Mitochondrial-Nuclear Epistasis Contributes to Phenotypic Variation and Coadaptation in Natural Isolates of *Saccharomyces cerevisiae*

Swati Paliwal, Anthony C. Fiumera, and Heather L. Fiumera¹

Department of Biological Sciences, Binghamton University, Binghamton, New York 13902

ABSTRACT Mitochondria are essential multifunctional organelles whose metabolic functions, biogenesis, and maintenance are controlled through genetic interactions between mitochondrial and nuclear genomes. In natural populations, mitochondrial efficiencies may be impacted by epistatic interactions between naturally segregating genome variants. The extent that mitochondrial-nuclear epistasis contributes to the phenotypic variation present in nature is unknown. We have systematically replaced mitochondrial DNAs in a collection of divergent *Saccharomyces cerevisiae* yeast isolates and quantified the effects on growth rates in a variety of environments. We found that mitochondrial-nuclear interactions significantly affected growth rates and explained a substantial proportion of the phenotypic variances under some environmental conditions. Naturally occurring mitochondrial-nuclear genome combinations were more likely to provide growth advantages, but genetic distance could not predict the effects of epistasis. Interruption of naturally occurring mitochondrial-nuclear genome combinations increased endogenous reactive oxygen species in several strains to levels that were not always proportional to growth rate differences. Our results demonstrate that interactions between mitochondrial and nuclear genomes generate phenotypic diversity in natural populations of yeasts and that coadaptation of intergenomic interactions likely occurs quickly within the specific niches that yeast occupy. This study reveals the importance of considering allelic interactions between mitochondrial and nuclear genomes when investigating evolutionary relationships and mapping the genetic basis underlying complex traits.

MITOCHONDRIAL energy production, which affects virtually every aspect of cellular fitness, requires the participation of two genomes. The mitochondrial genome encodes for essential components of the oxidative phosphorylation machinery and mitochondrial ribosomal RNAs and transfer RNAs. The nuclear genome encodes nearly 1000 proteins that are imported to the organelle where they compose the majority of the mitochondrial proteome. Specific interactions between components of both genomes are required at many levels, including mitochondrial DNA (mtDNA) replication, repair, and inheritance and transcription, translation, and assembly of the electron transport chain components. The respiratory complexes themselves are heterogeneous, composed of both nu-

clear and mitochondrially encoded proteins. Over evolutionary time, these interactions have been optimized, in part, to regulate production of the reactive oxygen species (ROS) that are the by-products of mitochondrial respiration.

Allelic variation in both genomes can affect mt-n interactions and alter mitochondrial fitness. These interactions have been shown to have direct consequences on health-related and life-history phenotypes in several taxa. In insects such as *Drosophila* and *Callosobruchus* (seed beetle), exchanging mtDNA variants between distinct populations has led to lowered metabolic rate (Arnqvist *et al.* 2010), decreased egg-to-adult survival (Dowling *et al.* 2007a; Montooth *et al.* 2010), and shortened life span (Clancy 2008; Zhu *et al.* 2014). Interpopulation hybrids of the marine copepod *Tigriopus* suffered compromised oxidative phosphorylation (OXPHOS) capacities (Ellison and Burton 2006, 2008b) and reduced mitochondrial transcription (Ellison and Burton 2008a), most likely influenced by interactions between alleles of the nuclear-encoded RNA polymerase and mtDNA variants (Ellison and Burton 2010).

Copyright © 2014 by the Genetics Society of America

doi: 10.1534/genetics.114.168575

Manuscript received July 18, 2014; accepted for publication August 21, 2014; published Early Online August 27, 2014.

Supporting information is available online at <http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.114.168575/-/DC1>.

¹Corresponding: 4400 Vestal Parkway East, Science 3, Binghamton University, Binghamton, NY 13902. E-mail: hfiumera@binghamton.edu

The importance of mt-n epistasis extends well past arthropods. In mice, mt-n epistasis is known to affect cognition (Roubertoux *et al.* 2003), ROS (Fetterman *et al.* 2013), and respiratory functions (Betancourt *et al.* 2014) as well as tissue-specific selection for mtDNA variants (Jenuth *et al.* 1997). Interactions between mt and n genomes likely contribute to cytoplasmic male sterility in plants (Hu *et al.* 2014) and disease presentation and aging in humans (Tranah 2011; Wallace and Chalkia 2013; Wolff *et al.* 2014), suggesting that mt-n epistasis is important in most eukaryotes.

Between species, incompatibilities between mitochondrial and nuclear genomes can be quite severe. Introgression of mtDNAs from *Drosophila simulans* to *D. melanogaster* had significant effects on respiratory complexes (Sackton *et al.* 2003), development (Montooth *et al.* 2010), and aging (Rand *et al.* 2006). Disrupted oxidative phosphorylation pathways were observed in F₂ male hybrids of *Nasonia* wasps (Ellison *et al.* 2008; Niehuis *et al.* 2008; Koevoets *et al.* 2012a). Activities of OXPHOS complexes in human cell lines harboring mtDNAs from other primates were reduced by as much as 90% (Barrientos *et al.* 2000). Similarly, respiratory capabilities of murine (Dey *et al.* 2000; Yamaoka *et al.* 2000; McKenzie *et al.* 2003) or amphibian (Liepins and Hennen 1977) xenomito-chondrial cybrids were drastically reduced. Interspecific mt-n incompatibilities between yeasts in the *Saccharomyces sensu stricto* genus can lead to complete respiratory deficiencies (Sulo *et al.* 2003; Lee *et al.* 2008; Chou *et al.* 2010; but see Prochazka *et al.* 2012). While incompatibilities between species generally leads to a decline in fitness, mt-n incompatibilities do not always tightly align with genetic distance; larger epistatic responses to mtDNA exchanges were observed within *D. melanogaster* populations than between species (Montooth *et al.* 2010). The Dobzhansky–Muller-type mt-n incompatibilities that sometimes exist between species may have contributed to speciation events (Trier *et al.* 2014; Wolff *et al.* 2014), although the dynamics of mt-n coevolution are not well enough understood to make substantial conclusions. Regardless, the trove of molecular sequence analyses repeatedly has shown evidence for coevolution of mt-n complexes (e.g., Bayona-Bafaluy *et al.* 2005; Parmakelis *et al.* 2013; Zhang and Broughton 2013), supporting the empirical data that mt-n incompatibilities generally increase with genetic distance.

The yeast *S. cerevisiae* is potentially an excellent model system to investigate mt-n epistasis. First, yeasts undergo both meiotic and mitotic replication. Biparental inheritance of mtDNAs, high levels of mitochondrial recombination, and maintenance of coadapted mt-n allelic pairs through high levels of sibling matings in nature should allow for selection to quickly evolve beneficial mt-n interactions. Indeed, mt-n epistasis was successfully evolved in the laboratory in as few as 2000 mitotic generations (Zeyl *et al.* 2005). Second, *S. cerevisiae* has long been a model for cell biology and genetic studies of mitochondrial functions because of a powerful genetic system and ease of laboratory manipulations. Owing to the ability of *S. cerevisiae* to survive in the absence of mtDNA via fermentation (rather than mitochondrial respiration), particular

advances in mitochondrial genetics, including mitochondrial transformations (Bonnefoy *et al.* 2007), have been possible. With next-generation sequencing technologies, *S. cerevisiae* yeasts are now being thrust into the limelight for population genetics and phenomics. As such, population structures for wild yeasts are better understood (Liti *et al.* 2009; Wang *et al.* 2012; Cromie *et al.* 2013; Hittinger 2013; Skelly *et al.* 2013) and an increasing number of laboratory-friendly isolates representing the genetic diversity of yeasts are now available (Cubillos *et al.* 2009; Louvel *et al.* 2014).

In this study, we have sought to determine if epistatic interactions between mitochondrial and nuclear genomes contribute to the phenotypic variation observed in *S. cerevisiae* yeasts. We generated 100 strains harboring unique mt-n genome combinations, representing each combination of 10 naturally occurring mtDNA and nuclear genome variants. These strains were created in the absence of nuclear recombination, thus allowing for the quantification of phenotypic effects due to mt-n epistasis. We found that mt-n epistasis determines how yeast strains respond to changing environmental conditions and that within a single environment mt-n epistasis can explain a surprisingly high proportion of phenotypic variance. We observed that, when grown at elevated temperature or in medium requiring respiration, strains containing their naturally occurring mtDNAs were generally more fit than when harboring alternative mtDNA variants. There was no evidence that these beneficial mt-n interactions followed genetic distances, suggesting that mt-n coadaptation occurs quickly within local populations of *S. cerevisiae* yeasts.

Materials and Methods

General yeast procedures

General yeast handling was performed through standard techniques (Guthrie and Fink 1991). Media included a fermentable media (CSM) containing 2% glucose, 6.7% yeast nitrogen base lacking amino acids, and a defined mix of amino acids (Sunrise Science) and nonfermentable media (EG) containing 2% peptone, 1% yeast extract, 3% ethanol, 3% glycerol, and 0.03% adenine hemisulfate. Selective minimal media used were CSM–arginine, CSM–uracil, and CSM–adenine (Sunrise Science). Media were supplemented with paraquat (Ultra Scientific) at concentrations indicated. To create strains devoid of mtDNA (p^0), cells were passaged twice through CSM medium containing 25 μ g/ml ethidium bromide and screened for respiratory deficiency, according to general protocols (Fox *et al.* 1991). mtDNAs were isolated as previously described (Defontaine *et al.* 1991), digested with *EcoRV*, and separated by 0.8% agarose gel electrophoresis. To chemically cure strains of prions, strains were passaged four times on rich medium containing 5 mM guanidine hydrochloride. Relevant genotypes for all strains are shown in [Supporting Information, Table S1](#).

