult, if not impossible. Yet, finding good conservation practices tailored to populations exposed to hybridization and introgression critically depends on a sound understanding of their long-term evolutionary potential. In an applied context, how do we assess whether supportive breeding is appropriate, if we do not know how outbred offspring respond to future environmental change? To forecast the impact of global warming on the long-term fitness and adaptation of recombined hybrids, it would be helpful to understand how the parental species' ecological and genomic backgrounds affect the hybrids' capacity to thermally adapt, across evolutionary time scales and ecologically relevant conditions.

Another question of major interest in evolutionary biology is how repeatable evolutionary responses to changing environments are. Experimental evolution studies have revealed parallelism at the gene and pathway level in bacteria and yeast (Ament-Velásquez et al., 2022; Harris et al., 2021; Kryazhimskiy et al., 2014.; Tenaillon 2012., 2014) but can we predict fitness outcomes of hybrid crosses as well? The response of hybrid populations to environmental stress can be extremely variable (Brice et al., 2021.; Gaworecki & Klaine, 2008; Lopandic, 2018; Oberhofer et al., 2014; Seehausen, 2004; Stelkens et al., 2014a; Stelkens et al., 2014b), suggesting that complex adaptations are based on different genes in different species, leading to additive, dominant and epistatic genetic interactions in hybrid genomes (Zhang et al., 2020). This highlights the importance of testing a large diversity of parental genomic and ecological backgrounds when generating predictions for the thermal adaptation of hybrids in response to climate change.

We used the microbial system budding yeast (*Saccharomyces* spp.) and experimental evolution across 200 (asexual) generations, to assess the long-term fitness evolution of interspecific hybrids in cold and hot temperatures. Yeast is well suited for this work for two main reasons. First, *Saccharomyces* yeast species vary widely in temperature preferences ranging from 15° to 35°C (Salvadó et al., 2011). Heat resistance and optimal growth temperatures are well known across the genus with some cryotolerant strains able to grow at 0°C, while some heat-resistant strains can grow up to 42°C (Belloch et al., 2008; Pérez-Torrado et al., 2015). Given that *Saccharomyces* species diverged 4-20M years ago (Borneman & Pretorius, 2014; Kellis et al., 2003; Shen et al., 2018) this suggests that thermal adaptation is a deeply diverged trait (Abrams et al., 2021; Weiss et al., 2018), providing interesting starting points for experimental evolution and a rich resource to investigate the long-term

fitness consequences of hybridization at different temperatures. Second, yeast can reproduce asexually by clonal 'budding', allowing for experiments with large population sizes (up to 200M cells), but also sexually by mating, followed by meiosis and recombination, which allows for controlled hybrid crosses between all species pairs despite their large evolutionary divergence (Bendixsen et al., 2022; R. Stelkens & Bendixsen, 2022). Hybridization is known to have substantially contributed to the origin and evolutionary history of *Saccharomyces* (Marcet-Houben & Gabaldón, 2015; Peris et al., 2017; Steensels et al., 2021) and interspecific yeast hybrids have been isolated from natural (D'Angiolo et al., 2020; Eberlein et al., 2019; Leducq et al., 2016) and industrial environments, e.g. from lager beer fermentation (Gallone et al., 2019; Gibson & Liti, 2015; Hutzler et al., 2023).

Here, we explore the fitness dynamics and evolutionary potential for thermal adaptation across four yeast species, which cover the entire range of temperature preferences and represent all major clades found in the *Saccharomyces* phylogeny (Bendixsen et al., 2021), and their recombined interspecific hybrid crosses at two extreme temperatures, 5±1°C and 31±1°C. The species *S. kudriavzevii*, and *S. uvarum* are considered cold-tolerant (Libkind et al., 2011; Naseeb et al., 2017; Salvadó et al., 2011). The Baker's yeast *S. cerevisiae* represents the other end of the temperature spectrum and is considered warm-tolerant, with an optimum growth temperature in the range of 30-39°C. The species *S. paradoxus* is considered a temperature generalist that grows well in a wide range of temperatures.

Materials and methods

Parental strains

We used two strains each of *S. cerevisiae* (XLY011 and Y55), *S. paradoxus* (YPS744 and Q89.8), *S. kudriavzevii* (ZP591 and FM1110), and *S. uvarum* (CBS7001 and JRY9189). These strains are representative of all major clades in the *Saccharomyces* phylogeny (Bendixsen et al., 2021), and have a wide range of different ecologies and temperature profiles (more details on strains and hybrids in Supplementary Table 1). All strains used in this study are derived from diploid, non-domesticated isolates from the wild with the exception of *S. cerevisiae* XLY011, which is a homothallic diploid derived from BY4742 and Y55 strain backgrounds commonly used in the laboratory. All strains are HO-/- (either ho:KanMX, ho:HYGMX, or ho:NATMX), i.e. they cannot switch mating type.

