Title

Differential gene expression and mitonuclear incompatibilities in fast- and slow-developing interpopulation Tigriopus californicus hybrids

Journal

Molecular Ecology, 32(12)

ISSN

0962-1083

Authors

Healy, Timothy M
Burton, Ronald S

Publication Date

2023-06-01

DOI

10.1111/mec.16917

Peer reviewed

Title Page

Title:
Differential gene expression and mitonuclear incompatibilities in fast- and slow-developing inter-population *Tigriopus californicus* hybrids

Running head:
Gene expression in inter-population hybrids

Authors:
Timothy M. Healy[a] and Ronald S. Burton[a]

Author affiliation:
[a]Marine Biology Research Division, Scripps Institution of Oceanography, University of California San Diego, 9500 Gilman Drive #0202, La Jolla, CA, USA

Corresponding author:
Timothy Healy
Email: healy.timothy.m@gmail.com

Keywords:
copepod, RNA-seq, coevolution, mitochondria, developmental rate, cytonuclear

Abstract

Mitochondrial functions are intimately reliant on proteins and RNAs encoded in both the nuclear and mitochondrial genomes, leading to inter-genomic coevolution within taxa. Hybridization can break apart coevolved mitonuclear genotypes, resulting in decreased mitochondrial performance and reduced fitness. This hybrid breakdown is an important component of outbreeding depression and early-stage reproductive isolation. However, the mechanisms contributing to mitonuclear interactions remain poorly resolved. Here we scored variation in developmental rate (a proxy for fitness) among reciprocal F_2 inter-population hybrids of the intertidal copepod *Tigriopus californicus*, and used RNA sequencing to assess differences in gene expression between fast- and slow-developing hybrids. In total, differences in expression associated with developmental rate were detected for 2,925 genes, whereas only 135 genes were differentially expressed as a result of differences in mitochondrial genotype. Up-regulated expression in fast developers was enriched for genes involved in chitin-based cuticle development, oxidation-reduction processes, hydrogen peroxide catabolic processes and mitochondrial respiratory chain complex I. In contrast, up-regulation in slow developers was enriched for DNA replication, cell division, DNA damage and DNA repair. Eighty-four nuclear-encoded mitochondrial genes were differentially expressed between fast- and slow-developing copepods, including twelve subunits of the electron transport system (ETS) which all had higher expression in fast developers than in slow developers. Nine of these genes were subunits of ETS complex I. Our results emphasize the major roles that mitonuclear interactions within the ETS, particularly in complex I, play in

45 hybrid breakdown, and resolve strong candidate genes for involvement in mitonuclear
46 interactions.

Introduction

Coevolved genetic interactions within a population or species can be disrupted by hybridization, and these disruptions may produce incompatibilities that cause loss of fitness in hybrids (i.e., hybrid breakdown; e.g., Burton, 1990; Burton et al., 2006; Ellison & Burton, 2008b; Meiklejohn et al., 2013), potentially contributing to outbreeding depression, early-stage reproductive isolation and speciation (Gershoni et al., 2009; Burton & Barreto, 2012; Hill, 2016, 2019; Sloan et al., 2017). In eukaryotic organisms, incompatibilities underlying these important evolutionary processes may disproportionately involve interactions between genes encoded in the mitochondrial genome and genes encoded in the nuclear genome (Burton & Barreto, 2012). Two major factors contribute to this potential bias for involvement of mitonuclear incompatibilities. First, rates of evolution are higher for mitochondrial DNA than for nuclear DNA (Lynch, 1997; Wallace, 2010), which increases divergence between the mitochondrial genomes of independent taxa at early stages of isolation; these differences in mitochondrial DNA give rise to intrinsic selection on nuclear-encoded genes to maintain compatible interactions (Burton & Barreto, 2012; Osada & Akashi, 2012; Barreto et al., 2018), resulting in taxon-specific mitonuclear coevolution. Second, mitochondrial functions are intimately reliant on mitonuclear interactions, and since mitochondria play critical roles in eukaryotic cells, including producing of the majority of cellular energy (i.e., ATP), mitochondrial dysfunction as a result of these incompatibilities is often associated with negative fitness consequences (Rand et al., 2004; Lane, 2005; Wallace, 2010; Hill, 2015; Hill et al., 2019).

Despite the potential impact of mitonuclear incompatibilities on hybrid breakdown, the physiological and genetic mechanisms underlying these effects are poorly resolved. Although the mitochondrial genome is small (typically ~16 kb encoding only 37 genes in most metazoans; e.g., Levin et al., 2014), approximately 1,500 nuclear-encoded genes function within mitochondria (Bar-Yaacov et al., 2012; Hill, 2014, 2017), and at least 180 of these genes closely interact with mitochondrial-encoded proteins or RNAs (Burton & Barreto, 2012; Burton et al., 2013; Hill, 2017). The best characterized examples of mitonuclear incompatibilities occur between protein subunits of the electron transport system (ETS; e.g., Ellison & Burton, 2006; Blier et al., 2001; Pichaud et al., 2019), or between a mitochondrial tRNA and its corresponding nuclear-encoded aminoacyl-tRNA synthetase (Meiklejohn et al., 2013). However, genetic incompatibilities in general can also result in changes in gene expression (Haerty & Singh, 2006; Landry et al., 2007) either directly through incompatible regulatory interactions or indirectly through physiological impacts of incompatibilities that alter the regulation of gene expression (Wittkopp et al., 2004; Graze et al., 2009; McManus et al., 2010; Barreto et al., 2015). Both of these possibilities are relevant to mitonuclear interactions, as nuclear-encoded polymerase complexes are responsible for mitochondrial DNA replication and RNA transcription (e.g., Ellison & Burton, 2008a), and variation in mitochondrial functions create regulatory signals that influence nuclear transcription as a part of 'crosstalk' between the genomes (Poyton & McEwen, 1996; Cannino et al., 2007; Horan et al., 2013). Therefore, assessing transcriptome-wide changes in gene expression associated with mitonuclear incompatibilities and hybrid breakdown is a promising

avenue to resolve not only pathways underlying the effects of incompatibilities, but also specific genes potentially involved in these interactions.

The intertidal copepod *Tigriopus californicus* is an ideal species to study the effects of mitonuclear incompatibilities. *T. californicus* are found in splashpools along the west coast of North America from Baja California, Mexico to Alaska, USA. Populations are restricted to specific rocky outcrops along the coast (Burton, 1997), which results in substantial mitochondrial and nuclear sequence divergence among populations (Burton & Lee, 1994; Burton 1997; Edmands, 2001; Peterson et al., 2013; Pereira et al., 2016; Barreto et al., 2018). Despite these high levels of divergence, inter-population crosses generate viable hybrid offspring in the laboratory (e.g., Burton, 1986), and signatures of inter-genomic coevolution have been detected for nuclear-encoded mitochondrial (N-mt) genes across several geographically isolated populations (Barreto et al., 2018). Specifically, effects of mitonuclear incompatibilities on oxidative phosphorylation (Ellison & Burton, 2006, 2008b; Healy & Burton, 2020; Han & Barreto, 2021), mitochondrial transcription (Ellison & Burton, 2008a), and the evolution of mitochondrial ribosomal proteins (Barreto & Burton, 2012) have been observed in *T. californicus* hybrids. Recent studies have demonstrated strong effects of mitonuclear incompatibilities by comparing nuclear-allele frequencies between reciprocal F_2 hybrids with fast or slow developmental rate (a proxy for fitness in *T. californicus*; Burton, 1990), and have identified chromosomes that likely contain loci responsible for these effects (Healy & Burton, 2020; Han & Barreto, 2021). However, the genes underlying these effects, and the relative influences of effects on different cellular pathways remain unknown.

In the current study, we examine physiological and genetic mechanisms underlying mitonuclear incompatibilities by using RNA sequencing (RNA-seq) to compare transcriptome-wide variation in gene expression between fast- and slow-developing F_2 *T. californicus* hybrids. Our goals were: (1) to assess the extent of variation in gene expression associated with differences in developmental rate, (2) to identify genes that were differentially expressed as a result of variation in mitochondrial genotype, (3) to determine biochemical pathways enriched for these differences in gene expression, and (4) to examine patterns of differential expression for both N-mt genes and mitochondrial-encoded genes.

Materials and Methods

Copepod collection, culturing and crossing

T. californicus adults were collected from supralittoral tidepools at San Diego, California (SD; 32° 45′ N, 117° 15′ W) and Santa Cruz, California (SC; 36° 56′ N, 122° 2′ W) in the summer of 2019. Large plastic pipettes were used to transfer copepods and tidepool water to 1 L plastic bottles. Bottles were transported to Scripps Institution of Oceanography, University of California San Diego within 24 h of collection, and population-specific laboratory cultures were initiated by dividing the collections into 400 mL glass beakers (250 mL per beaker). Cultures were maintained using filtered seawater (35 psu), and were held in incubators at 20 °C under a 12 h:12 h light:dark photoperiod. Powdered spirulina and live *Tetraselmis chuii* algal cultures were added to the cultures as food once per week, but copepods also consumed natural algal growth within their beakers. Laboratory cultures were maintained under these constant conditions for at least

12 months (~1 month per generation; e.g., Pereira et al., 2021) prior to the initiation of experimental crosses.

Four experimental hybrid lines were established for each reciprocal cross between the two populations: SD♀ x SC♂ (SDxSC; lines A-D) and SC♀ x SD♂ (SCxSD; lines E-H). Note that SDxSC and SCxSD lines differ in their mitochondrial genotype, which is generally maternally inherited in *T. californicus* (e.g., Burton et al., 2006, but see Lee & Willett, 2022), whereas population-specific contributions to nuclear genotypes are expected to be equal under a neutral assumption (e.g., Lima & Willett, 2018). Virgin females of each population were obtained by splitting mate-guarding pairs using a fine needle (Burton et al., 1981; Burton, 1985). Lines were started by adding 50 virgin females to 2.5 x 15 cm petri dishes containing ~200 mL filtered seawater and 50 males of the alternative population. Individuals were allowed to pair and mate haphazardly, and lines were maintained and fed as described above for the laboratory cultures. When gravid females were observed, they were transferred to new dishes (one dish per line), and 28 to 39 gravid P_0 females were obtained per line (Supplemental Table S1). F_1 offspring hatched naturally into the new dish, and once F_1 copepodids (juveniles) were visible without magnification, the P_0 females were removed resulting in an F_1-only dish for each line. *T. californicus* females produce multiple egg sacs from a single mating, and typically ~22-32 offspring hatch from each egg sac (e.g., Edmands & Harrison, 2003), meaning each F_1-only dish contained a minimum of many hundreds of offspring. F_1 individuals matured and mated haphazardly within their dishes, and the dishes were maintained until one week after gravid F_1 females were initially observed. At this time, F_2 developmental trials were started, which avoided inadvertently selecting only the fastest

developing F_1 females as parents in the F_2 trials and prevented any F_2 offspring that hatched in the F_1-only dish from reaching adulthood.

Developmental rate

Developmental rate measurements for F_2 offspring from each hybrid line were conducted similarly to those described in Healy & Burton (2020). In brief, mature (red) egg sacs were dissected from 30 haphazardly selected F_1 females for each line using a fine needle. Egg sacs were transferred individually into wells of 6-well plates containing ~8 mL filtered seawater. Powdered spirulina was added to the wells, and then the plates were placed in the incubators that were used for the F_1 crosses (at 20 °C; 12 h:12 h light:dark). F_2 egg sacs hatched overnight, and offspring development was monitored daily with additional spirulina added every other day. Development in *Tigriopus sp.* involves a distinct metamorphosis between the last naupliar (N6) stage and the first copepodid (C1) stage (Raisuddin et al., ~~2017~~2007), which can be used to score developmental rate as time to metamorphosis. As copepodids appeared in the experimental wells, days post hatch (dph) to metamorphosis was scored for each individual, and copepodids were grouped by dph to metamorphosis in line-specific 2 x 10 cm petri dishes containing ~50 mL filtered seawater. The total numbers of scored F_2 copepodids for each line are listed in Supplemental Table S1.

RNA isolation and RNA-seq

Stage 1 *Tigriopus sp.* copepodids are very small (~0.35 mm length [Raisuddin et al., 2007]), and obtaining sufficient RNA for standard RNA-seq library preparations from pools of large numbers of individuals at this stage is impractical. Thus, we allowed our scored F_2 hybrids to progress approximately two additional copepodid stages through

development to the C3 stage (~0.6 mm length) prior to RNA isolation. This progression was tracked by visual monitoring and by time, as under our experimental conditions stage 3 is reached ~4-5 days after initial metamorphosis to a stage 1 copepodid (Healy et al., *in prep.*). The 100 fastest and 100 slowest developing stage 3 copepodids from each line were snap frozen in liquid nitrogen and stored at -80 °C, and clear separation between fast and slow developers was achieved for every line (Supplemental Table S2). Note that monitoring developmental progression for copepodids that are not held individually is imprecise (Tsuboko-Ishii & Burton, 2018), but monitoring at the culture level was necessary given the number of copepodids scored in our study. As a result, it is possible that small numbers of frozen copepodids were at the C2 or C4 stages rather than the targeted C3 stage.

