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**Embryonic Heat Shock and its Effect on Larval and Adult Performance in *Drosophila*
*melanogaster***

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Abstract

Drosophila melanogaster live across a wide latitudinal range, thus resulting in divergence in thermal adaptation between populations. Despite differences in thermal tolerances, the effects of an embryonic thermal stress on development have not been studied. The goal of this experiment was to determine the effects of a brief embryonic heat shock on larval and adult performance in tropical versus temperate genotypes of *D. melanogaster*. Larval and adult performance were measured through pupation height and walking speed, respectively. I predicted that as embryonic thermal shock temperature increased, performance values would decrease, showing a physiological cost in response to the heat shock. Additionally, I expected that the tropical genotype would exhibit less of a plastic response to the thermal shock compared to the temperate genotype due to having a higher embryonic thermal tolerance. In response to increasing embryonic shock temperature, performance values decreased similarly in both genotypes. Given the tropical flies only walked at a slightly faster pace, it can be concluded that the thermal shocks resulted in a comparable plastic response in both genotypes. With global temperature rising and an increasing number of days reaching thermal extremes, these results suggest a possible decrease in survival and performance of *D. melanogaster* in the natural environment. Although there is a possibility of natural selection resulting in a less severe plastic response to embryonic thermal stress, based on these data, there may not be significant phenotypic variation among populations of this species on which natural selection can act.

Drosophila melanogaster is a species of fruit fly with a large latitudinal range from tropical to temperate environments (Reinhardt *et al.*, 2014). This pattern leads to a potential for divergence among populations of this species due to differing thermal habitats (David & Capy, 1988; Le Vinh Thuy *et al.*, 2016; Lockwood *et al.*, 2018). Globally, temperatures are on the rise, with an increase in the frequency of days in which thermal extremes are occurring (Meehl *et al.*, 2000). Given this, we can use information about developmental plasticity in response to thermal stress to investigate how fruit flies have previously diverged based on their geographical range and infer how they may continue to adapt based on projected climate patterns. With that, we can observe which traits are affected by natural selection in accordance with thermal stress.

Previous research has shown that embryos from tropical populations of *D. melanogaster* have a significantly higher thermal tolerance than embryos from temperate populations (Lockwood *et al.*, 2018). Tropical genotypes were shown to have a larger proportion of embryos survive after exposure to higher thermal extremes compared to temperate genotypes. However, in the adult stage, *D. melanogaster* from both temperate and tropical regions had similar thermal tolerances (Lockwood *et al.*, 2018). Thus, natural selection has led to higher thermal tolerance in the immobile, embryonic life stage but not the adult mobile stage when comparing tropical versus temperate genotypes. This study only looked at how heat stress affected a single stage, but it is still unknown how heat stress in one life stage may affect downstream development.

Physiological processes can also be affected by thermal stress through developmental plasticity, the process by which an individual's phenotype is changed due to outside pressures they are exposed to during development (Cooper *et al.*, 2012; Gilchrist, G., & Huey, R. 2001; Le Vinh Thuy *et al.*, 2016). Previously it has been shown that embryonic thermal heat stress can result in decreased larval performance as determined by a lowered pupation height (Lockwood *et al.*, 2017).

Pupation height refers to how high up in a vial larvae climb before they pupate. This can be used as a means of determining larval performance, as lower pupation heights are correlated with increased mortality rates during this life stage (Casares, P. *et al.*, 1997). Other research has shown that when *D. melanogaster* were reared in different long-term thermal conditions, the adult thermal tolerance changed through a plastic response to the environment (Cooper, B. S. *et al.*, 2012). *D. melanogaster* raised in warmer temperatures had higher thermal tolerances compared to *D. melanogaster* raised in lower temperature environments (Cooper, B.S. *et al.*, 2012). This shows that heat stress and the thermal environment experienced during development can result in a plastic response affecting later life stages. Given this plastic response, it is also plausible that an embryonic thermal shock may result in changes in larval and adult performance traits, such as pupation height and walking speed. In turn, these differences could be acted upon by natural selection, leading to further divergence between the temperate and tropical lineages based upon physiological processes other than hatching success.

From previous research we know that pupation height can be affected by an embryonic heat stress (Lockwood *et al* 2017). However, it is unclear how genotypes of *D. melanogaster* from different latitudinal ranges may differ in their response to a heat shock in terms of their pupation height. When exposed to a constant thermal environment, changes in adult walking speed have been noted (Gilchrist *et al.*, 1997). However, the effects of a brief thermal stress during embryonic development on adult walking speed is unknown. As walking speed in *D. melanogaster* can be used as a means of estimating overall fly performance, including survival and reproduction success, understanding the effects of an embryonic thermal stress on walking speed can provide insight into possible thermal effects on overall adult stage performance (Gilchrist *et al.*, 1997).