Construction of F1 and F2 hybrids

Haploid parents were grown overnight in liquid YEPD (1% yeast extract, 2% peptone, 2% dextrose) in a shaking incubator at 30°C and then mixed at equal volumes. Mating was induced by spinning down to concentrate cells and plating drops of 20µL on YEPD plates (2.5% agar), kept at room temperature for 12h. We then streaked out for single colonies on a fresh YEPD plate and incubated for 24 h at 30°C. Colonies were replica-plated to a sporulation plate (KAC; 2% potassium acetate, 2% agar) and incubated at room temperature for 5 days to induce sporulation. Diploid hybrids were identified by inspecting individual colonies for tetrads under the microscope. A pure F1 diploid hybrid colony from each interspecies pair was picked from the YEPD plate, previously used to streak out single colonies, then propagated clonally in liquid YEPD overnight, and frozen in 50% glycerol for later use. To obtain a genetically diverse, recombined F2 hybrid population, F1 hybrids were sporulated as follows. F1 hybrids were grown in liquid YEPD overnight, cells were pelleted by centrifugation and transferred to a 500 mL Erlenmeyer flask containing 50 mL of liquid KAC, which was

151 shaken at room temperature for 7 days to induce meiosis. After microscopic verification of
152 sporulation, we centrifuged and washed cells, and resuspended them in liquid YEPD to initiate
153 germination and population growth. These large, recombinant F2 hybrid populations (which
154 may still contain some unsporulated F1 hybrids) were frozen in 50% glycerol solution for later
155 use.
156 We used two strains of *S. cerevisiae*, a well-characterized model organism, and
157 crossed them with two strains of three other yeast species: *S. paradoxus*, the most genetically
158 similar; *S. uvarum*, one of the most distant; and *S. kudriavzevii*, representing an intermediate
159 genetic distance. Thus, the two *S. cerevisiae* strains were used as the reference with which
160 all other strains were crossed.
161
162 **Experimental evolution and fitness assays**
163 We used experimental evolution to mitotically propagate large diploid populations of all four
164 species (eight strains in total, in three or four independent replicate evolution lines each) and
165 six different interspecific F2 hybrids (in three independent replicate evolution lines each) at
166 two temperatures; 5±1°C and 31±1°C (Figure 1). We chose 31°C for the hot evolution regime
167 because this temperature still allows the two cold-tolerant species (*S. kudriavzevii*, and *S.*
168 *uvarum*) to grow. Note that replicate evolution lines of hybrid crosses are 'biological
169 replicates' in the sense that each population contains genetically different individuals
170 resulting from independent meiosis and recombination, while replicates of pure-species lines
171 are technical, i.e. they were isogenic at the start of evolution.
172 To initiate experimental evolution, we inoculated 100µL of overnight cell culture (from
173 frozen stock populations) into glass tubes containing 5 mL of liquid YPD. This corresponds to
174 a starting population size of approximately 3 x10^7 cells. All populations evolved in the same
175 incubator-shaker (one for each temperature) at the same time, i.e. they experienced the same
176 slight temperature fluctuations if present. Continuous population growth was achieved by
177 serially transferring 50µL of cell culture into 5mL fresh YPD to provide nutrients after 24 hours
178 at 31±1°C, and 96 hours at 5±1°C. This corresponds to approximately 20 generations in each
179 temperature regime, between serial transfers. Generation time was calculated using the
180 formula G= t / (3.3 log b/B) (G = generation time, B = number of cells at the beginning of time
181 interval, b = number of cells at the end of time interval). We used a total of ten serial transfers
182 to reach approximately 200 generations. This transfer protocol leads to periodic reductions
183 in population size but avoids bottlenecks to minimize the loss of beneficial mutations, i.e.,
184 selection is stronger than drift in these systems (Wein & Dagan, 2019).
185 To track population fitness across generations, we measured each population's optical
186 density (OD at 600nm, Biotek Epoch2), which quantifies the maximum biomass before each
187 transfer. OD_{600} was measured ten times for each evolving population across 200 generations
188 (i.e. every 20 generations), in three technical replicates. The dilutions were standardized to
189 approximately 0.5 OD_{600} at each serial transfer. We performed an unpaired t-test with
190 Bonferroni-Dunn correction (at 0.05 alpha-level), to test for differences between ancestors
191 and evolved populations. Relative fitness was calculated by dividing the fitness of evolved
192 populations at any generation by the initial fitness of their respective ancestor (i.e. at
193 generation 0).
194 In addition, we measured the fitness of populations from the endpoints of evolution
195 (after 200 generations) after reciprocally transplanting them into the opposite temperature,
196 i.e. populations evolved at 31°C were placed in the 5°C environment, and populations evolved
197 at 5°C were placed in the 31°C environment. The relative fitness of these reciprocal transplant

