4-2016

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The Effects of Mitochondrial DNA on the Fitness of the Marine Crustacean *Tigripus californicus*

by

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Submitted to the LSU Roger Hadfield Ogden Honors College in partial fulfillment of the Upper Division Honors Program.

April 2016

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Baton Rouge, Louisiana

Abstract

Mitochondrial DNA (mtDNA) has long been ignored when considering the genetic basis for the evolution of traits that confer a fitness advantage to an organism – this role has typically been accredited to nuclear DNA. However, recent research has shown that mtDNA may be under positive selection in organisms, indicating that mtDNA may in fact be involved in fitness. This study sought to test for mtDNA effects on the fitness of the copepod *Tigriopus californicus* by observing differences in maturation and respiration rates between lines of copepods with different mtDNA. Although the results did not provide evidence that mtDNA has an effect on respiration rate, they did show that the mtDNA affects the rate at which the different lines of copepods matured, indicating that the mtDNA affects the organism's fitness.

Introduction

Mitochondrial DNA (mtDNA) only accounts for about 0.00055% of all human DNA, but mitochondria account for about 90% of our energetic metabolism (Ballard and Whitlock 2004). Mitochondrial DNA encodes 37 genes, 24 of which are involved in the translation of the mitochondrial DNA itself and 13 of which encode protein subunits of the electron transport chain. Unlike nuclear DNA (nDNA), mtDNA is haploid and, in most organisms, is inherited only from the mother (Ballard and Whitlock 2004). Because mitochondria are the powerhouse of the cell, it is unsurprising that changes in this small genome can have profound impacts on the fitness of the entire organism. mtDNA has a very high mutation rate, and these mutations have been implicated in diseases such as Leber's hereditary optic neuropathy, chronic progressive external opthalmoplegia, and Kearns-Sayre syndrome (Wallace et al. 1988; Holt, Harding, and Morgan-Hughes 1988). In fact, there are over 50 metabolic diseases that can be directly traced to the mitochondrion (James and Ballard 2003).

Although in the past mtDNA has been used as a neutral genetic marker, recent research has shown that upwards of 45% of nonsynonymous substitutions seen in mtDNA could actually be the result of positive selection (James, Piganeau, and Eyre-Walker 2016). It makes sense that different haplotypes of mtDNA may be positively selected for if they cause an increase in fitness for that organism. In fact, several studies have shown this to be true. A study on bats showed that mtDNA genes involved in the oxidative phosphorylation pathway were positively selected for, increasing the fitness of bats, whose flights require more energy (Shen et al. 2010). On the other hand, in flightless birds, there was less mtDNA selection seen, likely due to their lower metabolic demands (Shen et al. 2009). Similar selective pressures on mtDNA were seen in snakes, insects, higher primates, and a number of other types of mammals (Castoe et al. 2008; Arnqvist et al. 2010; Brown, George, and Wilson 1979). Despite selective pressures due to differences in fitness of these organisms, positive selection rather than purifying selection is observed. Because of this, the original allele persists in the population. This is likely a result of trade-offs that occur when an adaptation that has a positive consequence on one aspect of an organism's fitness has a negative consequence on another. This is seen in the common killifish *Fundulus heteroclitus*. There are two different allozymes for lactate dehydrogenase in these killifish: LDHBa, which has a greater catalytic efficiency at temperatures less than 25°C, and LDHB^{b}, which has a greater catalytic efficiency at temperatures greater than 25°C. These enzymes affect the amount of ATP in

erythrocytes, which influences the amount of oxygen these blood cells can carry and can affect how fast the fish is able to swim. As a result, killifish homozygous for $LDHB^b$ were faster at lower temperatures than those homozygous for LDHBa (Powers and Schulte 1998). Although data was not collected at higher temperatures, homozygotes for $LDHB^a$ would likely have outperformed homozygotes for $LDHB^b$ in these conditions. Unsurprisingly, the $LDBH^b$ allozyme is more common in killifish in colder environments and lower in warmer environments. This is called a specialist-generalist tradeoff: neither genotype of killifish outperforms the other at all temperatures, but both outperform the other genotype in their own environments (Angilletta et al. 2003).

