2019

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**Recommended Citation**

Baker, EmilyClare P.; Peris, David; Moriarty, Ryan V.; Li, Xueying C.; Fay, Justin C.; and Hittinger, Chris T., "Mitochondrial DNA and temperature tolerance in lager yeasts." *Science Advances*. 5, 1. eaav1869. (2019).  
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Mitochondrial DNA and temperature tolerance in lager yeasts

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A growing body of research suggests that the mitochondrial genome (mtDNA) is important for temperature adaptation. In the yeast genus *Saccharomyces*, species have diverged in temperature tolerance, driving their use in high- or low-temperature fermentations. Here, we experimentally test the role of mtDNA in temperature tolerance in synthetic and industrial hybrids (*Saccharomyces cerevisiae* × *Saccharomyces eubayanus* or *Saccharomyces pastorianus*), which cold-brew lager beer. We find that the relative temperature tolerances of hybrids correspond to the parent donating mtDNA, allowing us to modulate lager strain temperature preferences. The strong influence of mitotype on the temperature tolerance of otherwise identical hybrid strains provides support for the mitochondrial climatic adaptation hypothesis in yeasts and demonstrates how mitotype has influenced the world’s most commonly fermented beverage.

**INTRODUCTION**

Temperature tolerance is a critical component of how species adapt to their environment. The mitochondrial climatic adaptation hypothesis (1) posits that functional variation between mitochondrial DNA (mtDNA) sequences (mitotypes) plays an important role in shaping the genetic adaptation of populations to the temperatures of their environments. Clines of mitotypes along temperature gradients and associations between mitotype and climate have been observed for numerous metazoan species, including humans (1, 2). Experiments in invertebrates have demonstrated directly that different mitotypes can alter temperature tolerance (3, 4) and that mitotype has been associated with adaption to temperature in natural environments (1, 5).

Recent work has suggested that mitotype can also play a role in temperature tolerance in the model budding yeast genus *Saccharomyces* (6–8). The eight known *Saccharomyces* species are broadly divided between cryotolerant and thermotolerant species (9–11). Thermotolerant strains (maximum growth temperature ≥ 36°C) form a clade that includes the model organism *Saccharomyces cerevisiae* (12), while the rest of the genus is more cryotolerant. Most previous research has focused on thermotolerance or the function of mitochondria under heat stress (~37°C), on mitotype differences within *S. cerevisiae* (6, 8), or on interspecies differences between *S. cerevisiae* and its moderately thermotolerant sister species, *Saccharomyces paradoxus* (13). The genetic basis of cryotolerance in *Saccharomyces* has been difficult to determine using conventional crosses focused on the nuclear genome (14–16). Nonetheless, given how common mitochondrial adaption to cold conditions is among arctic metazoan species (17–19), mitotype could conceivably influence cryotolerance in *Saccharomyces*.

In a companion study, Li et al. (20) found that the parent providing mtDNA in hybrids of *S. cerevisiae* and the cryotolerant species *Saccharomyces uvarum* had a large effect on temperature tolerance. Since *Saccharomyces eubayanus* is the sister species of *S. uvarum* but ~7% genetically divergent, we wondered whether the effect of mitotype would extend to industrial hybrids of *S. cerevisiae* × *S. eubayanus*, sometimes called *Saccharomyces pastorianus* or *Saccharomyces carlsbergensis* (21). While *S. cerevisiae* is well known for its role in human-associated fermentations, it is generally not used to produce lager-style beers, which are often brewed at colder temperatures than *S. cerevisiae* can tolerate. Instead, the world’s most commonly fermented beverage is brewed using cryotolerant *S. cerevisiae* × *S. eubayanus* hybrids (21) that inherited their mtDNA from *S. eubayanus* (22, 23). The recent discovery of nonhybrid strains of *S. eubayanus* (21) has sparked substantial interest in understanding the genetics of brewing-related traits to understand how lager strains were domesticated historically and to develop novel lager-brewing strains (24–28).