RNA was isolated from the pool~~s~~ of fast developers and the pool of slow developers from each line using TRI Reagent® (Sigma-Aldrich, St. Louis, MO, USA) following the manufacturer's instructions with modifications as described in Healy et al. (2019). Genomic DNA contamination was removed with an Invitrogen™ TURBO DNA-*free*™ kit (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions, and final RNA concentrations were determined with an Invitrogen™ Qubit™ 2.0 Fluorometer and RNA HS assay kit (Thermo Fisher Scientific). RNA samples were submitted to the University of California San Diego Institute for Genomic Medicine Genomics Center for preparation of mRNA stranded libraries for 100 base pair paired-end RNA-seq. The libraries were sequenced on an Illumina NovaSeq 6000 (Illumina Inc., San Diego, CA, USA), and between 18,165,204 and 31,441,512 paired-end reads were obtained for each sample (Supplemental Table S3).

206 *Data analysis and statistics*

207 All analyses were conducted in *R* v4.2.0 (R Core Team, 2022) with $\alpha = 0.05$.
208 Variation in log-transformed developmental rate as a result of variation in mitochondrial
209 genotype was assessed with a linear mixed-effects model using the *lmerTest* package
210 v3.1.3 (Kuznetsova et al., 2017) with mitochondrial genotype as a fixed factor and line as
211 a random factor.

212 RNA-seq reads were trimmed to remove any potential adapter sequences with
213 *Cutadapt* v3.4 (Martin, 2011), and were then mapped to a hybrid genome for SD and SC
214 *T. californicus* prepared using the *T. californicus* reference genome (SD reference
215 genome GenBank: GCA_007210705.1, Barreto et al., 2018), a SC-specific genome from
216 population re-sequencing (Healy & Burton, 2020), and published sequences for the SD
217 and SC mitochondrial genomes (GenBank: DQ913891.2 and DQ917374.1, respectively;
218 Burton et al., 2007). Note the SD and SC genomes were masked such that any 'N'
219 position in one genome was also 'N' in the other genome to avoid any potential mapping
220 biases due to incomplete re-sequencing (Healy & Burton, 2020); this masking procedure
221 between pairs of *T. californicus* populations generally affects less than 2% of coding
222 sequences (Barreto et al., 2018). Genomic feature annotations (i.e., gene models) for the
223 hybrid reference were prepared from previously published annotations for the reference
224 and mitochondrial genomes (Barreto et al., 2018). Sequencing reads were aligned to the
225 hybrid genome using *STAR* v2.7.8a (Dobin et al., 2013) allowing reads to map to a up to
226 two locations in the hybrid genome ("--outFilterMultimapNmax 2" option) so that reads
227 mapping to conserved regions between SD and SC would be included in expression
228 estimates. Overall mapping rates were between 90.67% and 92.94% per sample

(Supplemental Table S3) with the majority of reads mapping uniquely (82.30 ± 1.28%, μ ± σ), which is consistent with relatively high levels of sequence divergence across the transcriptome between SD and SC *T. californicus* (Barreto et al., 2011; Barreto et al., 2015). Reads were counted with *featureCounts* (Liao et al., 2014) from the *Subread* package v2.0.3 using fractional counting ("-M" and "--fraction" options; i.e., reads that mapped to two genes in the hybrid genome were counted as 0.5), and reads for homologous SD and SC genes in the hybrid genome were summed to allow comparisons of total expression for each gene among our RNA samples from pooled F_2 hybrids.

Variation in transcriptome-wide gene expression patterns was assessed with a principal component analysis (PCA) using the prcomp function from the *R* package *stats* v3.6.2, and 95% confidence ellipses for groups of samples (by developmental rate or mitochondrial genotype) were determined with the *FactoMineR* package v2.4 (Lê et al., 2008). Gene-wise differential expression was assessed by fitting negative bionomial models to the count data with the *edgeR* package v3.38.0 (Robinson et al., 2010) such that main effects of developmental rate and mitochondrial genotype, and interactive effects of these main factors could be tested as described in Lin et al. (2016). In brief, counts were normalized for library size using the relative log expression method (Anders & Huber, 2010; "RLE" option in *edgeR*), low expression genes were filtered with the filterByExp function, dispersions were estimated with the estimateGLMRobustDisp function (Zhou et al., 2014), and factor effects were tested by likelihood ratio tests using the glmFit and glmLRT functions. After filtering, the library sizes in *edgeR* ranged from 14,261,581 to 25,846,327 counts (Supplemental Table S3), and differential expression was assessed for 13,994 genes with two analytical approaches. First, we analysed the

252 complete dataset for all eight of our hybrid lines. However, because we pooled the 100
253 fastest or slowest developers in each line for RNA-seq, the ranges of developmental rate
254 for fast or slow developers had the potential to vary among lines. Thus, we compared fast
255 and slow developmental rates among lines using linear mixed-effects models (as
256 described above for effects of mitochondrial genotype), and performed a second
257 differential expression analysis using a subset of the lines that had similar developmental
258 rates across the copepodid pools sampled for RNA-seq (see Results below).
259 Previously published gene ontology (GO) functional annotations of the gene
260 models in the *T. californicus* genome were obtained from Barreto et al. (2018; 35,947
261 annotations for 9,362 genes). We expanded these annotations by running the
262 transcriptome through the *Trinotate* v3.2.2 pipeline (e.g., Bryant et al., 2017), and by
263 manually annotating any remaining unannotated mitochondrial-encoded genes using
264 information from other arthropods (*Aedes aegypti* and *Drosophila melanogaster*)
265 available in the UniProt database (www.uniprot.org). After combining annotations from
266 all sources and removing duplicate annotations, our GO database contained 152,010
267 annotations for 12,206 genes in the *T. californicus* genome. Functional enrichment
268 analyses were conducted for the differentially expressed (DE) genes with the *goseq*
269 package v1.48.0 (Young et al., 2010) in *R*. GO terms with less than 10 annotations in the
270 *T. californicus* genome were removed from the database (leaving 2,644 GO terms with
271 119,094 annotations for 12,160 genes) to provide robust tests for functional enrichments,
272 and analyses were run for genes up-regulated in fast developers, genes up-regulated in
273 slow developers, genes differentially expressed between mitochondrial genotypes, and

genes with a significant interaction between mitochondrial genotype and developmental rate.

False-discovery rate (FDR) corrections were made for all statistical results from the differential expression and functional enrichment analyses using the Benjamini-Hochberg method (Benjamini & Hochberg, 1995).

Results

Developmental rate

Across our hybrid lines metamorphosis was observed from 6 to 22 dph, and median time to metamorphosis ranged from 8 to 10 dph among lines (Supplemental Table S1). There was no significant effect of mitochondrial genotype on developmental rate overall ($p = 0.20$; Figure 1), and mitochondrial genotype also did not affect the developmental rates of the 100 fastest or 100 slowest developing copepodids ($p \leq 0.20$), which were each comprised of 10.9% to 21.4% of the total number of F_2 copepodids per line (Supplemental Table S1). However, despite the lack of effects of mitochondrial genotype, the observed ranges and distributions of developmental rate in "fast" or "slow" developers displayed variation among lines (Supplemental Table S2). In particular, for six lines the majority of fast developers metamorphosed by 7 dph (83, 100, 100, 60, 61 and 100% for lines C-H, respectively), whereas for two lines only 4% or 3% of fast developers metamorphosed by 7 dph (lines A and B, respectively). Comparing these two groups of lines, developmental rates in lines A and B were significantly different from developmental rates in lines C-H both overall ($p = 0.013$) and in fast developers ($p = 1.4 \times 10^{-3}$), but not in slow developers ($p = 0.060$; Supplemental Figure S1). Consequently, as

297 discussed above (see Materials and methods), we assessed differential gene expression
298 using all eight lines (hereafter the 8-line DE analysis), and using only lines C-H
299 (hereafter the 6-line DE analysis).

300 *Transcriptome-wide expression patterns*

301 Gene expression patterns associated with developmental rate were a dominant
302 source of variation across the transcriptome when examined by PCA. The first and
303 second principal components (PC1 and PC2) explained 30.0% and 14.7% of the variation
304 in expression among the RNA-seq samples, respectively, and the fast and slow
305 developers tended to separate along these two axes, particularly along PC1 (slight overlap
306 in 95% confidence ellipses with no overlap at a confidence level of ~93%; Figure 2a).
307 Interestingly, SDxSC lines A and B were the only two lines displaying a positive
308 trajectory from slow to fast developers on PC1 (Supplemental Table S4), which supports
309 the 6-line DE analysis based on variation in developmental rate among the hybrid lines in
310 our study. Consistent with this pattern, separation between the fast and slow developers
311 along PC1 and PC2 became particularly evident if confidence ellipses were re-calculated
312 for only the 6-line DE analysis RNA-seq samples (Supplemental Figure S2).

313 Unlike gene expression associated with developmental rate, little variation
314 associated with mitochondrial genotype was observed along PC1 and PC2 (Figure 2b).
315 Instead, samples from the SDxSC and SCxSD reciprocal lines were separated along PC3
316 and PC4, which explained 12.7% and 8.4% of the total variation in gene expression,
317 respectively (Supplemental Figure S3). This indicates relatively modest effects of
318 mitochondrial genotype on transriptome-wide gene expression patterns, particularly in
319 comparison to patterns associated with developmental rate.

320 *Differential expression associated with developmental rate*

321 Significant differences in gene expression between fast- and slow-developing *T.*
322 *californicus* were detected for 1,668 genes in the 8-line DE analysis and 2,850 genes in
323 the 6-line DE analysis (Figure 3; Supplemental Table S5). In both cases, these relatively
324 high proportions of differential expression across the transcriptome (11.9% or 20.3%)
325 were consistent with the patterns identified by PCA. In general, the 8-line and 6-line DE
326 analyses were highly congruent as 1,593 of the 1,668 DE genes in the 8-line analysis
327 (95.5%) were also detected as DE genes in the 6-line analysis, and there were no strong
328 biases for up- or down-regulation of gene expression in fast or slow developers in either
329 case (900:768 and 1,383:1,467 up-:down-regulated genes in fast developers in the 8-line
330 and 6-line analyses, respectively). However, the distribution of *p*-values versus fold
331 changes in expression was relatively symmetrical around a fold change of zero in the 6-
332 line analysis, whereas this was not the case in the 8-line analysis (Figure 3a,b). In total,
333 84 N-mt genes were detected as differentially expressed between fast- and slow-
334 developing copepodids (32 common to both the 8-line and 6-line DE analyses, and 1 and
335 51 unique to each analysis, respectively), whereas no protein-coding genes encoded in the
336 mitochondrial genome demonstrated differential expression associated with
337 developmental rate (FDR-adjusted $p \geq 0.36$).

338 Functional enrichment analyses found 47 GO terms enriched in the genes up-
339 regulated in fast developers (20 common, and 12 and 15 unique from the 8-line and 6-line
340 DE analyses, respectively), and 92 GO terms enriched in the genes up-regulated in slow
341 developers (38 common, and 1 and 53 unique from the 8-line and 6-line DE analyses,
342 respectively; Supplemental Table S6). There was variation in the specific GO terms that

were enriched among DE genes from the 8-line or 6-line analyses, but the overall patterns regarding cellular functions indicated by the enrichments were similar regardless of which set of DE genes was considered. These results are summarized below using the enrichments for GO biological processes and cellular components (see Supplemental Table S6 for GO molecular function results).

Enrichments in up-regulated genes were detected for 23 and 46 GO biological process terms in fast- and slow-developing hybrids, respectively (Figure 4). The clearest pattern among these results was the complete lack of overlap in significantly enriched terms between the fast and slow developers. For genes up-regulated in fast-developing copepodids, enrichments were detected related to chitin metabolism (e.g., GO:0040003 and GO:0006030), oxidation-reduction processes (GO:0055114), hydrogen peroxide metabolism (e.g., GO:0042744) and immune responses (e.g., GO:0045087). In contrast, enrichments related to DNA replication (e.g., GO:0006260 and GO:0006270), RNA processing (e.g., GO:0006364 and GO:0006402), cell division (e.g., GO:0051301 and GO:0007049) and DNA repair (e.g., GO:0006281 and GO:0036297) were detected for genes up-regulated in slow-developing copepodids. These major patterns were consistent across the DE genes from the 8-line and 6-line analyses, but additional enriched biological processes for carbohydrate and amino acid metabolism (e.g., GO:0006096 and GO:0006560) were only associated with up-regulation in fast developers using results from the 6-line DE analysis (see details in Figure 4).