198 populations was assessed as described above, by comparing to the fitness of the un-evolved
199 ancestors.
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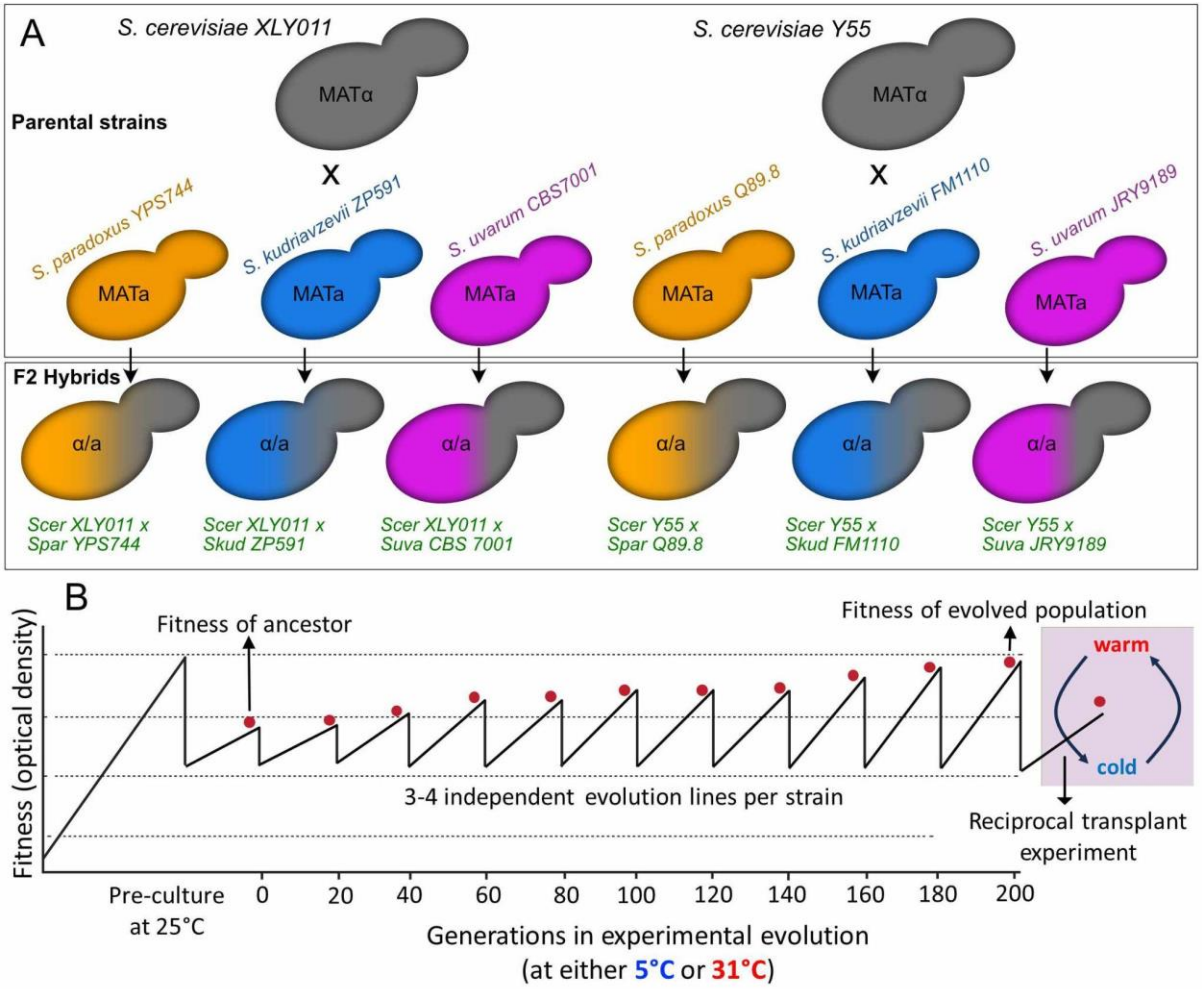
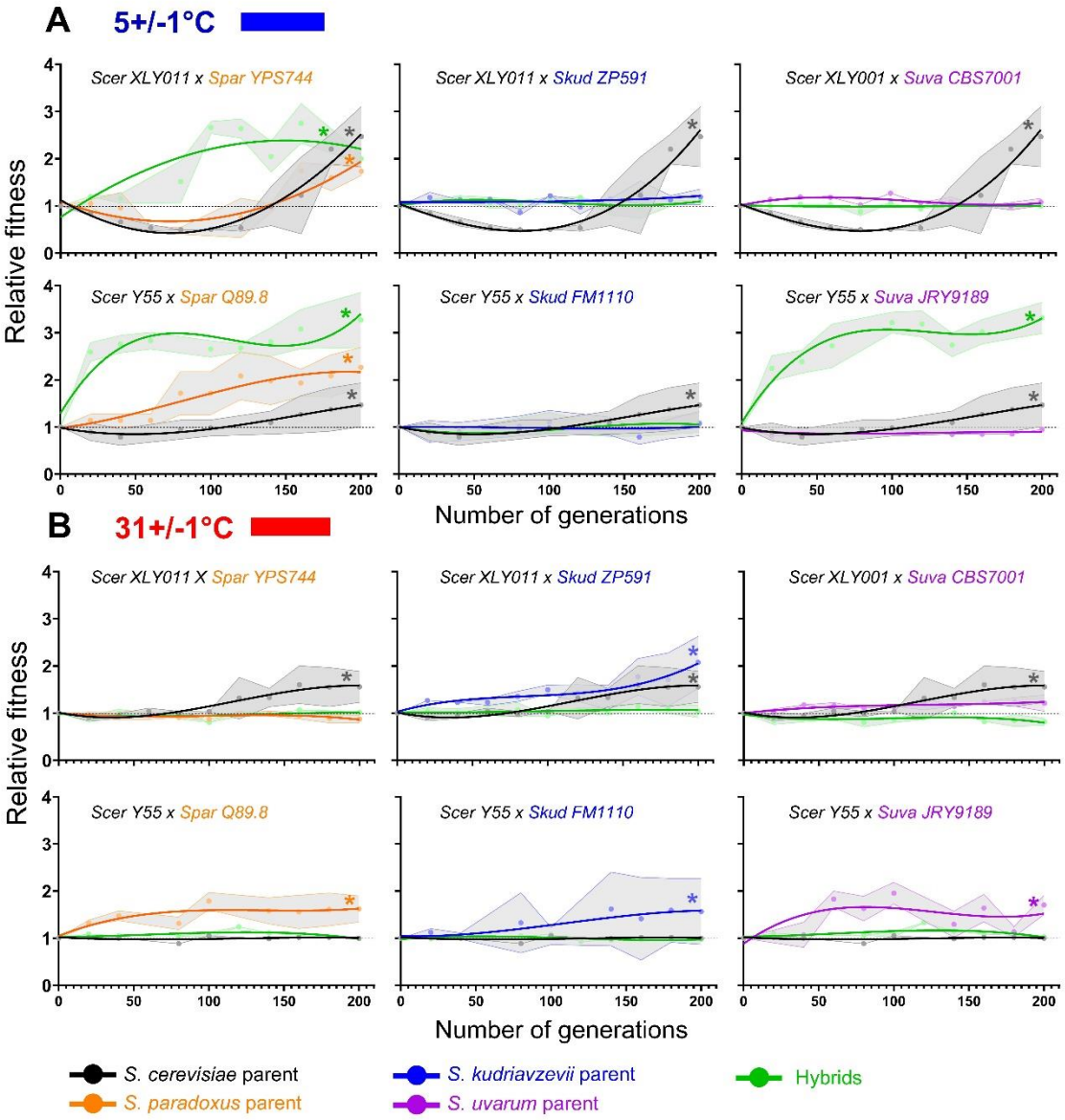


