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

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

Department of Biological Sciences, Binghamton University, Binghamton, NY, 13902
*Corresponding author

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 mtDNAs 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 in 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 (ROS) 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.

INTRODUCTION

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 rRNAs and tRNAs. The nuclear genome encodes nearly one thousand proteins that are imported to the organelle where they comprise the majority of the mitochondrial proteome. Specific interactions between components of both genomes are required at many levels, including 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 nuclear and mitochondrially encoded proteins. Over evolutionary time, these interactions have been optimized, in part, to regulate production of the reactive oxygen species that are the byproducts of mitochondrial respiration.

Allelic variation in both genomes can affect mitochondrial-nuclear (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 lifespan (CLANCY 2008; ZHU *et al.* 2014). Interpopulation hybrids of the marine copepods, *Tigriopus*, suffered compromised OXPHOS capacities (ELLISON and BURTON 2006; ELLISON and BURTON 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).

The importance of mt-n epistasis extends well past arthropods. In mice, mt-n epistasis is known to affect cognition (ROUBERTOUX et al. 2003), reactive oxygen species (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). Mt-n epistasis likely contributes 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 it is important in most eukaryotes.

Between species, incompatibilities between mitochondrial and nuclear genomes can be quite severe. Introgession of mtDNAs from Drosophila simulans to D. melanogaster had significant effects on respiratory complexes (SACKTON et al. 2003), development (MONTOTH 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; KOEVOETS et al. 2012a; NIEHUIS et al. 2008). Activities of OXPHOS complexes in human cells 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; MCKENZIE et al. 2003; YAMAOKA et al. 2000) or amphibian (LIEPINS and HENNEN 1977) xenomitochondrial cybrids were drastically reduced. Mt-n incompatibilities between yeasts in the Saccharomyces sensu stricto genus can lead to complete respiratory deficiencies (CHOU et al. 2010; LEE et al. 2008; SULO et al. 2003 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 (MONTOTH 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), though 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, Saccharomyces 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 (CROMIE et al. 2013; HITTINGER 2013; LITI et al. 2009; SKELLY et al. 2013; WANG et al. 2012) 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 sought to determine if epistatic interactions between mitochondrial and nuclear genomes contribute to 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 non-fermentable 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 (ρ₀), cells were passaged twice through CSM medium containing 25 µg/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 4 times on rich medium containing 5 mM guanidine hydrochloride. Relevant genotypes for all strains are shown in Table S1.

**Construction of Mitochondrial-Nuclear Strain Collection**

MtDNAs were exchanged between ten 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 YaroVoY 1977). First, haploid derivatives of each yeast isolate were mated to a strain devoid of mtDNA (i.e. ρ₀) carrying the dominant KAR1-1 mutation that inhibits nuclear fusion during zygote formation (strain NAB32 ρ₀). Following visual inspection of zygote formation in mating mixtures (~ 2-6 hours 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 MAT a tester strain, and no mating with a MAT α tester. MtDNAs were then transferred from the KAR1-1 mtDNA recipient strains to ρ⁺ 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 ρ₀ strains. RFLP analysis of mtDNAs isolated from a random sampling of the final strains matched expected patterns (not shown). See Fig. 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 minute intervals until reaching stationary phase (~48 hours). Growth in five different environmental conditions was monitored: CSM, CSM at 37°C, CSM + 200 µg/ml paraquat, EG, and EG + 50 µg/ml paraquat. Incubation was performed at 30°C unless otherwise indicated. To minimize the effects of anaerobic, non- respiratory growth due to oxygen limitation, microplate cultures were inoculated from actively dividing cells that were pre-cultivated in roller tubes in the appropriate medium (CSM or EG) and grown under continuous double orbital shaking. For culturing in non-fermentable media, 24-well plates were used to maximize oxygen concentrations, while 96-well plates were used for all other media. Maximal growth rates (Vₘₐₓ), 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 microtiter plates were washed in water, resuspended in 10 mM dichlorofluorescein diacetate (from a 5 mM stock in DMSO). Following a 30 minute incubation at 30°C, 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 OD_{600}.