There have been many studies exploring evolution and selection of mtDNA using comparisons among species, but there are currently not a large number of studies that examine the phenotypic effects of intraspecific variation in mtDNA and how these can increase or decrease the fitness of the organism in different environments, leading to evolution. However, the few studies that have been done on copepods, mice, and other organisms show that although small, mtDNA can have large consequences for the fitness of an organism (Schizas et al. 2001; Takeda et al. 2000). A 2003 study showed that 3 different mtDNA haplotypes in fruit flies (*Drosophila simulans*) exist and that these different haplotypes conveyed very different fitness advantages, such as the rate at which the flies matured and their activity levels (James and Ballard 2003). Another study on poikilotherms, organisms whose body temperatures fluctuate with their environment, showed how the external temperature affects mitochondrial activity. As a result, mtDNA would affect the fitness of these poikilotherms by altering their metabolic rate at different temperatures (Somero 2002). The mechanisms by which different mtDNA haplotypes affect fitness are complex and often affected by the environment, especially temperature. For example, a study examining the human mitochondrial genome found that the gene *ATPase6* has the highest amino acid sequence variation of any other gene in the genome, especially in human lineages from arctic regions. The mutant found in these regions is thought to reduce the coupling efficiency of the electron transport chain, which would decrease ATP production but increase heat production (Mishmar et al. 2003). In this case, the mtDNA genotype increases the fitness of individuals living in colder environments by reducing energy production in order to increase heat production.

Our study sought to add to the growing research on fitness effects of mtDNA sequence variation by studying the copepod *Tigriopus californicus*. We used maturation and respiration rates to evaluate differences in fitness and predicted that lines of copepods with different mtDNA would have different maturation and respiration rates. The Salt Point (North1) and Bodega Marine Reserve (North2) lines of copepods were from regions in northern California, while the Bird Rock (South) lines were from southern California (Figure 1) (Kelly, Sanford, and Grosberg 2012). Because the temperature is an average of 3.7°C lower in the North1 and North2 areas than in the South area ("Climate Data and Reports" 2016), we predict that copepods from the North1 and North2 populations will have a higher fitness at colder temperatures while those from the South line have a higher fitness at warmer temperatures. A faster maturation rate is indicative of higher fitness since it allows an organism to produce offspring more quickly, so we predict that copepods from the North1 and North2 lines will have a faster maturation rate at lower temperatures while those from the South lines have faster maturation rates at higher temperatures. Additionally, it has been shown that an organism's cellular

respiration rate has a maximum value at a particular temperature that depends on the organism (Precht 2013). Therefore, we predict that copepods from the South lines will have a maximum respiration rate at a higher temperature than those from the North1 and North2 lines. Furthermore, by performing controlled crosses, we intend to test whether differences in fitness can be attributed variation in the mitochondrial genome among populations of these copepods.

Methods

Maturity

To generate the desired crosses between different populations of copepods, mate guarding pairs were separated. Males and females from opposing populations were paired, and each pair was incubated in 35 ppt water at 20°C until the female became gravid. The incubators used for all experiments were on a light cycle involving 12 hours of light and 12 hours of darkness. The F_1 offspring of these crosses will contain nuclear DNA that is 50% from each parental population, but the mitochondrial DNA will come from only the mother. As a result, the nuclear genome will be the same for both crosses, but the mtDNA will depend on the mother's population of origin. This allows any observed differences in phenotype to be attributed to the mtDNA genotype. Additionally, sex determination in *T. califnicus* has been shown to have a polygenic basis rather than following the typical Mendelian segregation of sex chromosomes (Voordouw and Anholt 2002). The sex of the copepods is affected by factors in the environment, such as temperature, rather than the presence of sex chromosomes. The copepod's lack of typical genetic sex determining system allows us to ignore any effects from sex chromosomes, since they do not exist.