201
202 ***Figure 1. A)*** *Crosses between two S. cerevisiae strains and strains of S. paradoxus, S.*
203 *kudriavzevii and S. uvarum to obtain six different hybrids.* ***B)*** *Experimental evolution protocol*
204 *with hypothetical fitness dynamics. Maximum biomass was measured as optical density*
205 *(OD_{600}) every 20 generations as a proxy of fitness before each serial transfer (red dot). Every*
206 *population was evolved in warm (31°C) and cold (5°C) conditions, in three or 4 independent*
207 *evolution lines (replicates), for 200 mitotic generations. At the endpoint of evolution, a*
208 *reciprocal transplant experiment was performed (purple square) and the fitness of cold-*
209 *evolved populations was measured in warm conditions and vice versa after the sudden*
210 *temperature shift.*

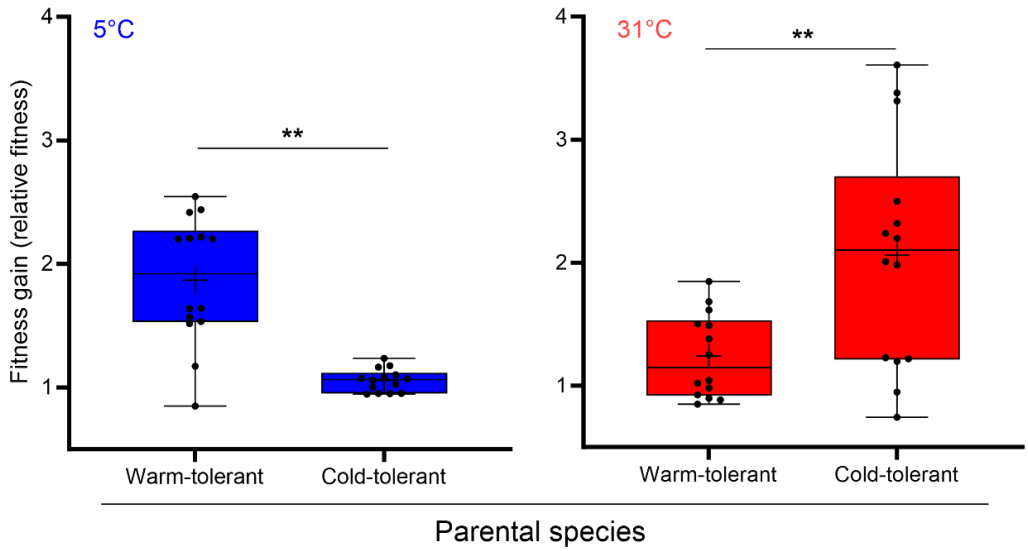
211
212 **Results**
213 **Thermal adaptation dynamics of *Saccharomyces* species**
214 We tracked the fitness dynamics and adaptation of four *Saccharomyces* species across 200
215 generations in cold (5±1°C) and warm environments (31±1°C). We found significant
216 differences in fitness gains between species at both temperatures (Figure 2). The largest
217 improvement in relative fitness (compared to the ancestor's fitness at generation 0) was
218 found in *S. cerevisiae* at cold temperature (Figure 2A) and *S. kudriavzevii* at warm temperature
219 (Figure 2B). *S. cerevisiae* XLY011 produced ~120% more biomass (averaged across replicates)

220 than the ancestor at the end of evolution in the cold environment (t = 13.24, p < 0.01). *S.*
221 *kudriavzevii ZP591* produced ~100% more biomass than the ancestor at the end of evolution
222 in the warm environment (t = 6.77, p < 0.01).



