2021

Investigation of Thermal Acclimation on Mitochondrial Membrane Fluidity in Tropical and Temperate Populations of Drosophila melanogaster

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Thesis Defense Date: May 6, 2021

Abstract

The fruit fly, *Drosophila melanogaster*, is an ectotherm with various populations occupying thermal environments across a wide latitudinal range. These populations are differentially thermally adapted to their natural environments, and thus have altered physiologies in response to thermal stress. It has been previously shown that populations of *D. melanogaster* alter the composition of their lipid constituents in response to thermal acclimation to preserve fluidity through a process known as homeoviscous adaptation (HA). The goals of this experiment were to determine if this process was occurring in the mitochondrial membranes of acclimated tropical and temperate genotypes of *D. melanogaster*. Mitochondrial membrane fluidity was measured through the use of the fluorescent probe 1-(4-Trimethylammoniumphenyl) -6-Phenyl-1,3,5-Hexatriene p-Toluenesulfonate (TMA-DPH) and fluorescence anisotropy. I predicted that the tropical genotype would have a more rigid membranes than the temperate genotype, the cold acclimation group would have more fluid membranes than the warm acclimation group, and that the temperate genotype would exhibit greater membrane plasticity across acclimation. For both genotypes, there was an increase in membrane fluidity with acclimation temperature, which is opposite to what would be expected if HA were occurring. Interestingly, genotypes exhibited greater plasticity when acclimated at temperatures that would be normally encountered in their natural environments but not outside that range. With global temperatures rising, it appears that neither genotype is especially better equipped to handle a rapidly changing environment. However, the greater plasticity shown by the tropical genotype at higher acclimation temperature could suggest that it is better suited to handle future increases in temperature.

Introduction

Ectothermic organisms encounter and must adapt to changing environmental temperatures. The maintenance and preservation of physiological functions within these ectotherms is paramount and often requires alterations in response to temperature fluctuations (Somero et al. 2017; Hazel and Williams 1990). In particular, the lipid bilayers of cells are incredibly sensitive to changes in temperature and have important consequences for physiological processes (Cooper et al. 2012; Cooper et al. 2014; Hazel and Williams 1990; Overgaard et al. 2008). Previous studies have shown that lipid membranes exhibit a high level of compensation in response to temperature in a phenomenon known as homeoviscous adaptation (HA) (Cooper et al. 2012; Cooper et al. 2014; Hazel 1995; Sinensky 1974). While many have investigated the role of temperature on bulk lipid constituents, relatively few studies have looked at the effect of temperature on the mitochondrial membrane fluidity (MMF) (Cossins et al. 1980; Dahlhoff and Somero 1993).

The fruit fly, *Drosophila melanogaster*, is an ectotherm that has various populations, which occupy widely different thermal environments from constantly warm tropical regions to thermally variable temperate regions (Lockwood et al. 2018). This represents the potential for differential response in these populations of the same species. Since these populations have evolved to their natural environment, they represent distinctly altered genotypes that can be studied to look at how variations in temperature alter MMF. Under the context of the modern-day rate of climate change, there is a disproportionately higher rate occurring in higher and southern latitudes compared to the equator (Deutsch et al. 2008). This means that *D. melanogaster* populations in more temperate locations must be able to rapidly adapt and exhibit a higher degree of plasticity compared to their tropical counterparts over time. However, it can be argued

that climate change can be even more disastrous for tropical populations. Despite the relatively lower rate of climate change occurring in the tropics, the insects in these areas are especially vulnerable because they have not experienced large-scale changes in temperature before and live closer to their upper thermal limit (Deutsch et al. 2008; Somero et al. 2017). Identifying how these organisms respond to such changes in temperature will be important in determining their adaptive capabilities in the face of an evolving climate. One way to test this is through lab acclimation experiments.