To test for effects of population of origin and temperature on maturation rate, one mate guarding pair, either from the pure population or F_1 individuals from crosses between populations, was put into each small plastic container filled with 35 ppt artificial sea water (Instant Ocean) and food (Spirulina, Aquadine). Half of the containers were incubated at 20°C and half were incubated at 25°C. For each container, the following data were recorded: the date the pair was added to the container, the date the pair separated, the date that larvae were first seen in the container, the date of the first gravid female offspring, and the number of juvenile copepods in the container at the date of first gravid juvenile. A visual inspection of the container was sufficient to obtain the date separated, first gravid female, and count of surviving juveniles. To check for larvae, the containers were observed under a microscope. Containers were checked 3 times per week. Food was added ad libitum. If the copepods died before data could be obtained, the dish was discarded.

Respiration

Mate guarding pairs from the North1, North2, South, North1^{X}South crosses, and North2^{X}South crosses were caught to measure their respiration rates. When the mate guarding pair separated, 5 mature females for each population and temperature being measured were collected and left in a 1.5 mL tube overnight without food. The next day, the 5 females were moved into the smaller plastic respiration tubes with NeoFox measuring equipment ("NeoFox Phase Fluorometer" 2010), taking as little water with the pair as possible. The tubes were then filled to the top with filter sterilized seawater,

leaving no room for excess air. The respiration tubes were weighed before and after adding the water and copepods to account for the amount of water in each tube.

Each tube was scanned using the NeoFox Phase Fluorometer to detect the initial oxygen content in each tube. Scans were done every 3 seconds for 30 seconds, and the average of the ten measurements was taken. Each tube was then incubated at its target temperature for 4.5 hours, with percent oxygen content being scanned again by the NeoFox system every 1.5 hours. This was done for each original and crossed copepod population at 12°C, 16°C, 20°C, 24°C, and 32°C. The data were used to calculate the change in oxygen content over time at each temperature. Control tubes containing the NeoFox equipment but no copepods were included, as well as tubes containing only water to control for temperature.

Statistical Methods

The number of days between separation of the mate-guarding pair and the presence of the first gravid female in the F1 population was used as a measure of maturity. For respiration rates, linear regressions were performed using Microsoft Excel ("Microsoft Excel for Mac 2011" 2010) to determine the rate of oxygen consumption over time. The slope of this line was used as a measure of oxygen consumption rate. For both experiments, R software ("R: A Language and Environment for Statistical Computing" 2015) was used to perform two-way ANOVAs to evaluate the effects of populations and temperatures on each response variable.

Results

Maturity

Our maturity experiment compared crosses between the North1 and South populations: one cross was performed using a North1 female and a South male (North1♀^{X}South♂), while the other was performed using a South female and a North1 male (South♀^{X}North1♂). The number of days between the separation of the parental mate-guarding pair and the presence of the first gravid female in the F_1 population was used as the measure of days to maturity. The days to maturation for each line at 20°C and 25°C was used to run a two-way ANOVA. Table 1a shows that there are significant differences between maturation rates for both the different temperatures and the different copepod lines. It is clear from the average maturation rates (Figure 2 and Table 1b) that both lines mature significantly faster at 25°C than at 20°C. However, the lack of significant interaction between the variables Temperature and Line indicates that the effect of temperature on maturation rate did not vary by line – the lines do not appear to be specialized to perform better at certain temperatures as we had predicted. Instead, the North1♀^{X}South♂ line matures faster than the South♀^{X}North1♂ line regardless of temperature.

Respiration

The data from the respiration experiment were plotted on a graph of O_2 concentration versus time. A linear regression line of the data was performed, and the slope of this line was used as a measure of respiration rate. The values of these slopes are shown in the bar graphs in Figure 3, which include error bars associated with each slope value. Oxygen consumption rates are used as the response variable in a two-way

ANOVA, testing for an effect of mtDNA on the respiration rates of the copepods (Table 2). The only significant difference revealed by this test was a difference in respiration rate according to the copepod line for the North2^{X}South crosses (Table 2b). After further manipulation of this data, it was found that the significant difference recorded was observed between the pure North2 and South lines, not between the crosses. However, the respiration data from the North1^{X}South experiments yielded no significant differences between lines (Table 2a). Additionally, none of the experiments showed that temperature had any significant effect on respiration rate.

