GENETICS

Mitochondrial DNA and temperature tolerance in lager yeasts

EmilyClare P. Baker[1,2], David Peris[1,3,4], Ryan V. Moriarty[1,3], Xueying C. Li[5,6,7,8], Justin C. Fay[5,6,7,8], Chris Todd Hittinger[1,2,3]*

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 climactic 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° 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

[1]Laboratory of Genetics, Genome Center of Wisconsin, Wisconsin Energy Institute, J. F. Crow Institute for the Study of Evolution, University of Wisconsin–Madison, Madison, WI, USA. [2]Microbiology Doctoral Training Program, University of Wisconsin–Madison, Madison, WI, USA. [3]DOE Great Lakes Bioenergy Research Center, University of Wisconsin–Madison, Madison, WI, USA. [4]Department of Food Biotechnology, Institute of Agrochemistry and Food Technology (IATA), CSIC, Paterna, Valencia, Spain. [5]Molecular Genetics and Genomics Program, Washington University, St. Louis, MO, USA. [6]Department of Genetics, Washington University, St. Louis, MO, USA. [7]Center for Genome Sciences and System Biology, Washington University, St. Louis, MO, USA. [8]Department of Biology, University of Rochester, Rochester, NY, USA.
*Corresponding author. Email: cthittinger@wisc.edu

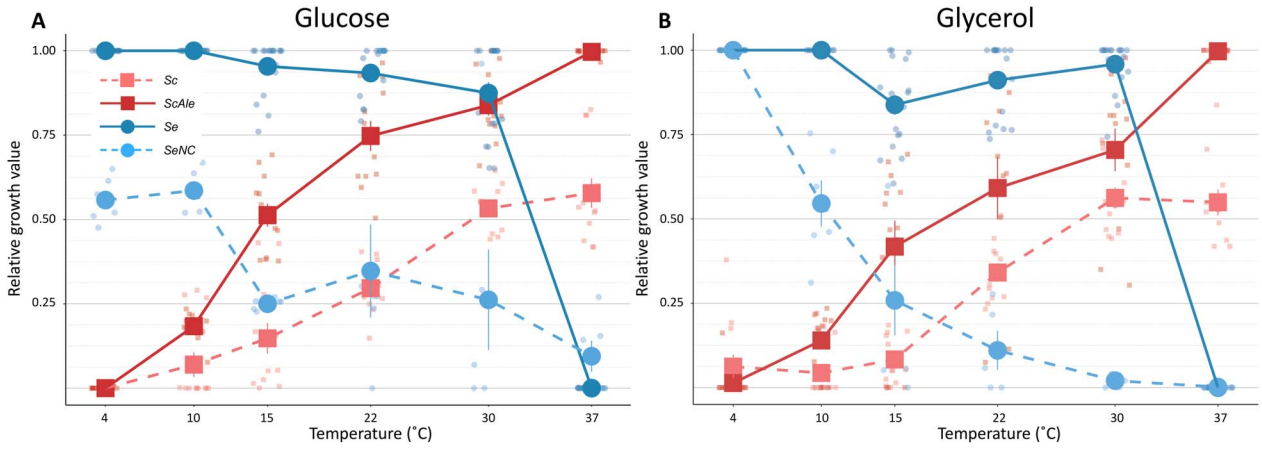

Fig. 1. Relative growth of *S. cerevisiae* and *S. eubayanus* strains. Relative growth scores of *S. cerevisiae* and *S. eubayanus* strains carrying their native mtDNA from 4° to 37°C combined from all tests on (**A**) glucose and (**B**) glycerol. Strains are *Sc*, *ScAle*, *Se*, and *SeNC*. Error bars represent SEs. Parents were not tested for significant differences.