Compared to biological processes, fewer GO cellular component terms were significantly enriched among DE genes associated with developmental rate. However, the 7 cellular components enriched in genes up-regulated in fast developers, and the 20

366 cellular components enriched in genes up-regulated in slow developers (Figure 5)
367 suggested similar overall results to those identified based on biological process
368 enrichments. Genes up-regulated in fast developers demonstrated enrichments involving
369 extracellular regions (e.g., GO:0005576 and GO:0005615), whereas genes up-regulated
370 in slow developers were enriched for cellular components related to the nucleus (e.g.,
371 GO:0005634 and GO:0005730), a ribosome assembly complex (GO:0032040) and the
372 DNA polymerase complex (GO:0042575). Additionally, among genes significantly up-
373 regulated in fast developers in the 6-line DE analysis, there was an enrichment for the
374 mitochondrial respiratory chain complex I cellular component (GO:0005747). As a result,
375 we specifically examined the DE N-mt genes encoding subunits of the electron transport
376 system (ETS). Twelve nuclear-encoded subunits of the ETS were differentially expressed
377 between fast- and slow-developing hybrid copepodids (FDR-adjusted $p \leq 0.048$ and fold
378 change in expression 1.21-1.37 for all), and nine of these were subunits of ETS complex I
379 (Table 1).
380 *Differential expression associated with mitochondrial genotype*
381 As in most metazoans, mitochondrial DNA is generally maternally inherited in *T.*
382 *californicus*; however, Lee and Willett (2022) recently detected substantial paternal
383 leakage of mitochondrial DNA in hybrids between some pairs of populations. In contrast,
384 there was virtually no evidence of paternal leakage in our hybrid lines, with 99.8 ± 0.6%
385 ($\mu \pm \sigma$) of the read counts matching the maternal genotype across all samples, suggesting
386 mitochondrial genotypes were maternally inherited in our F_2 hybrids between the SD and
387 SC populations. In the 8-line DE analysis, 135 genes were significantly differentially
388 expressed between hybrids with a SD mitochondrial genotype (SDxSC) and hybrids with

389 a SC mitochondrial genotype (SCxSD; Figure 6a,b; Supplemental Table S7), and 21
390 genes demonstrated a significant interaction between mitochondrial genotype and
391 developmental rate (Figure 6c; Supplemental Table S8). Differences in gene expression
392 as a result of variation in mitochondrial genotype were not evaluated in the 6-line DE
393 analysis, because there was a low sample size for SDxSC lines (2 lines) and an
394 unbalanced design with respect to mitochondrial genotype (2 SDxSC lines [C and D] and
395 4 SCxSD lines [E-H]).

396 There were no significant functional enrichments among the DE genes between
397 the SD and SC mitochondrial genotype hybrids, but there was a clear bias in the direction
398 of variation in expression as 107 genes were expressed at higher levels in SDxSC hybrids
399 than in SCxSD hybrids, whereas only 28 genes displayed the opposite pattern (Figure
400 6a,b). One N-mt gene, 3-hydroxyisobutyrate dehydrogenase (*hibadh*), had higher
401 expression levels in hybrids carrying the SD mitochondrial genotype than in hybrids
402 carrying the SC mitochondrial genotype, and this was the only N-mt gene demonstrating
403 a significant effect of mitochondrial genotype on gene expression. In contrast, 8 protein-
404 coding genes (*mt-nd1*, *mt-nd3*, *mt-nd4*, *mt-nd6*, *mt-cyb*, *mt-co1*, *mt-co3* and *mt-atp8*) and
405 2 rRNA genes (*mt-rnr1* and *mt-rnr2*) encoded in the mitochondrial genome were
406 differentially expressed between SDxSC and SCxSD hybrids (Table 2). Directions of
407 expression differences among these mitochondrial-encoded genes did not reflect the
408 overall bias of up-regulation of genes in SDxSC hybrids, as 6 DE genes were expressed
409 at higher levels in hybrids with the SC mitochondrial genotype than in hybrids with the
410 SD mitochondrial genotype and 4 DE genes displayed the opposite pattern. The
411 mitochondrial-encoded protein-coding genes all produce subunits of the ETS complexes,

412 but differences in up- or down-regulation for these genes between the two mitochondrial
413 genotypes also did not group consistently based on ETS complex membership (see Table
414 2).

415 In general, genes with expression profiles that were significantly affected by an
416 interaction between mitochondrial genotype and developmental rate demonstrated a
417 consistent pattern when comparing SDxSC and SCxSD hybrids. For all 21 genes with
418 significant interaction effects (Figure 6c), the fold change in expression associated with a
419 difference in developmental rate was higher in hybrids with the SC mitochondrial
420 genotype (7.6 ± 3.5X, mean ± SEM) than in hybrids with the SD mitochondrial genotype
421 (2.7 ± 1.1X), but whether a gene was up- or down-regulated between fast and slow
422 developers was the same regardless of mitochondrial genotype. Genes displaying
423 interactive effects of mitochondrial genotype and developmental rate were significantly
424 enriched for three GO terms: lipid transporter activity (GO:0005319; FDR-adjusted p =
425 2.8×10^{-5}), extracellular region (GO:0005576; FDR-adjusted $p = 0.014$) and lipid
426 transport (GO:0006869; FDR-adjusted $p = 0.032$); however, these enrichments should be
427 interpreted with some caution given the low number of genes affected by these
428 interactions. No N-mt genes or mitochondrial-encoded genes had differences in
429 expression consistent with effects of mitochondrial genotype by developmental rate
430 interactions.

431

432 **Discussion**

433 The expression of genetic incompatibilities in hybrid organisms can result in
434 changes in gene expression through either direct effects of incompatible regulatory

interactions or indirect responses to functional consequences of incompatibilities (e.g., Landry et al., 2007; Barreto et al., 2015). The current study reveals high levels of differential gene expression associated with variation in a fitness-related trait (developmental rate) among inter-population F_2 *T. californicus* hybrids, and recent work in this species has demonstrated that differences in mitonuclear compatibility ~~has a~~play a major ~~strong effect~~role underlying ~~on variation in developmental rate~~fast- versus slow-developing phenotypes ~~among~~ in these F_2 hybrids (Healy & Burton, 2020; Han & Barreto, 2021). Additionally, the slowest developing *T. californicus* hybrids display extreme developmental rates that are outside the ranges of developmental rates observed for offspring from within-population crosses (Healy & Burton, 2020; Han & Barreto, 2021), and consequently the differences in gene expression presented here provide potential insight into not only the wide range of mechanisms underlying mitonuclear interactions, but also the physiological consequences of these interactions that result in hybrid breakdown.

Variation in developmental rate among hybrid T. californicus

The developmental rates of inter-population F_2 *T. californicus* hybrids typically display hybrid breakdown (Burton, 1990; Ellison & Burton, 2008b; Healy & Burton, 2020; Han & Barreto, 2021), and as in previous studies (Ellison & Burton, 2008b; Healy & Burton, 2020), we found that the reciprocal SDxSC and SCxSD hybrids have, on average, similar developmental rates. Despite this, we detected variation in developmental rate among the 8 hybrid lines in the current study (line A and B versus lines C to H). Since only 30 families contributed to each line, the variation in developmental rate among lines may be the result of inheritance of different epigenetic

modifications or genetic effects such as random sampling of recombination events or intra-population allelic variants (e.g., Pereira et al., 2016). Alternatively, environmental effects such as differences in algal growth or offspring density may have contributed to the observed variation among lines. Environmental effects on developmental rate and mitonuclear interactions are common in hybrid organisms in general (Hoekstra et al., 2013, 2018; Baris et al., 2016; Mossman et al., 2016a, 2017; Drummond et al., 2019; Rand & Mossman, 2020; Rand et al., 2022); however, variation in algal growth among lines was minimized manually in the current study, and effects of density dependence on developmental rate are typically minor under our experimental conditions (Healy et al., *in prep.*). Regardless of the cause of variation among lines, strong effects of mitonuclear incompatibilities within lines of hybrid *T. californicus* are expected to result in high degrees of mitonuclear compatibility in fast-developing (high-fitness) hybrids (Healy & Burton, 2020; Han & Barreto, 2021).

Gene expression differences between high- and low-fitness hybrids

~~Although ~~Approximately 1,500 nuclear gene products are potentially imported into mitochondria, and 599 N-mt genes have been annotated in the *T. californicus* genome (Barreto et al., 2018). Although not all effects of mitonuclear incompatibilities will necessarily result in changes in gene expression,~~, and~~ 84 of these N-mt genes were differentially expressed between fast- and slow-developing F_2 *T. californicus* hybrids. The majority of these (51 genes) were detected only in the 6-line DE analysis, but this is not surprising, because strong signatures of coevolution are only observed in fast-developing hybrids (Healy & Burton, 2020), and developmental rates for the fast developers in the 6-line analysis (lines C-H) were clearly faster than those from the other

two lines (lines A and B; Supplemental Figure S1 and Table S2). Among N-mt genes, there are four groups that most likely involve interactions between mitochondrial- and nuclear-encoded genes: mitochondrial DNA and RNA polymerases, mitochondrial aminoacyl-tRNA synthetases, mitochondrial ribosomal proteins and ETS complex subunits (Burton & Barreto, 2012; Hill, 2015, 2017; Hill et al., 2019). Of the DE N-mt genes in the current study, the clearest association with these groups was the 12 DE genes encoding subunits of the ETS complexes. All of these subunits were expressed at higher levels in fast developers than in slow developers (Table 2), which is consistent with previous studies positively associating ATP synthesis capacity with high fitness in *T. californicus* hybrids (Ellison & Burton, 2006, 2008b; Healy & Burton, 2020; Han & Barreto, 2021). In addition, six other N-mt genes either directly involved in the tricarboxylic acid (TCA) cycle (*idh3b* and *aco2*) or involved in pathways delivering substrates to the TCA cycle (*acss1*, *pc*, *mut* and *T05H10.6*) were also all up-regulated in fast developers compared to slow developers.

Changes in the proportions of even a small number of interacting mitochondrial proteins can have substantial functional consequences (e.g., Herrmann et al., 2003; Chae et al., 2013), and consequently our results highlight not only specific ETS subunits potentially involved in incompatibilities, but also the key role that dysfunction at complex I may play in hybrid breakdown. Nine of the 12 DE ETS genes encoded subunits of complex I, and the GO cellular component mitochondrial respiratory chain complex I (GO:0005747) was enriched among genes that were up-regulated in fast developers in the 6-line DE analysis. Compared to the other ETS complexes, complex I has the highest number of nuclear-encoded and mitochondrial-encoded subunits (38 and

7 subunits in mammals, respectively; Zhu et al., 2016), and thus is a particularly likely target for the formation of mitonuclear incompatibilities (e.g., Pichaud et al., 2019; Moran et al., 2021). Furthermore, although negative effects of mitonuclear incompatibilities have been demonstrated for complexes I, III and IV in *T. californicus* hybrids (Edmands & Burton, 1999; Willett & Burton, 2001, 2003; Rawson & Burton, 2002; Harrison & Burton, 2006; Ellison & Burton, 2006, 2008b), signatures of divergent selection (i.e., elevated dN/dS) among populations of *T. californicus* are modestly, but significantly, higher for complex I than the other ETS complexes (Barreto et al., 2018).

Potential effects of mitonuclear interactions on mitochondrial transcription (Ellison & Burton, 2008a) and translation (i.e., mitochondrial ribosomal proteins; Barreto & Burton, 2012) have also been detected in *T. californicus*, but relatively few N-mt genes that were differentially expressed between high- and low-fitness hybrids were involved in these functions in the current study. Two mitochondrial ribosomal proteins were differentially expressed between the fast- and slow-developing copepodids with *mrpl19* up-regulated in slow developers and *mrps18c* up-regulated in fast developers, but note neither of these genes were annotated as N-mt genes in Barreto et al. (2018). One mitochondrial aminoacyl-tRNA synthetase (*kars*) and the mitochondrial poly(A) polymerase, *mtpap*, were expressed at higher levels in slow developers than in fast developers, as were N-mt genes involved in mitochondrial DNA replication (*twnk* and *polg*) and translation regulation (*ptcd1* and *guf1*). Additionally, five N-mt genes involved in protein and RNA import into the mitochondria were also all up-regulated in slow developers (*hsp60*, *tim14*, *roe1*, *timm23* and *pnpt1*). Although changes in mRNA levels for individual genes are not necessarily directly related to functional differences at the

protein-level (e.g., Hack, 2004), consistent patterns of expression across genes from similar pathways or genes with similar functions are more likely to be indicative of functional effects in the cell. Beyond the differences in gene expression between fast and slow developers discussed above, allele-specific expression patterns may also contribute to the effects of mitonuclear interactions in F_2 hybrids. The pooled sequencing approach utilized in the current study produces the potential to confound genetic variation with allele-specific expression, which precludes informative allele-specific analyses. However, at least in F_1 hybrids between the SD and SC *T. californicus* populations, Tangwancharoen et al. (2020) observed limited evidence for allele-specific expression patterns, suggesting biased expression of population-specific alleles at heterozygous loci is unlikely to play a major role underlying the effects of mitonuclear interactions in these crosses.