Discussion

Both crosses used to generate the lines of copepods used in the maturity experiment included one parent from the North1 line and one parent from the South line, so the nuclear DNA of both the North1♀^{X}South♂ line and the South♀^{X}North1♂ line is 50% from the North1 copepods and 50% from the South copepods. The only genetic difference between the two lines is their mtDNA passed down from the mother – the North1♀^{X}South♂ line contains only North1 mtDNA while the South♀^{X}North1♂ line contains only South mtDNA. Because the nuclear DNA of the two lines is the same, then, any differences in performance between the two lines is due to their different mtDNA. In fact, the data from this experiment shows a significant difference in maturation rate between the two lines of copepods: the North1♀^{X}South♂ line matured more quickly than the South♀^{X}North1♂ line at both temperatures tested, indicating that this line has a higher fitness at both temperatures. It can be concluded, then, that the North1 mtDNA conferred a fitness advantage to the North1♀^{X}South♂ line of copepods that caused it to mature more quickly than the South♀^{X}North1♂ copepod line at both temperatures tested.

The results of the maturity experiment also indicate that both copepod lines mature faster at 25°C than they do at 20°C. Both temperatures used in this experiment are closer to the average temperature of the South collection site than the North1 collection site ("Climate Data and Reports" 2016), yet the line containing the North1 mtDNA matured faster at both temperatures, indicating that it has a higher fitness at these temperatures than the line containing South mtDNA. Additionally, there was no significant interaction detected between the Line and Temperature variables, indicating that the South♀^{X}North1♂ line does not perform better at warmer temperatures while the North1♀^{X}South♂ line performs better at cooler temperatures as we had expected. It appears that neither line is specialized to perform better at a specific temperature – instead, the North1♀^{X}South♂ line performs better at both temperatures. However, maturation rate is not the only measure of fitness of an organism, so it is possible that this lack of interaction is due to a trade-off – for example, the South♀^{X}North1♂ line may mature more slowly but produce more offspring per brood than the North1♀^{X}South♂ line. Further experiments using different measures of fitness could explore this idea.

The only significant result obtained from the respiration experiments is a difference in respiration rate between the pure North2 and pure South copepod populations (Table 2b). From the data shown in Figure 3b, it is evident that the South population has a higher respiration rate than the North2 population at all 5 temperatures tested. However, since these two populations have different nuclear as well as mitochondrial DNA, it is unclear whether this difference is a result of the nuclear or mitochondrial genomes.

As in the maturation experiments, the South♀^{X}North1♂ and North1♀^{X}South♂ lines both contain 50% South and 50% North1 nuclear DNA, differing only in their mtDNA. Similarly, the North2♀^{X}South♂ and South♀^{X}North2♂ lines both contain 50% North2 and 50% South nuclear DNA, differing only in their mtDNA. For both of these crosses, one line of copepods has mtDNA derived from a copepod population accustomed to warmer temperatures (South♀^{X}North 2♂ and South♀^{X}North1♂ lines), while the other has mtDNA derived from a copepod population accustomed to cooler temperatures (North1♀^{X}South♂ and North2♀^{X}South♂ lines). The majority of the genes on mtDNA code for products that are directly involved in cellular respiration, such as enzymes involved in oxidative phosphorylation, tRNA, and rRNA ("Mitochondrial DNA" 2016). It is surprising that lines of copepods with different mtDNA derived from parents accustomed to different climates would show no significant difference in respiration rate, since the genes encoded by mtDNA are so integral in cellular respiration. Some of the respiration rate measurements have high error (Figure 3) and a low R^2 value for the linear regression line, signifying that the slopes obtained for these data points are probably unreliable and are not a good indicator of respiration. However, even when these data points are removed from the data set, no significant differences are seen between populations with the same nuclear DNA but different mtDNA. The data could be improved by running these tests again to get a better measurement of respiration rate.