While there have been numerous studies looking at potential homeoviscous adaptation through acclimation (Cooper et al. 2012; Cooper et al. 2014), relatively few have focused on the mitochondrial membrane. Of the ones that have, the comparisons made were between closely related species (Dahlhoff and Somero 1993) or used PE:PC ratio to estimate the degree of fluidity (Chung et al. 2018). The PE:PC ratio describes the relative amounts of phosphatidylethanolamine (PE) to phosphatidylcholine (PC) and allows researchers to infer the degree of plasticity of membranes. No studies have yet looked at the acclimatory effects on MMF in intraspecific populations of *D. melanogaster* and directly measured membrane fluidity. In this present study, I made measurements of MMF of acclimated temperate and tropical populations of *D. melanogaster* using fluorescence anisotropy (FA). Previous studies have measured MMF through the similar quantification of fluorescence polarization (FP) (Dahlhoff and Somero 1993). Both of the measurements represent the same phenomena, but they are calculated using slightly different equations (Zhang et al. 2016). Since membranes are not inherently fluorescent, a fluorescent probe is used, and its movement within the membrane is quantified based on the ratio of the intensity of polarized light it emits. I used the fluorescent probe, 1-(4-Trimethylammoniumphenyl)-6-Phenyl-1,3,5-Hexatriene p-Toluenesulfonate

(TMA-DPH). A more fluid membrane allows TMA-DPH to move more freely within the membrane and thus results in a lower intensity of emitted polarized light and FA value. A more rigid membrane limits the movement of TMA-DPH and thus increases the amount of emitted polarized light and FA value (do Canto et al. 2016). The experiment conducted by Dahlhoff and Somero (1993) found that warm-adapted species of abalone had more rigid membranes than those from colder areas. This finding was consistent with HA.

I investigated the patterns of MMF through the acclimation of differentially thermally adapted populations of temperate and tropical fruit flies. The temperate genotype was from Vermont (VT) and the tropical genotype was from Saint Kitts (SK). Following acclimation at both warm and cold temperatures, I tested the fluidity of mitochondrial membranes through fluorescence anisotropy. I found that the opposite was occurring in regards to HA. The cold acclimate group had more rigid membranes than the warm acclimate group. There was no significant difference between genotype. These results suggest that homeoviscous adaptation is not occurring and that the organisms are not remodeling their mitochondrial membranes to preserve an optimal fluidity. Under certain conditions, the trends seen could be maladaptive and have serious consequences with the current rapidly changing climate.

Methods

Fly Stocks

For this experiment, I used two isogenic female lines of *Drosophila melanogaster* fruit flies that were present in our lab from a previous study (Lockwood et al. 2017). One line was collected from East Calais, VT (VT8) and gifted to our lab by B.S. Cooper and K.L. Montooth. This line was established by a single female founder and inbred for subsequent generations in order to limit the genetic variability and decrease chances of in-lab evolution. The Vermont line

was maintained at controlled densities of 50-100 adult flies per vial. I obtained the other isofemale line used in the experiment from the Drosophila Species Stock Center at the University of California, San Diego (UCSD). In particular, the obtained flies were captured from Monkey Hill, Saint Kitts (SK). Similar to the Vermont line, the Saint Kitts line from UCSD was established by a single female founder and kept at controlled densities. I maintained flies under common-garden conditions on cornmeal-yeast-molasses medium at 25°C on a 12:12 light cycle (Lockwood et al. 2018).

Experimental Treatments

In the experiment, I subjected eggs from each of the lines to acclimation. Stock flies mated at 25°C, and I collected eggs hourly. The eggs were placed in vials with controlled density and placed in one of two incubators. One incubator was at 18°C (cold) and the other at 28°C (warm). Both lines were subjected to both treatments. The eggs were kept in the incubators until the larvae eclosed as adults. Since flies in the warm treatment group developed much faster than the cold treatment (Imasheva et al. 1998; Robinson and Partridge 2001), I staggered the incubation period. To have adults from the two acclimation groups eclose on the same day, I collected eggs for the cold acclimation group 21 days prior to collecting eggs for the warm acclimation group so that eclosion was synchronized. The resulting adult flies were used for mitochondrial extraction and measurement of fluidity by fluorescence anisotropy.

Mitochondria Membrane Fluidity Assay

For mitochondrial extraction, I followed the protocol used by Meiklejohn et al., 2013. I gently homogenized 30 female flies from each acclimation group using a glass teflon dounce