Mitonuclear incompatibilities and mitochondrial dysfunctions in hybrids have been linked to increased production of reactive oxygen species (ROS) from the ETS (Du et al., 2017; Pichaud et al., 2019), and hybrid lines of *T. californicus* have elevated levels of oxidative damage compared to within-population lines (Barreto & Burton, 2013). The ETS produces ROS as a byproduct of oxidative metabolism, particularly at complex I and III (Brand, 2010; Andreyev et al., 2015), and although ROS can have important functions in cellular signaling, including 'crosstalk' between the mitochondria and nucleus (Yun & Finkel, 2014; Shadel & Horvath, 2015), excessive ROS production leads to oxidative stress that is harmful for macromolecules such as DNA (e.g., Temple et al., 2005). In the current study, GO terms associated with antioxidant defense processes such as hydrogen peroxide catabolic process and peroxidase reaction were enriched among genes up-

550 regulated in fast developers, whereas GO terms associated with DNA damage and repair
551 including, for example, DNA repair, cellular response to DNA damage stimulus and
552 interstrand cross-link repair were enriched among genes up-regulated in slow developers.
553 These differences suggest there may be variation in oxidative stress mitigation and
554 damage between the fast and slow developers, potentially indicating additional
555 consequences of mitonuclear incompatibilities associated with the ETS. Consistent with
556 this possibility, hallmark antioxidant enzymes (e.g., Yoo et al., 2020) such as superoxide
557 dismutase (*sod1*) and glutathione *S*-transferase (*mgst1*) were up-regulated in fast-
558 developing copepodids in the current study, and high-fitness *T. californicus* hybrids tend
559 to have lower oxidative damage and higher mitonuclear compatibility than low-fitness
560 hybrids (Barreto & Burton, 2013; Healy & Burton, 2020).
561 Other functional enrichments among the DE genes in the current study were less
562 clearly connected to potential effects of mitonuclear incompatibilities. For example,
563 enriched up-regulation of cuticle proteins in fast developers could be plausibly connected
564 to the chitin-based exoskeleton in *T. californicus* and the five moults required to reach
565 adulthood from the C1 stage, but connections between chitin metabolic processes and
566 mitonuclear interactions are not readily apparent, despite transgressive expression levels
567 of these proteins generally in *T. californicus* hybrids (Barreto et al., 2015). Cytosolic
568 ribosomal proteins also display transgressive expression patterns in *T. californicus*
569 (Barreto et al., 2015), but no cytosolic ribosomal proteins were differentially expressed
570 between fast and slow developers in the current study. This is at least somewhat
571 surprising~~ly~~ given the substantial impacts of rates of protein synthesis and variation in
572 ribosomal protein expression on rapid growth during development in Pacific oyster

(*Crassostrea gigas*; Hedgecock et al., 2007; Meyer & Manahan, 2010; Pan et al., 2018), and the key role of ribosome abundance in high growth rates in *Saccharomyces sp.* (Warner, 1999; Regenberg et al., 2006; Airoldi et al., 2009).

Taken together, the genes differentially expressed between fast- and slow-developing F_2 *T. californicus* hybrids suggest a consistent hypothesis for the mechanisms underlying ~~strong~~ effects of mitonuclear incompatibilities in this species. Oxidative phosphorylation functions efficiently at high rates in high-fitness hybrids, whereas incompatible mitonuclear genotypes in low-fitness hybrids cause ETS dysfunction that is associated with increased signals of oxidative damage and potentially signals of compensatory increases in mitochondrial translation and protein import. Although clearly this is largely speculative based on the results of the current study alone, physiological studies in *T. californicus* generally support various aspects of this hypothesis (e.g., Ellison & Burton, 2006, 2008b; Barreto & Burton, 2013; Healy & Burton, 2020), suggesting that further functional work to test these ideas, particularly in the context of variation in developmental rate, is warranted.

Effects of mitochondrial genotype on the transcriptome

Relatively few genes were differentially expressed between hybrids carrying a SD or SC mitochondrial genotype, which is unlike the substantial variation in nuclear gene expression associated with mitochondrial substitutions in *Drosophila sp.* (Mossman et al., 2016b, 2017, 2019) or differences in mitochondrial genotype in horseshoe bats (*Rhinolophus affinis*; Ding et al., 2021). However, modest effects of mitochondrial genotype on the transcriptome are observed in Atlantic killifish (*Fundulus heteroclitus*) from natural populations with genetic admixture between two subspecies (Flight et al.,

2011; Healy et al., 2017), and only small numbers of genes are differentially expressed between *T. californicus* from the SD and SC populations (Barreto et al., 2015). Interestingly, mitochondrial-encoded genes also display similar expression levels between the SD and SC populations (differences only for *mt-co1* and *mt-cyb* which were more highly expressed in SC than in SD; Barreto et al., 2015), whereas in the current study the clearest group of DE genes between SDxSC and SCxSD hybrids were 10 mitochondrial-encoded genes. Given this difference, it is possible that nuclear genetic background alters mitochondrial transcription in *T. californicus*, as in other species (e.g., Mossman et al., 2016b), which may include direct effects of incompatible mitonuclear regulatory interactions similar to those detected by Ellison & Burton (2008a).

Mechanisms underlying mitonuclear interactions

Several studies have investigated genetic mechanisms underlying mitonuclear interactions by assessing variation in nuclear allele frequencies in reciprocal *T. californicus* hybrids (Pritchard et al., 2011; Foley et al., 2013; Lima & Willett, 2018; Lima et al., 2019; Healy & Burton, 2020; Han & Barreto, 2020; Pereira et al., 2021). In general, allele frequency deviations away from neutral expectations (i.e., 0.5) reveal consequences of both nuclear-nuclear and mitonuclear interactions with a bias towards the latter, especially in recent studies that focus on variation between reciprocal high-fitness hybrids (Healy & Burton, 2020; Han & Barreto, 2021; Pereira et al., 2021). In particular, fitness differences among hybrids between SD and SC, scored by developmental rate, highlight strong effects of mitonuclear interactions involving loci on chromosomes 1 to 5 (Healy & Burton, 2020). There are 249 annotated N-mt genes ~~are~~ located on chromosomes 1 to 5 in *T. californicus* (47, 71, 42, 46 and 43 in order for

chromosomes 1 to 5), and 42 were differentially expressed between fast and slow developers in the results presented here (14, 5, 4, 9 and 10 in order for chromosomes 1 to 5). Differences in allele frequencies among the pools of copepods in the current study were not assessed to avoid both the potential confound between genetic variation and allele-specific gene expression, and the inaccuracy of allele frequency estimation from pooled sequencing at the relatively low sequencing depths required to estimate gene expression. However, given the major association between mitonuclear compatibility on chromosomes 1 to 5 with variation in F_2-hybrid developmental rate detected by Healy and Burton (2020), it is likely that differentially expressed N-mt genes on these chromosomes may reflect direct or indirect consequences of mitonuclear incompatibilities. ~~Additionally, t~~The main findings of the current study ~~indicate~~ suggest that mitonuclear incompatibilities in the ETS complexes likely play key roles underlying variation in developmental rate among F_2 hybrids between SD and SC, and 22 N-mt ETS subunits are encoded on chromosomes 1 to 5 of which 7 were differentially expressed between the fast and slow developers. Although they are unlikely to be the only nuclear genes involved in mitonuclear interactions, the 6 of these genes that are not subunits of complex II, which has no mitochondrial-encoded subunits (e.g., Saraste, 1999), are currently ~~the strongest~~excellent candidate genes to underlie mitonuclear incompatibilities in *T. californicus* (complex I: *ndufa6*, *ndufv2*, *ndufa8*, *ndufa5* and *ndufb3*, and complex III: *uqcrq*; Table 1). The relatively large number of genes encoding subunits of complex I among these results further highlight the potential key impacts of incompatibilities in this ETS complex. Therefore, it is possible that interacting subunits of complex I, including

641 the specific subunits identified here, may be potential candidates underlying mitonuclear
642 interactions in eukaryotic organisms more generally as well.
643
644 **Acknowledgements**
645 Funding for the current study was provided by a National Science Foundation
646 grant to RSB (IOS1754347). This publication includes data generated at the UC San
647 Diego IGM Genomics Center utilizing an Illumina NovaSeq 6000 that was purchased
648 with funding from a National Institutes of Health SIG grant (#S10 OD026929).
649 The authors thank Alexis Cody Hargadon for assistance with copepod maintenance, and
650 Dr. Kevin Olsen for comments on the manuscript.

References

Airoldi, E. M., Huttenhower, C., Gresham, D., Lu, C., Caudy, A. A., Dunham, M. J., Broach, J. R., Botstein, D., & Troyanskaya, O. G. (2009). Predicting cellular growth from gene expression signatures. *PLoS Computational Biology*, *5*(1), e1000257. https://doi.org/10.1371/journal.pcbi.1000257

Andreyev, A. Y., Kushnareva, Y. E., Murphy, A. N., & Starkov, A. A. (2015). Mitochondrial ROS metabolism: 10 years later. *Biochemistry (Moscow)*, *80*(5), 517-531. https://doi.org/10.1134/S0006297915050028

Baris, T. Z., Blier, P. U., Pichaud, N., Crawford, D. L., & Oleksiak, M. F. (2016). Gene by environmental interactions affecting oxidative phosphorylation and thermal sensitivity. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *311*(1), R157-R165. https://doi.org/10.1152/ajpregu.00008.2016

Barreto, F. S., & Burton, R. S. (2012). Evidence for compensatory evolution of ribosomal proteins in response to rapid divergence of mitochondrial rRNA. *Molecular Biology and Evolution*, *30*(2), 310-314. https://doi.org/10.1093/molbev/mss228

Barreto, F. S., & Burton, R. S. (2013). Elevated oxidative damage is correlated with reduced fitness in interpopulation hybrids of a marine copepod. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1767), 20131521. https://doi.org/10.1098/rspb.2013.1521

Barreto, F. S., Moy, G. W., & Burton, R. S. (2011). Interpopulation patterns of divergence and selection across the transcriptome of the copepod *Tigriopus californicus*. *Molecular Ecology*, *20*(3), 560-572. https://doi.org/10.1098/rspb.2013.1521

Barreto, F. S., Pereira, R. J., & Burton, R. S. (2015). Hybrid dysfunction and physiological compensation in gene expression. *Molecular Biology and Evolution*, *32*(3), 613-622. https://doi.org/10.1093/molbev/msu321

Barreto, F. S., Watson, E. T., Lima, T. G., Willett, C. S., Edmands, S., Li, W., & Burton, R. S. (2018). Genomic signatures of mitonuclear coevolution across populations of *Tigriopus californicus*. *Nature Ecology & Evolution*, *2*(8), 1250-1257. https://doi.org/10.1038/s41559-018-0588-1

Bar-Yaacov, D., Blumberg, A., & Mishmar, D. (2012). Mitochondrial-nuclear co-evolution and its effects on OXPHOS activity and regulation. *Biochimica et Biophysica Acta-Gene Regulatory Mechanisms*, *1819*(9-10), 1107-1111. https://doi.org/10.1016/j.bbagrm.2011.10.008