Construction of mitochondrial-nuclear strain collection

We exchanged mtDNAs between 10 divergent *S. cerevisiae* isolates to create strains with each possible combinations of 10

nuclear and 10 mtDNAs (100 unique mt-n combinations) using a series of karyogamy-deficient matings (Zakharov and Yarovsky 1977). First, haploid derivatives of each yeast isolate were mated to a strain devoid of mtDNA (*i.e.*, ρ^0) carrying the dominant *KAR1-1* mutation that inhibits nuclear fusion during zygote formation (strain NAB32 ρ^0). Following visual inspection of zygote formation in mating mixtures (~2–6 hr post mating), cells were plated to CSM-ura. Colonies were replica-plated to EG, CSM-ade, and CSM-arg to identify respiring Ade[−] Arg[−] Ura⁺ colonies that contained the *KAR1-1* nuclear background from NAB32 and mtDNAs from the divergent yeast isolates. The *KAR1-1* ρ^+ strains demonstrated reduced mating efficiencies with a *MATa* tester strain, and no mating with a *MATα* tester. The mtDNAs were then transferred from the *KAR1-1* mtDNA recipient strains to ρ^0 derivatives of each of the 10 isolates through similar strategies [identifying the final mt-n strains as Ura[−], Arg⁺, Ade⁺, respiring (*i.e.*, EG⁺) colonies that mated with *MAT α* testers]. Biological replicates from independent matings for each of 100 unique mtDNA-nuclear genomic combinations were isolated. We also recreated the original mt-n genome combinations by reintroducing the mtDNAs from the *KAR1-1* mtDNA carrier strain back to the parental ρ^0 strains. RFLP analysis of mtDNAs isolated from a random sampling of the final strains matched expected patterns (not shown). See Figure S1 for a schematic of the mating strategies.

Phenotyping

Approximately 2500 growth curves were recorded for strains cultivated in microplates using Biotek Eon microplate spectrophotometers. Optical densities (600 nm) were sampled at 15-min intervals until reaching stationary phase (~48 hr). Growth under five different environmental conditions was monitored: CSM, CSM at 37°, CSM + 200 μg/ml paraquat, EG, and EG + 50 μg/ml paraquat. Incubation was performed at 30° unless otherwise indicated. To minimize the effects of anaerobic, nonrespiratory growth due to oxygen limitation, microplate cultures were inoculated from actively dividing cells that were precultivated in roller tubes in the appropriate medium (CSM or EG) and grown under continuous double orbital shaking. For culturing in nonfermentable media, 24-well plates were used to maximize oxygen concentrations, while 96-well plates were used for all other media. Maximal growth rates ($V_{\max}$), determined as the highest slope of regression lines modeled over sliding windows of 5 or 10 data points, were extracted from growth curves and normalized to the behavior of 13 (in 96-well plates) or 4 (in 24-well plates) replicates of a reference strain, D273-10B, included on each plate. Strains were tested in replicate ($n = 3$ –8; average $n = 6$).

ROS assays

Endogenous ROS levels were determined by a fluorescence assay adapted from Doudican *et al.* (2005). Basically, equivalent volumes of mid-log phase cells grown in microtiter plates were washed in water and resuspended in 10 μM dichlorofluorescein diacetate (from a 5 mM stock in DMSO).

Following a 30-min incubation at 30°, cells were lysed using glass-bead disruptions in 1% SDS, 2% TritonX-100, 10 mM NaCl, 1 mM EDTA, and 10 mM Tris, pH 8.0. Fluorescence of the soluble fraction was measured with an excitation of 485 nm and emission at 520 nm using a Synergy Mx (Biotek). Fluorescence readings were normalized to OD₆₀₀.

Statistical analyses

A SNP table generated from nuclear genome sequences was obtained from the Sanger Institute FTP repository (Liti *et al.* 2009). Sequences of mitochondrial coding sequences were obtained from the Yeast Resource Center (<http://www.yeastrc.org/g2p/home.do>) (Skelly *et al.* 2013). Distance matrices and unrooted neighbor-joining phylogenetic trees were created using PHYLIP (Felsenstein 2005).

Statistical analyses were performed in R 2.15.2 (R Core Team 2012) or Minitab Statistical Software. Ten ANOVAs were used to test for differences between the original yeast isolates, recreated native mt-n genome pairings, and prion-cured strains, nesting the biological replicate within each treatment category according to the following equation: $y_{ijk} = \mu + O_i + (\text{BR within } O_{i(i)}) + \varepsilon_{ijk}$, where O refers to the origin of the mt-n genotype (*i.e.*, the original, recreated mt-n combination or prion-cured derivative) and BR is the biological replicate nested within original. Random effects models (lme4 using REML) were used to test for the significance of mitotypes, nuclear backgrounds, environment, and their interactions using the following full model: $y_{ijk} = \mu + N_i + Mt_j + E_k + (N^*M)_{ij} + (N^*E)_{ik} + (M^*E)_{jk} + (N^*M^*E)_{ijk} + \varepsilon_{ijk}$, where y_{ijk} is the $V_{\max}$ fitness estimate, and N_i , Mt_j , and E_k are random effects factors representing nuclear genetic background, mitotype, and environmental condition, respectively, and the remaining terms representing the two- and three-way interactions. Within individual environments, random effects models were used to test for the significance and contributions of mitotype, nuclear genotype, and their interactions to phenotypic variances using the full model, $y_{ikj} = \mu + N_i + Mt_j + (NM)_{ij} + \varepsilon_{ijk}$. The main mt (or n) effects were evaluated by considering a single nuclear (or mitochondrial) background at a time. Where appropriate, *post hoc* analyses were completed using Tukey HSD.

Fitness advantage of naturally occurring mt-n combinations was evaluated by comparing the average growth rates of strains with naturally occurring (native) or non-native mt-n genome combinations using the ANOVA model, $V_{\max_{ijk}} = m + A_i + (I \text{ within } A_{j(i)}) + \varepsilon_{ijk}$, where A is the status of the mt-n genome combination (native or non-native) and I is the individual strain identification nested within the status.

To assay the frequency of epistasis, data were restricted to combinations of four strains. For each four-strain combination, the significance of mt-n epistasis was tested using a fixed effects model (lm). Differences in the frequency of epistasis were tested using contingency tables. To consider the consequences of interrupting native mt-n genomes, tests were performed on four strains that included two strains harboring naturally occurring mt-n combinations and the

mitochondrial replacement strains (e.g., strains C₁ and C₂ with their original mitotypes, strain C₁ harboring the C₂ mitotype, and strain C₂ harboring the C₁ mitotype). To consider the consequences of interrupting non-native mt-n genome combinations, tests considered each set of four strains representing two unique nuclear and two unique mitotypes (and no native mt-n combinations).

Adaptation was tested using fixed effects models treating ecotype and native or non-native mt-n status as factors. Differences in the frequency of epistasis were tested using contingency tables. Effects of genetic distance on likelihood or phenotypic value of epistasis were tested using glm (binomial family) or lm, respectively.

Results

mtDNA variation in *S. cerevisiae*

To study the impact of mt-n epistasis on phenotypic variation, we selected 10 strains from the *Saccharomyces* Genome Resequencing Project (Liti *et al.* 2009) that represent much of the genetic diversity present in natural isolates and for which haploid derivatives were available (Cubillos *et al.* 2009). Genotypes and parental origins are provided in Table S1. A phylogenetic tree created from previously determined nuclear SNP differences (Liti *et al.* 2009) is provided in Figure S2.

Complete mitochondrial genome sequences are not yet available for the numerous *S. cerevisiae* isolates that have been sequenced via next-generation technologies, likely due to the high AT content and repetitive nature of yeast mtDNA. To verify that the mtDNAs from the 10 isolates were genetically distinct, mtDNAs were isolated and subjected to RFLP analysis (Figure 1A). Nine unique banding patterns indicated that sequence polymorphisms exist between most of the strains. mtDNAs from C₁ and C₄ showed an identical RFLP pattern. Partial mtDNA sequences for 7 of the 10 strains used in this study, including C₁ and C₄, were obtained through deep sequencing (Skelly *et al.* 2013). Using these available mtDNA sequences, we aligned 6684 bp (of the ~86,000-bp genome) from eight mitochondrially encoded ORFs, determined SNP differences, and constructed an unrooted phylogenetic tree (Figure 1B). Based on three SNP differences, we found that C₁ and C₄ are closely related yet are not identical, similar to the relationship seen by nuclear SNP differences (Figure S2). The genetic relatedness of the mtDNAs from these strains is generally similar to those determined by nuclear genomes, suggesting that mitochondrial genome divergence approximately parallels nuclear sequence divergence. For this study, the mtDNA from each strain will be referred to as an independent mitotype (haplotype).