223
224
225 ***Figure 2.*** *Relative fitness dynamics of eight strains of four Saccharomyces yeast species and*
226 *six hybrid populations evolved for 200 generations in* ***A)*** *a cold (5±1°C) and* ***B)*** *a warm (31±1°C)*
227 *environment. Lines show mean relative fitness across three or four independently evolved*
228 *replicate populations per strain background. Grey-shaded areas show the standard deviations*
229 *of replicates. Values above 1 represent fitness gains, values below 1 represent fitness losses,*
230 *relative to the ancestral strain's fitness. Asterisks indicate significant differences between the*
231 *ancestral and the evolved strains after 200 generations.*
232
233 Both of the cold-tolerant species, *S. kudriavzevii*, and *S. uvarum,* showed only a small
234 or no fitness gain in the cold environment, but significant fitness improvements in the warm
235 environment. The warm-tolerant *S. cerevisiae* also improved in fitness in the warm
236 environment, but not to the same extent as in the cold environment. The thermo-generalist

237 *S. paradoxus* showed mixed outcomes, with both *S. paradoxus* strains increasing in fitness in
238 the cold, but only one strain improving in the warm environment.
239 This suggests that the long-term evolutionary potential to thermally adapt differs
240 between temperature conditions and among species backgrounds. Comparing warm-tolerant
241 with cold-tolerant species showed that warm-tolerant species generally showed higher
242 evolutionary potential at low temperatures (t-test with Welch correction: $t_{13,83}$= 5.76; p <
243 0.001), whereas cold-tolerant species showed higher evolutionary potential at warm
244 temperatures (t-test with Welch correction: $t_{16,45}$ = 3.77, p= 0.0065; Figure 3). Note, that as
245 expected, cold-tolerant species (*S. kudriavzevii*, and *S. uvarum*) had higher absolute starting
246 fitness than warm-tolerant species at cold temperature (Supplementary Figure 1A, test
247 results in Supplementary Table S2), whereas at warm temperature, warm-tolerant species (*S.*
248 *cerevisiae* and *S. paradoxus)* showed higher absolute starting fitness (Supplementary Figure
249 1B).



250
251 ***Figure 3*** *Comparing the relative fitness gains after evolution in cold (5±1°C) and warm*
252 *(31±1°C) environments between warm-tolerant or generalist (S. cerevisiae and S. paradoxus)*
253 *and cold-tolerant (S. kudriavzevii and S. uvarum) species. Whiskers show the smallest and*
254 *largest values, lines in the middle of the boxes represent the median and + represents the*
255 *mean. A t-test with Welch correction shows significant differences in cold and warm*
256 *environments (p< 0.01**).*
257
258
259 **Thermal adaptation dynamics of interspecific hybrids**
260 We also tracked the fitness evolution of six interspecific F2 hybrid populations, each
261 containing large amounts of genetic variation, across 200 mitotic generations at 5±1°C and
262 31±1°C. In the cold environment, three hybrid crosses significantly improved in relative fitness
263 (Figure 2A). As opposed to their parents, the largest improvements in hybrid fitness were seen
264 in the first 100 generations of experimental evolution, where both *S. cerevisiae* x *S. paradoxus*
265 hybrid crosses, plus the *S. cerevisiae Y55* x *S. uvarum JRY9189* hybrid, vastly exceeded both
266 their parents in relative fitness. In the warm environment, hybrids changed overall less in
267 fitness (Figure 2B). *S. cerevisiae* x *S. paradoxus* and *S. cerevisiae* x *S. kudriavzevii* hybrid crosses
268 did not change significantly. One of the *S. cerevisiae* x *S. uvarum* hybrids tended to lose fitness
269 at warm temperature (*S. cerevisiae XL011* x *S. uvarum CBS7001*).

Hybrids had low absolute starting fitness in the cold (often lower than both parents individually) but improved over the course of evolution, often doubling or tripling in absolute fitness (Supplementary Figure 1). This was less pronounced in the warm environment, where the starting fitness of the hybrids was not as low as in the cold, but intermediate or similar to that of their parents. Absolute fitness values and results of unpaired t-tests comparing evolved lines with their respective ancestral strains are shown in Supplementary Figure 1 and Table S2.

Comparing the thermal evolutionary potential of parents and hybrids

To directly compare the fitness of parents and hybrids after experimental evolution in the warm and cold environment, we plotted the relative fitness gains at 31±1°C against the relative fitness gains at 5±1°C across all populations. We found that by far the largest improvements in fitness occurred in hybrids in the cold environment, with some populations nearly quadrupling in fitness (Figure 4). The majority of the parental populations (grey dots) gained fitness at both temperatures, but not to the same extent as the hybrids. Confirming the patterns found above, hybrids showed the highest improvements at cold, particularly both *S. cerevisiae* and *S. paradoxus* hybrid crosses (Figure 5, orange dots) and *S. cerevisiae Y55* x *S. uvarum JRY9189* (purple dots). Hybrid crosses between *S. cerevisiae* and *S. kudriavzevii* fell in the centre, showing no fitness gains in either the warm or cold environment (blue dots).