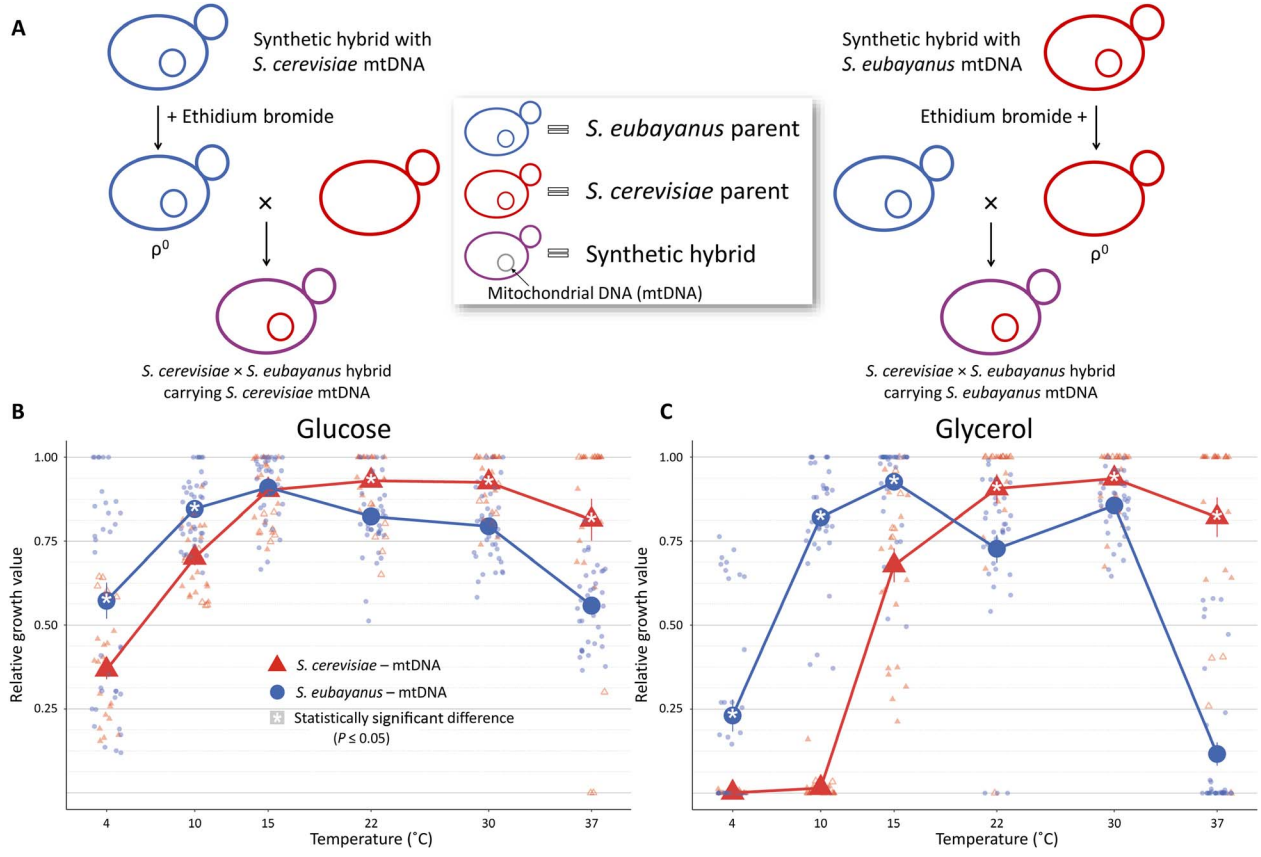

Fig. 2. Mitotype affects temperature tolerance in synthetic lager hybrids. (**A**) Outline of the procedure to control the mitotype of synthetic *S. cerevisiae* × *S. eubayanus* hybrids. Yeast cells represent nuclear genomes, and inner circles represent mtDNA. Red and blue indicate genetic material of *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* × *SeNC*, 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 ($P \leq 0.05$) 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 ρ^0 strains lacking mtDNA and ρ^+ strains retaining their native mtDNA (Fig. 2A). The generation of ρ^0 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 ρ^0 strains of each parent in triplicate and used each independently generated ρ^0 strain to make synthetic hybrids. We further verified, by analysis of variance (ANOVA) of ρ^0 relative growth scores, that variation between ρ^0 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. eubayanus* 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 adaption to lagering conditions, much of which are 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.