**RESULTS AND DISCUSSION**

**Temperature tolerance of *S. cerevisiae* and *S. eubayanus***

To establish the temperature tolerance of *S. cerevisiae* and *S. eubayanus*, we calculated relative growth scores at temperatures ranging from 4°C to 37°C. Two strains of *S. cerevisiae* [a laboratory strain (Sc) and a strain used to brew ale-style beers (ScAle)] and two strains of *S. eubayanus* [a derivative of the taxonomic type strain (Se) from Patagonia (21) and a strain isolated from North Carolina (SeNC) that is closely related to the ancestor of lager yeasts (29)] were tested (see table S1A for a complete list of strains and genotypes). Strains were spotted onto plates containing either glucose (a fermentable carbon source) or glycerol (a nonfermentable carbon source that requires respiration to assimilate) and were grown for several days (high temperatures) or up to 2 months (low temperatures).

*S. eubayanus* and *S. cerevisiae* had reciprocal temperature responses. *S. eubayanus* strains grew at all temperatures, except 37°C, while *S. cerevisiae* strains began to decline in relative growth at 15°C and were completely unable to grow at 4°C (Fig. 1, A and B, and figs. S1 to S4). Strain-specific differences were also apparent. The Sc and the...
Fig. 1. Relative growth of *S. cerevisiae* and *S. eubayanus* strains. Relative growth scores of *S. cerevisiae* and *S. eubayanus* strains carrying their native mtDNA from 4° to 37°C combined from all tests on (A) glucose and (B) glycerol. Strains are Sc, ScAle, Se, and SeNC. Error bars represent SEs. Parents were not tested for significant differences.

Fig. 2. Mitotype affects temperature tolerance in synthetic lager hybrids. (A) Outline of the procedure to control the mitotype of synthetic *S. cerevisiae × S. eubayanus* hybrids. Yeast cells represent nuclear genomes, and inner circles represent mtDNA. Red and blue indicate genetic material of *S. cerevisiae* origin and of *S. eubayanus* origin, respectively, and purple indicates hybrid nuclear material. (B) On glucose and (C) glycerol, relative growth scores of *S. cerevisiae × S. eubayanus* synthetic hybrids with alternate mitotypes from 4° to 37°C combined across all experiments (tiny circles and triangles). Each hybrid of each mitotype is represented in the above graphs. Mean data for synthetic hybrids carrying *S. eubayanus* mtDNA are represented by large blue circles, and mean data for synthetic hybrids with *S. cerevisiae* mtDNA are represented by large red triangles. Parent strains are Sc, ScAle, Se, and SeNC. Synthetic hybrids are as follows: Sc × Se, ScAle × Se, Sc × SeNC, SeAle × SeNC, ScAle × ScAle, and Sc × SeNC. Hybrids carrying *S. cerevisiae* mtDNA, for which only single biological replicates of crosses were available, are represented by open tiny triangles. Statistically significant differences (*P* ≤ 0.05) in relative growth between mitotypes are indicated by an asterisk.
SeNC strains grew relatively weakly compared to conspecific strains. For Sc, relatively poor growth was likely driven by multiple auxotrophies and differences in growth rates between diploid and haploid yeast strains. The reason for SeNC’s poor performance is unknown.

**Influence of mitotype in synthetic lager hybrids**

To directly test the role of mtDNA in temperature tolerance, we constructed a panel of synthetic hybrids of *S. cerevisiae* × *S. eubayanus*, controlling the source of mtDNA using crosses between ρ⁰ strains lacking mtDNA and ρ⁺ strains retaining their native mtDNA (Fig. 2A). The generation of ρ⁰ strains for crosses requires treating parent strains with ethidium bromide, a known mutagen. To control for possible variation in growth as a result of spurious nuclear mutations, we generated ρ⁰ strains of each parent in triplicate and used each independently generated ρ⁰ strain to make synthetic hybrids. We further verified, by analysis of variance (ANOVA) of ρ⁰ relative growth scores, that variation between ρ⁰ replicates across temperatures was limited and did not affect conclusions (fig. S5 and Materials and Methods).