In this research, I sought to determine if thermal adaptation in tropical fruit fly genotypes only effects immobile life stage thermal tolerance, or if there is a difference in plastic response between tropical and temperate lines that also could influence thermal adaptation. Larval performance was determined based on pupation height, and adult performance was determined based on average walking speed. I used two fly genotypes, one from Vermont, USA, and one from Chiapas, Mexico, to represent both a temperate and a tropical lineage. I predicted the flies from Vermont would show significant changes in performance at lower heat-shock temperatures compared to those from Chiapas, though a decrease in pupation height and walking speed would occur in both genotypes. An effect on the fruit fly performance in either the larval stage or adult stage would support the hypothesis that the embryonic heat stress caused a change in the expected phenotype of the individuals. The results would show the potential evolutionary path of fruit flies from different latitudes in terms of what factors potentially had a role in thermal adaptation.

I found that for both the Vermont and Chiapas genotypes, the survival rates were inversely related to temperature. Survival decreased as temperature increased across the three life stages: embryonic, larval, and adult. As the temperature of the embryonic heat shock increased, the average pupation height of the larvae decreased for both genotypes. There was no significant difference in pupation heights between the two genotypes. Adult walking speed decreased in response to increasing embryonic thermal stress temperature, with the Vermont genotype walking at a subtly slower rate compared to Chiapas. These results suggest that thermal stress during the embryonic stage resulted in a plastic response that caused decreased performance in larval and adult life stages. The differences in declining performance ability may be acted upon by natural selection due to differing thermal environments, leading to a subtle divergence between temperate and tropical genotypes. With this research, we can see if larval and adult performance could have

influenced natural selection for thermal tolerance. Looking forward, developmental plasticity in *D. melanogaster* with respect to heat shock and thermal adaptation is important as we face significant temperature changes across the globe.

Materials and Methods

Fly strains

I used two isofemale genetic lines previously obtained by the Lockwood Laboratory. The first line was from Vermont, USA, and the second line was from Chiapas, Mexico. These two lines were selected to represent temperate and tropical populations, respectively. Previously, these lineages showed statistically significant differences in embryonic thermal tolerance but no difference in adult thermal tolerance (Lockwood *et al.*, 2018). I stored flies in vials under common-garden conditions in an incubator set to 25 °C with a 12:12-hour day/night light cycle. Each vial contained a cornmeal-yeast-molasses mixture as the food supply.

Embryonic Heat Shock

I obtained eggs from the flies by first transferring them into a chamber that contained a grape juice agar plate with yeast paste. The flies were allowed to acclimate to the condos for one day before embryos were collected. I used 0-1 hour old embryos for the heat shocks. To obtain embryos within this range, I put a new agar plate in the chamber and immediately moved the chamber into the incubator. The flies were allowed to mate for one hour before the egg plates were removed.

I ran the heat shocks immediately after the egg plates were removed from the chambers. I conducted heat shocks by submerging the egg plates in a hot water bath for 45 minutes. I covered

each egg plate with a lid and wrapped them in Parafilm to prevent water from getting in to the egg plates. It has been previously found that tropical flies have an embryonic thermal tolerance, as measured by LT_{50} , of 35.8 ± 0.45 °C and temperate embryos have thermal tolerance of 34.88 ± 0.18 °C (Lockwood *et al.*, 2018). LT_{50} is the temperature at which 50% of the embryos did not survive the heat stress, thus higher values of LT_{50} indicate enhanced heat tolerance. Eggs that did not hatch were considered to have not survived the thermal shock. Given the thermal tolerance of each genotype, I ran heat shocks at temperatures ranging from 32 °C to 36 °C, at 1 °C increments, as the range encompasses both thermal tolerance temperatures. I removed the egg plates from the water bath after the heat shock was completed and transferred the eggs from the agar plates to new food vials in groups of 20. At the beginning of the experiment, there were 10 vials from Vermont at 32 °C, 14 at 33 °C, 17 at 33 °C, 17 at 35 °C and 10 at 36 °C. For the Chiapas genotype, there were 9 vials at 32 °C, 13 at 33 °C, 14 at 33 °C, 16 at 35 °C and 10 at 36 °C. There was variation in the number of vials at each temperature due to differences in the number of eggs that were laid on each agar plate. The food vials were stored in the incubator for the remainder of the experiment.

Performance Testing

I determined the hatching success by counting the number of eggs that hatched in each vial after 48 hours. I calculated the proportion of eggs that hatched in each vial. Larval performance was determined based pupation height. In the laboratory setting, when larvae prepare to pupate they migrate up the sides of the vials away from the food. In order to measure larval performance, I measured the distance from the top of the food in the vial to the lowest part of each pupa. Individuals that were touching the food or that pupated directly on the food were scored as 0 cm. From the Vermont genotype, there were 129 pupae at 32 °C, 127 pupae at 33 °C, 73 pupae at 34

°C, 26 at 35 °C and 1 at 36 °C. For the Chiapas genotype, there were 150 pupae at 32 °C, 167 pupae at 33 °C, 103 pupae at 34 °C, 47 at 35 °C and 11 at 36 °C. The differences in sample size for each genotype at each temperature were due to differences in survival rates.