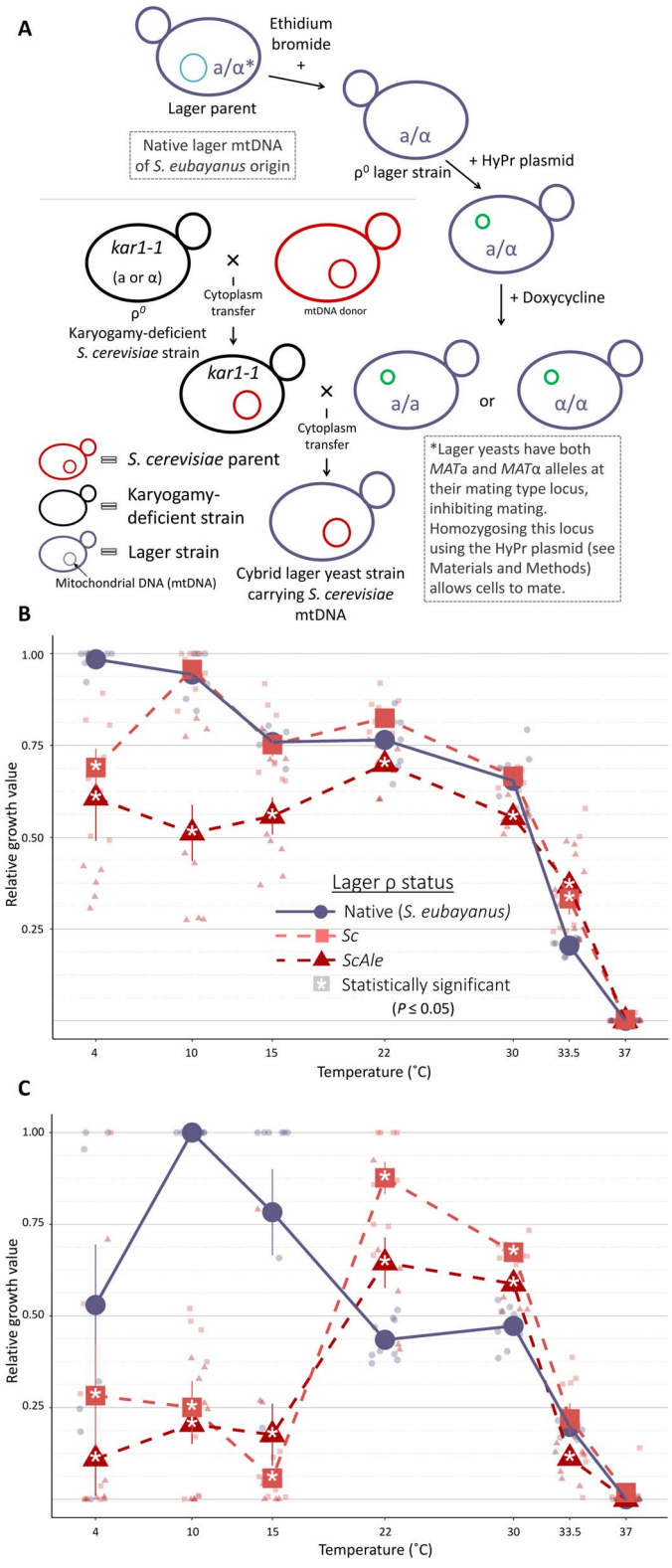

Fig. 3. *S. cerevisiae* mtDNA increases the thermotolerance and decreases the cryotolerance of an industrial lager strain. (**A**) Outline of crosses and strain engineering to produce lager cybrids. Yeast cells, large inner circles, and small green inner circles represent the nuclear genome, the mtDNA, and the HyPr plasmid (*50*), 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 ($P \leq 0.05$).