**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 (RCORETEAM 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 biological replicate within each treatment category according to the equation, $y_{ijk} = \mu + O_i + (BR$ within $O_{ij}) + e_{ijk}$, 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 biological replicate nested within origin. Random effects models (lme4 using REML) were used to test for the significance of mitotypes, nuclear backgrounds, environment, and their interactions using the full model, $y_{ijk} = \mu + N_i + M_t + E_k + (N*M)_i + (N*E)_k + (M*E)_j + (N*M*E)_{ijk} + e_{ijk}$, where $y_{ijk}$ is the V_max fitness estimate, and $N_i$, $M_t$, and $E_k$ are random effect 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, $y_{ijk} = \mu + N_i + M_t + (N*M)_i + e_{ijk}$. The main mt (or n) effects were evaluated by considering a single nuclear (or mitochondrial) background at a time. Where appropriate, posthoc 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, $V_{max_{ijk}} = m + A_i + (l$ within $A_{ji}) + e_{ijk}$, 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 was 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 2 unique nuclear and 2 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 mitochondrial-nuclear (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 Fig. 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 ten isolates were genetically distinct, mtDNAs were isolated and subjected to RFLP analysis (Fig. 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 seven of the ten 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 bps (of the ~86000 bp genome) from 8 mitochondrially-encoded ORFs, determined SNP differences, and constructed an unrooted phylogenetic tree (Fig. 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 (Fig. S2). The genetic relatedness of the mtDNAs from these strains are 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 strain creation methodology, we compared the growth rates of the ten original strains to growth rates of strains in which mitotypes were reintroduced to the appropriate parental backgrounds, and of strains cured of prions (Fig. S3). Individual ANOVAS were performed on growth rates (n ≥ 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 (HALFMAN 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 coincidently 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 x N x E (G x G x E) Effects**

To measure of 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 non-fermentable carbons, ethanol and glycerol (EG) that supported growth exclusively through aerobic respiration. Strains were also grown in conditions that stress mitochondrial functions (MORANO et al. 2012) including CSM medium at elevated temperature (37°C) 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 x n x e, P < 0.0001) indicate that specific combinations of mitochondrial and nuclear genomes responded to media conditions differently than others. Phenotypic variation was also influenced by gene x environment interactions, as seen through significant interactions between the environment and nuclear genotype (n x e, P < 0.0001) or mitotype (mt x 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 mitochondrial (mt) and nuclear (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 autosomal genetic background was significant.
in all growth conditions \( (P < 0.0001) \). Mitotypes had significant effects in CSM and at 37°C \( (P < 0.0001) \), but were non-significant 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 x n) had 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 (Fig. 2). In these interaction plots, each line connects the growth rates \( (V_{\text{max}}) \) of a single nuclear genotype as it was paired with the different mtDNAs. No autosomal 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°C when paired with any of the C1, E1, F1, F2 and F5 autosomal backgrounds (Fig. 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°C did not necessarily confer fitness advantages in other media, thus demonstrating mt x n x e interactions.

It is important to note that \( V_{\text{max}} \) 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 \( V_{\text{max}} \) values do not reflect the inability of a strain to grow under a particular condition, but its relative growth to a robust laboratory strain.

### Nuclear genetic backgrounds are influenced by mitotypes.

The strong mt x 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 discernable 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°C, 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°C or PQ, or the F1 nuclear 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 2-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 (Fig. 3A-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°C and 37°C, nuclear genetic backgrounds formed 7 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 x 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. (Fig. 3F-J). At elevated temperature, the means across mitotypes were similar (Fig. 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 x e effects. The phenotypic ranges of individual nuclear and mitochondrial genetic backgrounds varied (as an example, in Fig. 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 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 (Fig. 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 in 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°C, these proportions were expanded to 16% and 10%, respectively. In non-fermentable medium (EG) or when ROS-inducing agents were added to fermentable or non-fermentable media, interactions between mitotypes and genetic background explained a significant proportion of the variances, while independent effects of mitotypes were negligible. Mt-n 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.**