Synthetic hybrids tolerated an increased range of temperatures compared to their parents, regardless of mitotype (Fig. 2, B and C, and figs. S1 to S4). These results support a strong role for the nuclear genome in temperature tolerance and indicate some level of codominance between alleles supporting thermotolerance and cryotolerance. Despite generally robust growth across temperatures, synthetic hybrids with different mitotypes displayed clear and consistent differences in relative growth. At higher temperatures, *S. cerevisiae* mitotypes permitted increased growth relative to *S. eubayanus* mitotypes, while the same was true for *S. eubayanus* mitotypes at lower temperatures. Relative growth was typically high for both mitotypes on glucose, but we detected statistically significant differences at five of six temperatures when data were considered in aggregate (Fig. 2B). On glycerol, the impact of mitotype was exaggerated (Fig. 2C), and the differences in growth were significant at all temperatures. We also observed subtle background-specific effects, including a growth defect at 37°C for the ScAle × SeNC hybrid carrying ScAle mtDNA (fig. S1). Arrhenius plots approximated using the relative growth data displayed the same overall trends (fig. S6).

Because we encountered challenges forming hybrids with a *S. cerevisiae* × SeNC nuclear background and an *S. cerevisiae* mitotype, hybrids of Sc × SeNC with Sc mtDNA and of ScAle × SeNC with ScAle mtDNA were both represented by single biological replicates. The behavior of these strains suggests that incompatibilities related to mitochondrial function may exist in these hybrids. To confirm that our results were not being driven by the unusual behavior of these hybrids, we excluded these data and again compared the growth of synthetic hybrids with *S. cerevisiae* and *S. eubayanus* mtDNA (fig. S7). Analyses on this restricted dataset had slightly less power, but they still suggested that the *S. cerevisiae* mtDNA conferred vigorous growth at colder temperatures, while the *S. cerevisiae* mtDNA conferred vigorous growth at warmer temperatures.

The challenges obtaining *S. cerevisiae* × SeNC hybrids with *S. cerevisiae* mtDNA suggest that dominant cytonuclear incompatibilities may exist between some strains of *S. cerevisiae* and *S. eubayanus* (see Materials and Methods). Recessive cytonuclear incompatibilities are common both within and between *Saccharomyces* species (7, 8), but dominant cytonuclear incompatibilities affecting hybrids could explain why *Saccharomyces* interspecies hybrids tend to lose more nuclear genetic material from the parental genome that did not contribute mtDNA (30, 31). Another group recently described a separate strain-specific incompatibility between *S. cerevisiae* and *S. eubayanus* (28), and the companion manuscript of Li et al. (20) also describes potential dominant interactions between hybrid genomes and mtDNA in crosses between *S. cerevisiae* and *S. uvarum*. More research is needed to better characterize this class of cytonuclear incompatibilities.

**Influence of mitotype in industrial lager cybrids**

To test whether mtDNA still plays a role in temperature tolerance in industrial lager-brewing hybrids that have been evolving under lagering conditions for many generations, we replaced the native lager mtDNA of *S. eubayanus* origin (23) with *S. cerevisiae* mtDNA from Sc and ScAle, creating lager cybrids (Fig. 3A). Consistent with results for synthetic hybrids, lager cybrids carrying *S. cerevisiae* mtDNA had greater growth at higher temperatures and decreased growth at colder temperatures, especially on glycerol (Fig. 3, B and C, and fig. S8). On glucose, strain-specific differences between lager cybrids were particularly apparent. At 30°C and below, lager cybrids carrying ScAle mtDNA grew significantly less than the parental lager strain with its native mtDNA (from the lager *S. eubayanus* parent; Fig. 3B and fig. S8, A and B), while there was no difference in growth between the parental lager strain and the cybrids carrying Sc mtDNA, except at temperature extremes (4° and 33.5°C; Fig. 3B and fig. S8, A and B). On glycerol, both lager cybrids grew significantly less than the industrial strain at 15°C and below, while they grew significantly more at 22° and 30°C (Fig. 3C and fig. S8, A and C), displaying a shift from lager-brewing toward ale-brewing temperatures. Approximate Arrhenius growth plots revealed similar trends (fig. S9). These results show that the strong effect of mtDNA on temperature tolerance seen in synthetic hybrids extends to industrial lager strains under at least some conditions.