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 Holarctic 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*). WLP530B (*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 (*Se*) is a monosporic derivative of the taxonomic type strain of *S. eubayanus*, CBS 12357^T (*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*, *Se*, 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^0 strain of one species with a ρ^+ 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 yHEB1638 (*SeNC* ρ^0) and three between yHWA117 (*Sc* ρ^+) and yHEB1638 (*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^+ 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 yHEB1638, as even hybrids with this strain took multiple attempts to achieve. Because the ethidium bromide used to generate ρ^0 strains is broadly mutagenic, yHEB1638 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 yHEB1638 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^{Sc} 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* mitotype 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* ρ^{ScAle} crosses. Even if the defect is specific to strain yHEB1638, it was only detrimental in the *ScAle* background, as the *Sc* × *SeNC* ρ^{Sc} hybrid did not have the same sensitivity to 37°C, despite sharing the same *SeNC* ρ^0 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* ρ^0 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. S7, A 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° 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° and 15°C, while strains with *S. cerevisiae* mtDNA had an advantage from 22° 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 hybrids. 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 *ScAle* × *S. eubayanus* Holarctic strains with *ScAle* mtDNA, then hybrids that inherited *S. eubayanus* mtDNA could have had another important advantage beyond superior growth at lower temperatures by being able to colonize hot wort earlier than hybrids with *ScAle* mtDNA.

Mitochondrial transfers

To produce strains with an industrial lager yeast nuclear background and *S. cerevisiae* mtDNA (cybrids), karyogamy-deficient (*kar1-1*) ρ^0 strains (*47*–*49*) were used to transfer mitochondria from a donor *S. cerevisiae* strain to a ρ^0 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* ρ^0 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 *MAT*a and *MAT*α at their mating type locus, mating does not usually occur. To mate polyploid lagers to the *kar1-1* ρ^+ strains for mitochondria transfer, the *MAT* locus had to first be homozygosed or otherwise reduced to a single mating type. The *MAT* locus of lager ρ^0 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 ρ^0 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* ρ^0 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 each had three technical replicates at each temperature. Consequently, these hybrids (*Sc* × *SeNC* ρ^{ScAle} and *ScAle* × *SeNC* ρ^{ScAle}) only 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 *Sc* × *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 ρ^0 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 defloculation buffer (20 mM citrate and 5 mM EDTA) and resuspended in either SC (without carbon) or defloculation buffer to an optical diameter at 600 nm (OD_{600}) of 1 ± 0.05. Defloculation 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°, 10°, 15°, 22°, 30°, 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

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 ρ^0 strains

Of the *S. eubayanus* and *S. cerevisiae* ρ^0 strains, only a single replicate pair at a single temperature (*S. eubayanus* ρ^0 replicate A versus replicate B at 30°C) had a statistically significant difference in the values between replicates (fig. S5). For lager ρ^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 ρ^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 0 as 10^{-15}. For t, we used the average time for each growth assay at each temperature for each carbon source. We confirmed that other approximations of 0 (10^{-10} and 10^{-5}) did not change our interpretation of results.

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 ρ^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.

SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/5/1/eaav1869/DC1

Fig. S1. Growth assay for *ScAle* × *SeNC* hybrids and parental strains.
Fig. S2. Growth assay for *Sc* × *SeNC* hybrids and parental strains.
Fig. S3. Growth assay for *Sc* × *Se* hybrids and parental strains.
Fig. S4. Growth assay for *ScAle* × *Se* strain hybrids and parental strains.
Fig. S5. Relative growth scores and box plots of ρ^0 biological replicates.
Fig. S6. Approximation of Arrhenius growth plots for synthetic hybrid growth assays.
Fig. S7. Analysis of synthetic hybrid relative growth scores using restricted dataset.
Fig. S8. Growth assay for lager cybrids and parental strains.
Fig. S9. Approximation of Arrhenius growth plots for lager cybrid growth assays.
Table S1. Strains, plasmid, and oligonucleotides used in this work.