The hypothesis that, in copepod lines containing the same nuclear DNA, lines with mtDNA derived from copepods accustomed to higher temperatures (South^{X}North1♂ and South♀^{X}North2♂) would reach a maximum respiration rate at a higher temperature than those with mtDNA derived from copepods accustomed to lower temperatures (North2♀^{X}South♂ and North1♀^{X}South♂) is difficult to evaluate from the data available. Figure 3b shows that the North2♀^{X}South♂ line has a maximum respiration rate at 32°C while the South♀^{X}North2♂ line has a maximum respiration rate at 24°C; Figure 3a shows that the South♀^{X}North1♂ and North1♀^{X}South♂ lines both have a maximum respiration rate at 28°C. None of these results are as expected, and the divergence from the hypothesis could be due to the data points with high error values mentioned above. Again, running these data points again could help to evaluate the dataset. Additionally, the ANOVA performed was limited by the available data set to evaluate respiration rate by line and by temperature individually, but not the interaction of the two variables. Supplementing the dataset, possibly by performing multiple trials for each line at each temperature, would allow for analysis of interaction between the two variables of line and temperature and would allow for a more thorough analysis of the dataset.

Although the data from these experiments did not yield the expected results, they do provide some evidence that mtDNA is involved in an organism's fitness as we had predicted. The mtDNA of the copepods in the respiration experiments clearly affected the fitness of the copepods by affecting the time it takes for the copepods to mature. The data collected during the respiration experiments indicate that the mtDNA did not have an effect on the respiration rate of the copepods. However, the shortcomings of the data provide a multitude of ideas for future research to expand upon the hypotheses presented and evaluate the role of mtDNA in other measures of an organism's fitness.

Figures & Tables

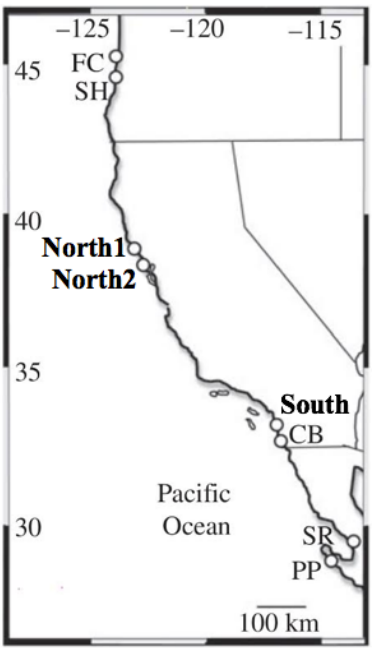


Figure 1. Locations copepods were collected from: Salt Point, CA (North1) 38°20’ N, 123°33’ W; Bodega Marine Reserve, CA (North2) 38°04’ N, 123°19’W; and Bird Rock, CA (South) 32°49’ N, 117°16’ W.