A novel yeast population allows for assessment of mt-n epistasis

We exchanged the mtDNAs between the yeast isolates to generate 100 strains representing each combination of the 10 nuclear and 10 mitochondrial genomes. To assess our

Figure 1 mtDNA polymorphisms in *S. cerevisiae*. (A) RFLP analysis of purified mtDNA from 10 isolates restricted with *EcoRV* separated by agarose gel electrophoresis (0.8%). Nine unique banding patterns were observed. Banding patterns of strains C₁ and C₄ are similar. No bands were observed from a ρ^0 control, demonstrating specificity of mtDNA purification. (B) Neighbor-joining phylogenetic tree based on 6684 bp of available mtDNA sequences (Skelly *et al.* 2013). (mtDNA sequences not available for C₃, F₁, and F₂). Scale bar indicates frequency of base-pair differences.

strain creation methodology, we compared the growth rates of the 10 original strains to growth rates of strains in which mitotypes were reintroduced to the appropriate parental backgrounds and of strains cured of prions (Figure S3). Individual ANOVAS were performed on growth rates ($n \geq 4$) of strains containing identical mt-n genome combinations. The growth rates of prion-cured derivatives of strains E₂ and E₄ were statistically different from the strains containing

recreated mt-n genome combinations ($P = 0.01$ and 0.02 , respectively), but not after applying a Bonferroni correction (cutoff of $P = 0.005$). However, the prion-cured versions of these strains were not different from the original strains ($P > 0.05$), consistent with previous studies that did not find evidence of prions in the parental versions of these strains (Halfmann *et al.* 2012). For all other strains, no significant differences were found in the growth rates between the original, reconstituted mt-n genotypes or prion-cured strains (nesting biological replicates within each grouping). If prions do indeed exist, they would have been cytoplasmically transferred coincidentally with the mtDNAs, and their modest effects on phenotype would appear as mitochondrial effects in our analyses. While the absence of novel mutations in any of the newly created mt-n strains cannot be ruled out entirely, these analyses provide confidence that our strain collection can be used to accurately assess the phenotypes attributed to nuclear and mitochondrial genomes or interactions between the two.

Mitochondrial-nuclear interactions demonstrate $mt \times n \times e$ ($G \times G \times E$) effects

To measure the relative fitness of each strain, we grew cells in a variety of media (environmental conditions) and extracted maximal growth rates from growth curves. Each strain was phenotyped in media that supported growth predominantly through fermentation (CSM) and in media containing the nonfermentable carbons, ethanol, and glycerol (EG) that supported growth exclusively through aerobic respiration. Strains were also grown under conditions that stress mitochondrial functions (Morano *et al.* 2012) including CSM medium at elevated temperature (37°) and CSM and EG media containing the ROS-inducing agent, paraquat (PQ and EG + PQ, respectively).

Numerous studies have shown that phenotypic variances in yeast are dependent on environmental conditions (Kvitek *et al.* 2008; Liti *et al.* 2009; Skelly *et al.* 2013). We first performed an analysis to determine whether the phenotypic responses of strains were impacted by environment-dependent interactions between their mitochondrial and nuclear genomes. We compared an ANOVA model containing environment (e), nuclear genotype (n), and mitotype (mt) terms as independent and interacting factors, with models lacking each term. All terms were treated as random factors (Table 1). A highly significant three-way interaction between the media, nuclear background, and mitotype ($mt \times n \times e$, $P < 0.0001$) indicates that specific combinations of mitochondrial and nuclear genomes responded to media conditions differently than others. Phenotypic variation was also influenced by gene $\times$ environment interactions, as seen through significant interactions between the environment and nuclear genotype ($n \times e$, $P < 0.0001$) or mitotype ($mt \times e$, $P < 0.0001$). Media (*i.e.*, environment) also had a pronounced effect on growth rates ($P = 0.0003$), consistent with the fact that yeast utilize different metabolic pathways in different sugars and temperatures.

Within-environment mitochondrial-nuclear epistasis

The impact of mt and n genotypes and mt-n interactions on cellular fitness in each medium was estimated from the normally distributed data using random effects ANOVAs (Table 2). The nuclear genetic background was significant under all growth conditions ($P < 0.0001$). Mitotypes had significant effects in CSM and at 37° ($P < 0.0001$), but were nonsignificant when mitochondrial functions were taxed during respiratory growth (EG) or upon endogenous ROS generation (PQ and EG + PQ). Epistatic interactions between mt and n genomes ($mt \times n$) had highly significant effects ($P < 2.2 \times 10^{-16}$) in each growth environment.

To visualize mt-n epistasis in each environment, the response of each nuclear genetic background across each of 10 mitotypes is shown (Figure 2). In these interaction plots, each line connects the growth rates ($V_{\max}$) of a single nuclear genotype as it was paired with the different mtDNAs. No nuclear background was completely unaffected by mitotype, demonstrating that mitochondrial variants influenced growth rates. Notably, the mitotypes from C₁, E₁, F₁, F₂, and F₅ conferred highest growth rates at 37° when paired with any of the C₁, E₁, F₁, F₂, and F₅ autosomal backgrounds (Figure 2C), suggesting that these mitotypes provided temperature advantages. However, we also observed that these mitotypes did not confer a temperature advantage in all nuclear genomes. Furthermore, the mitotype-n combinations resulting in high growth rates at 37° did not necessarily confer fitness advantages in other media, thus demonstrating $mt \times n \times e$ interactions.

It is important to note that $V_{\max}$ values were normalized to a common laboratory strain used for mitochondrial studies whose genetic background and mitotype were not included in the strains analyzed here. Thus, low normalized $V_{\max}$ values do not reflect the inability of a strain to grow under a particular condition, but rather its relative growth to a robust laboratory strain.

Nuclear genetic backgrounds are influenced by mitotypes

The strong $mt \times n$ interactions may have affected the ability to accurately assess significance of main (mt or n) effects in the random effects ANOVA models. We therefore examined the independent effects of mtDNA variants by testing whether the growth rates of each nuclear background were altered by pairing it with variable mtDNAs through a series of individual ANOVAs (Table S2). We observed that mitotypes affected the growth of each nuclear background in at least one environment, with statistically discernible effects in 38 of 45 scenarios. In only a few instances did mitotypes not influence growth rates for a nuclear genotype. Mitotypes did not significantly affect fitness of the C₂ autosomal background in CSM at 37° , but did impact fitness under lower temperature (CSM), respiratory (EG), or oxidative stress (PQ and EG + PQ) conditions. Additionally, mitotypes did not impact fitness of the C₃ nuclear background in CSM or EG, the E₄ nuclear background at 37° , PQ, or the F₁ nuclear

Table 1 Mitochondrial $\times$ nuclear $\times$ environment interactions (mt $\times$ n $\times$ e)

	d.f.	χ^2	P-value	Model comparisons
Nuclear background (n)	1	3.739	0.05316	Full, n
Mitotype (mt)	1	0	1	Full, mt
Media (e)	1	12.926	0.0003241	Full, e
mt $\times$ n interaction	1	0	1	Full, mt $\times$ n
n $\times$ e interaction	1	393.04	<2.2e-16	Full, n $\times$ e
mt $\times$ e interaction	1	65.492	5.84e-16	Full, mt $\times$ e
mt $\times$ n $\times$ e	1	513.72	<2.2e-16	Full, mt $\times$ n $\times$ e

To determine the significance of each term, ANOVAs comparing the full model with a model lacking the indicated term were evaluated. Each factor was treated as a random effect. Full model: n + mt + e + (mt $\times$ n) + (n $\times$ e) + (mt $\times$ e) + (mt $\times$ n $\times$ e).

background in EG + PQ. These instances, however, are the exceptions to the general trend that mitotypes carried fitness consequences. Similar tests were performed to show that nuclear genotypes influenced the fitness of each mitotype (Table S2; $P < 0.001$ in all tests), consistent with the strong nuclear effects seen in the two-way ANOVAs (Table 2).

Tukey *post hoc* analyses were used to group each nuclear genetic background according to the distributions of their phenotypes when paired with all mitotypes (Figure 3, A–E). Despite the large variability in fitness values, the nuclear backgrounds were organized into a number of distinct Tukey groups. During fermentative growth at 30° and 37°, nuclear genetic backgrounds formed seven distinct groups. The fewest groupings (four) were observed when cells were grown in EG + PQ. Importantly, the members of groups changed across different environments, consistent with significant n $\times$ e influences. Similar analyses were performed on the fitness of each mitotype when averaged across all nuclear backgrounds. Generally, larger phenotypic variances and fewer Tukey groupings were observed, consistent with strong nuclear effects on growth. (Figure 3, F–J). At elevated temperature, the means across mitotypes were similar (Figure 3H), but the phenotypic ranges allowed for two distinct groups to be formed. This raises the possibility that low and high fitness mitochondrial haplotypes for elevated temperature may exist in nature. Members of each mitotype group also changed across environments, consistent with mt $\times$ e effects. The phenotypic ranges of individual nuclear and mitochondrial genetic backgrounds varied (as an example, in Figure 3C, compare the sizes of the boxes for nuclear genotypes F₅, E₁, C₁, and F₂ with the size of the boxes for E₂ and E₄). These phenotypic distributions are consistent with the idea that mt-n epistasis is more significant in some genetic backgrounds than in others.

The contributions of n, mt, and mt-n interactions to phenotypic variation in each environment were quantified using variance component analyses of the full ANOVA models (Figure 4). Elevated temperature induced the most phenotypic variation among these yeasts. The majority of this variance could be explained by nuclear genotypes

(69%). The overall variances under all other conditions were considerably lower, and similarly, the autosomal genetic background explained the majority of the variances. In the presence of glucose and oxygen, *Saccharomyces* and other “Crabtree” positive yeasts limit mitochondrial respiration, preferring alcoholic fermentations. Despite this, we found that 3% of the phenotypic variances observed in CSM could be explained by mt-n epistasis and 2% by mitotype. At 37°, these proportions were expanded to 16 and 10%, respectively. In nonfermentable medium (EG) or when ROS-inducing agents were added to fermentable or nonfermentable media, interactions between mitotypes and genetic background explained a significant proportion of the variances, while independent effects of mitotypes were negligible. Interorganellar epistasis explained 40% of the overall variances when cells were forced to grow via mitochondrial respiration. In all environments, mt-n epistasis had a larger impact on phenotypic variances than mt haplotypes alone.