REFERENCES AND NOTES

1. M. F. Camus, J. N. Wolff, C. M. Sgrò, D. K. Dowling, Experimental support that natural selection has shaped the latitudinal distribution of mitochondrial haplotypes in Australian *Drosophila melanogaster*. *Mol. Biol. Evol.* **34**, 2600–2612 (2017).
2. D. Mishmar, E. Ruiz-Pesini, P. Golik, V. Macaulay, A. G. Clark, S. Hosseini, M. Brandon, K. Easley, E. Chen, M. D. Brown, R. I. Sukernik, A. Olckers, D. C. Wallace, Natural selection shaped regional mtDNA variation in humans. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 171–176 (2003).
3. N. Pichaud, J. W. O. Ballard, R. M. Tanguay, P. U. Blier, Mitochondrial haplotype divergences affect specific temperature sensitivity of mitochondrial respiration. *J. Bioenerg. Biomembr.* **45**, 25–35 (2013).
4. C. S. Willett, The nature of interactions that contribute to postzygotic reproductive isolation in hybrid copepods. *Genetica* **139**, 575–588 (2011).
5. S. D. Dingley, E. Polyak, J. Ostrovsky, S. Srinivasan, I. Lee, A. B. Rosenfeld, M. Tsukikawa, R. Xiao, M. A. Selak, J. J. Coon, A. S. Hebert, P. A. Grimsrud, Y. Joon Kwon, D. J. Pagliarini, X. Gai, T. G. Schurr, M. Hüttemann, E. Nakamaru-Ogiso, M. J. Falk, Mitochondrial DNA variant in *COX1* subunit significantly alters energy metabolism of geographically divergent wild isolates in *Caenorhabditis elegans*. *J. Mol. Biol.* **426**, 2199–2216 (2014).
6. J. F. Wolters, G. Charron, A. Gaspary, C. R. Landry, A. C. Fiumera, H. L. Fiumera, Mitochondrial recombination reveals mito-mito epistasis in yeast. *Genetics* **209**, 307–319 (2018).
7. M. Špírek, S. Poláková, K. Jatzová, P. Sulo, Post-zygotic sterility and cytonuclear compatibility limits in *S. cerevisiae* xenomitochondrial cybrids. *Front. Genet.* **5**, 454 (2014).
8. S. Paliwal, A. C. Fiumera, H. L. Fiumera, Mitochondrial-nuclear epistasis contributes to phenotypic variation and coadaptation in natural isolates of *Saccharomyces cerevisiae*. *Genetics* **198**, 1251–1265 (2014).
9. S. Naseeb, S. A. James, H. Alsammar, C. J. Michaels, B. Gini, C. Nueno-Palop, C. J. Bond, H. McGhie, I. N. Roberts, D. Delneri, *Saccharomyces jurei* sp. nov., isolation and genetic identification of a novel yeast species from *Quercus robur*. *Int. J. Syst. Evol. Microbiol.* **67**, 2046–2052 (2017).
10. G. Liti, D. B. H. Barton, E. J. Louis, Sequence diversity, reproductive isolation and species concepts in *Saccharomyces*. *Genetics* **174**, 839–850 (2006).
11. C. T. Hittinger, *Saccharomyces* diversity and evolution: A budding model genus. *Trends Genet.* **29**, 309–317 (2013).
12. P. Gonçalves, E. Valério, C. Correia, J. M. G. C. F. de Almeida, J. P. Sampaio, Evidence for divergent evolution of growth temperature preference in sympatric *Saccharomyces* species. *PLOS ONE* **6**, e20739 (2011).