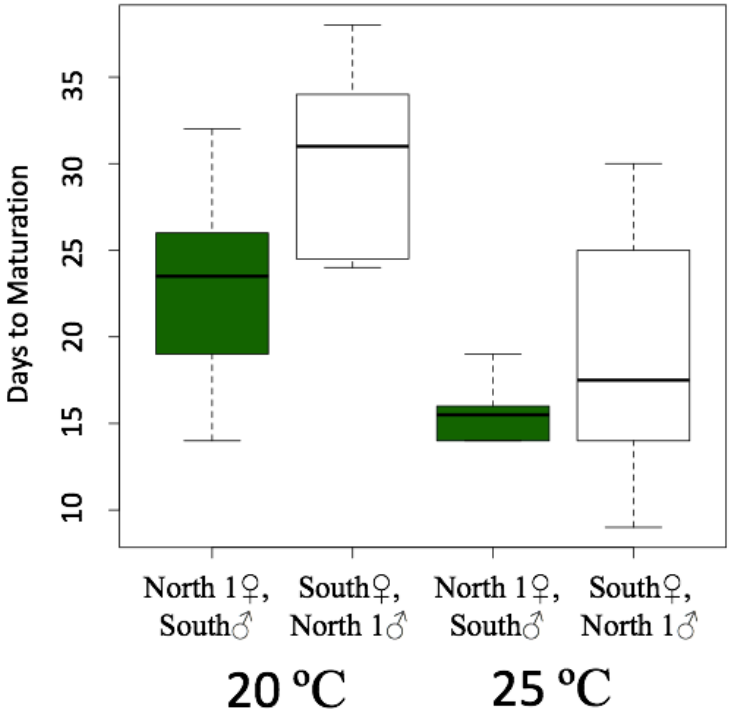
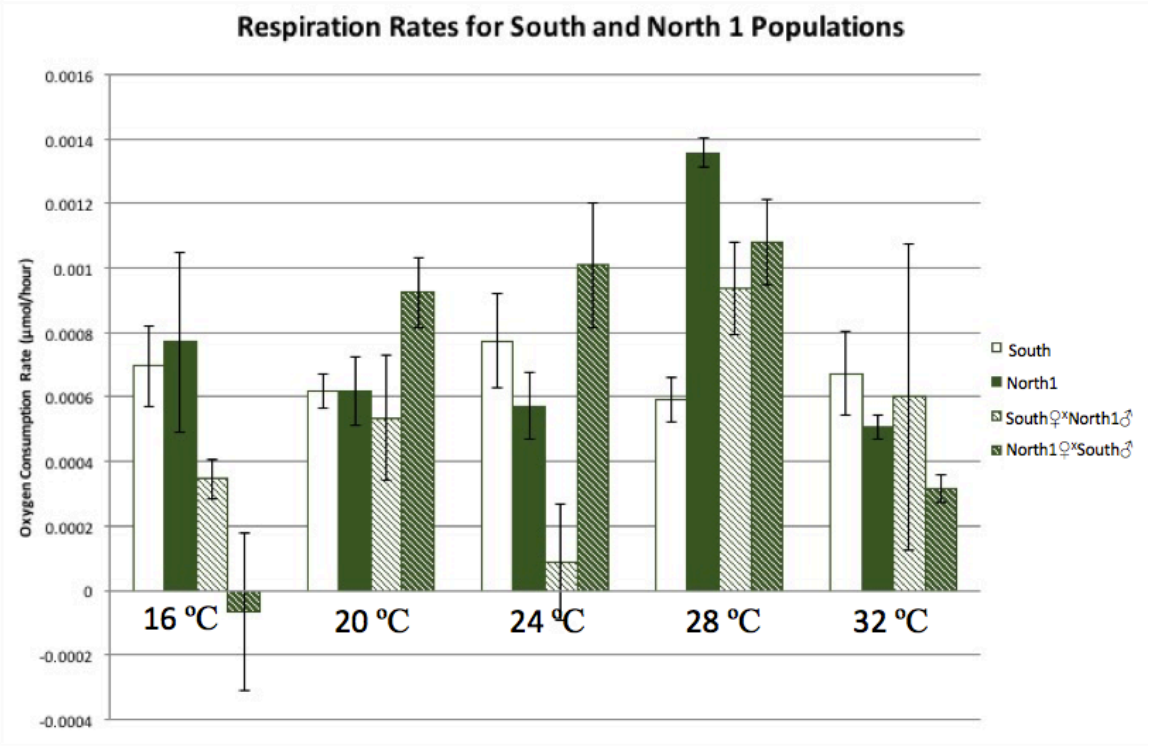


Figure 2. Boxplot of data from the maturity experiment showing average days to maturation as a function of the specific copepod lines at their temperature of incubation.

a)



b)