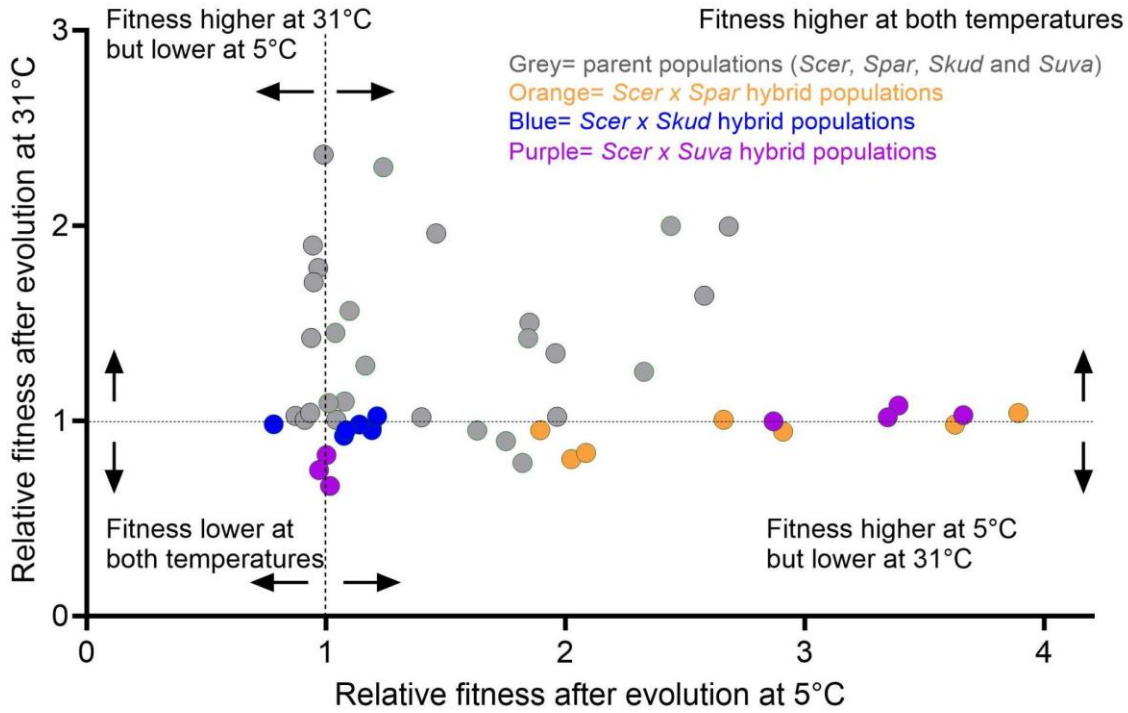


***Figure 4**. Relative fitness of parental strains and their six interspecific F2 hybrids after 200 generations of experimental evolution at 31±1°C, plotted against relative fitness after evolution at 5±1°C. Each data point is an independently evolved line.*

Effects of sudden temperature shifts on the fitness of evolved populations

After 200 generations of experimental evolution, we measured the fitness of all warm-evolved populations (at 31±1°C) in the cold environment (5±1°C), and the fitness of cold-

evolved populations in the warm environment, akin to a reciprocal transplant experiment. The transition from warm to cold caused significant fitness decreases in six of the eight evolved parent populations (Supplementary Figure 2). The two cold-tolerant species (*S. kudriavzevii* and *S. uvarum*) were most affected, with significantly lower fitness than the warm-tolerant parents after the sudden temperature shift (ANOVA: $F_{2,11} = 4.80$; Tukey's test: p= 0.025; Figure 5A left). One strain of the thermo-generalist *S. paradoxus* was unaffected while the other strain (*YPS744*) showed a significant fitness increase. The transition from warm to cold also significantly decreased the fitness of most hybrid crosses (Supplementary Figure 2). Overall, hybrid fitness after the temperature shift to cold was intermediate between that of the parents, with no significant differences to either cold- or warm-tolerant parents (Tukey's test vs. cold-tolerant: p= 0.23; vs. warm-tolerant p= 0.26).

The shift from cold to warm only caused significant fitness decreases in three of the eight evolved parent populations and only the two warm-tolerant species were affected (*S. cerevisiae* and *S. paradoxus*; Supplementary Figure 2). The growth of the two cold-tolerant species (*S. uvarum* and *S. kudriavzevii*) remained mostly unaffected by the sudden temperature transition to warm. A comparison between the fitness of warm-tolerant and cold-tolerant species after the shift to warm was non-significant (ANOVA: $F_{2,11}= 3.48$; Tukey's test: p= 0.06; Figure 5A right). The transition from cold to warm caused a significant fitness decrease in five of the six hybrid populations. Again, hybrid fitness was overall intermediate between that of the parents, with no significant differences to either cold- or warm-tolerant parents (Tukey's test vs. cold-tolerant: p= 0.19; vs. warm-tolerant p= 0.62).

We then tested if the original thermal performance of the strains before experimental evolution predicts how much their fitness is impacted by sudden temperature shifts. We found that the absolute fitness of ancestral hybrid strains before evolution predicted their fitness after the temperature shift when transplanting populations from warm to cold (linear regression; $R^2= 0.94$, $p <0.01$ Figure 6B left), but not when transplanting from cold to warm conditions ($R^2= 0.41$, p= 0.17; Figure 6B right). This was not the case for the parent populations, which showed no correlation between the ancestor's fitness and the temperature-shifted populations in either direction ($R^2=0.08$, p = 0.49 in cold, $R^2=0.16$, p = 0.32 in warm). This suggests that hybrids may have greater resilience when faced with sudden temperature shifts from warm to cold, and remain similar in fitness to their hybrid ancestor.