13. J.-B. Leducq, M. Henault, G. Charron, L. Nielly-Thibault, Y. Terrat, H. L. Fiumera, B. J. Shapiro, C. R. Landry, Mitochondrial recombination and introgression during speciation by hybridization. *Mol. Biol. Evol.* **34**, 1947–1959 (2017).
14. H. Yamagishi, S. Ohnuki, S. Nogami, T. Ogata, Y. Ohya, Role of bottom-fermenting brewer's yeast *KEX2* in high temperature resistance and poor proliferation at low temperatures. *J. Gen. Appl. Microbiol.* **56**, 297–312 (2010).
15. C. M. Paget, J.-M. Schwartz, D. Delneri, Environmental systems biology of cold-tolerant phenotype in *Saccharomyces* species adapted to grow at different temperatures. *Mol. Ecol.* **23**, 5241–5257 (2014).
16. L. M. Steinmetz, H. Sinha, D. R. Richards, J. I. Spiegelman, P. J. Oefner, J. H. McCusker, R. W. Davis, Dissecting the architecture of a quantitative trait locus in yeast. *Nature* **416**, 326–330 (2002).
17. A. D. Foote, P. A. Morin, J. W. Durban, R. L. Pitman, P. Wade, E. Willerslev, M. T. P. Gilbert, R. R. da Fonseca, Positive selection on the killer whale mitogenome. *Biol. Lett.* **7**, 116–118 (2011).
18. J. Melo-Ferreira, J. Vilela, M. M. Fonseca, R. R. da Fonseca, P. Boursot, P. C. Alves, The elusive nature of adaptive mitochondrial DNA evolution of an arctic lineage prone to frequent introgression. *Genome Biol. Evol.* **6**, 886–896 (2014).
19. M. R. Garvin, J. P. Bielawski, A. J. Gharrett, Positive Darwinian selection in the piston that powers proton pumps in complex I of the mitochondria of Pacific salmon. *PLOS ONE* **6**, e24127 (2011).
20. X. C. Li, D. Peris, C. T. Hittinger, E. A. Sia, J. C. Fay, Mitochondria-encoded genes contribute to evolution of heat and cold tolerance in yeast. *Sci. Adv.* **5**, eaav1848 (2019).
21. D. Libkind, C. T. Hittinger, E. Valério, C. Gonçalves, J. Dover, M. Johnston, P. Gonçalves, J. P. Sampaio, Microbe domestication and the identification of the wild genetic stock of lager-brewing yeast. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 14539–14544 (2011).
22. M. Okuno, R. Kajitani, R. Ryusui, H. Morimoto, Y. Kodama, T. Itoh, Next-generation sequencing analysis of lager brewing yeast strains reveals the evolutionary history of interspecies hybridization. *DNA Res.* **23**, 67–80 (2016).
23. E. Baker, B. Wang, N. Bellora, D. Peris, A. B. Hulfachor, J. A. Koshalek, M. Adams, D. Libkind, C. T. Hittinger, The genome sequence of *Saccharomyces eubayanus* and the domestication of lager-brewing yeasts. *Mol. Biol. Evol.* **32**, 2818–2831 (2015).
24. C. T. Hittinger, J. L. Steele, D. S. Ryder, Diverse yeasts for diverse fermented beverages and foods. *Curr. Opin. Biotechnol.* **49**, 199–206 (2018).