homogenizer in chilled isolation buffer (225 mM mannitol, 75 mM sucrose, 10 mM MOPS, 1 mM EGTA, 0.5% fatty acid-free BSA, pH 7.2). I then centrifuged the samples at 300 g at 4°C for 5 minutes. The resulting supernatant was collected and centrifuged at 6,000 g at 4°C for 10 minutes. I resuspended the resulting mitochondrial pellet in 200µL of resuspension buffer (225 mM mannitol, 75 mM sucrose, 10 mM KCl, 10 mM Tris-HCl, 5 mM KH_2PO_4, pH 7.2). In order to standardize the concentration of mitochondria in each sample, I performed a Quick Start™ Bradford protein assay (Bio-Rad). I followed the protocol for a microplate assay and used 5µL of each sample with 250µL of Bradford dye. I created standards with concentrations of 0, 125, 250, 500, 750, 1,000, 1,500, and 2000 µg/mL of BSA. I measured the absorbance using the in-lab TECAN plate reader at 595 nm. After creating a BSA standard curve, I calculated the concentrations of the samples and adjusted them to 0.5mg/mL with additional respiration buffer as needed.

1-(4-Trimethylammoniumphenyl) -6-Phenyl-1,3,5-Hexatriene p-Toluenesulfonate (TMA-DPH) in phosphate buffer (final concentration 3µM) was then added to the mitochondrial resuspensions. I wrapped the resulting mixtures in aluminum foil and incubated them at 37°C with continuous stirring for 30 minutes as outlined (García-Ruiz et al. 2003). The resulting incubated samples were allowed to cool at room temperature for 5 minutes before being brought to temperatures of either 18°C or 28°C via water baths for 25 minutes. Upon the samples reaching the desired temperature, I loaded 200µL of each into the wells of TECAN black 96 microplates. Along with the samples, I prepared and loaded blanks containing TMA-DPH in phosphate buffer solution.

I measured FA using a TECAN microplate reader. The excitation wavelength for TMA-DPH was set to 360 nm and the emission was 425 nm. The machine was set to the

temperature that corresponded with the treatments being tested at the time. For example, the machine was set to 18°C for samples that were at 18°C after the water bath. This was done to keep samples at the desired temperature while measuring FA. After running the samples, I looked at anisotropy units as a measure of the degree of membrane fluidity present.

Statistical Analyses

For data analysis, I used R statistical programming language (R version 3.5.1). I used a 3-way analysis of variance (ANOVA) to analyze the effects of acclimation, measurement temperature, and genotype on FA. I created an interaction plot to visualize the mean FA values for each of the treatment groups.

Results

There was not a significant difference in anisotropy between the Vermont and St. Kitts genotypes with the mean anisotropy of 155.5 ± 4.117 for VT and 161.2 ± 5.407 for SK (Figure 1; Table 1; p=0.2589). The mean anisotropy for Vermont was generally lower across acclimation and measurement temperature groups except for the 28°C measurement at an acclimation of 28°C. Here, the Vermont mean anisotropy was actually higher than SK. Within the 18°C acclimate group, the SK flies appear to have higher anisotropy values than the VT flies, although it is still not statistically significant (Figure 1; Table 1; p=0.24).

Table 1. 3-way ANOVA results for the factors of genotype, acclimation, and measurement temperature effect on anisotropy and MMF. There was a significant effect of acclimation and measurement temperature on anisotropy (p=0.0003 and p=0.0475, respectively) and no significant effect of genotype (p=0.2589). There was also no significant interaction between any of the factors.

Anisotropy ANOVA					
Factor	Sum Sq.	Df	F-value	P-value	Significance
Genotype	508.8	1	1.3098	0.2589	
Acclimation	6058.6	1	15.5972	0.0003	***
Measurement Temperature	1619.5	1	4.1692	0.0475	*
Genotype: Acclimation	1089.2	1	2.8040	0.1015	
Genotype: Measurement Temp.	0.2	1	0.0006	0.9801	
Acclimation: Measurement Temp.	43.2	1	0.1128	0.7387	
Genotype: Acclimation: Measurement Temp.	611.2	1	1.5733	0.2167	

There was a significant effect of acclimation on anisotropy between the 18°C and 28°C acclimation groups with a mean anisotropy of 169.4 ± 3.607 for 18°C and 145.9 ± 4.604 for 28°C (Figure 1; Table 1; p=0.0003). Surprisingly, there were higher anisotropy values, meaning greater membrane rigidity, for the cold acclimate group in comparison to the warm acclimate group. This is contrary to what would be expected if homeoviscous adaptation were occurring in these populations. There was also no significant interaction occurring between genotype and acclimation (Figure 1; Table 1; p=0.1015).