**Origin of the mitotype of industrial lager yeasts**

Compared with ale strains or new hybrids carrying *S. cerevisiae* mtDNA, the increased cold tolerance conferred on new interspecies hybrids carrying *S. eubayanus* mtDNA would have provided an immediate selective advantage at the lower temperatures at which lagers are brewed. It is likely that additional changes occurred that affected temperature tolerance during adaptation to lagering conditions, much of which is likely attributable to changes within the nuclear genome. Even so, our data suggest that mitotype had a disproportionate impact on temperature tolerance, considering the limited number of genes encoded by mtDNA. Along with previous research suggesting that hybrid lager yeasts acquired most of their aggressive fermentation traits from *S. cerevisiae* (25, 27, 28), our results suggest that they acquired their cold tolerance from *S. eubayanus* in large part by retaining *S. eubayanus* mtDNA. Our results and methods provide a road map for constructing designer lager strains where temperature tolerance can be controlled. Shifting the temperature preference of synthetic or industrial lager strains to warmer fermentation temperatures could substantially reduce the cost of lager brewing by reducing production time and infrastructure requirements. The strain-specific differences observed further suggest that the *S. cerevisiae* parent, the *S. eubayanus* parent, and any cytonuclear incompatibilities (32) should all be considered during strain construction. Along with the companion study of Li et al. (20), the identification of a role for mtDNA in temperature tolerance of these yeasts extends support for the mitochondrial climatic adaptation hypothesis (1) to fungi and suggests that the outsized role of mtDNA in controlling temperature tolerance may be general to eukaryotes.

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Fig. 3. *S. cerevisiae* mtDNA increases the thermotolerance and decreases the cryotolerance of an industrial lager strain. (A) Outline of crosses and strain engineering to produce lager cybrids. Yeast cells, large inner circles, and small green inner circles represent the nuclear genome, the mtDNA, and the HyPr plasmid (S0), respectively. Lowercase “a” and “α” indicate mating types. Karyogamy-deficient (*kar1-1*) strains can be of either mating type and are mated to the opposite mating type. Black, red, purple, and blue indicate genetic material from the *S. cerevisiae kar1-1* strain, from a *S. cerevisiae* parent, a hybrid (i.e., lager) nuclear genome, and mtDNA of *S. eubayanus* origin, respectively. (B) On glucose and (C) glycerol, growth of a lager strain with native mtDNA (inherited from *S. eubayanus* lager parent) and lager cybrids with *S. cerevisiae* mtDNA. Error bars represent SEs, and asterisks indicate statistically significant differences in growth between the cybrid and the lager with native mtDNA (*P* ≤ 0.05).
**MATERIALS AND METHODS**

**Yeast strains and strain construction**

Not all strains within a species are equally thermotolerant or cryotolerant, and different strains of *S. cerevisiae* can differ by 4°C or more between their optimum growth temperatures (33). Since mitotype has been found to be important, at least at temperature extremes (6–8, 13), when determining thermotolerance in different strains of *S. cerevisiae*, we decided to include strains from different populations in our study. In addition to a laboratory strain of *S. cerevisiae* and a monosporic derivative of the taxonomic type strain of *S. eubayanus*, an ale-brewing strain of *S. cerevisiae* and a strain of *S. eubayanus* isolated from North Carolina were also included (21, 29, 34). These two additional strains were chosen because the parents of industrial lager-brewing yeast hybrids included an ale-brewing strain of *S. cerevisiae* and a member of the Holartic lineage of *S. eubayanus*, which includes isolates from North Carolina and Tibet, but not Patagonia (29).

Specifically, FM1283 (Sc) is descended from BY4724, which is itself a derivative of S288C (34, 35). WLP300 (ScAle) is a commercial ale strain; its pure *S. cerevisiae* background was confirmed by whole-genome sequencing and assembly of reads to a concatenated pan-*Saccharomyces* reference genome using the program sppIDer (36). FM1318 (Sc) is a monosporic derivative of the taxonomic type strain of *S. eubayanus*, CBS 12357T (21). The strain yHRVM108 (SeNC) was isolated from North Carolina and identified as being a close relative of the *S. eubayanus* parent of lager-brewing yeast hybrids (29). W34/70 (Weihenstephan 34/70 or yHAB47) is an industrial strain belonging to the Frohberg lineage of lager-brewing yeast hybrids (29). All strains used in this study are listed in table S1A.