Naturally occurring mt-n genome combinations provide fitness advantages in certain environments

Pairings of mt and n genomes that lead to fitness advantages should provide a selective advantage. To determine if selection on mt-n epistasis has occurred in yeast, we compared the fitness of strains harboring the 10 original, potentially coadapted, mt-n genome combinations (referred to as “native”) with strains in which the original mtDNAs were replaced with alternative mitotypes (referred to as “non-native”) (Figure 5A). The average growth rate of strains with native mt-n genome pairs was significantly higher than that of strains with non-native combinations at 37° ($V_{\max} = 1.9$ vs. 1.5 milli absorbance (mOD)/min, corresponding to a 27% increase, $P < 0.001$). Native mt-n genome pairings also conferred growth advantages when cells were grown under respiratory conditions ($V_{\max} = 0.95$ vs. 0.78 mOD/min, 22% increase, $P < 0.001$). Strains harboring non-native genome combinations grew, on average, slightly better than strains with naturally occurring mt-n genome combinations in minimal glucose-containing medium ($V_{\max} = 0.90$ vs. 0.88 mOD/min, 2.5% increase, $P = 0.003$) and upon the generation of endogenous ROS in fermentable ($V_{\max} = 0.79$ vs. 0.74 mOD/min, 7% increase, $P = 0.001$) or non-fermentable ($V_{\max} = 0.52$ vs. 0.47 mOD/min, 10% increase, $P < 0.001$) media.

Disruption of naturally occurring mt-n genomes increases the frequency of observing mt-n epistasis

Coadapted mt-n genome combinations should have a beneficial outcome on fitness. In theory, the fitness consequences of disrupting coadapted genomes should increase the likelihood of generating observable epistatic interactions as compared to when disrupting non-native combinations. We first performed fixed effect, two-way ANOVAs on fitness values from four strains representing two native mt-n and the two strains that resulted from exchanging their mtDNAs,

Table 2 Mitochondrial × nuclear interactions (mt × n) within single environments

Effect	Model	d.f.	Media (environment)									
			CSM		EG		37°		PQ		EG + PQ	
			χ^2	<i>P</i>	χ^2	<i>P</i>	χ^2	<i>P</i>	χ^2	<i>P</i>	χ^2	<i>P</i>
mt	Full mt	1	16.4	5.1e-05	0	1	25.4	4.6e-07	0	1	0	1
n	Full n	1	221.4	<2.2e-16	38.4	5.8e-10	132.1	<2.2e-16	121.4	<2.2e-16	81.8	<2.2e-16
mt × n	Full (mt × n)	1	77.2	<2.2e-16	409.9	<2.2e-16	184.3	<2.2e-16	134.2	<2.2e-16	126.8	<2.2e-16

Full model: n + mt + (mt × n).

resulting in 45 independent tests under each condition. These results are shown in Figure 5B, in which the exchange of mtDNAs between two strains leading to a significant mt-n term ($P < 0.05$) is indicated by a solid black line. When cells were grown via fermentation in glucose, interrupting native mt-n pairs led to a significant mt-n interaction term 22% of the time. This increased to 36% when endogenous ROS production was induced and to 64% at elevated temperature. When cells were grown under strict respiratory conditions, disrupting native mt-n combinations generated mt-n epistasis in nearly every test (96%). This frequency decreased to 48% in respiring cells exposed to paraquat. The disruption of each native mt-n genome combination resulted in epistatic interactions in more than one environment.

Similar ANOVAs were performed to determine the frequencies of observing mt-n epistasis when exchanging mtDNAs between strains harboring non-native genome combinations (an additional 1500 possible combinations of four strains representing two unique nuclear and two unique mitochondrial haplotypes with no naturally occurring mt-n pairings). The frequencies of discerning epistasis when disrupting native and non-native combinations were compared (Figure 5C). Disrupting native mt-n genome combinations more frequently produced epistatic effects during respiratory growth and temperature stress (EG: 96 vs. 64%, $P = 0.001$; 37°: 64 vs. 42%, $P = 0.003$, respectively). While mt-n epistasis was seen more frequently when exchanging mitotypes between strains with non-native genome combinations in PQ (36% of tests involving strains with native mt-n genome pairings vs. 66% of tests involving strains with all non-native genome pairings), the difference was not statistically significant ($P = 0.7$). The relative frequencies of observing mt-n epistasis were roughly equal when comparing native and non-native mt-n genome disruptions in CSM and EG + PQ ($P > 0.6$).

We also determined the magnitude of epistatic responses following the exchange of mtDNAs (Figure 5D). In each ANOVA test revealing a significant mt-n interaction term, the magnitude of the epistatic fitness effect was determined as the absolute difference in slopes from two-way interaction plots ($\Delta\Delta V_{\max}$). Epistatic effects were greatest during temperature stress when disrupting both native and non-native mt-n genome combinations compared with other conditions. These effects were significantly higher when native mt-n genome pairs were disrupted ($P = 0.006$). Similarly,

interruption of native mt-n combinations had a larger magnitude during respiratory growth (EG, $P < 2.2e^{-16}$). Epistatic effects for disrupting non-native genome combinations were higher than when disrupting non-native combinations in CSM + PQ, but only slightly significant ($P = 0.01$).

Genetic distance vs. habitat

Others have reported phenotypic correlations with genetic distances between the yeast strains used here (as part of a larger collection) when exposing yeasts to extreme environments (Liti *et al.* 2009; Halfmann *et al.* 2012). Under the conditions used in this study, we found no correlation between the pairwise phenotypic differences and genetic distance among the 10 original isolates (Pearson's correlation coefficients = -0.1 to 0.2 , $P \geq 0.1$, Figure S4). We also found no correlation between the relative strength of the epistatic responses ($\Delta\Delta V_{\max}$) when exchanging mtDNAs between the 10 original strains and their genetic distances (Pearson's correlation coefficients = -0.1 to 0.2 , $P \geq 0.1$, Figure S5). That mt-n epistasis is observed frequently across all strains, with uncorrelated fitness response to genetic distance, suggests that mt-n epistasis does not scale to intra-specific molecular divergence.

Strains living in similar environments may undergo similar selective pressures leading to selection of similar phenotypes. Here, we considered only strains the mt and n genomes of which originated from similar isolation habitats (clinical, environmental, or fermentation). Ecotype did have a significant effect on growth rate in CSM, EG, and at 37° (strains with fermentation, clinical, or environmental n and mt genomes showed significantly different growth rates; $P < 0.001$, Figure S6). We did not consider media conditions containing paraquat because it is unlikely to represent an environment observed in nature. Clinical strains grew better when they were paired with their original mtDNAs than when paired with other clinically derived mtDNAs at 37° ($P = 0.006$, *post hoc* test) and in EG medium ($P = 0.02$, *post hoc* test). Domesticated strains from fermentation sources outperformed environmental and clinical ecotypes at 37° and in EG ($P < 0.001$, *post hoc* test) and performed equally as well as environmental ecotypes (and better than clinical ecotypes; $P < 0.001$, *post hoc* test) in CSM (Figure S6). In EG, there was a strong interaction ($P = 0.008$) between

Figure 2 Fitness effects of 10 mitotypes on 10 nuclear backgrounds. Changes in fitness are presented as interaction plots, where each colored line follows the changes in fitness ($V_{\max}$) of a single nuclear genetic background when paired with 10 different mt haplotypes, as indicated on the x-axis, under different media conditions. The ordering of mitotypes does not reflect genetic relatedness. (A) Fermentable glucose media (CSM). (B) Non-fermentable media (EG). (C) CSM at elevated temperature (37°). (D) CSM + paraquat (CSM + PQ). (E) EG + PQ. Note that the scale of the axes are different for each media condition.

native status and ecotype with native fermentation strains growing the best (Figure 6). These results are consistent with intense selection for robust performance through human activities and suggest that mt-n interactions contribute to the overall fitness benefits of these domesticated yeasts.

If niche-specific environments consistently selected for common mt-n alleles, then fewer mt-n incompatibilities should arise between strains from similar ecotypes. We did not find differences in the frequencies of observing mt-n epistasis when considering the exchange of mtDNAs between two strains from similar or different isolation sources (clinical, fermentation, or environmental) ($P \geq 0.2$ for all five media conditions) nor in the overall magnitude of the epistatic responses ($P \geq 0.3$).

Interruption of coadapted mt-n genomes results in increased ROS

The mitochondrial respiratory chain is the major intracellular source of ROS. We more closely examined the consequence of interrupting potentially coadapted mt-n complexes through measurements of endogenous ROS levels in strains that showed large epistatic fitness responses at 37°. Exchanging mitotypes between strains C₁ and C₄ showed epistatic effects on growth rates (Figure 7A) and endogenous ROS levels (Figure 7B). Strain C₁ with its original mitotype had

a higher growth rate and lower ROS than when harboring the mitotype from C₄ ($P < 0.001$, *post hoc* tests). Similarly, C₄ with its native mitotype had a higher $V_{\max}$ and a lower ROS than C₄ with the C₁ mitotype ($P \leq 0.01$, *post hoc* tests). Similarly, when exchanging mitotypes between strains C₁ and F₂, mt-n interactions affected both growth rates (Figure 7C) and ROS (Figure 7D). In the C₁ nuclear background, the F₂ mitotype did not show a change in growth rate, but did result in a large increase in ROS ($P < 0.001$, *post hoc* tests). In the F₂ nuclear background, the C₁ mitotype resulted in slower growth ($P < 0.001$, *post hoc* tests), but ROS levels were unaffected.