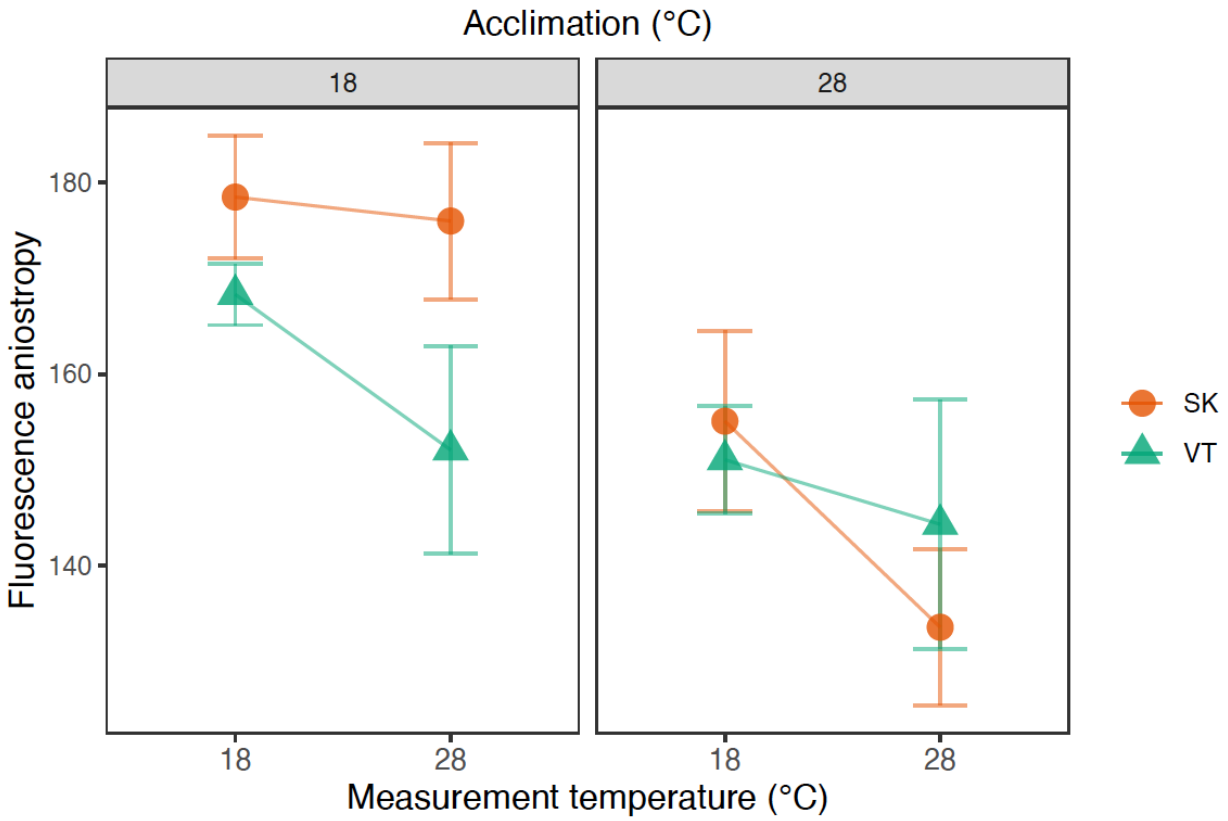

Figure 1. Interaction plot for fluorescence anisotropy values for Vermont and Saint Kitts genotypes acclimated to either 18°C or 28°C across measurement temperatures of either 18°C or 28°C. Points represent mean (n=50) anisotropy values for each treatment group. The error bars represent one standard error of measurement both above and below each mean. Each line connects the mean of one group to its counterpart at the other measurement temperature and allows visualization of the degree of change in anisotropy. Anisotropy was not significantly different based on genotype (p=0.2589). Anisotropy was significantly different for acclimation and measurement temperature (p=0.0003 and p=0.0475, respectively). There were no significant interactions between variables.

There was a significant effect of measurement temperature on anisotropy between the 18°C and 28°C measurement temperature groups with a mean anisotropy of 164.2 ± 3.409 for 18°C and 150.4 ± 5.875 for 28°C (Figure 1; Table 1; p=0.0475). There was an overall higher degree of rigidity present in samples measured at 18°C compared to 28°C. This is consistent with the effect of temperature on membranes. While not a significant result, there was this trend that VT showed higher MMF plasticity after acclimation at 18°C and SK showed higher MMF plasticity after acclimation at 28°C (Figure 1; Table 1; p=0.2167). 18°C acclimation of SK did not show a large change in fluidity with a change in measurement temperature while VT did.