To facilitate strain crossing, stable haploid ScAle, Sc, and SeNC strains were generated by replacing one allele of the *HO* locus with a selectable marker by standard lithium acetate transformation (37, 38). The following modifications were made for transforming *S. eubayanus*: (i) *S. eubayanus* transformation reactions were heat-shocked at 34°C, rather than 42°C typical for *S. cerevisiae*; (ii) after 55 min of heat shock, 100% ethanol was added to 10% total volume; and (iii) the reaction was heat-shocked for another 5 min before recovery and selection for transformants. Successful replacement of the *HO* locus was confirmed by polymerase chain reaction with primers specific to the *HO* locus (table S1B). The resultant strains were sporulated, and individual tetrads were dissected using a Singer SporePlay. ScAle was sporulated in liquid sporulation medium (1% potassium acetate and 0.005% zinc acetate) and grown at room temperature (~22°C) before dissecting spores after 4 to 5 days. To sporulate Se and SeNC, 200 µl of saturated culture was plated onto a YPD (1% yeast extract, 2% peptone, and 2% glucose) plate and grown at room temperature for 3 to 5 days before dissecting tetrads. Strains lacking the *HO* coding sequence were selected for by growth on YPD and antibiotic, and the mating type was determined by mating with tester strains.

**Synthetic hybrids**

To test the effect of mitotype on temperature tolerance in *S. cerevisiae × S. eubayanus* hybrids, we made sets of hybrids containing mtDNA from one parent or the other. When two *ρ*+ yeast cells mate, the mtDNAs of both parents are present in the zygote, but a single mtDNA haplotype is rapidly fixed after only a few cell divisions (39). Which mtDNA haplotype is fixed is often nonrandom (30, 40, 41), and recombinant mtDNAs are also possible, even common (6, 39). To control the inheritance of mtDNA in synthetic hybrids, we generated *ρ*0 (mtDNA completely absent) strains to mate with *ρ*+ strains, so that mtDNA from only the *ρ*+ parent would be present in hybrids (Fig. 2A). *ρ*0 strains were generated by treating *ρ*+ parent strains with ethidium bromide (42). Respiratorily deficient strains were screened for by the absence of growth on glycerol, and the complete removal of mtDNA was confirmed by 4,6-diamidino-2-phenylindole staining (43). Because of the mutagenic nature of ethidium bromide, to control for the effect of any spurious nuclear mutations, we generated *ρ*0 strains of each parent strain in triplicate.

Hybrids were made by mating a *ρ*+ strain of one species with a *ρ*0 strain of the opposite mating type of the other species. Mating was performed by mixing the parent strains together on a YPD plate and letting them mate overnight. Allowing mating to occur for one or two more days and/or at 30°C sometimes improved mating efficiency. Hybrids were selected by growth on glycerol and resistance to the appropriate antibiotics. When appropriate drug selection markers were not present in the parental genomes, zygotes were picked manually and tested for growth on glycerol to confirm retention of functional mitochondria. The hybrid nature of all strains was confirmed by internal transcribed spacer sequencing (table S1B) (44, 45). To ensure the maintenance of functional mitochondria, hybrid strains were grown only on media with glycerol as the sole carbon source, except during experiments.

**Putative strain-specific dominant cytonuclear incompatibilities**

In general, the different *S. cerevisiae* and *S. eubayanus* backgrounds and mitotypes readily formed hybrids, although mtDNA could be lost if hybrids were not grown on nonfermentable media. The exception was for crosses attempted between SeNC *ρ*0 strains and Sc and ScAle *ρ*+ strains. Only a single SeNC *ρ*0 strain was able to successfully form a small number of respiratorily competent hybrids, although hybrids between SeNC *ρ*0 and Sc and ScAle *ρ*+ strains were attempted multiple times (>50 attempts total) with six independently generated SeNC *ρ*0 strains. Of these attempts, only four respiratorily competent hybrids were formed, one between yHEB1528 (ScAle *ρ*+) and yHBE1638 (SeNC *ρ*0) and three between yHWA117 (Sc *ρ*0) and yHBE1638 (SeNC *ρ*0). For other cross attempts, it is not known whether mtDNA was lost in hybrids or was retained but nonfunctional.