The interaction plots of growth rates in Figure 7, A and C, present new data collected for biological and technical replicates collected on the same cultures used for the ROS assays. These growth rates follow the same patterns observed in Figure 2C.

Discussion

Intraspecific mt-n epistasis

We provide evidence that epistasis interactions between mitochondrial and nuclear polymorphisms found in 10 natural isolates of *S. cerevisiae* yeasts are important contributors to phenotypic variance. In this study, we replaced mtDNAs

Figure 3 Growth rate distributions of nuclear genotypes and mitochondrial haplotypes. (A–E) Box plots showing the distributions of individual nuclear backgrounds with all 10 mitotypes. (F–J) Box plots showing the distributions of each mitotype across all nuclear genotypes. Each box plot shows the combined data for 10 strains and indicates the 25th percentile (lower edge of the box), median (solid line in the box), 75th percentile (upper edge of the box), and 90th percentile (whisker). The fitness scale on the y-axis is the same for each plot. Tukey groupings are shown in blue across the top of each plot. (A and F) CSM. (B and G) EG. (C and H) 37°. (D and I) CSM + PQ. (E and J) EG + PQ.

to create 100 unique mt-n genome combinations using a series of karyogamy-deficient matings. While similar mating strategies are routinely used to study cytoplasmic inheritance in yeast (Guthrie and Fink 1991; Bonnefoy *et al.*

2007; Li and Kowal 2012), rare events such as nuclear mutations, transfer of naturally occurring yeast prions (Halfmann *et al.* 2012), or internuclear exchange of chromosomes (Dutcher 1981) may occur. To reduce the likelihood

Figure 4 Phenotypic variance under five environmental conditions. The components of variance due to mitotype (yellow), nuclear genetic background (white), and mt-n epistasis (red) are indicated in each bar graph. The proportion of variance contributed by each component is shown in pie charts, noting significance. *** $P < 0.001$.

of selecting strains that were genetic recombinants or aneuploid, each strain was created in replicate, phenotyped at six independent genetic markers (five nuclear and one mitochondrial), and cured for endogenous prions. While a rare mutation affecting phenotype cannot be completely ruled out, the robust experimental design used here should allow for unambiguous assignment of phenotypes due to mitochondrial or nuclear effects or epistatic interactions between the two.

We measured growth rates in five different environmental conditions and determined the impact of nuclear genotype, mitochondrial haplotypes, and mt-n epistatic interactions on fitness. We found that each mtDNA variant strongly impacted fitness when paired with each nuclear genotype under at least one condition. The effect of each mitotype depended on the autosomal background; *i.e.*, epistatic interactions between mitochondrial and nuclear genomes contributed to phenotypic variances.

We found that the frequency of observing mt-n epistasis was overall very high (Figure 5B) and dependent on the environment. We most frequently observed epistatic interactions in nonfermentable medium (96% of all tests), suggesting that the efficiencies of OXPHOS energy production are heavily influenced through interactions of naturally occurring mt and nuclear genome variants. Epistatic interactions were observed even when cells were grown in fermentable, glucose-containing medium (22% of all tests), despite the fact that catabolite repression limits mitochondrial respiration in this environment. Thus, mitochondrial energy production, altered through allelic variation, is im-

portant even when the majority of energy is obtained through alternate metabolic pathways. This is consistent with the fact that respiratory-deficient yeasts show a “petite” phenotype when grown on fermentable sugars. These results suggest the ubiquity of mt-n epistasis in natural populations of yeasts.

Stress responses, such as high temperature, are known to reveal substantial phenotypic variation among wild isolates of *S. cerevisiae* yeasts (Kvitek *et al.* 2008; Li *et al.* 2012). We observed the largest amount of phenotypic variance when cells were grown under high temperature stress (37°) and that mt-n epistasis explained a significant component of this phenotypic variance (Figure 4). In yeast, high temperature increases oxidative stress produced by the electron transport chain (Davidson and Schiestl 2001). We found that endogenous levels of ROS were influenced by mt-n interactions for a number of strains at high temperature (Figure 7). This is consistent with the idea that elevated temperatures increase the oxidative damage produced by incompatible mt-n complexes. It has also been noted that temperature exacerbates the phenotypic consequences of mt-n incompatibilities in arthropods (Dowling *et al.* 2007a; Arnqvist *et al.* 2010; Koevoets *et al.* 2012b; Hoekstra *et al.* 2013). It is thought that temperature stress destabilized substrates of heat-shock proteins in yeast, taxing their phenotypic buffering capacity (Jarosz and Lindquist 2010). While speculative, our results are also consistent with the idea that destabilization of incompatible mt-n protein complexes overwhelmed the innate chaperone and quality control systems.

Figure 5 Evidence for coadapted mt-n genomes. (A) Average growth rates of strains with native (red) or non-native (gray) mt-n genome combinations under different media conditions. (B) Strains containing native mt-n genome combinations are shown as circles color-coded by isolation habitat. Circles are connected by bold lines when significant mt-n epistasis was noted following an exchange of mtDNA between the two strains (two-way ANOVA, $P < 0.05$). mt haplotype exchanges that did not reveal epistasis are shown as gray lines. The percentage of all significant epistasis tests is shown for each condition. Missing data, due to occasional strain flocculation, prevented certain tests from being performed and are indicated by an absence of lines or gray circles: orange (clinical), blue (environmental), green (fermentation). (C) The frequency of observed mito-nuclear epistasis (two-way ANOVA, $P < 0.05$) when interrupting native (red) or non-native (gray) mt-n genome combinations. (D) The average magnitude of the epistatic response ($\Delta\Delta V_{\max}$) when interrupting native (red) or non-native (gray) mt-n genome combinations. $\Delta\Delta V_{\max}$ was measured as the absolute value of the difference in slopes from two-way interaction plots containing four different mt-n combinations. Native tests contain two native mito-nuclear combinations and the corresponding non-natives while non-native tests contain four strains containing two unique mt and n genomes with non-native mito-nuclear combinations. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

We observed much less phenotypic variance when cells were grown under strict respiratory conditions than when grown at high temperature. This was consistent with comparable colony sizes of all strains on solid EG medium (not shown). Thus, every mt-n pairing was capable of producing functional OXPHOS complexes. Presumably, defects in respiratory growth would offer a large selective disadvantage and would purge the strongest incompatibilities that arise in natural populations. Still, phenotypic variation did exist under respiratory conditions, and mt-n epistasis explained 40% of this variance. In nature, these segregating mt and n genome variants likely lead to selectable differences in respiratory growth.

Mitochondrial and nuclear genome interactions had subtle, but statistically significant, effects when cells were grown by fermentation. Here, mt-n epistasis explained only 3% of the phenotypic variance, consistent with the fact that mitochondrial functions are reduced during catabolite repression. When oxidative stress was induced by the addition of paraquat in fermenting cells, the epistatic contribution increased to 16%, consistent with the role of mitochondria in ROS production. In respiring cells, addition of paraquat actually decreased the contribution of mt-n epistasis to phenotypic variances. As ROS is produced through the respiratory electron transport chain, it is possible that paraquat increased ROS levels to a threshold that induced expression of nuclear-encoded stress response systems, thus

increasing the observed contribution of nuclear genotypes to phenotypic variances. Transcriptional responses in these yeasts have not yet been explored. We also observed that phenotypic variances were low in media containing paraquat, in line with previous observations that wild yeasts displayed low levels of phenotypic variances in similar oxidative stress conditions (Kvitek *et al.* 2008).

In all environments, we found that interactions between mt and n genomes had a larger influence on growth than mitotypes alone. Main effects of mitotype were most pronounced at elevated temperatures, explaining 10% of the overall fitness variances. This seems to be influenced by a number of mitotypes (from strains C₁, E₁, D₁, F₂, and F₅) that provided strong fitness benefits in certain nuclear backgrounds (Figure 2C). These strong fitness benefits were not uniform across all nuclear backgrounds and in some cases had a striking negative effect (e.g., C₄ nuclear background with mtDNA from E₁), indicating that mt-n epistasis can overwhelm even strong mt haplotype effects. While independent effects of *Drosophila* mitotypes are frequently noted (Christie *et al.* 2011; Pichaud *et al.* 2012, 2013), experiments designed to examine multiple mitotypes in different nuclear backgrounds have found that mt-n epistasis is a more important predictor of phenotype (Dowling *et al.* 2007b; Montooth *et al.* 2010). The impact of mt-n epistasis may explain why fitness effects associated with human mt haplotypes are often difficult to replicate.

Figure 6 Interaction plot showing $V_{\max}$ for native and non-native mt-n genome combinations from different ecotypes grown in EG media. Native combinations outperformed non-native combinations for fermentation and clinical strains ($P < 0.001$). Native fermentation strains performed the best with a strong interaction between native status and ecotype ($P = 0.008$).

Coadaptation of mt-n complexes

The reduced variances that we observed under strict respiratory growth conditions suggested that selection promotes coadaptation of harmonious mt-n complexes. We found that naturally occurring mt-n genomic combinations offered large fitness advantages during respiratory growth and temperature stress (Figure 5A), possibly indicating the presence of coadapted gene pools. There were small, but statistically discernible, fitness disadvantages of native mt-n combinations under other conditions. While the natural environments experienced by yeast likely include temperature shifts and changes in sugar densities, the physiological relevance of each environment is not entirely clear, and so it is difficult to interpret the importance of this potential fitness trade-off. The reciprocal exchange of mitotypes between strains C_1 and C_4 led to decreased fitness and increased ROS (Figure 7), supporting the idea that coadapted mt-n complexes exist in yeast and that selection in mt-n interactions occurs at high temperature.