Conversely, in the 28°C acclimation group, VT did not show a large change in fluidity with a change in measurement temperature while SK did (Figure 1).

Discussion

The goal of my research was to determine if thermal acclimation had an effect on the mitochondrial membrane fluidity of temperate and tropical populations of *D. melanogaster*. In order to test this, I acclimated both genotypes to temperatures of either 18°C and 28°C and measured their mitochondrial membrane fluidity using fluorescence anisotropy via the fluorophore TMA-DPH. I predicted that these flies would exhibit HA and compensate for the thermal pressure by altering the fluidity of their membranes, SK flies would have more rigid membranes than VT flies, and that VT flies would exhibit a greater plastic response from acclimation.

The measurements of fluorescence anisotropy indicated that the cold acclimate flies had a significantly more rigid membrane in comparison to the warm acclimate flies. This result is directly contrary to what I would expect to happen if HA were occurring. Previously, Dahlhoff and Somero (1993) found that there was an inverse relationship between MMF and FP in thermally acclimated species of abalone. In other words, they found that the colder acclimation groups had more fluid membranes while the warmer acclimation groups had more rigid membranes for all species of abalone. These findings were consistent with the paradigm of HA. There have been other experiments that have shown that there is no significant HA in certain tissues or species (Rais et al. 2010; Fudge et al. 1998). Rais et al. (2010) found that there was no significant effect of acclimation temperature on FA in membranes of intertidal snails. Fudge et al. (1998) found no significant evidence of HA occurring in the heterothermic rete mirabile

tissue of tuna. However, the lack of significant HA found in these experiments is not the same as what I saw in my experiment. There was a significant thermally induced change in MMF through acclimation that exhibited a trend opposite of what would be expected based on homeoviscous theory. Under the context of my research, the lack of HA and the subsequent rigidification or fluidization from cold or warm acclimation, respectively, points toward a maladaptive response in these populations. This response is maladaptive in the sense that a failure to alter MMF in response to temperature has negative consequences on the membrane-associated reaction mechanisms that take place during aerobic respiration in the mitochondria. Based on previous research, that notion is debatable. The enzymatic reactions that take place and are associated with the mitochondrial membrane, may (Gibbs and Somero 1990) or may not (Raynard and Cossins 1991; Trigari et al. 1992; van den Thillart & Modderkolk 1978) be linked with the fluidity or lipid composition of the membrane itself in response to thermal acclimation. Most notably, Raynard and Cossins (1991) found that the transmembrane sodium pump enzyme of trout erythrocytes and the associated membranes did exhibit compensation, but the degree of compensation was variable depending on the temperature, which suggested that the membrane's effect on embedded proteins was not entirely clear. Based on the lack of strong correlation between membrane fluidity and the function of the linked enzymatic proteins from thermal acclimation, the lack of HA may not be as ecologically maladaptive as originally presumed. It is possible that the mitochondria in the flies are still able to sufficiently carry out their biological functions following acclimation without the necessity for preserving an optimal level of fluidity. Instead, the mitochondrial membranes may be changing more as a function of temperature rather than a function of preservation. To see if this were actually the case, I would have to conduct a lipid assay on the mitochondrial membranes and measure the PE:PC ratio in order to determine

whether lipids were actually being significantly exchanged during the acclimation process or if it was not significant and more of a function of temperature on the membranes. The PE:PC ratio measures the relative abundance of the two most common phospholipids, PE and PC, to draw conclusions about membrane fluidity. Cooper et al. (2014) looked at this PE:PC ratio in response to thermal acclimation in temperate and warmer-climate populations of *D. melanogaster* from the United States. They found that there was an inverse relationship between PE:PC ratio and acclimation temperature. As the acclimation temperature increased, there was a higher degree of PC, a less fluid-inducing phospholipid, in comparison to PE. This finding was consistent with homeoviscous adaptation. However, the experiment did not investigate the mitochondrial membranes of these acclimated fruit fly populations. The study looked at the whole lipid pool, drew inferences about the cellular membrane, and found evidence of HA occurring. However, the comparison between a bulk lipid assay and a mitochondrial membrane assay have different implications with regard to physiological contexts. The bulk lipid study takes into account lipid stores as well that are used as a mobilizable energy source, which must be regulated. The way this regulation takes place may or may not be similar to how metabolic rate is changed through the alteration of the mitochondrial membrane. No studies as of yet have investigated potential phospholipid changing of mitochondrial membranes. The results from such an experiment would shed additional light on my results and how exactly the mitochondrial membrane is becoming less or more fluid depending on cold or warm acclimation, respectively.