There was no similar difficulty producing the same hybrids with *S. eubayanus* mitochondrial genomes; for comparison, every attempt to hybridize either of the *S. cerevisiae* strains with Se *ρ*0 or *ρ*0 strains or the SeNC *ρ*− parent produced hybrids. It is not clear whether the ability to form respiratorily competent hybrids is unique to yHBE1638, as even hybrids with this strain took multiple attempts to achieve. Because the ethidium bromide used to generate *ρ*0 strains is broadly mutagenic, yHBE1638 may have one or more mutations differentiating it from the other SeNC *ρ*0 strains we generated. It is possible that one of these changes allowed yHBE1638 to maintain functional mtDNA in hybrids with *S. cerevisiae* carrying *S. cerevisiae* mtDNA, whereas other SeNC *ρ*0 strains could not. Thus, the simplest model is that a mutation inactivated a gene involved in a dominant cytonuclear incompatibility affecting interspecies hybrids, although that model remains to be tested.

Difficulty forming hybrids was not the only unusual characteristic of the *S. cerevisiae × SeNC* hybrids with *S. cerevisiae* mtDNA. While the Sc × SeNC *ρ*0 hybrid had high relative growth at 37°C, like other hybrids carrying *S. cerevisiae* mitochondria, relative growth for the ScAle × SeNC *ρ*ScAle hybrid plummeted at 37°C (figs. S1 and S2). Even with this severe temperature-related growth defect, the ScAle mtmitotype still supported greater growth at 37°C on glycerol than the SeNC mitotype (fig. S1). Because we were only able to form *S. cerevisiae* mtDNA carrying hybrids with one SeNC *ρ*0 strain, it is unclear whether this
temperature-dependent growth defect is specific to the yHEB1638 background or general to all ScAle × SeNC p
ScAle crosses. Even if the defect is specific to strain yHEB1638, it was only detrimental in the ScAle background, as the Sc × SeNC p
ScAle hybrid did not have the same sensitivity to 37°C, despite sharing the same SeNC p
ScAle parent. We included the results of growth assays with the hybrids made using yHEB1638, which follow the same general trends as other hybrid strains, with the caveat that the results from these experiments could not be verified by hybrids made from other independently generated SeNC p
ScAle strains (Fig. 1 and fig. S8).

We found that excluding SeNC hybrids with S. cerevisiae mtDNA, for which we only had single biological replicates, resulted in only a single temperature on glucose changing in significance between hybrids with S. cerevisiae versus S. eubayanus mtDNA (fig. S7A and B). When excluding all hybrids with SeNC as the S. eubayanus parent from analyses of growth on glucose (fig. S7C), there was no significant difference in growth between hybrids at 4°C and 10°C, but hybrids with S. cerevisiae mtDNA had greater relative growth at all other temperatures. On glycerol, when excluding SeNC hybrids, strains with S. eubayanus mtDNA again had a significant growth advantage between 4°C and 15°C, while strains with S. cerevisiae mtDNA had an advantage from 22°C to 37°C (fig. S7D), consistent with our other analyses (Fig. 2, B and C).

An interesting consideration is that interspecies incompatibilities might also have been a factor in the retention of S. eubayanus mtDNA in industrial lager yeasts. Of our synthetic hybrids, the ScAle × SeNC hybrids are the most genetically similar to the strains that gave rise to industrial lager yeasts. Similar to other hybrids, the S. eubayanus mitotype had a growth advantage over the S. cerevisiae mitotype at low temperatures. Although it could be a strain-specific defect, unlike other hybrids, the ScAle × SeNC hybrids with ScAle mtDNA had a severe growth defect at 37°C. Historically, wort was cooled after boiling in open troughs (46), exposing it to the microbes that would ferment the wort into beer. If a high-temperature growth defect is common to industrial lager hybrids. Similar to other hybrids, the hybrids are the most genetically similar to the strains that gave rise to industrial lager yeasts. If a high-temperature growth defect is common to industrial lager yeasts. Similar to other hybrids, the hybrids are the most genetically similar to the strains that gave rise to industrial lager yeasts. With some exceptions. Since there was only one SeNC p
ScAle strain with which we were able to successfully form hybrids containing S. cerevisiae mtDNA, only one biological replicate for each S. cerevisiae strain was formed with SeNC, which had three technical replicates at each temperature. Consequently, these hybrids (ScAle × SeNC p
ScAle and ScAle × SeNC p
ScAle) had three replicates total at each temperature. In addition, because of contamination or poor-quality photographs, a small number of replicates (n = 5) had to be discarded. These were as follows: two for ScAle × SeNC experiments on glycerol (one at 22°C and one at 37°C) and three for lager cybrid experiments at 4°C (one replicate growing on glucose and two growing on glycerol).