25. M. Hebly, A. Brickwedde, I. Bolat, M. R. M. Driessen, E. A. F. de Hulster, M. van den Broek, J. T. Pronk, J.-M. Geertman, J.-M. Daran, P. Daran-Lapujade, *S. cerevisiae* × *S. eubayanus* interspecific hybrid, the best of both worlds and beyond. *FEMS Yeast Res.* **15**, fov005 (2015).
26. B. Gibson, J.-M. A. Geertman, C. T. Hittinger, K. Krogerus, D. Libkind, E. J. Louis, F. Magalhães, J. P. Sampaio, New yeasts—New brews: Modern approaches to brewing yeast design and development. *FEMS Yeast Res.* **17**, fox038 (2017).
27. K. Krogerus, F. Magalhães, V. Vidgren, B. Gibson, New lager yeast strains generated by interspecific hybridization. *J. Ind. Microbiol. Biotechnol.* **42**, 769–778 (2015).
28. S. Mertens, J. Steensels, V. Saels, G. De Rouck, G. Aerts, K. J. Verstrepen, A large set of newly created interspecific *Saccharomyces* hybrids increases aromatic diversity in lager beers. *Appl. Environ. Microbiol.* **81**, 8202–8214 (2015).
29. D. Peris, Q. K. Langdon, R. V. Moriarty, K. Sylvester, M. Bontrager, G. Charron, J.-B. Leducq, C. R. Landry, D. Libkind, C. T. Hittinger, Complex ancestries of lager-brewing hybrids were shaped by standing variation in the wild yeast *Saccharomyces eubayanus*. *PLOS Genet.* **12**, e1006155 (2016).
30. G. Marinoni, M. Manuel, R. F. Petersen, J. Hvidtfeldt, P. Sulo, J. Piškur, Horizontal transfer of genetic material among *Saccharomyces* yeasts. *J. Bacteriol.* **181**, 6488–6496 (1999).
31. D. Peris, R. Pérez-Torrado, C. T. Hittinger, E. Barrio, A. Querol, On the origins and industrial applications of *Saccharomyces cerevisiae* × *Saccharomyces kudriavzevii* hybrids. *Yeast* **35**, 51–69 (2018).
32. J.-Y. Chou, J.-Y. Leu, Speciation through cytonuclear incompatibility: Insights from yeast and implications for higher eukaryotes. *BioEssays* **32**, 401–411 (2010).
33. Z. Salvadó, F. N. Arroyo-López, J. M. Guillamón, G. Salazar, A. Querol, E. Barrio, Temperature adaptation markedly determines evolution within the genus *Saccharomyces*. *Appl. Environ. Microbiol.* **77**, 2292–2302 (2011).
34. C. T. Hittinger, S. B. Carroll, Gene duplication and the adaptive evolution of a classic genetic switch. *Nature* **449**, 677–681 (2007).
35. C. B. Brachmann, A. Davies, G. J. Cost, E. Caputo, J. Li, P. Hieter, J. D. Boeke, Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: A useful set of strains and plasmids for PCR-mediated gene disruption and other applications. *Yeast* **14**, 115–132 (1998).
36. Q. K. Langdon, D. Peris, B. Kyle, C. T. Hittinger, spplDer: A species identification tool to investigate hybrid genomes with high-throughput sequencing. *Mol. Biol. Evol.* **35**, 2835–2849 (2018).
37. D. R. Gietz, R. A. Woods, Transformation of yeast by lithium acetate/single-stranded carrier DNA/polyethylene glycol method. *Methods Enzymol.* **350**, 87–96 (2002).
38. W. G. Alexander, D. T. Doering, C. T. Hittinger, High-efficiency genome editing and allele replacement in prototrophic and wild strains of *Saccharomyces*. *Genetics* **198**, 859–866 (2014).
39. K. H. Berger, M. P. Yaffe, Mitochondrial DNA inheritance in *Saccharomyces cerevisiae*. *Trends Microbiol.* **8**, 508–513 (2000).
40. S. G. Zweifel, W. L. Fangman, A nuclear mutation reversing a biased transmission of yeast mitochondrial DNA. *Genetics* **128**, 241–249 (1991).