693 Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: a practical
694 and powerful approach to multiple testing. *Journal of the Royal Statistical Society: Series*
695 *B*, *57*(1), 289-300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x
696
697 Blier, P. U., Dufresne, F., & Burton, R. S. (2001). Natural selection and the evolution of
698 mtDNA-encoded peptides: Evidence for intergenomic co-adaptation. *Trends in*
699 *Genetics*, *17*(7), 400-406. https://doi.org/10.1016/S0168-9525(01)02338-1
700
701 Brand, M. D. (2010). The sites and topology of mitochondrial superoxide production.
702 *Experimental Gerontology*, *45*(7-8), 466-472. https://doi.org/10.1016/j.exger.2010.01.003
703
704 Bryant, D. M., Johnson, K., DiTommaso, T., Tickle, T., Couger, M. B., Payzin-Dogru,
705 D., Lee, T. J., Leigh, N. D., Kuo, T. H., Davis, F. G., Bateman, J., Bryant, S.,
706 Guzikowski, A. R., Tsai, S. L., Coyne, S., Ye, W. W., Freeman Jr., R. M., Peshkin, L.,
707 Tabin, C. J., ... Whited, J. L. (2017). A tissue-mapped axolotl de novo transcriptome
708 enables identification of limb regeneration factors. *Cell Reports*, *18*(3), 762-776.
709 https://doi.org/10.1016/j.celrep.2016.12.063
710
711 Burton, R. S. (1985). Mating system of the intertidal copepod *Tigriopus californicus*.
712 *Marine Biology*, *86*(3), 247-252. https://doi.org/10.1007/BF00397511
713
714 Burton, R. S. (1986). Evolutionary consequences of restricted gene flow among natural
715 populations of the copepod, *Tigriopus californicus*. *Bulletin of Marine Science*, *39*(2),
716 526-535.
717
718 Burton, R. S. (1990). Hybrid breakdown in developmental time in the copepod *Tigriopus*
719 *californicus*. *Evolution*, *44*(7), 1814-1822. https://doi.org/10.1111/j.1558-
720 5646.1990.tb05252.x
721
722 Burton, R. S. (1997). Genetic evidence for long term persistence of marine invertebrate
723 populations in an ephemeral environment. *Evolution*, *51*(3), 993-998.
724 https://doi.org/10.2307/2411174
725
726 Burton, R. S., & Barreto, F. S. (2012). A disproportionate role for mtDNA in
727 Dobzhansky–Muller incompatibilities?. *Molecular Ecology*, *21*(20), 4942-4957.
728 https://doi.org/10.1111/mec.12006
729
730 Burton, R. S., Byrne, R. J., & Rawson, P. D. (2007). Three divergent mitochondrial
731 genomes from California populations of the copepod *Tigriopus californicus*. *Gene*,
732 *403*(1-2), 53-59. https://doi.org/10.1016/j.gene.2007.07.026
733
734 Burton, R. S., Ellison, C. K., & Harrison, J. S. (2006). The sorry state of F_2 hybrids:
735 consequences of rapid mitochondrial DNA evolution in allopatric populations. *The*
736 *American Naturalist*, *168*(S6), S14-S24. https://doi.org/10.1086/509046
737

738 Burton, R. S., Feldman, M. W., & Swisher, S. G. (1981). Linkage relationships among
739 five enzyme-coding gene loci in the copepod *Tigriopus californicus*: A genetic
740 confirmation of achiasmatic meiosis. *Biochemical Genetics*, *19*(11), 1237-1245.
741 https://doi.org/10.1007/BF00484576
742
743 Burton, R. S., & Lee, B. N. (1994). Nuclear and mitochondrial gene genealogies and
744 allozyme polymorphism across a major phylogeographic break in the copepod *Tigriopus*
745 *californicus*. *Proceedings of the National Academy of Sciences*, *91*(11), 5197-5201.
746 https://doi.org/10.1073/pnas.91.11.5197
747
748 Burton, R. S., Pereira, R. J., & Barreto, F. S. (2013). Cytonuclear genomic interactions
749 and hybrid breakdown. *Annual Review of Ecology, Evolution, and Systematics*, *44*(1),
750 281-302. https://doi.org/10.1146/annurev-ecolsys-110512-135758
751
752 Cannino, G., Di Liegro, C. M., & Rinaldi, A. M. (2007). Nuclear–mitochondrial
753 interaction. *Mitochondrion*, *7*(6), 359-366. https://doi.org/10.1016/j.mito.2007.07.001
754
755 Chae S, Ahn BY, Byun K, Cho YM, Yu M-H, Lee B, Hwang D, Park KS. (2013). A
756 systems approach for decoding mitochondrial retrograde signaling pathways. *Science*
757 *Signaling*, *6*(264), rs4. https://doi.org/10.1126/scisignal.2003266
758
759 Ding, Y., Chen, W., Li, Q., Rossiter, S. J., & Mao, X. (2021). Mitonuclear mismatch
760 alters nuclear gene expression in naturally introgressed *Rhinolophus* bats. *Frontiers in*
761 *Zoology*, *18*(1), 1-14. https://doi.org/10.1186/s12983-021-00424-x
762
763 Dobin, A., Davis, C. A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P.,
764 Chaisson, M., & Gingeras, T. R. (2013). STAR: ultrafast universal RNA-seq
765 aligner. *Bioinformatics*, *29*(1), 15-21. https://doi.org/10.1093/bioinformatics/bts635
766
767 Drummond, E., Short, E., & Clancy, D. (2019). Mitonuclear gene X environment effects
768 on lifespan and health: How common, how big?. *Mitochondrion*, *49*, 12-18.
769 https://doi.org/10.1016/j.mito.2019.06.009
770
771 Du, S. N., Khajali, F., Dawson, N. J., & Scott, G. R. (2017). Hybridization increases
772 mitochondrial production of reactive oxygen species in sunfish. *Evolution*, *71*(6), 1643-
773 1652. https://doi.org/10.1111/evo.13254
774
775 Edmands, S. (2001). Phylogeography of the intertidal copepod *Tigriopus californicus*
776 reveals substantially reduced population differentiation at northern latitudes. *Molecular*
777 *Ecology*, *10*(7), 1743-1750. https://doi.org/10.1046/j.0962-1083.2001.01306.x
778
779 Edmands, S., & Burton, R. S. (1999). Cytochrome *c* oxidase activity in interpopulation
780 hybrids of a marine copepod: A test for nuclear-nuclear or nuclear-cytoplasmic
781 coadaptation. *Evolution*, *53*(6), 1972-1978. https://doi.org/10.1111/j.1558-
782 5646.1999.tb04578.x

Edmands, S., & Harrison, J. S. (2003). Molecular and quantitative trait variation within and among populations of the intertidal copepod *Tigriopus californicus*. *Evolution*, *57*(10), 2277-2285. https://doi.org/10.1111/j.0014-3820.2003.tb00239.x

Ellison, C. K., & Burton, R. S. (2006). Disruption of mitochondrial function in interpopulation hybrids of *Tigriopus californicus*. *Evolution*, *60*(7), 1382-1391. https://doi.org/10.1111/j.0014-3820.2006.tb01217.x

Ellison, C. K., & Burton, R. S. (2008a). Genotype-dependent variation of mitochondrial transcriptional profiles in interpopulation hybrids. *Proceedings of the National Academy of Sciences*, *105*(41), 15831-15836. https://doi.org/10.1073/pnas.0804253105

Ellison, C. K., & Burton, R. S. (2008b). Interpopulation hybrid breakdown maps to the mitochondrial genome. *Evolution*, *62*(3), 631-638. https://doi.org/10.1111/j.1558-5646.2007.00305.x

Foley, B. R., Rose, C. G., Rundle, D. E., Leong, W., & Edmands, S. (2013). Postzygotic isolation involves strong mitochondrial and sex-specific effects in *Tigriopus californicus*, a species lacking heteromorphic sex chromosomes. *Heredity*, *111*(5), 391-401. https://doi.org/10.1038/hdy.2013.61

Flight, P. A., Nacci, D., Champlin, D., Whitehead, A., & Rand, D. M. (2011). The effects of mitochondrial genotype on hypoxic survival and gene expression in a hybrid population of the killifish, *Fundulus heteroclitus*. *Molecular Ecology*, *20*(21), 4503-4520. https://doi.org/10.1111/j.1365-294X.2011.05290.x

Gershoni, M., Templeton, A. R., & Mishmar, D. (2009). Mitochondrial bioenergetics as a major motive force of speciation. *Bioessays*, *31*(6), 642-650. https://doi.org/10.1002/bies.200800139

Graze, R. M., McIntyre, L. M., Main, B. J., Wayne, M. L., & Nuzhdin, S. V. (2009). Regulatory divergence in *Drosophila melanogaster* and *D. simulans*, a genomewide analysis of allele-specific expression. *Genetics*, *183*(2), 547-561. https://doi.org/10.1534/genetics.109.105957

Hack, C. J. (2004). Integrated transcriptome and proteome data: The challenges ahead. *Briefings in Functional Genomics*, *3*(3), 212-219. https://doi.org/10.1093/bfgp/3.3.212

Haerty, W., & Singh, R. S. (2006). Gene regulation divergence is a major contributor to the evolution of Dobzhansky–Muller incompatibilities between species of *Drosophila*. *Molecular Biology and Evolution*, *23*(9), 1707-1714. https://doi.org/10.1093/molbev/msl033

Han, K. L., & Barreto, F. S. (2021). Pervasive mitonuclear coadaptation underlies fast development in interpopulation hybrids of a marine crustacean. *Genome Biology and Evolution*, *13*(3), evab004. https://doi.org/10.1093/gbe/evab004

Harrison, J. S., & Burton, R. S. (2006). Tracing hybrid incompatibilities to single amino acid substitutions. *Molecular Biology and Evolution*, *23*(3), 559-564. https://doi.org/10.1093/molbev/msj058

Healy, T. M., Bock, A. K., & Burton, R. S. (2019). Variation in developmental temperature alters adulthood plasticity of thermal tolerance in *Tigriopus californicus*. *Journal of Experimental Biology*, *222*(22), jeb213405. https://doi.org/10.1242/jeb.213405

Healy, T. M., & Burton, R. S. (2020). Strong selective effects of mitochondrial DNA on the nuclear genome. *Proceedings of the National Academy of Sciences*, *117*(12), 6616-6621. https://doi.org/10.1073/pnas.1910141117

[dataset] Healy, T. M., & Burton, R. S. (2022). Data from: Differential gene expression and mitonuclear incompatibilities in fast- and slow-developing inter-population *Tigriopus californicus* hybrids. Dryad Digital Repository. https://doi.org/10.5061/dryad.qrfj6q5kd

Healy, T. M., Bryant, H. J., & Schulte, P. M. (2017). Mitochondrial genotype and phenotypic plasticity of gene expression in response to cold acclimation in killifish. *Molecular Ecology*, *26*(3), 814-830. https://doi.org/10.1111/mec.13945

Healy, T. M., Hargadon, A. C., & Burton, R. S. (*in prep.*). Developmental rate displays effects of inheritance but not of sex in inter-population hybrids of *Tigriopus californicus*.

Hedgecock, D., Lin, J. Z., DeCola, S., Haudenschild, C. D., Meyer, E., Manahan, D. T., & Bowen, B. (2007). Transcriptomic analysis of growth heterosis in larval Pacific oysters (*Crassostrea gigas*). *Proceedings of the National Academy of Sciences*, *104*(7), 2313-2318. https://doi.org/10.1073/pnas.0610880104

Herrmann, P. C., Gillespie, J. W., Charboneau, L., Bichsel, V. E., Paweletz, C. P., Calvert, V. S., Kohn, E. C., Emmert-Buck, M. R., Liotta, L. A., & Petricoin III, E. F. (2003). Mitochondrial proteome: Altered cytochrome *c* oxidase subunit levels in prostate cancer. *Proteomics*, *3*(9), 1801-1810. https://doi.org/10.1002/pmic.200300461

Hill, G. E. (2014). Cellular respiration: The nexus of stress, condition, and ornamentation. *Integrative and Comparative Biology*, *54*(4), 645-657. https://doi.org/10.1093/icb/icu029

Hill, G. E. (2015). Mitonuclear ecology. *Molecular Biology and Evolution*, *32*(8), 1917-1927. https://doi.org/10.1093/molbev/msv104

Hill, G. E. (2016). Mitonuclear coevolution as the genesis of speciation and the mitochondrial DNA barcode gap. *Ecology and Evolution*, *6*(16), 5831-5842. https://doi.org/10.1002/ece3.2338

Hill, G. E. (2017). The mitonuclear compatibility species concept. *The Auk: Ornithological Advances*, *134*(2), 393-409. https://doi.org/10.1642/AUK-16-201.1

Hill, G. E. (2019). Reconciling the mitonuclear compatibility species concept with rampant mitochondrial introgression. *Integrative and Comparative Biology*, *59*(4), 912-924. https://doi.org/10.1093/icb/icz019

Hill, G. E., Havird, J. C., Sloan, D. B., Burton, R. S., Greening, C., & Dowling, D. K. (2019). Assessing the fitness consequences of mitonuclear interactions in natural populations. *Biological Reviews*, *94*(3), 1089-1104. https://doi.org/10.1111/brv.12493

Hoekstra, L. A., Julick, C. R., Mika, K. M., & Montooth, K. L. (2018). Energy demand and the context-dependent effects of genetic interactions underlying metabolism. *Evolution Letters*, *2*(2), 102-113. https://doi.org/10.1002/evl3.47

Hoekstra, L. A., Siddiq, M. A., & Montooth, K. L. (2013). Pleiotropic effects of a mitochondrial –nuclear incompatibility depend upon the accelerating effect of temperature in *Drosophila*. *Genetics*, *195*(3), 1129-1139. https://doi.org/10.1534/genetics.113.154914

Horan, M. P., Gemmell, N. J., & Wolff, J. N. (2013). From evolutionary bystander to master manipulator: The emerging roles for the mitochondrial genome as a modulator of nuclear gene expression. *European Journal of Human Genetics*, *21*(12), 1335-1337. https://doi.org/10.1038/ejhg.2013.75

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. (2017). lmerTest package: tests in linear mixed effects models. *Journal of Statistical Software*, *82*, 1-26. https://doi.org/10.18637/jss.v082.i13

Landry, C. R., Hartl, D. L., & Ranz, J. M. (2007). Genome clashes in hybrids: insights from gene expression. *Heredity*, *99*(5), 483-493. https://doi.org/10.1038/sj.hdy.6801045

Lane, N. (2005). *Power, sex, suicide: mitochondria and the meaning of life*. Oxford University Press.