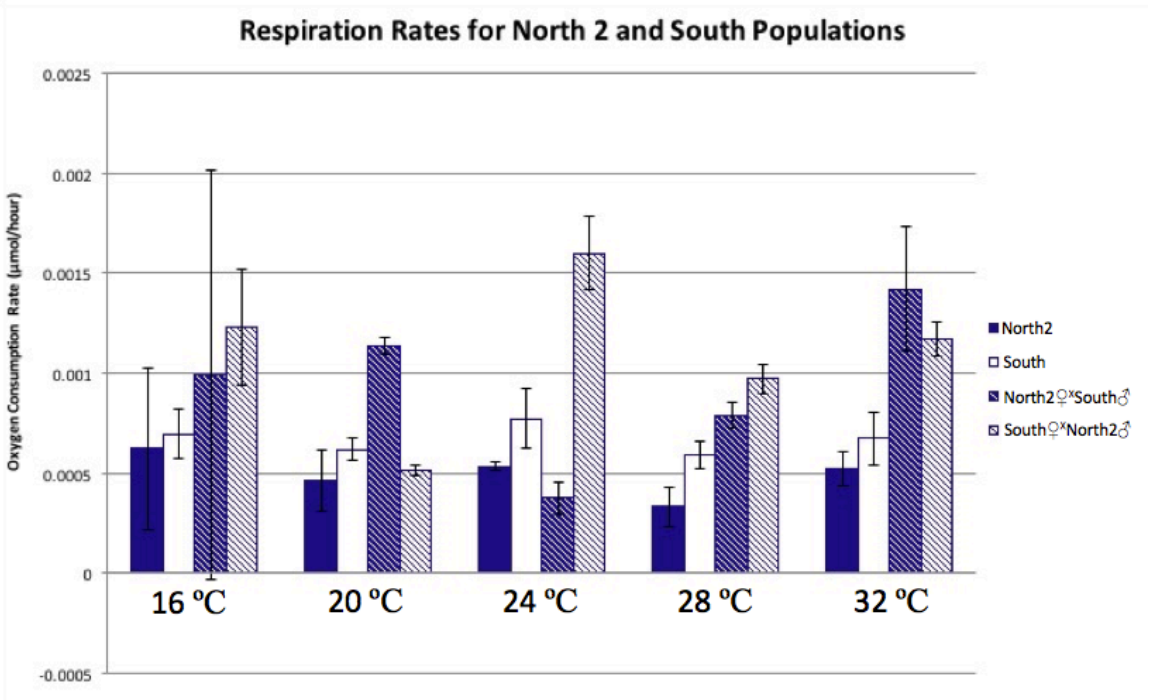


Figure 3. Plots of the slopes of the regression lines created using respiration data, which serve as a measure of respiration rate. Data includes the respiration rates measured at 5 different temperatures (16°C, 20°C, 24°C, 28°C, and 32°C) for a) the pure North1, pure South, and North 1^{X}South copepod lines and b) the pure North2, pure South, and North2^{X}South copepod lines, as well as the error associated with each slope value.

Table 1. Data collected during the maturity experiments for North1 x South crosses at 20°C and 25°C. a) ANOVA results for Days to Maturation as a function of Temperature, Line, and the interaction between Temperature and Line. Values significant at the 0.05 level are indicated with an asterisk. b) Average dates of maturation for each line of copepods at each temperature tested.

a)

	F value	Pr(>F)
Temperature	35.569	5.81e-07*
Line	11.026	0.00196*
Temperature:Line	1.074	0.30649

b)

Temperature (ºC)	Line	Average # Days to Maturation
20	North1♀^{X}South♂	22.9
25	North1♀^{X}South♂	15.5
20	South♀^{X}North1♂	29.7
25	South♀^{X}North1♂	18.9

Table 2. ANOVA from the data from the respiration rate experiments. Both data sets include respiration measurements from the native populations as well as from the crosses for comparison. a) ANOVA results for the respiration experiment involving North1 and SD populations. b) ANOVA results for the respiration experiment involving North2 and SA populations. Values significant at the 0.05 level are indicated with an asterisk.

a)

	F value	Pr(>F)
Temperature	2.666	0.0842
Line	0.576	0.6419

b)

	F value	Pr(>F)
Temperature	1.259	0.3386
Line	3.999	0.0346*

Bibliography

Angilletta, Michael J., Robbie S. Wilson, Carlos A. Navas, and Rob S. James. 2003. "Tradeoffs and the Evolution of Thermal Reaction Norms." *Trends in Ecology and Evolution* 18(5): 234–40.

Arnqvist, Goran et al. 2010. "Genetic Architecture of Metabolic Rate: Environment Specific Epistasis between Mitochondrial and Nuclear Genes in an Insect." *Evolution* 64(12): 3354–63.