Yeast strains were grown in liquid synthetic complete (SC) medium (0.17% yeast nitrogen base, 0.5% ammonium sulfate, and 0.2% complete drop-out mix). Strains containing their native mtDNA and p
ScAle strains were grown with 2% glucose, while hybrids and cybrids were grown with 2% glycerol and 2% ethanol to force the maintenance of mtDNA. After reaching saturation, cells were washed in either water or deflocculation buffer (20 mM citrate and 5 mM EDTA) and resuspended in either SC (without carbon) or deflocculation buffer to an optical diameter at 600 nm (OD
600 of 1 ± 0.05). Deflocculation was necessary for the extremely flocculent strain ScAle, so for experiments involving this strain, all other strains were treated identically for consistency. Yeast strains were plated in a dilution series of OD
600 of 1.0, 10⁻¹, 10⁻², 10⁻³, and 10⁻⁴. Dilutions were plated onto SC plates containing either 2% glucose or 2% glycerol as the sole carbon source. Plates were grown at 4°C, 10°C, 15°C, 22°C, 30°C, and 37°C. Lager cybrids were also grown at 33.5°C. Plates were grown until at least one strain on a plate showed growth at all five dilutions or after they had been allowed to grow for more than 2 months, whichever came first.

Analysis of growth assays
To determine how well different strains grew relative to each other, the photographic intensities (a composite of relevant fitness components, including growth rates, survival, and stress responses) of the first and second dilutions (OD
600 of 1 and 10⁻¹) were measured using custom

Mitochondrial transfers
To produce strains with an industrial lager yeast nuclear background and S. cerevisiae mtDNA (cybrids), karyogamy-deficient (kar1-1) p
ScAle strains (47–49) were used to transfer mitochondria from a donor S. cerevisiae strain to a p
ScAle lager strain (Fig. 3A), which were constructed as described above. Briefly, the lack of karyogamy in crosses with kar1-1 mutants allows the mixing of cytoplasm between mated cells while preventing fusion between the nuclear genomes, ultimately leading to progeny with mixed cytoplasm but with only one nuclear background. In this manner, donor mitochondria from S. cerevisiae strains were transferred into the kar1-1 p
ScAle strains by mating yeast as above and by selecting for functional mtDNA (by growth on glycerol, a nonfermentable carbon source) and the kar1-1 background while selecting against the donor strain background (Fig. 3A). Since the S. cerevisiae–ale strain WLP530B (ScAle) and its derivatives are prototrophic and the kar1-1 strains (MCC109 and MCC123) are auxotrophic for ura3, we were able to select for the kar1-1 background and simultaneously select against the ScAle background by selecting for resistance to 5-fluoroorotic acid. To select for kar1-1 background strains carrying mtDNA from the S. cerevisiae–laboratory strain FM1283 (Sc), strains were grown on minimal media supplemented with adenine. Because the kar1-1 strains are ade2 auxotrophs and Sc is auxotrophic for lys2, this medium selected for the kar1-1 nuclear genetic background. The medium was also supplemented with uracil, for which both the kar1-1 strains and Sc are auxotrophic (ura3).

Because lager yeasts contain both MATa and MATa at their mating type locus, mating does not usually occur. To mate polyplloid lagers to the kar1-1 p
ScAle hybrids for mitochondria transfer, the MAT locus had to first be homozygosed or otherwise reduced to a single mating type. The MAT locus of lager p
ScAle strains was manipulated using a HyPr (Hybrid Production) plasmid (pHCT2, table S1A) to induce mating type switching (50). Cybrids, strains with a single nuclear background and mitochondria from a donor strain, were selected for by selecting against the kar1-1 nuclear genetic background. To confirm that only lager genetic material was present in the resulting cybrids, three loci throughout the lager genome were sequenced to confirm that they contained only lager alleles (table S1B) (51). As with hybrids, cybrids were also cultured on glycerol, except for during experiments, to ensure maintenance of mtDNA.