The interruption of potentially coadapted mt-n complexes did not always lead to predictable phenotypes. The mitotypes from C_1 and F_2 belong to the “high fitness” Tukey grouping from mtDNAs (Figure 3) and may share common mtDNA sequences leading to strong main effects that diminish negative epistatic effects. Consistent with this, replacing the mitotype in strain C_1 with the F_2 mitotype had no effect on growth rates (Figure 7C). However, despite no change in growth, ROS levels were greatly increased (Figure 7D). In parallel, replacing the mitotype in strain F_2 with the C_1 mitotype led to a fitness decrease, but no significant difference in intracellular ROS. It is likely that coadapted mt-n complexes will belong to a variety of pathways, not all leading to imbalances in ROS homeostasis. Previously mapped interspecific mt-n incompatibilities have exposed numerous mechanisms, including mitochondrial transcription (Gaspari

et al. 2004), intron processing (Chou *et al.* 2010), protein translation (Lee *et al.* 2008; Meiklejohn *et al.* 2013), and post-translational modifications (Chou *et al.* 2010).

Genetic diversity and global population structures of *S. cerevisiae* yeasts have been revealed through numerous large-scale sequencing projects (Liti *et al.* 2009; Wang *et al.* 2012; Cromie *et al.* 2013). We did not find evidence that the genetic distances between the original strains correlated with their phenotypes (Figure S4). This is in contrast to previous studies that observed correlations of these same strains as part of larger collections (Liti *et al.* 2009; Halfmann *et al.* 2012). This discrepancy is most likely due to the fact that conditions resulting in extreme phenotypic variances (such as metal toxicities and high molar salt stress) were included in their studies, but could also be due to the lack of power in detecting correlations with the relatively small number of original strains used here. We also found no correlation between the likelihood of observing mt-n epistasis, or between the magnitudes of the epistatic effect, with genetic distances (Figure S5). Indeed, one of the strongest epistatic responses was seen between the closely related strains C_1 and C_4 at 37°. Similarly, genetic distance has not been a reliable predictor of epistatic effects in *Drosophila* (Montooth *et al.* 2010).

These strains used in this study were originally isolated from a number of habitats and include environmental strains from tree or soil sources (E_1 , E_2 , E_4), clinical strains from human patients (C_1 – C_4), and domesticated strains isolated from commercial fermentations (F_1 , F_2 , F_5). We did not find evidence that niches have selected for common mt-n alleles; there was no difference in the frequency of observing epistasis or magnitude of epistatic effects when mtDNAs were exchanged between strains isolated from similar or different habitats. Low statistical power diminishes the impact of these nonconclusive observations, but it is interesting to note that the fitness consequences of disrupting the native mt-n genome combinations in fermentation strains were greater than similar disruptions in clinical or environmental isolates (Figure 6). It is likely that strong selection imposed by human activities has led to coadapted mt-n complexes that provide improved respiratory growth in addition to improved fermentations. *S. cerevisiae* has been isolated from immune-compromised patients, but is generally not considered a pathogen. In support of this, we did not find evidence of niche-specific temperature adaptation in the clinical strains studied here, consistent with earlier observations (Liti *et al.* 2009).

Because the exchange of mtDNAs between genetically similar strains led to strong epistatic effects, it is tempting to think that a small number of mt-n interactions are involved in the epistatic response. In hybrids, mt-n incompatibilities have been mapped to single loci (Lee *et al.* 2008; Barr and Fishman 2010; Chou *et al.* 2010; Gibson *et al.* 2013; Meiklejohn *et al.* 2013), consistent with the idea that intergenomic incompatibilities are due to a small

Figure 7 Coadapted mt-n genome pairs influence ROS production. Interaction plots of (A) growth rates at 37° of C₁ and C₄ nuclear backgrounds paired with C₁ and C₄ mitotypes; (B) endogenous ROS values at 37° for strains in A; (C) growth rates of C₁ and F₂ nuclear backgrounds paired with C₁ and F₂ mitotypes; and (D) ROS values for strains in C. The *P*-values are provided for the nuclear (P_n), mitochondrial (P_{mt}), and interaction (P_{mtxn}) components of two-way ANOVAs, nesting biological replicate within the interaction term. ****P* < 0.001, ***P* < 0.01, **P* < 0.05.

number of interactions with large effect. There are only two published complete mtDNA sequences for *S. cerevisiae* yeasts (Foury *et al.* 1998; Wei *et al.* 2007), and so the numbers of sequence polymorphisms between the mitotypes used in this study are unknown. The mitochondrial genomes of yeasts are much larger than those of other eukaryotes (except plants) and are thought to be highly polymorphic, varying widely in intron content and intergenic nucleotide sequences. Population structures of *Saccharomyces* mtDNAs are not known, and while our analysis of mtDNA-coding sequences suggests that mtDNAs follow similar (but faster) divergence patterns as nuclear genomes, mtDNAs with variable RFLP patterns can be found within a single niche (R. Futia and H. Fiumera unpublished observations). It will be important to develop mapping strategies to identify the mt and nuclear loci involved in these intraspecific mt-n epistatic responses.

Understanding how mitochondrial haplotypes interact with nuclear genotypes and environmental conditions will greatly improve mitochondrial disease diagnoses and the development of beneficial treatments in addition to understanding coevolutionary processes. Our study introduces *S. cerevisiae* yeasts as a model for future studies on mt-n epistasis by revealing that, in natural populations, intergenomic interactions are frequent phenomena that lead to selectable, quantitative trait differences.

Acknowledgments

We thank Thomas D. Fox for providing the *KAR1-1* and D273-10B reference strains, Susan L. Bane for use of a fluorescence reader, and members of the H.L.F. lab for discussions. This work was supported by National Institutes of Health grant GM10132 (to H.L.F.).

Literature Cited

- Arnqvist, G., D. K. Dowling, P. Eady, L. Gay, T. Tregenza *et al.*, 2010 Genetic architecture of metabolic rate: environment specific epistasis between mitochondrial and nuclear genes in an insect. *Evolution* 64: 3354–3363.
- Barr, C. M., and L. Fishman, 2010 The nuclear component of a cytonuclear hybrid incompatibility in *Mimulus* maps to a cluster of pentatricopeptide repeat genes. *Genetics* 184: 455–465.
- Barrientos, A., S. Muller, R. Dey, J. Wienberg, and C. T. Moraes, 2000 Cytochrome *c* oxidase assembly in primates is sensitive to small evolutionary variations in amino acid sequence. *Mol. Biol. Evol.* 17: 1508–1519.
- Bayona-Bafaluy, M. P., S. Muller, and C. T. Moraes, 2005 Fast adaptive coevolution of nuclear and mitochondrial subunits of ATP synthetase in orangutan. *Mol. Biol. Evol.* 22: 716–724.
- Betancourt, A. M., A. L. King, J. L. Fetterman, T. Millender-Swain, R. D. Finley *et al.*, 2014 Mitochondrial-nuclear genome interactions in non-alcoholic fatty liver disease in mice. *Biochem. J.* 461: 223–232.