Lê, S., Josse, J., & Husson, F. (2008). FactoMineR: an R package for multivariate analysis. *Journal of Statistical Software*, *25*, 1-18. https://doi.org/10.18637/jss.v025.i01

Lee, J., & Willett, C. S. (2022). Frequent paternal mitochondrial inheritance and rapid haplotype frequency shifts in copepod hybrids. *Journal of Heredity*, *113*(2), 171-183. https://doi.org/10.1093/jhered/esab068

Levin, L., Blumberg, A., Barshad, G., & Mishmar, D. (2014). Mito-nuclear co-evolution: The positive and negative sides of functional ancient mutations. *Frontiers in Genetics*, *5*, 448. https://doi.org/10.3389/fgene.2014.00448

Liao, Y., Smyth, G. K., & Shi, W. (2014). featureCounts: An efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics*, *30*(7), 923-930. https://doi.org/10.1093/bioinformatics/btt656

Lima, T. G., Burton, R. S., & Willett, C. S. (2019). Genomic scans reveal multiple mito-nuclear incompatibilities in population crosses of the copepod *Tigriopus californicus*. *Evolution*, *73*(3), 609-620. https://doi.org/10.1111/evo.13690

Lima, T. G., & Willett, C. S. (2018). Using Pool-seq to search for genomic regions affected by hybrid inviability in the copepod *T. californicus*. *Journal of Heredity*, *109*(4), 469-476. https://doi.org/10.1093/jhered/esx115

Lin, Y., Golovnina, K., Chen, Z. X., Lee, H. N., Negron, Y. L. S., Sultana, H., Oliver, B., & Harbison, S. T. (2016). Comparison of normalization and differential expression analyses using RNA-Seq data from 726 individual *Drosophila melanogaster*. *BMC genomics*, *17*(1), 1-20. https://doi.org/10.1186/s12864-015-2353-z

Lynch, M. (1997). Mutation accumulation in nuclear, organelle, and prokaryotic transfer RNA genes. *Molecular Biology and Evolution*, *14*(9), 914-925. https://doi.org/10.1093/oxfordjournals.molbev.a025834

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.journal*, *17*(1), 10-12. https://doi.org/10.14806/ej.17.1.200

McManus, C. J., Coolon, J. D., Duff, M. O., Eipper-Mains, J., Graveley, B. R., & Wittkopp, P. J. (2010). Regulatory divergence in *Drosophila* revealed by mRNA-seq. *Genome Research*, *20*(6), 816-825. https://doi.org/10.1101/gr.102491.109

Meiklejohn, C. D., Holmbeck, M. A., Siddiq, M. A., Abt, D. N., Rand, D. M., & Montooth, K. L. (2013). An incompatibility between a mitochondrial tRNA and its nuclear-encoded tRNA synthetase compromises development and fitness in *Drosophila*. *PLoS Genetics*, *9*(1), e1003238. https://doi.org/10.1371/journal.pgen.1003238

Meyer, E., & Manahan, D. T. (2010). Gene expression profiling of genetically determined growth variation in bivalve larvae (*Crassostrea gigas*). *Journal of Experimental Biology*, *213*(5), 749-758. https://doi.org/10.1242/jeb.037242

Moran, B. M., Payne, C. Y., Powell, D. L., Iverson, E. N., Banerjee, S. M., Langdon, Q. K., Gunn, T. R., Liu, F., Matney, R., Singhai, K., Leib, R. D., Hernandez-Perez, O.,

962 Corbett-Detig, R., Frydman, J., Schartl, M., Havird, J. C., & Schumer, M. (2021). A
963 lethal genetic incompatibility between naturally hybridizing species in Mitochondrial
964 Complex I. *BioRxiv*. https://doi.org/10.1101/2021.07.13.452279
965
966 Mossman, J. A., Biancani, L. M., & Rand, D. M. (2019). Mitochondrial genomic
967 variation drives differential nuclear gene expression in discrete regions of *Drosophila*
968 gene and protein interaction networks. *BMC Genomics*, *20*(1), 1-23.
969 https://doi.org/10.1186/s12864-019-6061-y
970
971 Mossman, J. A., Biancani, L. M., Zhu, C. T., & Rand, D. M. (2016a). Mitonuclear
972 epistasis for development time and its modification by diet in *Drosophila*. *Genetics*,
973 *203*(1), 463-484. https://doi.org/10.1534/genetics.116.187286
974
975 Mossman, J. A., Tross, J. G., Jourjine, N. A., Li, N., Wu, Z., & Rand, D. M. (2017).
976 Mitonuclear interactions mediate transcriptional responses to hypoxia in *Drosophila*.
977 *Molecular Biology and Evolution*, *34*(2), 447-466.
978 https://doi.org/10.1093/molbev/msw246
979
980 Mossman, J. A., Tross, J. G., Li, N., Wu, Z., & Rand, D. M. (2016b). Mitochondrial-
981 nuclear interactions mediate sex-specific transcriptional profiles in *Drosophila*. *Genetics*,
982 *204*(2), 613-630. https://doi.org/10.1534/genetics.116.192328
983
984 Osada, N., & Akashi, H. (2012). Mitochondrial–nuclear interactions and accelerated
985 compensatory evolution: Evidence from the primate cytochrome *c* oxidase complex.
986 *Molecular Biology and Evolution*, *29*(1), 337-346.
987 https://doi.org/10.1093/molbev/msr211
988
989 Pan, T. C. F., Applebaum, S. L., Frieder, C. A., & Manahan, D. T. (2018). Biochemical
990 bases of growth variation during development: A study of protein turnover in pedigreed
991 families of bivalve larvae (*Crassostrea gigas*). *Journal of Experimental Biology*, *221*(10),
992 jeb171967. https://doi.org/10.1242/jeb.171967
993
994 Pereira, R. J., Barreto, F. S., Pierce, N. T., Carneiro, M., & Burton, R. S. (2016).
995 Transcriptome-wide patterns of divergence during allopatric evolution. *Molecular*
996 *Ecology*, *25*(7), 1478-1493. https://doi.org/10.1111/mec.13579
997
998 Pereira, R. J., Lima, T. G., Pierce-Ward, N. T., Chao, L., & Burton, R. S. (2021).
999 Recovery from hybrid breakdown reveals a complex genetic architecture of mitonuclear
1000 incompatibilities. *Molecular Ecology*, *30*(23), 6403-6416.
1001 https://doi.org/10.1111/mec.15985
1002
1003 Peterson, D. L., Kubow, K. B., Connolly, M. J., Kaplan, L. R., Wetkowski, M. M.,
1004 Leong, W., Phillips, B. C., & Edmands, S. (2013). Reproductive and phylogenetic
1005 divergence of tidepool copepod populations across a narrow geographical boundary in

Baja California. *Journal of Biogeography*, *40*(9), 1664-1675. https://doi.org/10.1111/jbi.12107

Pichaud, N., Bérubé, R., Côté, G., Belzile, C., Dufresne, F., Morrow, G., Tanguay, R. M., Rand, D. M., & Blier, P. U. (2019). Age dependent dysfunction of mitochondrial and ROS metabolism induced by mitonuclear mismatch. *Frontiers in Genetics*, *10*, 130. https://doi.org/10.3389/fgene.2019.00130

Poyton, R. O., & McEwen, J. E. (1996). Crosstalk between nuclear and mitochondrial genomes. *Annual Review of Biochemistry*, *65*(1), 563-607. https://doi.org/10.1146/annurev.bi.65.070196.003023

Pritchard, V. L., Dimond, L., Harrison, J. S., S Velázquez, C. C., Zieba, J. T., Burton, R. S., & Edmands, S. (2011). Interpopulation hybridization results in widespread viability selection across the genome in *Tigriopus californicus*. *BMC genetics*, *12*(1), 1-13. https://doi.org/10.1186/1471-2156-12-54

R Core Team. (2022). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Raisuddin, S., Kwok, K. W., Leung, K. M., Schlenk, D., & Lee, J. S. (2007). The copepod *Tigriopus*: A promising marine model organism for ecotoxicology and environmental genomics. *Aquatic Toxicology*, *83*(3), 161-173. https://doi.org/10.1016/j.aquatox.2007.04.005

Rand, D. M., Haney, R. A., & Fry, A. J. (2004). Cytonuclear coevolution: The genomics of cooperation. *Trends in Ecology & Evolution*, *19*(12), 645-653. https://doi.org/10.1016/j.tree.2004.10.003

Rand, D. M., & Mossman, J. A. (2020). Mitonuclear conflict and cooperation govern the integration of genotypes, phenotypes and environments. *Philosophical Transactions of the Royal Society B*, *375*(1790), 20190188. https://doi.org/10.1098/rstb.2019.0188

Rand, D. M., Mossman, J. A., Spierer, A. N., & Santiago, J. A. (2022). Mitochondria as environments for the nuclear genome in *Drosophila*: Mitonuclear G×G×E. *Journal of Heredity*, *113*(1), 37-47. https://doi.org/10.1093/jhered/esab066

Rawson, P. D., & Burton, R. S. (2002). Functional coadaptation between cytochrome c and cytochrome c oxidase within allopatric populations of a marine copepod. *Proceedings of the National Academy of Sciences*, *99*(20), 12955-12958. https://doi.org/10.1073/pnas.202335899

Regenberg, B., Grotkjær, T., Winther, O., Fausbøll, A., Åkesson, M., Bro, C., Hansen, L. K., Brunak, S., & Nielsen, J. (2006). Growth-rate regulated genes have profound impact

on interpretation of transcriptome profiling in *Saccharomyces cerevisiae*. *Genome Biology*, *7*(11), 1-13. https://doi.org/10.1186/gb-2006-7-11-r107

Robinson, M. D., McCarthy, D. J., & Smyth, G. K. (2010). edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics*, *26*(1), 139-140. https://doi.org/10.1093/bioinformatics/btp616

Saraste, M. (1999). Oxidative phosphorylation at the fin de siecle. *Science*, *283*(5407), 1488-1493. https://doi.org/10.1126/science.283.5407.1488

Shadel, G. S., & Horvath, T. L. (2015). Mitochondrial ROS signaling in organismal homeostasis. *Cell*, *163*(3), 560-569. https://doi.org/10.1016/j.cell.2015.10.001

Sloan, D. B., Havird, J. C., & Sharbrough, J. (2017). The on-again, off-again relationship between mitochondrial genomes and species boundaries. *Molecular Ecology*, *26*(8), 2212-2236. https://doi.org/10.1111/mec.13959

Tangwancharoen, S., Semmens, B. X., & Burton, R. S. (2020). Allele-specific expression and evolution of gene regulation underlying acute heat stress response and local adaptation in the copepod *Tigriopus californicus*. *Journal of Heredity*, *111*(6), 539-547. https://doi.org/10.1093/jhered/esaa044

Temple, M. D., Perrone, G. G., & Dawes, I. W. (2005). Complex cellular responses to reactive oxygen species. *Trends in Cell Biology*, *15*(6), 319-326. https://doi.org/10.1016/j.tcb.2005.04.003

Tsuboko-Ishii, S., & Burton, R. S. (2018). Individual culturing of *Tigriopus* copepods and quantitative analysis of their mate-guarding behavior. *Journal of Visualized Experiments*, 139, e58378. https://doi.org/10.3791/58378

Wallace, D. C. (2010). Mitochondrial DNA mutations in disease and aging. *Environmental and Molecular Mutagenesis*, *51*(5), 440-450. https://doi.org/10.1002/em.20586

Warner, J. R. (1999). The economics of ribosome biosynthesis in yeast. *Trends in Biochemical Sciences*, *24*(11), 437-440. https://doi.org/10.1016/S0968-0004(99)01460-7

Willett, C. S., & Burton, R. S. (2001). Viability of cytochrome c genotypes depends on cytoplasmic backgrounds in *Tigriopus californicus*. *Evolution*, *55*(8), 1592-1599. https://doi.org/10.1111/j.0014-3820.2001.tb00678.x