Ballard, J. William O, and Michael C. Whitlock. 2004. "The Incomplete Natural History of Mitochondria." *Molecular Ecology* 13(4): 729–44.

Brown, Wesley M., Matthew George, and Allan C. Wilson. 1979. "Rapid Evolution of Animal Mitochondrial DNA." *Proceedings of the National Academy of Sciences of the USA* 76(4): 1967–71.

Castoe, Todd A et al. 2008. "Adaptive Evolution and Functional Redesign of Core Metabolic Proteins in Snakes." *PLoS One* 3(5): e2201.

"Climate Data and Reports." 2016. *National Oceanic and Atmospheric Administration*.

Dwyer, Marge. 2015. "No Title." *Harvard School of Public Health*.

Holt, Ian J., Anita E. Harding, and John A. Morgan-Hughes. 1989. "Deletions of Muscle Mitochondrial DNA in Mitochondrial Myopathies: Sequence Analysis and Possible Mechanisms." *Nucleic Acids Research* 17(12): 4465–69.

James, Avis C., and J. William O Ballard. 2003. "Mitochondrial Genotype Affects Fitness in Drosophila Simulans." *Genetics* 164(1): 187–94.

James, Jennifer E., Gwenael Piganeau, and Adam Eyre-Walker. 2015. "The Rate of Adaptive Evolution in Animal Mitochondria." *Molecular Ecology* 25: 67–78.

Kelly, Morgan W, Eric Sanford, and Richard K Grosberg. 2011. "Limited Potential for Adaptation to Climate Change in a Broadly Distributed Marine Crustacean." *Proceedings of the Royal Society* 279: 349–56.

"Microsoft Excel for Mac 2011." 2010.

"Mitochondrial DNA." 2016. *Genetics Home Reference, A Service of the U.S. National Library of Medicine*.

Mishmar, Dan et al. 2003. "Natural Selection Shaped Regional mtDNA Variation in Humans." *Proceedings of the National Academy of Sciences* 100(1): 171–76.

"NeoFox Phase Fluorometer." 2010.

Powers, Dennis A., and Patricia M. Schulte. 1998. "Evolutionary Adaptations of Gene Structure and Expression in Natural Populations in Relation to a Changing Environment: A Multidisciplinary Approach to Address the Million-Year Saga of a Small Fish." *The Journal of Experimental Zoology* 282: 71–94.

Precht, Herbert. 2013. *Temperature and Life*. Springer Science & Business Media.

"R: A Language and Environment for Statistical Computing." 2015.

Schizas, Nikolaos V. et al. 2001. "Differential Survival of Three Mitochondrial Lineages of a Marine Benthic Copepod Exposed to a Pesticide Mixture." *Environmental Science and Technology* 35(3): 535–38.

Shen, Yong Yi, Peng Shi, Yan Bo Sun, and Ya Ping Zhang. 2009. "Relaxation of Selective Constraints on Avian Mitochondrial DNA Following the Degeneration of Flight Ability." *Genome Research* 19(10): 1760–65.

Shen, Yong-Yi et al. 2010. “Adaptive Evolution of Energy Metabolism Genes and the Origin of Flight in Bats.” *Proceedings of the National Academy of Sciences of the United States of America* 107(19): 8666–71.
Somero, George N. 2002. “Thermal Physiology and Vertical Zonation of Intertidal Animals: Optima, Limits, and Costs of Living.” *Integrative and Comparative Biology* 42: 780–89.
Takeda, Kumiko et al. 2000. “Replicative Advantage and Tissue-Specific Segregation of RR Mitochondrial DNA between C57BL/6 and RR Heteroplasmic Mice.” *Genetics* 155(2): 777–83.
Voordouw, Maarten J, and Bradley R. Anholt. 2002. “Heritability of Sex Tendency in a Harpacticoid Copepod, Tigriopus Californicus.” *Evolution* 56(9): 1754–63.
Wallace, Douglas C et al. 1988. “Mitochondrial DNA Mutation Associated with Leber’s Hereditary Optic Neuropathy.” *Science* 242(4884): 1427–30.