While fluorescence anisotropy and fluorescence polarization are calculated in slightly different ways, both terms still give the same information regarding membrane fluidity and are comparable (Owicki 2000). The Dahlhoff and Somero (1993) experiment uses FP to compare relative mitochondrial fluidities, while I used FA. The FP values found in their study ranged

about 200 mFP, while the ones in mine ranged only 80 mFA, meaning that there were overall smaller differences in fluidity between the populations in my study compared to theirs. This smaller gap in fluidity may be important regarding why HA is not occurring in these populations. It is possible that the small gap does not represent one that is necessarily biologically disadvantageous or relevant. The fluidities could exist in a range that is acceptable even with thermal stress and therefore does not require alteration via HA. Moreover, the Dahlhoff and Somero (1993) study had acclimation temperatures that ranged from 5°C to 26°C. If I expanded my acclimation range to a similar range of 20°C (8°C - 28°C), I may see a similar broadening of my FA range to 160 mFA instead. These results would then be more comparable to the findings in the Dahlhoff and Somero (1993) study.

Another key difference between my study and the one conducted by Dahlhoff and Somero (1993) is the model organism used and their environmental and physiological histories. Abalone are marine mollusks that are found underwater and experience a usually stable thermal environment. Fruit flies, on the other hand, live in much more thermally variable climates. More importantly, abalone are slow moving organisms while fruit flies are hyperactive and have consequent higher energy needs. These factors suggest that the mechanisms and homeoviscous strategy employed by abalone to alter the fluidity and functionality of their mitochondria are different from those employed in *Drosophila*. In addition to this, another consideration to take into account is the upper thermal limits of the organism as measured by LT_{50} and whether or not it is dependent on the upper thermal limit of their mitochondria as measured by mitochondrial Arrhenius break temperature (ABT). It appears that this is the case for abalone (Somero 1992), but not for fruit flies as *Drosophila* die at temperatures well below their mitochondrial ABT (Lockwood 2018). This suggests that increases in temperature for fruit flies are not necessarily

maladaptive up to a point. Fewer constraints on mitochondrial function may allow fruit flies to sustain their high metabolic rate at warmer temperatures to support a hyperactive lifestyle.

Looking more at the trends seen in response to acclimation and measurement temperature highlights some consistencies with those seen in previous studies and HA. The SK flies appear to have more rigid membranes when looking at the 18°C acclimation group. This trend is consistent with what is expected regarding homeoviscous adaptation. Since SK flies are from a historically warmer climate, it appears that they have diverged to have more rigid membranes in comparison to their Vermont counterparts. This is similar to the trends seen in Dahlhoff and Somero (1993) and Cooper et al. (2014). However, Dahlhoff and Somero (1993) found that the warm adapted species consistently had more rigid mitochondrial membranes in comparison to the cold adapted ones for all acclimation temperatures. Cooper et al., on the other hand, found that there was no difference between the warm and cold adapted populations when acclimated to a cold temperature, but there was a difference at the higher acclimation temperature. That difference was that Vermont flies had a significant more rigid membrane by PE:PC ratio in comparison to the warmer populations. More importantly, the Vermont flies exhibited a higher degree of change in fluidity or plasticity between acclimations. While in my present experiment it appears that SK might have greater plasticity in terms of difference between acclimation groups, the notion of plasticity is more apparent when comparing measurement temperatures. Specifically, VT exhibits greater plasticity, steeper slope between means of anisotropy across measurement temperature, for the 18°C acclimation group and less so for the 28°C group. Conversely, SK exhibits higher plasticity in the 28°C group and less so in the 18°C group. This is intriguing as it ties back to the findings in Dahlhoff and Somero (1993), where membrane fluidity changes only occurred across the range of temperature at which the species persists. While Vermont flies regularly experience

temperatures of 18°C, the SK flies rarely do. As a result, it appears the SK membranes become rigid and remain that way after acclimating to 18°C regardless of the measurement temperature. VT membranes exhibit a more plastic change after acclimating to 18°C. The opposite is true of the 28°C acclimate group. While it does reach 28°C during the summer months in Vermont, it is not consistent over periods of time. Saint Kitts, on the other hand, does experience 28°C often. As a result, the VT membranes do not change fluidity much in response to different measurement temperatures and have a slope similar to that of SK membranes in the 18°C acclimation. SK membranes at 28°C acclimation, however, exhibit greater plasticity much like the VT membranes acclimated at 18°C. These observations suggest that the degree of membrane plasticity is influenced by the natural range of temperatures the populations normally encounter.