Mt-n pairings 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 co-adapted, 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”) (Fig. 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°C ($V_{max} = 1.9$ vs. $1.5$ 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 increase 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 by exchanging their mtDNAs, resulting in 45 independent tests in each condition. These results are shown in Fig. 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 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 4 strains representing 2 unique nuclear and 2 unique mitochondrial haplotypes with no naturally-occurring mt-n pairings). The frequencies of discerning epistasis when disrupting native and non-native combinations were compared (Fig. 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°C: 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 (Fig. 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 a two-way interaction plots (ΔΔVmax). Epistatic effects were greatest during temperature stress when disrupting both native and non-native mt-n genome combinations than in 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 (HALFMAANN et al. 2012; LITI et al. 2009). In the conditions used in this study, we found no correlation between the pair wise phenotypic differences and genetic distance among the 10 original isolates (Pearson’s correlation coefficients = -0.1 to 0.2, P ≥ 0.1, Fig. S4). We also found no correlation between the relative strength of the epistatic responses (ΔΔVmax) when exchanging mtDNAs between the 10 original strains and their genetic distances (Pearson’s correlation coefficients = -0.1 to 0.2, P ≥ 0.1, Fig. 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 intraspecific molecular divergence.

Strains living in similar environments may undergo similar selective pressures leading to selection of similar phenotypes. Here, we only considered strains whose mt and n genomes originated from similar isolation habitats (clinical, environmental, or fermentation). Ecotype did have a significant effect on growth rate in CSM, EG, and at 37°C (strains with fermentation, clinical, or environmental n and mt genomes showed significantly different growth rates, P < 0.001, Fig. 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°C (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°C 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 (Fig. S6). In EG, there was a strong interaction (P = 0.008) between native status and ecotype with native fermentation strains growing the best (Fig. 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 ≥ 0.2 for all 5 media conditions), nor in the overall magnitude of the epistatic responses (P ≥ 0.3).

**Interruption of coadapted mt-n genomes results in increased ROS**

The mitochondrial respiratory chain is the major intracellular source of reactive oxygen species (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°C. Exchanging mitotypes between strains C1 and C4 showed epistatic effects on growth rates (Fig. 7A) and endogenous ROS levels (Fig. 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 Vmax and lower ROS than C4 with the C1 mitotype (P ≤0.01, post-hoc tests). Similarly, when exchanging mitotypes between strains C1 and F2, mt-n interactions affected both growth rates (Fig. 7C) and ROS (Fig. 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 Fig. 7A 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 Fig. 2C.

DISCUSSION
Intraspecific mt-n epistasis
We provide evidence that epistasis interactions between mitochondrial and nuclear polymorphisms found in ten natural isolates of *S. cerevisiae* yeasts are important contributors to phenotypic variance. In this study, we replaced mtDNAs 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 (BONNEFOY et al. 2007; GUTHRIE and FINK 1991; 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 of selecting strains that were genetic recombinants or aneuploid, each strain was created in replicate, phenotyped at 6 independent genetic markers (5 nuclear and 1 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 genotypes in 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 (Fig. 5B), and dependent on the environment. We most frequently observed epistatic interactions in non-fermentable 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 important 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 (37ºC), and that mt-n epistasis explained a significant component of this phenotypic variance (Fig. 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 (Fig. 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 (ARNQVIST et al. 2010; DOWLING et al. 2007a; HOEKSTRA et al. 2013; KOEVOETS et al. 2012b). It is thought that temperature stress destabilized substrates of heat shock proteins in yeast, taxing their phenotypic buffering capacity (JAROSZ and LINQUIST 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.

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.

Mt-n epistasis 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 mtotypes alone. Main effects of mtotype were most pronounced at elevated temperatures, explaining 10\% of the overall fitness variances. This seems to be influenced by a number of mtotypes (from strains C1, E1, D1, F2, and F5) that provided strong fitness benefits in certain nuclear backgrounds (Fig. 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; PICHAUD et al. 2013), experiments designed to examine multiple mtotypes in different nuclear backgrounds have found that mt-n epistasis is a more important predictor of phenotype (DOWLING et al. 2007b; MONTTOOTH et al. 2010). The impact of mt-n epistasis also explains why fitness effects associated with human mt haplotypes are often difficult to replicate.

Coadaptation of mt-n complexes
The reduced variances 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 (Fig. 5A), potentially indicating the presence of coadapted gene pools. There were small, but statistically discernible, fitness disadvantages of native mt-n combinations in 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 tradeoff. The reciprocal exchange of mtotypes between strains C1 and C4 led to decreased fitness and increased ROS (Fig. 7), supporting the idea that coadapted mt-n complexes exist in yeast, and that selection on mt-n interactions occurs at high temperature.