Willett, C. S., & Burton, R. S. (2003). Environmental influences on epistatic interactions: viabilities of cytochrome *c* genotypes in interpopulation crosses. *Evolution*, *57*(10), 2286-2292. https://doi.org/10.1111/j.0014-3820.2003.tb00240.x

Wittkopp, P. J., Haerum, B. K., & Clark, A. G. (2004). Evolutionary changes in cis and trans gene regulation. *Nature*, *430*(6995), 85-88. https://doi.org/10.1038/nature02698

Yoo, J., Cho, H., & Lee, Y. M. (2020). Modulation of glutathione *S*-transferase and superoxide dismutase in response to heavy metals in brackish water flea *Diaphanosoma celebensis*. *Toxicology and Environmental Health Sciences*, *12*(1), 83-90. https://doi.org/10.1007/s13530-020-00017-x

Young, M. D., Wakefield, M. J., Smyth, G. K., & Oshlack, A. (2010). Gene ontology analysis for RNA-seq: Accounting for selection bias. *Genome Biology*, *11*(2), 1-12. https://doi.org/10.1186/gb-2010-11-2-r14

Yun, J., & Finkel, T. (2014). Mitohormesis. *Cell Metabolism*, *19*(5), 757-766. https://doi.org/10.1016/j.cmet.2014.01.011

Zhou, X., Lindsay, H., & Robinson, M. D. (2014). Robustly detecting differential expression in RNA sequencing data using observation weights. *Nucleic Acids Research*, *42*(11), e91. https://doi.org/10.1093/nar/gku310

Zhu, J., Vinothkumar, K. R., & Hirst, J. (2016). Structure of mammalian respiratory complex I. *Nature*, *536*(7616), 354-358. https://doi.org/10.1038/nature19095

Data Accessibility and Benefit-Sharing

RNA-seq reads will be uploaded to the National Center for Biotechnology Information Sequence Read Archive prior to publication of the manuscript. All datasets, the full RNA-seq and enrichment statistical outputs, our annotated hybrid reference for SD and SC, our *T. californicus* GO database, and the genomic locations of the annotated N-mt genes in *T. californicus* have been uploaded to the Dryad Digital Repository and will be made available once the manuscript has been assigned an identifier.

Benefits Generated: Benefits from this research accrue from the sharing of our data and results on public databases as described above.

Author Contributions

TMH and RSB conceived and designed the experiments; TMH conducted all experiments and analyses; RSB supervised the study; TMH prepared the figures, and TMH and RSB wrote the manuscript.

Tables and Figures

Table 1. Differences in gene expression between fast- and slow-developing copepodids for the 12 differentially expressed nuclear genes encoding subunits of electron transport system complexes.

Gene	ETS complex	Chromosome	Counts per million (μ ± SEM)		FDR-adjusted *p* value[†]
			Slow developers	Fast developers	
ndufs8	I	10	66.2 ± 1.3	81.0 ± 2.9	2.0×10^{-2}
sdhd	II	5	47.8 ± 1.4	59.4 ± 2.7	2.1×10^{-2}
ndufv2	I	2	77.2 ± 2.0	95.8 ± 4.7	2.4×10^{-2}
ndufa10	I	6	169.3 ± 5.0	213.9 ± 10.0	2.6×10^{-2}
atpsyno	V	10	200.1 ± 6.1	257.8 ± 15.0	2.6×10^{-2}
ndufa5	I	5	41.8 ± 1.1	50.4 ± 1.8	2.7×10^{-2}
uqcrq	III	3	91.0 ± 2.4	115.7 ± 7.1	3.0×10^{-2}
ndufs7	I	10	127.1 ± 2.9	159.2 ± 8.7	3.3×10^{-2}
ndufb3	I	5	53.1 ± 2.1	64.8 ± 2.7	3.4×10^{-2}
ndufa8	I	3	79.9 ± 2.3	109.3 ± 13.6	3.8×10^{-2}
ndufa6	I	1	55.3 ± 1.3	69.2 ± 4.6	4.2×10^{-2}
ndufb11	I	10	72.1 ± 2.4	87.5 ± 4.0	4.8×10^{-2}

[†] from the 6-line DE analysis

Table 2. Expression levels of mitochondrial-encoded protein- and rRNA-coding genes in SDxSC and SCxSD F_2 hybrid *T. californicus*.

Gene†	Reads per kilobase per million reads (μ ± SEM)				Mitotype FDR-adjusted *p* value
	SDxSC slow developers	SDxSC fast developers	SCxSD slow developers	SCxSD fast developers	
mt-nd1	4567 ± 308	5001 ± 109	2680 ± 124	2690 ± 256	2.5×10^{-10}
mt-nd2	5483 ± 378	5604 ± 204	5725 ± 210	5699 ± 362	0.93
mt-nd3	2068 ± 157	2180 ± 93	2958 ± 150	2980 ± 444	3.7×10^{-2}
mt-nd4	2235 ± 161	2336 ± 18	4356 ± 325	3698 ± 276	2.5×10^{-9}
mt-nd4l	3763 ± 338	4045 ± 116	4818 ± 203	4565 ± 411	0.29
mt-nd5	2621 ± 169	2773 ± 44	3451 ± 169	3127 ± 288	0.22
mt-nd6	3913 ± 298	4248 ± 131	5769 ± 308	5122 ± 457	3.0×10^{-2}
mt-cyb	3798 ± 236	3777 ± 112	6519 ± 280	6888 ± 406	1.3×10^{-11}
mt-co1	8320 ± 445	8792 ± 249	6071 ± 199	6669 ± 542	8.5×10^{-3}
mt-co2	6670 ± 468	6787 ± 286	7493 ± 422	7587 ± 505	0.64
mt-co3	4098 ± 355	4507 ± 238	8113 ± 331	7370 ± 437	4.1×10^{-10}
mt-atp6	10245 ± 773	10884 ± 503	11840 ± 865	11874 ± 1410	0.70
mt-atp8	65 ± 9	92 ± 14	247 ± 33	152 ± 28	1.1×10^{-8}
mt-rnr1	219 ± 21	240 ± 18	105 ± 11	219 ± 58	4.6×10^{-4}
mt-rnr2	206370 ± 13569	223208 ± 5510	109165 ± 4133	130732 ± 17155	2.3×10^{-11}

† *nd* – complex I subunit, *cyb* – complex III subunit, *co* – complex IV subunit, *atp* – complex V subunit, and *rnr* – rRNA

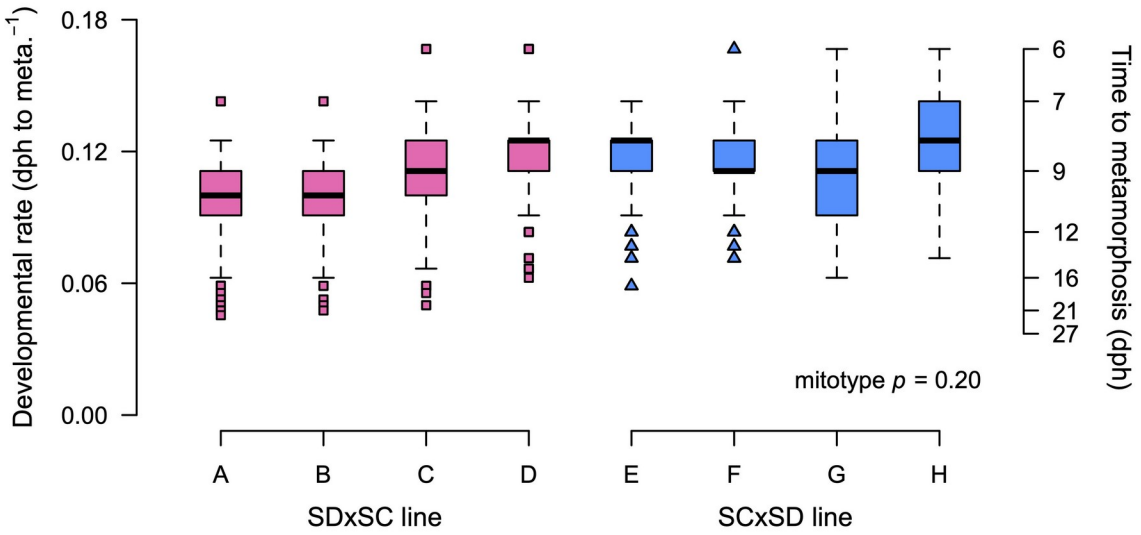


Figure 1. Developmental rate box plots for eight reciprocal F_2 hybrid lines from crosses between San Diego (SD) and Santa Cruz (SC), USA ~~of~~ *Tigriopus*~~.~~ *californicus* (lines A-D: SDxSC, pink, squares; lines E-H: SCxSD, blue, triangles). Developmental rate is shown both as a rate (left axis) and as the days post hatch (dph) to stage 1 copepodid metamorphosis (right axis).

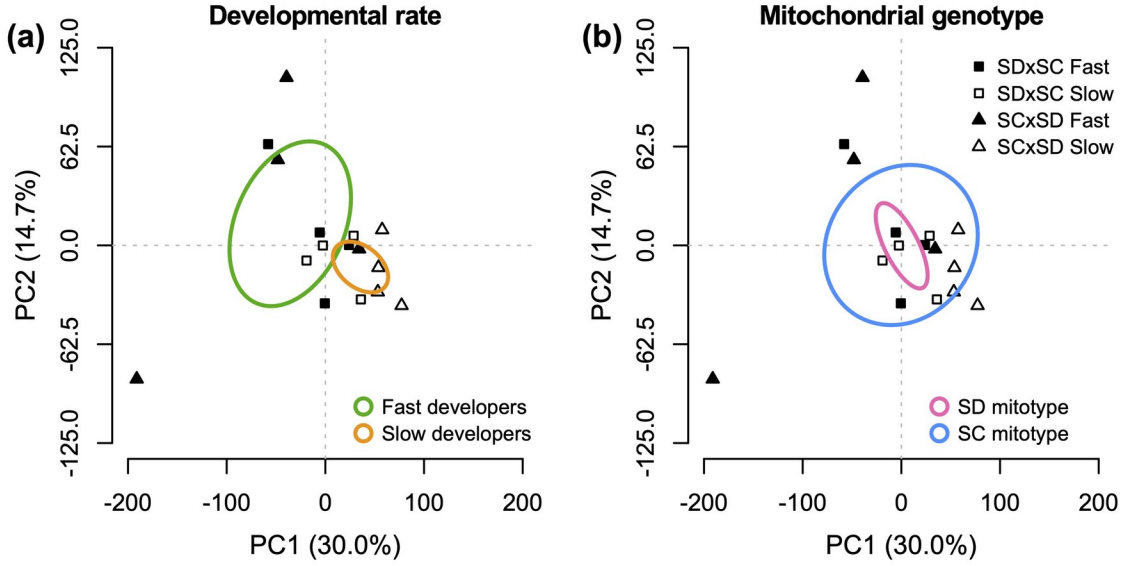
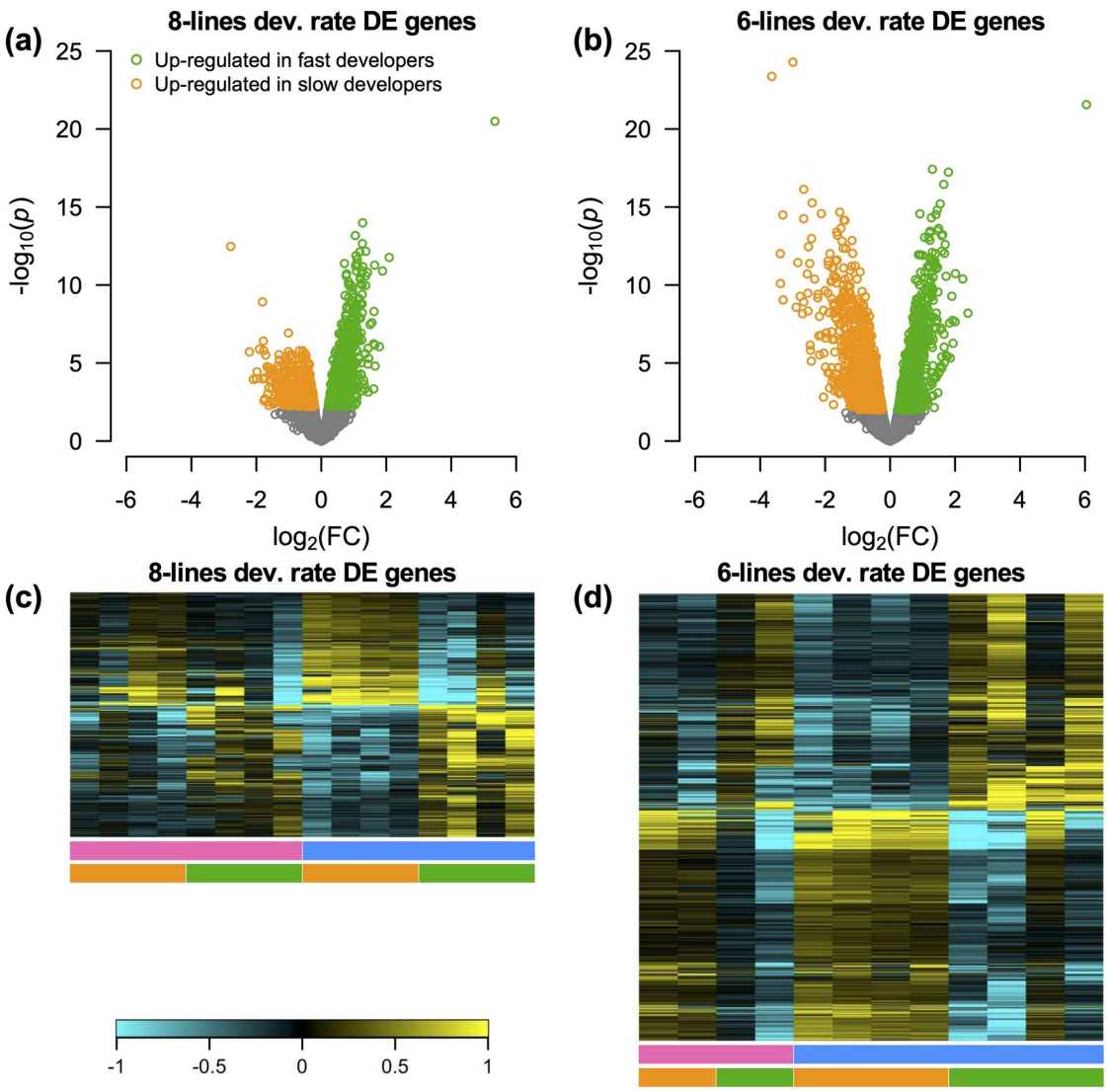