The alterations in membrane fluidity due to measurement temperature also serve as a proof of concept. While HA is a paradigm that may or not may not be observed, the reaction of membranes to temperature has remained consistent. In the presence of warm temperature, a membrane will tend to become more fluid. In the presence of cold temperature, a membrane will tend to become more rigid (Quinn 1988; Lee and Chapman 1987). The measurements of FA in this experiment are consistent with this trend. At higher measurement temperatures, the FA values generally decreased, indicating a more fluid membrane. At the lower membrane temperatures, there were higher FA values, indicating a more rigid membrane. This observed and expected trend indicates that the other observed trends in this experiment are reflective of the actual fluidities present in the treatments.

In regard to the current rate of climate change, neither genotype appears to be better suited to adapt to it than the other. It does appear, however, that each genotype is thermally adapted and shows HA as SK has a higher membrane rigidity in the 18°C acclimation. The effect

of acclimation alone was not enough to reflect the HA that occurs over long periods of evolutionary adaptation to a particular climate. Consideration of metabolic rate is also important when looking at temperature. Since fruit flies are ectotherms, their metabolic rate is fundamentally influenced by changes in temperature (Berrigan & Partridge 1997). Looking specifically at the 18°C acclimate group, both genotypes are going to have reduced metabolic rates. Since the VT mitochondrial membranes exhibit more fluid membranes compared to SK, it suggests that VT flies may be able to better maintain a relatively higher level of metabolism to compensate for the decrease in temperature.

In conclusion, I demonstrated that homeoviscous adaptation is not occurring in response to thermal acclimation in temperate and tropical populations of *D. melanogaster*. The mitochondrial membranes of flies in the 18°C thermal acclimation group exhibited higher fluorescence anisotropy than the mitochondrial membranes of flies in the 28°C thermal acclimation group. These findings are contrary to what would be expected if HA were occurring as cold acclimated flies have more rigid membranes than their warm acclimated counterparts. However, it is possible that this observation is not maladaptive as there is not a strong correlation between mitochondrial membrane fluidity and linked protein functionality (Gibbs and Somero 1990; Raynard and Cossins 1991; Trigari et al. 1992;). Furthermore, the fluidity seen in my experiment exists within a narrow range, which may not be wide enough to have a measurable impact on the organism. I also observed a higher degree of plasticity in the genotypes depending on whether or not they were in the acclimation group that contained a temperature the population encounters in its natural habitat. In the face of climate change, it does not appear that either genotype is especially better suited to handle a rapidly evolving climate. However, evidence of more fluid mitochondrial membranes in VT flies as compared to SK flies acclimated at 18°C

may suggest greater compensatory ability with regard to metabolic rate. With global temperatures on the rise, it becomes important to see how ectotherms may or may not be able to adapt to such rapidly changing conditions. From this study, it appears that *D. melanogaster* populations are not readily equipped to handle such increasing changes in temperature with respect to their mitochondrial membranes.

Acknowledgements

I would like to thank Dr. Lockwood for his consistent mentorship and for allowing me to complete my thesis research in his lab. I would also like to thank Dr. Emily Mikucki for the incredible time and effort she put in to aid me with my research. I am grateful to all the members of the Lockwood lab for their continuous support and feedback over the years.

This work was supported by NSF grant OIA-1826689 to Brent Lockwood

Abbreviations Used

Homeoviscous adaptation (HA)
1-(4-Trimethylammoniumphenyl) -6-Phenyl-1,3,5-Hexatriene p-Toluenesulfonate (TMA-DPH)
Mitochondrial membrane fluidity (MMF)
Phosphatidylethanolamine (PE)
Phosphatidylcholine (PC)
Fluorescence anisotropy (FA)
Fluorescence polarization (FP)
Vermont (VT)
Saint Kitts (SK)
Arrhenius break temperature (ABT)

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