- Bonnefoy, N., C. Remacle, and T. D. Fox, 2007 Genetic transformation of *Saccharomyces cerevisiae* and *Chlamydomonas reinhardtii* mitochondria. *Methods Cell Biol.* 80: 525–548.
- Chou, J. Y., Y. S. Hung, K. H. Lin, H. Y. Lee, and J. Y. Leu, 2010 Multiple molecular mechanisms cause reproductive isolation between three yeast species. *PLoS Biol.* 8: e1000432.
- Christie, J. S., A. Picornell, A. Moya, M. M. Ramon, and J. A. Castro, 2011 Mitochondrial DNA effects on fitness in *Drosophila subobscura*. *Heredity (Edinb)* 107: 239–245.
- Clancy, D. J., 2008 Variation in mitochondrial genotype has substantial lifespan effects which may be modulated by nuclear background. *Aging Cell* 7: 795–804.
- Cromie, G. A., K. E. Hyma, C. L. Ludlow, C. Garmendia-Torres, T. L. Gilbert *et al.*, 2013 Genomic sequence diversity and population structure of *Saccharomyces cerevisiae* assessed by RAD-seq. *G3 (Bethesda)* 3: 2163–2171.
- Cubillos, F. A., E. J. Louis, and G. Liti, 2009 Generation of a large set of genetically tractable haploid and diploid *Saccharomyces* strains. *FEMS Yeast Res.* 9: 1217–1225.
- Davidson, J. F., and R. H. Schiestl, 2001 Mitochondrial respiratory electron carriers are involved in oxidative stress during heat stress in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 21: 8483–8489.
- Defontaine, A., F. M. Lecocq, and J. N. Hallet, 1991 A rapid miniprep method for the preparation of yeast mitochondrial DNA. *Nucleic Acids Res.* 19: 185.
- Dey, R., A. Barrientos, and C. T. Moraes, 2000 Functional constraints of nuclear-mitochondrial DNA interactions in xenomito-chondrial rodent cell lines. *J. Biol. Chem.* 275: 31520–31527.
- Doudican, N. A., B. Song, G. S. Shadel, and P. W. Doetsch, 2005 Oxidative DNA damage causes mitochondrial genomic instability in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 25: 5196–5204.
- Dowling, D. K., K. C. Abiega, and G. Arnqvist, 2007a Temperature-specific outcomes of cytoplasmic-nuclear interactions on egg-to-adult development time in seed beetles. *Evolution* 61: 194–201.
- Dowling, D. K., U. Friberg, F. Hailer, and G. Arnqvist, 2007b Intergenomic epistasis for fitness: within-population interactions between cytoplasmic and nuclear genes in *Drosophila melanogaster*. *Genetics* 175: 235–244.
- Dutcher, S. K., 1981 Internuclear transfer of genetic information in *kar1-1/KAR1* heterokaryons in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 1: 245–253.
- Ellison, C. K., and R. S. Burton, 2006 Disruption of mitochondrial function in interpopulation hybrids of *Tigriopus californicus*. *Evolution* 60: 1382–1391.
- Ellison, C. K., and R. S. Burton, 2008a Genotype-dependent variation of mitochondrial transcriptional profiles in interpopulation hybrids. *Proc. Natl. Acad. Sci. USA* 105: 15831–15836.
- Ellison, C. K., and R. S. Burton, 2008b Interpopulation hybrid breakdown maps to the mitochondrial genome. *Evolution* 62: 631–638.
- Ellison, C. K., and R. S. Burton, 2010 Cytonuclear conflict in interpopulation hybrids: the role of RNA polymerase in mtDNA transcription and replication. *J. Evol. Biol.* 23: 528–538.
- Ellison, C. K., O. Niehuis, and J. Gadau, 2008 Hybrid breakdown and mitochondrial dysfunction in hybrids of *Nasonia* parasitoid wasps. *J. Evol. Biol.* 21: 1844–1851.
- Felsenstein, J., 2005 *PHYLIP (Phylogeny Inference Package) version 3.6*. Distributed by the author, Department of Genome Sciences, University of Washington, Seattle.
- Fetterman, J. L., B. R. Zelikson, L. W. Johnson, D. R. Moellering, D. G. Westbrook *et al.*, 2013 Mitochondrial genetic background modulates bioenergetics and susceptibility to acute cardiac volume overload. *Biochem. J.* 455: 157–167.
- Foury, F., T. Roganti, N. Lecrenier, and B. Purnelle, 1998 The complete sequence of the mitochondrial genome of *Saccharomyces cerevisiae*. *FEBS Lett.* 440: 325–331.
- Fox, T. D., L. S. Folley, J. J. Mulero, T. W. McMullin, P. E. Thorsness *et al.*, 1991 Analysis and manipulation of yeast mitochondrial genes. *Methods Enzymol.* 194: 149–165.
- Gaspari, M., M. Falkenberg, N. G. Larsson, and C. M. Gustafsson, 2004 The mitochondrial RNA polymerase contributes critically to promoter specificity in mammalian cells. *EMBO J.* 23: 4606–4614.
- Gibson, J. D., O. Niehuis, B. R. Peirson, E. I. Cash, and J. Gadau, 2013 Genetic and developmental basis of F2 hybrid breakdown in *Nasonia* parasitoid wasps. *Evolution* 67: 2124–2132.
- Guthrie, C., and G. R. Fink, 1991 Guide to yeast genetics and molecular biology. *Methods Enzymol.* 194: 1–863.
- Halfmann, R., D. F. Jarosz, S. K. Jones, A. Chang, A. K. Lancaster *et al.*, 2012 Prions are a common mechanism for phenotypic inheritance in wild yeasts. *Nature* 482: 363–368.
- Hittinger, C. T., 2013 *Saccharomyces* diversity and evolution: a budding model genus. *Trends Genet.* 29: 309–317.
- Hoekstra, L. A., M. A. Siddiq, and K. L. Montooth, 2013 Pleiotropic effects of a mitochondrial-nuclear incompatibility depend upon the accelerating effect of temperature in *Drosophila*. *Genetics* 195: 1129–1139.
- Hu, J., W. Huang, Q. Huang, X. Qin, C. Yu *et al.*, 2014 Mitochondria and cytoplasmic male sterility in plants. *Mitochondrion*. DOI:10.1016/j.mito.2014.02.008.
- Jarosz, D. F., and S. Lindquist, 2010 Hsp90 and environmental stress transform the adaptive value of natural genetic variation. *Science* 330: 1820–1824.
- Jenuth, J. P., A. C. Peterson, and E. A. Shoubridge, 1997 Tissue-specific selection for different mtDNA genotypes in heteroplasmic mice. *Nat. Genet.* 16: 93–95.
- Koevoets, T., O. Niehuis, L. van de Zande, and L. W. Beukeboom, 2012a Hybrid incompatibilities in the parasitic wasp genus *Nasonia*: negative effects of hemizygoty and the identification of transmission ratio distortion loci. *Heredity (Edinb)* 108: 302–311.
- Koevoets, T., L. van de Zande, and L. W. Beukeboom, 2012b Temperature stress increases hybrid incompatibilities in the parasitic wasp genus *Nasonia*. *J. Evol. Biol.* 25: 304–316.
- Kvitek, D. J., J. L. Will, and A. P. Gasch, 2008 Variations in stress sensitivity and genomic expression in diverse *S. cerevisiae* isolates. *PLoS Genet.* 4: e1000223.
- Lee, H. Y., J. Y. Chou, L. Cheong, N. H. Chang, S. Y. Yang *et al.*, 2008 Incompatibility of nuclear and mitochondrial genomes causes hybrid sterility between two yeast species. *Cell* 135: 1065–1073.
- Li, C. M., J. N. Tzeng, and H. M. Sung, 2012 Effects of cis and trans regulatory variations on the expression divergence of heat shock response genes between yeast strains. *Gene* 506: 93–97.
- Li, L., and A. S. Kowal, 2012 Environmental regulation of prions in yeast. *PLoS Pathog.* 8: e1002973.
- Liepins, A., and S. Hennen, 1977 Cytochrome oxidase deficiency during development of amphibian nucleocytoplasmic hybrids. *Dev. Biol.* 57: 284–292.
- Liti, G., D. M. Carter, A. M. Moses, J. Warringer, L. Parts *et al.*, 2009 Population genomics of domestic and wild yeasts. *Nature* 458: 337–341.
- Louvel, H., A. Gillet-Markowska, G. Liti, and G. Fischer, 2014 A set of genetically diverged *Saccharomyces cerevisiae* strains with markerless deletions of multiple auxotrophic genes. *Yeast* 31: 91–101.
- McKenzie, M., M. Chiotis, C. A. Pinkert, and I. A. Trounce, 2003 Functional respiratory chain analyses in murine xenomito-chondrial cybrids expose coevolutionary constraints of cyto-

- chrome b and nuclear subunits of complex III. *Mol. Biol. Evol.* 20: 1117–1124.
- Meiklejohn, C. D., M. A. Holmbeck, M. A. Siddiq, D. N. Abt, D. M. Rand *et al.*, 2013 An incompatibility between a mitochondrial tRNA and its nuclear-encoded tRNA synthetase compromises development and fitness in *Drosophila*. *PLoS Genet.* 9: e1003238.
- Montooth, K. L., C. D. Meiklejohn, D. N. Abt, and D. M. Rand, 2010 Mitochondrial-nuclear epistasis affects fitness within species but does not contribute to fixed incompatibilities between species of *Drosophila*. *Evolution* 64: 3364–3379.
- Morano, K. A., C. M. Grant, and W. S. Moye-Rowley, 2012 The response to heat shock and oxidative stress in *Saccharomyces cerevisiae*. *Genetics* 190: 1157–1195.
- Niehuis, O., A. K. Judson, and J. Gadau, 2008 Cytonuclear genic incompatibilities cause increased mortality in male F2 hybrids of *Nasonia giraulti* and *N. vitripennis*. *Genetics* 178: 413–426.
- Parmakelis, A., P. Kotsakiozi, and D. Rand, 2013 Animal mitochondria, positive selection and cyto-nuclear coevolution: insights from pulmonates. *PLoS ONE* 8: e61970.
- Pichaud, N., J. W. Ballard, R. M. Tanguay, and P. U. Blier, 2012 Naturally occurring mitochondrial DNA haplotypes exhibit metabolic differences: insight into functional properties of mitochondria. *Evolution* 66: 3189–3197.
- Pichaud, N., J. W. Ballard, R. M. Tanguay, and P. U. Blier, 2013 Mitochondrial haplotype divergences affect specific temperature sensitivity of mitochondrial respiration. *J. Bioenerg. Biomembr.* 45: 25–35.
- Prochazka, E., F. Franko, S. Polakova, and P. Sulo, 2012 A complete sequence of *Saccharomyces paradoxus* mitochondrial genome that restores the respiration in *S. cerevisiae*. *FEMS Yeast Res.* 12: 819–830.
- Rand, D. M., A. Fry, and L. Sheldahl, 2006 Nuclear-mitochondrial epistasis and drosophila aging: introgression of *Drosophila simulans* mtDNA modifies longevity in *D. melanogaster* nuclear backgrounds. *Genetics* 172: 329–341.
- R Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL <http://www.R-project.org/>.
- Roubertoux, P. L., F. Sluyter, M. Carlier, B. Marcet, F. Maarouf-Veray *et al.*, 2003 Mitochondrial DNA modifies cognition in interaction with the nuclear genome and age in mice. *Nat. Genet.* 35: 65–69.
- Sackton, T. B., R. A. Haney, and D. M. Rand, 2003 Cytonuclear coadaptation in *Drosophila*: disruption of cytochrome c oxidase activity in backcross genotypes. *Evolution* 57: 2315–2325.
- Skelly, D. A., G. E. Merrihew, M. Riffle, C. F. Connelly, E. O. Kerr *et al.*, 2013 Integrative phenomics reveals insight into the structure of phenotypic diversity in budding yeast. *Genome Res.* 23: 1496–1504.
- Sulo, P., M. Spirek, A. Soltesova, G. Marinoni, and J. Piskur, 2003 The efficiency of functional mitochondrial replacement in *Saccharomyces* species has directional character. *FEMS Yeast Res.* 4: 97–104.
- Tranah, G. J., 2011 Mitochondrial-nuclear epistasis: implications for human aging and longevity. *Ageing Res. Rev.* 10: 238–252.
- Trier, C. N., J. S. Hermansen, G. P. Saetre, and R. I. Bailey, 2014 Evidence for mito-nuclear and sex-linked reproductive barriers between the hybrid Italian sparrow and its parent species. *PLoS Genet.* 10: e1004075.
- Wallace, D. C., and D. Chalkia, 2013 Mitochondrial DNA genetics and the heteroplasmy conundrum in evolution and disease. *Cold Spring Harb. Perspect. Biol.* 5: a021220.
- Wang, Q. M., W. Q. Liu, G. Liti, S. A. Wang, and F. Y. Bai, 2012 Surprisingly diverged populations of *Saccharomyces cerevisiae* in natural environments remote from human activity. *Mol. Ecol.* 21: 5404–5417.
- Wei, W., J. H. McCusker, R. W. Hyman, T. Jones, Y. Ning *et al.*, 2007 Genome sequencing and comparative analysis of *Saccharomyces cerevisiae* strain YJM789. *Proc. Natl. Acad. Sci. USA* 104: 12825–12830.
- Wolff, J. N., E. D. Ladoukakis, J. A. Enriquez, and D. K. Dowling, 2014 Mitonuclear interactions: evolutionary consequences over multiple biological scales. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 369:
- Yamaoka, M., K. Isobe, H. Shitara, H. Yonekawa, S. Miyabayashi *et al.*, 2000 Complete repopulation of mouse mitochondrial DNA-less cells with rat mitochondrial DNA restores mitochondrial translation but not mitochondrial respiratory function. *Genetics* 155: 301–307.
- Zakharov, I. A., and B. P. Yarovoy, 1977 Cytofusion as a new tool in studying the cytoplasmic heredity in yeast. *Mol. Cell. Biochem.* 14: 15–18.
- Zeyl, C., B. Andreson, and E. Weninck, 2005 Nuclear-mitochondrial epistasis for fitness in *Saccharomyces cerevisiae*. *Evolution* 59: 910–914.
- Zhang, F., and R. E. Broughton, 2013 Mitochondrial-nuclear interactions: Compensatory evolution or variable functional constraint among vertebrate oxidative phosphorylation genes? *Genome Biol. Evol.* 5: 1781–1791.
- Zhu, C. T., P. Ingelmo, and D. M. Rand, 2014 GxGxE for lifespan in *Drosophila*: mitochondrial, nuclear, and dietary interactions that modify longevity. *PLoS Genet.* 10: e1004354.