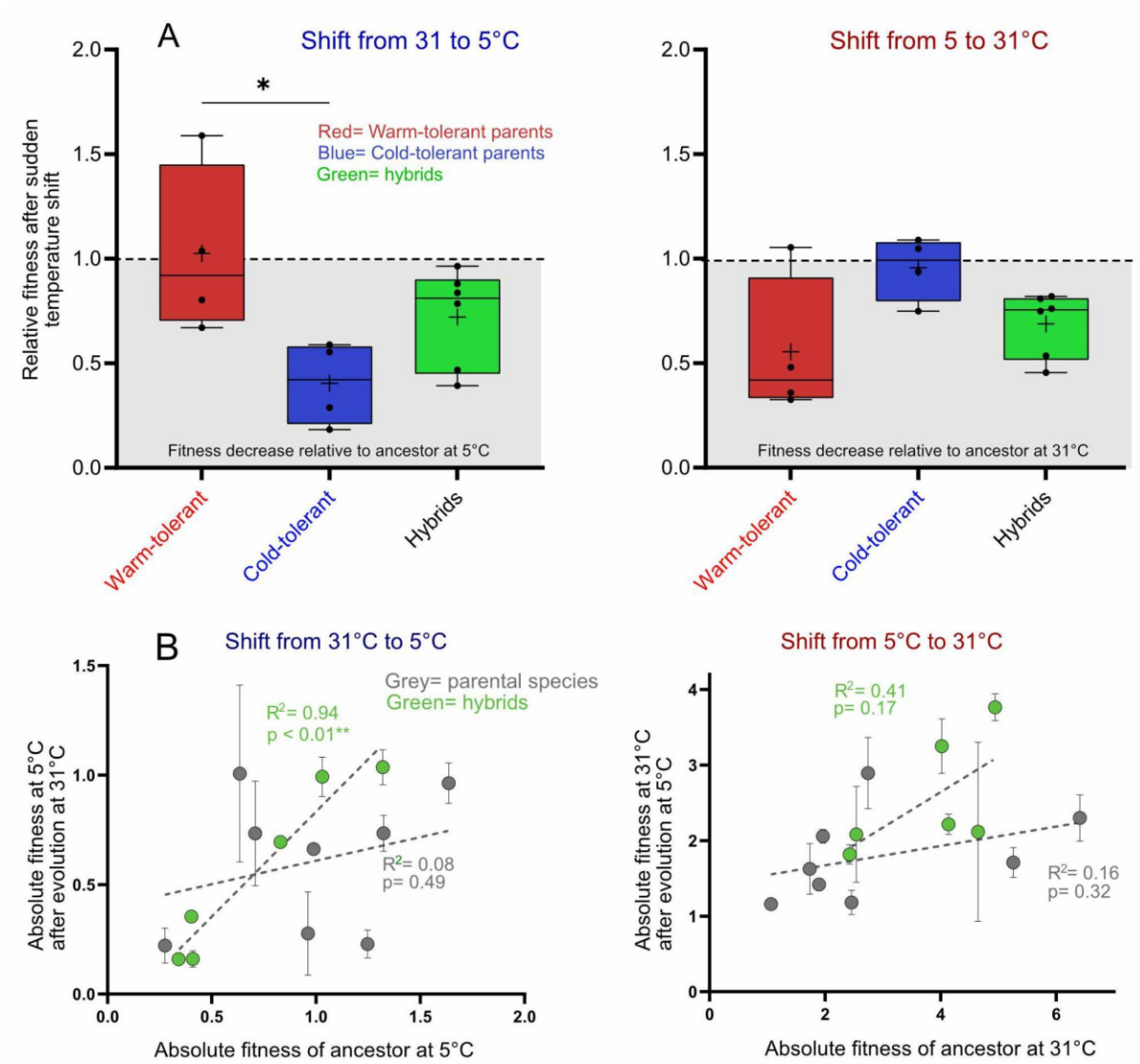


***Figure 5 A**) Left: Comparing the relative fitness of warm-tolerant species, cold-tolerant species, and their hybrids, after evolution in the warm environment (31±1°C), when suddenly exposed to the cold (5±1°C). Right: Comparing the relative fitness of these three groups after evolution in the cold environment (5±1°C), when suddenly exposed to the warm (31±1°C). Whiskers show the smallest and largest values, lines in the middle of the boxes represent the median and + represents the mean. Asterisk indicates significant difference in ANOVA. **B**) Linear regression of the absolute fitness of the evolved populations after sudden temperature shifts plotted against the fitness of their ancestors. Asterisks indicate significant correlation coefficient.*

Discussion

In times of global warming and extreme climate events (Perkins-Kirkpatrick & Gibson, 2017), the effect of outbreeding on thermal adaptation is of particular interest to evolutionary biologists, conservation managers, and agriculturalists alike. Genetic exchange in the form of gene flow, introgression and hybridization occurs in the majority of eukaryotic taxa and may become intensified through climate change and globalization (Chunco, 2014; McFarlane & Pemberton, 2019). Outbreeding across large genetic distances is a conservation concern because it can lead to offspring with poor fitness (Stelkens et al., 2015), maladaptation (Coyne & Orr, 2004), or produce more potent pathogens (King et al., 2015). But genetic exchange

with divergent populations or species can also be a 'saving grace', particularly for endangered, genetically eroded populations that face stressful environments and require fast rates of adaptation to avoid extinction (Abbott et al., 2013; Brauer et al., 2023). However, predicting the long-term fitness effects of hybridization remains a major challenge in evolutionary and conservation biology to date.