41. Y.-Y. Hsu, J.-Y. Chou, Environmental factors can influence mitochondrial inheritance in the *Saccharomyces* yeast hybrids. *PLOS ONE* **12**, e0169953 (2017).
42. T. D. Fox, L. S. Folley, J. J. Mulero, T. W. McMullin, P. E. Thorsness, L. O. Hedin, M. C. Costanzo, Analysis and manipulation of yeast mitochondrial genes. *Methods Enzymol.* **194**, 149–165 (1991).
43. N. Eckert-Boulet, R. Rothstein, M. Lisby, Cell biology of homologous recombination in yeast. *Methods Mol. Biol.* **745**, 523–536 (2011).
44. M. J. McCullough, K. V. Clemons, J. H. McCusker, D. A. Stevens, Intergenic transcribed spacer PCR ribotyping for differentiation of *Saccharomyces* species and interspecific hybrids. *J. Clin. Microbiol.* **36**, 1035–1038 (1998).
45. K. Sylvester, Q.-M. Wang, B. James, R. Mendez, A. B. Hulfachor, C. T. Hittinger, Temperature and host preferences drive the diversification of *Saccharomyces* and other yeasts: A survey and the discovery of eight new yeast species. *FEMS Yeast Res.* **15**, fov002 (2015).
46. R. W. Unger, *Beer in the Middle Ages and the Renaissance* (University of Pennsylvania Press, 2004).
47. P. E. Thorsness, T. D. Fox, Nuclear mutations in *Saccharomyces cerevisiae* that affect the escape of DNA from mitochondria to the nucleus. *Genetics* **134**, 21–28 (1993).
48. M. C. Costanzo, T. D. Fox, Suppression of a defect in the 5′ untranslated leader of mitochondrial *COX3* mRNA by a mutation affecting an mRNA-specific translational activator protein. *Mol. Cell. Biol.* **13**, 4806–4813 (1993).
49. J. Conde, G. R. Fink, A mutant of *Saccharomyces cerevisiae* defective for nuclear fusion. *Proc. Natl. Acad. Sci. U.S.A.* **73**, 3651–3655 (1976).
50. W. G. Alexander, D. Peris, B. T. Pfannenstiel, D. A. Opulente, M. Kuang, C. T. Hittinger, Efficient engineering of marker-free synthetic allotetraploids of *Saccharomyces*. *Fungal Genet. Biol.* **89**, 10–17 (2016).
51. D. Peris, C. A. Lopes, A. Arias, E. Barrio, Reconstruction of the evolutionary history of *Saccharomyces cerevisiae* x *S. kudriavzevii* hybrids based on multilocus sequence analysis. *PLOS ONE.* **7**, e45527 (2012).
52. M. R. Lamprecht, D. M. Sabatini, A. E. Carpenter, CellProfiler: Free, versatile software for automated biological image analysis. *Biotechniques* **42**, 71–75 (2007).
53. T. A. McMeekin, J. Olley, D. A. Ratkowsky, in *Physiological Models in Microbiology*, M. J. Bazin, J. I. Prosser, Eds. (CRC Press, 2018), pp. 75–89.
54. R R Development Core Team, *R: A Language and Environment for Statistical Computing* (2017).
55. Y. Benjamini, Y. Hochberg, Controlling the false discovery rate: A practical and powerful approach to multiple testing. *J. R. Stat. Soc. Series. B.* **57**, 289–300 (1995).

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-FC02-07ER64494). 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 Sklodowska-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. constructed 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

Citation: E. P. Baker, D. Peris, R. V. Moriarty, X. C. Li, J. C. Fay, C. T. Hittinger, Mitochondrial DNA and temperature tolerance in lager yeasts. *Sci. Adv.* **5**, eaav1869 (2019).

Mitochondrial DNA and temperature tolerance in lager yeasts

EmilyClare P. Baker, David Peris, Ryan V. Moriarty, Xueying C. Li, Justin C. Fay and Chris Todd Hittinger

Sci Adv **5** (1), eaav1869.
DOI: 10.1126/sciadv.aav1869