Communicating editor: J. B. Wolf

GENETICS

Supporting Information

<http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.114.168575/-/DC1>

Mitochondrial-Nuclear Epistasis Contributes to Phenotypic Variation and Coadaptation in Natural Isolates of *Saccharomyces cerevisiae*

Swati Paliwal, Anthony C. Fiumera, and Heather L. Fiumera

Figure S1 Karyogamy-deficient matings were used to transfer mitochondria between genetic backgrounds. Nuclear backgrounds are depicted as colored rings. Mitochondria are depicted as curved grey lines and MtDNA as red dots within the mitochondrial networks. (A) A strain containing mitotype, Mt-1, was mated to a *KAR1-1* ρ^0 strain (lacking mtDNA). The *KAR1-1* allele reduces nuclear fusion in the zygotes. Cytoductants containing mtDNA from Nuclear-1 and the *KAR1-1* nuclear background were identified through selection and screening of appropriate auxotrophies. (B) Mitotype Mt-1 is subsequently transferred to a new genetic background (Nuclear-2) through similar strategies.

Figure S2 Phylogenetic relationships between yeast isolates are shown through a neighbor-joining tree based on nuclear SNP differences (LITI et al. 2009). Isolation sources are indicated. Scale bar indicates frequency of base-pair differences.

Figure S3 Growth rate distributions in CSM media ($n \geq 4$) of ten *S. cerevisiae* strains (white boxes) compared to strains where the mt-n genome combinations were recreated (light gray boxes) or cured of prions (dark gray boxes). Box plots show the 25th percentile (lower edge), median (solid line), 75th percentile (upper edge), and 90th percentile (whisker). Outliers are shown as empty circles.

Figure S4 Phenotypic differences (ΔV_{max}) between strains plotted by genetic distance. The scales in each plot are the same to show the difference in overall phenotypic variation. The non-significant Pearson's correlation coefficients are given.

Figure S5 Magnitude of epistatic effects do not correlate with genetic distance. Magnitude was determined as the absolute difference of the slopes from interaction plots ($\Delta\Delta V_{\max}$), considering each possible exchange of mtDNAs between the original 10 strains. The non-significant Pearson's correlation coefficients are provided.

Figure S6 Growth rate distributions of strains containing native or non-native mt-n genome pairs, separated by isolation habitat. Each box indicates the 25th percentile (lower edge), median (solid line), 75th percentile (upper edge) and 90th percentile (whisker). Note the that fitness scale on the y axis is not the same for each plot. Significant differences between native and non-native mt-n genome combinations, as determined by ANOVAs are indicated. ***<0.001, **<0.01 (A) CSM (B) EG (C) 37°C

Table S1 Strain Table

Strain Designation	Ecotype	Strain Name	Parental Strain	Genotype	Source
C1	Clinical	NCYC3594 (CUBILLOS <i>et al.</i> 2009)	YJM975 (LITI <i>et al.</i> 2009)	<i>MAT a</i> <i>ho::HygMX</i> <i>ura3::KanMX</i>	National Collection of Yeast Cultures (NCYC)
C2	Clinical	NCYC3585 (CUBILLOS <i>et al.</i> 2009)	273614N (LITI <i>et al.</i> 2009)	<i>MAT a</i> <i>ho::HygMX</i> <i>ura3::KanMX</i>	NCYC
C3	Clinical	NCYC3587 (CUBILLOS <i>et al.</i> 2009)	322134S (LITI <i>et al.</i> 2009)	<i>MAT a</i> <i>ho::HygMX</i> <i>ura3::KanMX</i>	NCYC
C4	Clinical	NCYC3593 (CUBILLOS <i>et al.</i> 2009)	YJM981 (LITI <i>et al.</i> 2009)	<i>MAT a</i> <i>ho::HygMX</i> <i>ura3::KanMX</i>	NCYC
E1	Environmental	NCYC3606 (CUBILLOS <i>et al.</i> 2009)	YPS606 (LITI <i>et al.</i> 2009)	<i>MAT a</i> <i>ho::HygMX</i> <i>ura3::KanMX</i>	NCYC
E2	Environmental	NCYC3595 (CUBILLOS <i>et al.</i> 2009)	DBVPG1373 (LITI <i>et al.</i> 2009)	<i>MAT a</i> <i>ho::HygMX</i> <i>ura3::KanMX</i>	NCYC
E4	Environmental	NCYC3588 (CUBILLOS <i>et al.</i> 2009)	Y55 (LITI <i>et al.</i> 2009)	<i>MAT a</i> <i>ho::HygMX</i> <i>ura3::KanMX</i>	NCYC
F1	Fermentation	NCYC3600 (CUBILLOS <i>et al.</i> 2009)	DBVPG6044 (LITI <i>et al.</i> 2009)	<i>MAT a</i> <i>ho::HygMX</i> <i>ura3::KanMX</i>	NCYC
F2	Fermentation	NCYC3599 (CUBILLOS <i>et al.</i> 2009)	L-1528 (LITI <i>et al.</i> 2009)	<i>MAT a</i> <i>ho::HygMX</i> <i>ura3::KanMX</i>	NCYC
F5	Fermentation	NCYC3605 (CUBILLOS <i>et al.</i> 2009)	Y12 (LITI <i>et al.</i> 2009)	<i>MAT a</i> <i>ho::HygMX</i> <i>ura3::KanMX</i>	NCYC
		D273-10B		<i>MAT α</i>	ATCC 24657
		NAB32 ρ^0		<i>MAT α</i> <i>KAR1-1 ade2</i> <i>arg8 ρ⁰</i>	Gift from T.D. Fox

Table S2 Independent Effects of Mitochondrial Haplotypes and Nuclear Backgrounds.

Condition	CSM	37°C	PQ	EG	EG+PQ
<i>P values</i>					
A tests for mt effects					
n C1	***	***	***	***	***
n C2	*	0.079	***	***	***
n C3	0.246	*	na	0.469	na
n C4	***	*	**	***	***
n E1	***	***	***	***	***
n E2	***	*	***	***	***
n E4	*	0.274	0.073	***	***
n F1	*	***	**	***	0.067
n F2	***	***	*	***	***
n F5	***	***	**	na	na
B tests for n effects					
mt C1	***	***	***	***	***
mt C2	***	***	***	***	***
mt C3	***	***	***	***	***
mt C4	***	***	***	***	***
mt E1	***	***	***	***	***
mt E2	***	***	***	***	***
mt E4	***	***	***	***	***
mt F1	***	***	***	***	***
mt F2	***	***	***	***	***
mt F5	***	***	***	***	***

Significance codes: <0.001 ***, < .01 **, < 0.05 *

(A) The extent that mitochondrial haplotypes altered the growth rates of each nuclear genotype was evaluated through ANOVAs, considering the effect of mt on fitness of each nuclear background. Mitotypes were treated as random effects. (B) Similar analyses were performed to show that nuclear genetic backgrounds influenced growth rates afforded by each mitotype.