Here, we tracked the fitness dynamics and thermal adaptation of four *Saccharomyces* yeast species and their genetically recombined F2 hybrids across 200 generations in a cold (5±1°C) and a warm environment (31±1°C). We found that species significantly vary in their long-term evolutionary potential for thermal adaptation. Warm-tolerant species showed larger fitness improvements at cold temperature (Figure 2A, Figure 3) and cold-tolerant species showed larger fitness improvements at warm temperature (Figure 2B, Figure 3). When comparing the adaptive potential of parent species against that of their hybrids, we found that by far the largest relative fitness improvements occurred in hybrids, with some populations nearly quadrupling in fitness in the cold environment, especially in the first 100 generations of experimental evolution (Figure 2A). Notably, three of the six hybrids significantly exceeded both of their parents in their potential to adapt to growing in the cold.

Interestingly, some hybrids made from parent species with opposite thermal preferences showed no fitness gains in either warm or cold evolution conditions (Figure 4) . This is consistent with theory (Barton, 2001; Chevin et al., 2014; Rieseberg et al., 1999) and empiricism (Chhina et al., 2022; Stelkens & Seehausen, 2009) suggesting that F2 hybrids with parents with a history of divergent selection for ecologically important traits (like thermotolerance) often display intermediate (or 'mismatched') phenotypes, because there are fewer options for complementation or epistasis of parental alleles with opposite signs at quantitative trait loci (which can otherwise result in transgressive traits).

Our reciprocal transplant experiment revealed that sudden transitions from warm to cold temperatures have larger fitness costs than *vice versa*, especially for cold-tolerant species that had been forced to adapt to warm conditions (Figure 5A). Shifts from cold to warm only negatively affected warm-tolerant species and thermo-generalists, which had been forced to adapt to cold conditions (Figure 5A). These results suggest that beneficial mutations and molecular mechanisms leading to high-temperature adaptation may have deleterious fitness effects at cold temperatures (consistent with antagonistic pleiotropy). Alternatively, when evolving in warm environments, cold-tolerant species may accumulate loss-of-function mutations in genes normally needed for growth at cold temperatures, rendering them ill-adapted to their cold 'home' environment. The fact that the sudden shift from cold to warm left the two cold-tolerant species unaffected (*S. kudriavzevii*, and *S. uvarum*) suggests that the genes and mechanisms underlying cold adaptation do not have detrimental effects on fitness at high temperatures.

Interestingly, hybrids showed an overall greater resilience to sudden temperature changes than the parent species, especially from warm to cold conditions (Figure 5B). Even after 200 generations of evolution under opposite temperature conditions, hybrids still showed a similar performance as their ancestors: hybrids initially growing well at 5°C still grew well at 5°C, even after long-term evolution at 31°C, and hybrids initially growing well at 31°C still had high fitness at 31°, even after evolution at 5°C. Recombined hybrid genetic backgrounds may allow for better (or faster) compensation of sudden environmental shifts than parental genomes, for instance, due to the altered expression of parental genes (Runemark et al., 2024). For instance, gene expression analysis of ortholog genes in a domesticated hybrid yeast strain that is used for brewing lager beer (*S. pastorianus*) showed

that alleles inherited from the cold-tolerant *S. eubayanus* parent were significantly overrepresented compared to the warm-tolerant *S. cerevisiae* alleles at cold temperatures (Timouma et al., 2021). Alternatively, hybrid genomes may have had lower access to adaptive mutations over the course of experimental evolution, due to lower rates of loss of heterozygosity (LOH), which can result from gene conversion or mitotic crossover in yeast (Smukowski Heil, 2023). LOH can allow beneficial mutations, even when recessive or only partially dominant, to sweep to fixation quickly in homozygous parental genomes, but may be masked by heterozygosity in the hybrids, as recently demonstrated in yeast hybrids challenged to evolve in an environment containing a UV mimetic drug (Bautista et al., 2023). This is consistent with our finding that hybrids adapted more slowly (or to a lesser extent) than the parents to the warm environment (Figure 2B). Thus, upon temperature reversal and exposure to the cold, hybrids may be better equipped to still grow well in the cold 'ancestral' environment as their cold-adapted alleles were preserved.

Recent work on natural hybrids between climate generalists and species with more narrow climatic ranges has shown that hybrids have reduced vulnerability to environmental change due to the fitness benefits of adaptive introgression (Brauer et al., 2023). Our findings confirm that hybridization alters the fitness outcomes of long-term adaptation to extreme environments and may contribute to population resilience when challenged by sudden temperature changes. More experimental studies on hybrids with different genetic and ecological parental backgrounds are needed if we want to accurately predict the long-term adaptive outcomes of hybridization, which is equally important for the breeding of climate-resilient hybrid crops with improved (a)biotic stress resistance (Cooper & Messina, 2023; Ter Steeg et al., 2022) and the conservation of endangered, climate-sensitive populations.

Author contributions

J.P. conducted the experimental and analytical work and drafted the figures. J.P. and R.S. jointly wrote the manuscript.

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Conflict of interest statement

The authors declare no conflicts of interest.

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