Growth assays
Each hybrid and cybrid was constructed three times with an independently generated p
ScAle parent. Each of these independent hybrids was tested three times at each temperature. In total, combining biological and technical replicates, each hybrid cross was tested a total of nine times at each temperature, with some exceptions. Since there was only one SeNC p
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CellProfiler pipelines (www.cellprofiler.org) (52), and the values were combined. To compare growth between plates, which may have differences in absolute intensities, growth on each plate was normalized by dividing by the strain with the highest combined intensity on each plate. This procedure created a relative growth score for each strain that was used to compare growth across different replicates.

ANOVA of \( p^0 \) strains

Of the S. eubayanus and S. cerevisiae \( p^0 \) strains, only a single replicate pair at a single temperature (S. eubayanus \( p^0 \) replicate A versus replicate B at 30°C) had a statistically significant difference in the values between replicates (fig S5). For larger \( p^0 \) replicates, there were more temperatures and pairwise replicate comparisons where variation was found to be significant, but the predicted interaction between temperature and mitotype was still readily apparent (fig S5). While some of this variation may be a result of mutations induced by ethidium bromide, the absence of a strong trend for any specific \( p^0 \) strain in the resulting cybrids or synthetic hybrids suggests that these differences more likely reflect experimental variation or the additional genome instability of the allopolyploid lager strains.

Arrhenius growth plots

The specific growth rate constant, \( k \), for Arrhenius growth plots was determined by the equation

\[
\frac{dN}{dt} = kN
\]

where \( N \) is the concentration of cells and \( t \) is time (53). We approximated the starting concentration of cells as a value near 0 and the final concentration as the normalized relative growth score for each strain. Since the Arrhenius equation requires a nonzero starting value, we approximated concentration as the normalized relative growth score for each strain. Since where

\[
t \quad \text{for Arrhenius growth plots was}
\]

Statistical analysis

For growth assays, statistically significant differences in relative growth were assessed using the Wilcoxon rank sum test, as implemented in R version 3.4.3 (54); we corrected for multiple tests using the Benjamini-Hochberg procedure (55). To compare variance between biological replicates of \( p^0 \) strains, we used ANOVA of replicate data and performed pairwise comparisons using Tukey’s “honest significant difference” method as implemented in R version 3.4.3 (54). Multiple test–corrected \( P \leq 0.05 \) were considered significant.

REFERENCES AND NOTES


Acknowledgments: We thank T. D. Fox, D. Libkind, and J. P. Sampaio for sharing yeast strains used in this study. *Funding:* This work was supported by the USDA National Institute of Food and Agriculture ( Hatch project no. 1003258), the NSF (grant no. DEB-1253634), and in part by the DOE Great Lakes Bioenergy Research Center (DOE BER Office of Science; nos. DE-SC0018409 and DE-FC02-07ER64404). E.P.B. was supported by a Louis and Elsa Thomsen Wisconsin Distinguished Graduate Fellowship. C.T.H. is a Pew Scholar in the Biomedical Sciences and a Vilas Faculty Early Career Investigator, supported by the Pew Charitable Trusts and the Vilas Trust Estate. D.P. is a Marie Skłodowska-Curie fellow of the European Union’s Horizon 2020 research and innovation programme (grant agreement no. 747775). J.C.F. was supported by the NIH (no. GM080669). *Author contributions:* E.P.B., D.P., X.C.L., J.C.F., and C.T.H. conceptualized the study. E.P.B., D.P., and C.T.H. designed experiments. E.P.B. and R.V.M. conducted strains. E.P.B. conducted experiments and analyzed data. D.P. and C.T.H. supervised R.V.M. C.T.H. supervised the project. E.P.B. and C.T.H. wrote the manuscript with input and approval from all authors. *Competing interests:* E.P.B., D.P., and C.T.H. are inventors on a provisional patent application related to this work filed by the Wisconsin Alumni Research Foundation (no. 62/715498; filed 7 August 2018). D.P. and C.T.H. are inventors on a pending patent related to this work filed by the Wisconsin Alumni Research Foundation (no. 15/805950; filed 7 November 2017). All other authors declare that they have no competing interests. *Data and materials availability:* All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. The strains and constructs can be provided by C.T.H., pending a completed material transfer agreement. Requests for the strains and constructs should be submitted to the corresponding author. Additional data related to this paper may be requested from the authors.

Submitted 22 August 2018
Accepted 3 January 2019
Published 30 January 2019
10.1126/sciadv.aav1869

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DOI: 10.1126/sciadv.aav1869

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