Figure 2. Results from a principal component analysis of transcriptome-wide gene expression for principal components one (PC1) and two (PC2) scores (SDxSC: squares; SCxSD: triangles; fast-developing copepodids: filled symbols; slow-developing copepodids: open symbols). 95% confidence ellipses are shown for developmental rate groups (a – fast developers: green; slow developers: orange) and mitochondrial genotypes (b – SD mitochondrial genotype: pink; SC mitochondrial genotype: blue).



1153 **Figure 3.** Differences in gene expression between fast- and slow-developing F_2 hybrid *T.*
1154 *californicus*. Volcano plots (a – 8-line DE analysis; b – 6-line DE analysis) display the
1155 negative logarithm of the *p*-value versus the fold change in expression for each gene
1156 (green – up-regulated in fast-developing copepodids; orange – up-regulated in slow-
1157 developing copepodids; grey – not differentially expressed). Heat maps (c – 8-line DE
1158 analysis; d – 6-line DE analysis) display relative variation in expression (higher: yellow;
1159 lower: turquoise) among samples (columns) for each differentially expressed gene (rows).
1160 Developmental rates and mitochondrial genotypes for each sample are indicated by the
1161 horizontal bars below each heat map (developmental rate – fast: green, slow: orange;
1162 mitochondrial genotype – SD: pink, SC: blue).

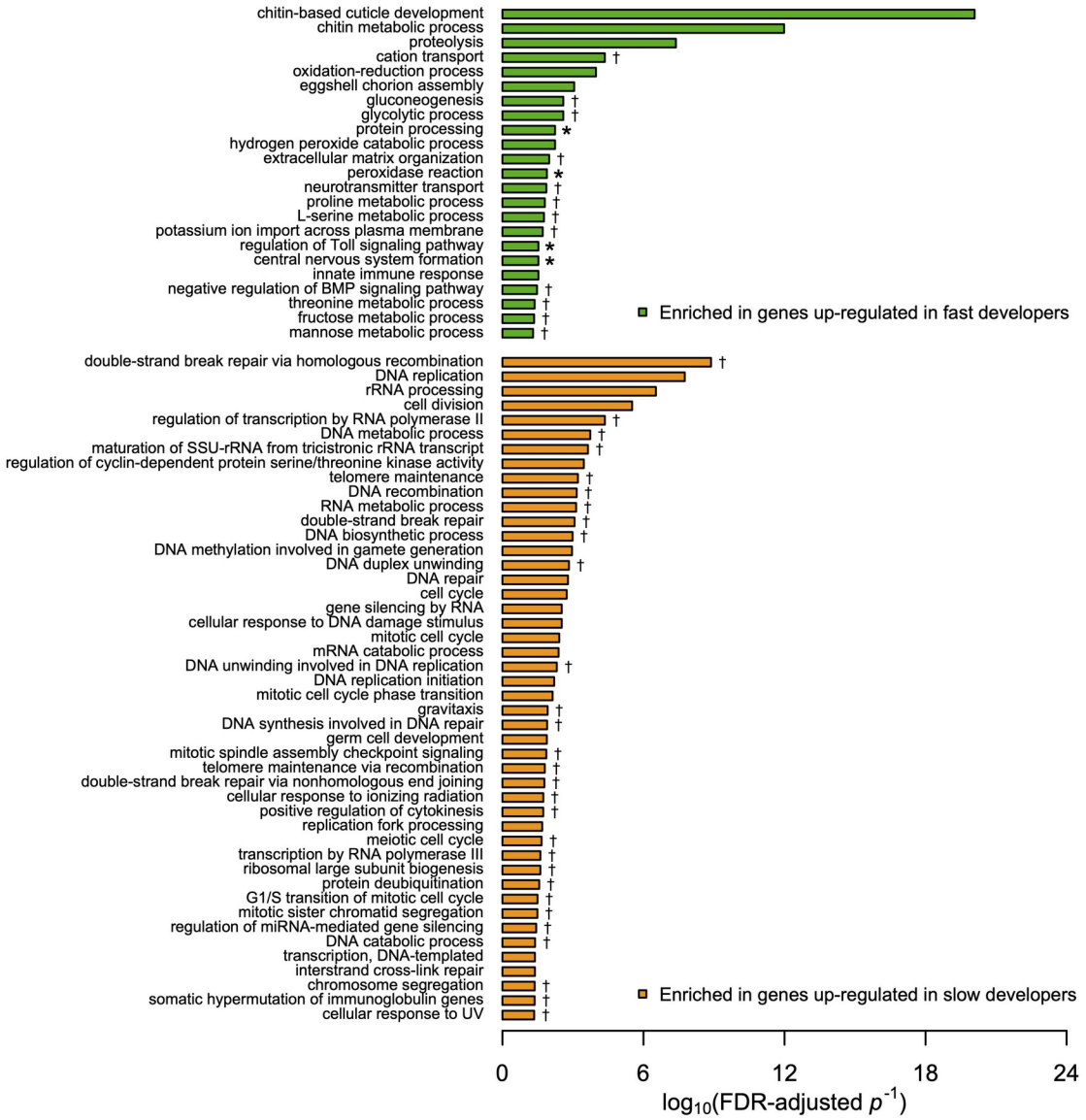


Figure 4. Significantly enriched gene ontology biological process terms among genes up-regulated in fast-developing (green) and slow-developing (orange) copepodids. Bar length indicates the negative logarithm of the FDR-adjusted *p*-value for the enrichment (asterisk – significant for the 8-line DE analysis genes; dagger – significant for the 6-line DE analysis genes; no symbol – significant in genes from both DE analyses; for terms detected in both analyses *p*-values shown are from the 8-line DE analysis).

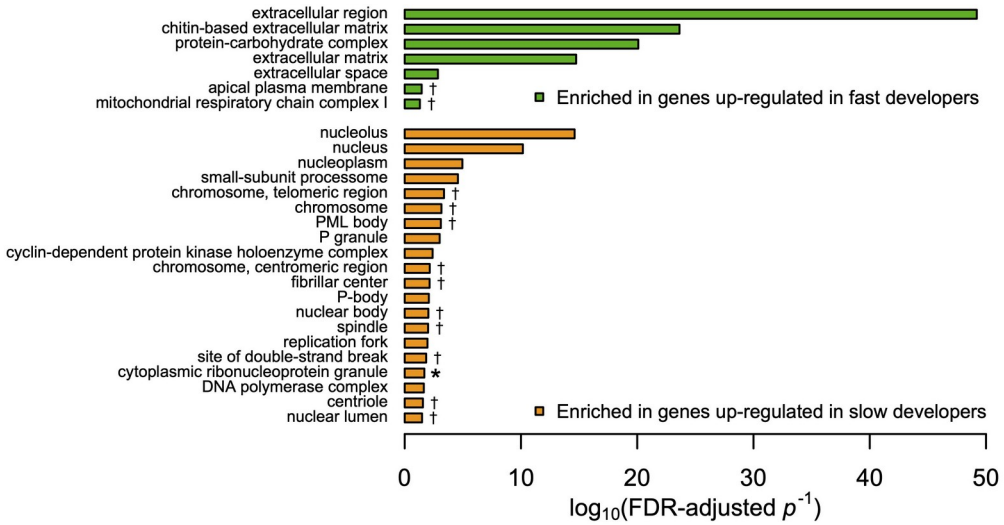


Figure 5. Significantly enriched gene ontology cellular component terms among genes up-regulated in fast-developing (green) and slow-developing (orange) copepodids. Bar length indicates the negative logarithm of the FDR-adjusted *p*-value for the enrichment (asterisk – significant for the 8-line DE analysis genes; dagger – significant for the 6-line DE analysis genes; no symbol – significant in genes from both DE analyses; for terms detected in both analyses *p*-values shown are from the 8-line DE analysis).

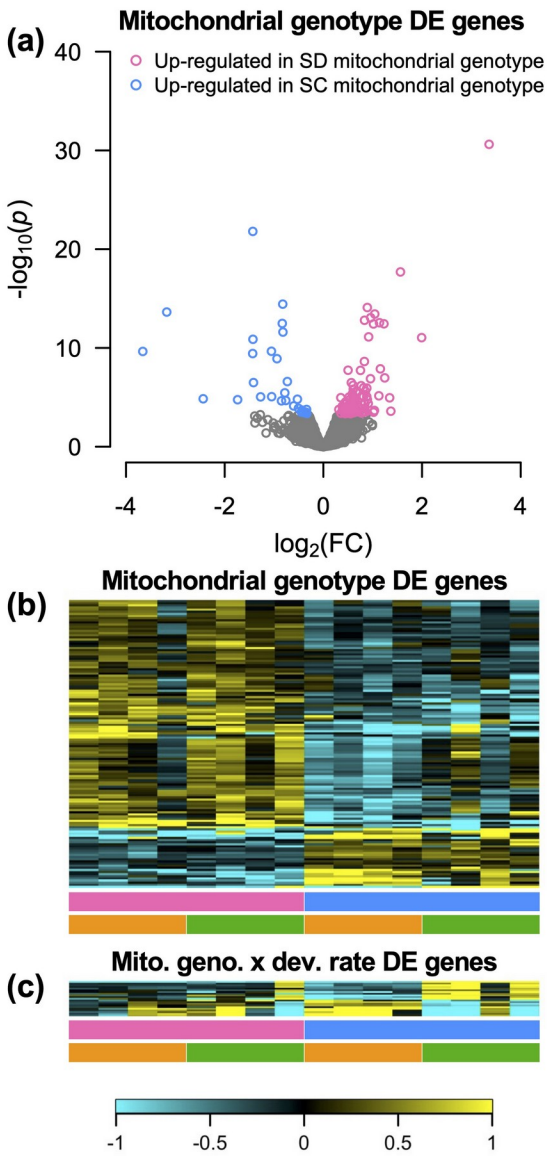


Figure 6. Mitochondrial genotype and interaction effects on gene expression in F_2 hybrid *T. californicus*. The volcano plot (a) displays the negative logarithm of the *p*-value versus the fold change in expression for each gene differentially expressed between hybrids with SD or SC mitochondrial genotypes (pink – up-regulated in SD mitochondrial genotype; blue – up-regulated SD mitochondrial genotype; grey – not differentially expressed). Heat maps display relative variation in expression (higher: yellow; lower: turquoise) among samples (columns) for each gene (rows) with expression patterns affected by mitochondrial genotype (b) or by a mitochondrial genotype x developmental rate interaction (c). Mitochondrial genotypes and developmental rates for each sample are indicated by the horizontal bars below each heat map (mitochondrial genotype – SD: pink, SC: blue; developmental rate – fast: green, slow: orange).