The interruption of potentially coadapted mt-n complexes did not always lead to predictable phenotypes. The mtotypes from C1 and F2 belong to the “high fitness” Tukey grouping from mtDNAs (Fig. 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 C1 with the F2 mitotype had no effect on growth rates (Fig. 7C). However, despite no change in growth, ROS levels were greatly increased (Fig. 7D). In parallel, replacing the mitotype in strain F2 with the C1 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 (CROMIE et al. 2013; LITI et al. 2009; WANG et al. 2012). We did not find evidence that the genetic distances between the original strains correlated with their phenotypes (Fig. S4). This is in contrast to previous studies that observed correlations of these
same strains, as part of larger collections (HALFMANN et al. 2012; LITI et al. 2009). 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 (Fig. S5). Indeed, one of the strongest epistatic responses was seen between the closely related strains, C1 and C4, at 37°C. Similarly, genetic distance has not been a reliable predictor of epistatic effects in Drosophila (MONTTOOTH 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 (E1, E2, E4), clinical strains from human patients (C1-C4), and domesticated strains isolated from commercial fermentations (F1, F2, F5). 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 non-conclusive 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 (Fig. 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 (BARR and FISHMAN 2010; CHOU et al. 2010; GIBSON et al. 2013; LEE et al. 2008; MEIKLEJOHN et al. 2013), consistent with the idea that intergenomic incompatibilities are due to a small 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 (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.

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Figure 1  MtDNA polymorphisms in *S. cerevisiae*. (A) RFLP analysis of purified mtDNA from ten isolates restricted with *Eco*RV separated by agarose gel electrophoreses (0.8%). Nine unique banding patterns were observed. Banding patterns of strains C1 and C4 are similar. No bands were observed from a $\rho^0$ control, demonstrating specificity of mtDNA purification. (B) Neighbor-joining phlogenetic 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.
Figure 2  Fitness effects of ten mitotypes on ten nuclear backgrounds. Changes in fitness are presented as interaction plots, where each colored line follows the changes in fitness (Vmax) of a single nuclear genetic background when paired with ten different mt haplotypes, as indicated on the x axis, in 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°C) (D) CSM+paraquat (CSM+PQ) (E) EG + PQ. Note that the scale of the axes are different for each media condition.
Figure 3  Growth rate distributions of nuclear genotypes and mitochondrial haplotypes. The box plots in (A)-(E) show the distributions of individual nuclear backgrounds with all ten mitotypes and the box plots in (F)-(J) show the distributions of each mitotype across all nuclear genotypes. Each box plots show 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°C; (D) and (I) CSM + PQ; (E) and (J) EG + PQ.
Figure 3, continued.
Figure 4  Phenotypic variance in 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. Significance codes: <0.001 ***
Figure 5  Evidence for coadapted mt-n genomes. (A) Average growth rates of strains with native (red) or non-native (grey) mt-n genome combinations in 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 2 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 grayed 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 (grey) mt-n genome combinations. (D) The average magnitude of the epistatic response (ΔΔVmax) when interrupting native (red) or non-native (grey) mt-n genome combinations. ΔΔVmax 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 2 native mito-nuclear combinations and the corresponding non-natives while non-native tests contain 4 strains containing two unique mt and n genomes with non-native mito-nuclear combinations. Significance codes: ***<0.001, **<0.01, *<0.05
Figure 6  Interaction plot showing Vmax 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 ($P = 0.008$).
Figure 7  Coadapted mt-n genome pairs influence ROS production. Interaction plots of (A) growth rates at 37°C of C1 and C4 nuclear backgrounds paired with C1 and C4 mitotypes, (B) endogenous ROS values at 37°C 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. Significance codes: ***< 0.001, **< 0.01, *<0.05.
### Table 1  Mitochondrial x Nuclear x Environment Interactions (mt x n x e)

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To determine 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: $n + mt + e + (mt \times n) + (n \times e) + (mt \times e) + (mt \times n \times e)$
Table 2  Mitochondrial x nuclear Interactions (mt x n) within single environments

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full model: n + mt + (mt x n)