Once the adult flies emerged from the pupae, I exposed the flies to exercise trials to determine adult performance. The performance of the flies was measured in terms of their walking speed to get from the bottom to the top of the vial. In order to conduct each trial, I tapped each vial firmly on the benchtop to knock all of the flies in the vial down to the food. When startled, such as when the vials are tapped on the bench top, fruit flies naturally respond by walking/flying upward. I then timed how long the flies took to walk back up to the top of the vial (Gilbert *et al.* 2001). I measured the distance from the food to the top of the vial prior to completing trials for each vial to determine the overall distance the flies were walking. I completed ten trials for each vial and calculated the average walking speed per vial. I counted the number of adult flies that were in each vial after completing the 10 trials to determine if there was an effect of the number of individuals in a vial on the average walking speed.

At 32 °C, there were 10 vials from the Vermont lineage and 9 from the Chiapas lineage. At 33 °C there were 14 from Vermont and 13 from Chiapas, at 34 °C, there were 16 vials from Vermont and 14 vials from Chiapas. At 35 °C there were 12 vials from the Vermont genotype and 14 from the Chiapas genotype. For the 36 °C embryonic heat shock there was 1 vial from the Vermont genotype and 6 vials from the Chiapas genotype. There was variation in the number of vials used at each temperature for each genotype due to differences in survival rates at each thermal shock temperature and between the life stages.

Statistical Analyses

To complete the data analysis I used RStudio and GraphPad Prism. I determined the hatching success, pupation success, and eclosion success using a non-linear regression fitted to a logarithmic curve. The inflection point represented the LT_{50} , or the embryonic thermal shock temperature at which half of the individuals did not survive in each life stage respectively. I used a 2-way analysis of variance (ANOVA) to analyze the results of the effects of temperature and genotype on pupation height. I used a 3-way ANOVA to analyze the effects of temperature, genotype and number of individuals in a vial on the walking speed. As there was no significant interaction between the number of individuals in the vial and the genotype or temperature on fruit fly walking speed, I used a reduced model to complete a 2-way ANOVA looking at the effects of temperature and genotype on walking speed. I considered a higher pupation height to be indicative of greater larval performance and a faster average walking speed to mean more successful adult performance.

RESULTS

Survival

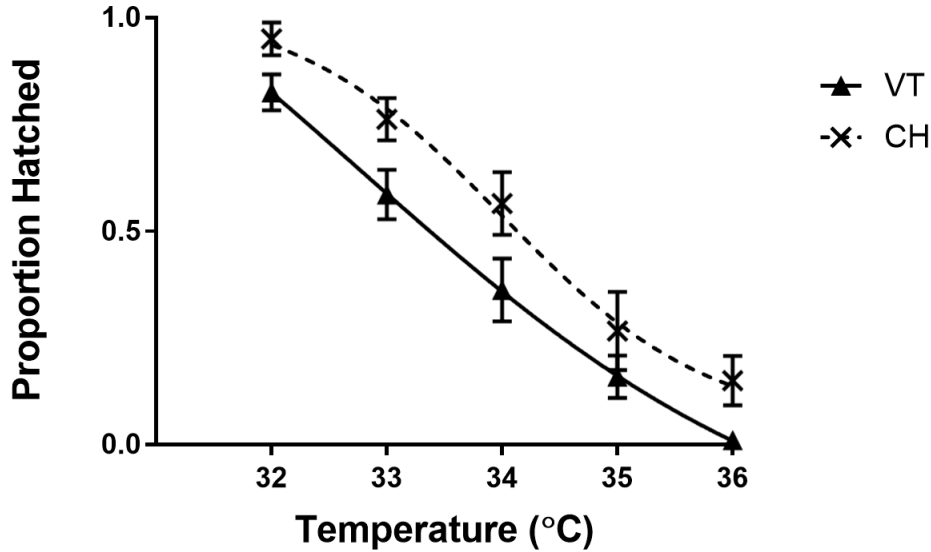


Figure 1: Proportion of eggs that hatched from the Vermont and Chiapas genotypes across five embryonic thermal shock temperatures: 32 °C, 33 °C, 34 °C, 35 °C and 36 °C. Points represent the average proportion of eggs that hatched in each vial for each genotype and temperature. The error bars represent the 95% confidence intervals. LT_{50} between the two genotypes was not significantly different: VT = 33.43 ± 0.27 °C, CH = 34.01 ± 0.25 °C (Nonlinear regression logistic model, $F_{(1, 124)} = 2.156$, $p = 0.1445$).

There was not a significant difference in the LT_{50} between the Vermont and Chiapas genotypes with the LT_{50} for the Vermont genotype being 33.43 ± 0.27 °C and for the Chiapas genotype 34.01 ± 0.25 °C (Figure 1; Non-linear regression logistic model, Fisher's exact test, $F_{(1, 124)} = 2.156$, $p = 0.1445$). As the temperature of the embryonic thermal shock increased, the proportion of eggs that successfully hatched decreased. Additionally, the genotype from Vermont had lower hatching success compared to the genotype from Chiapas across all of the temperatures.

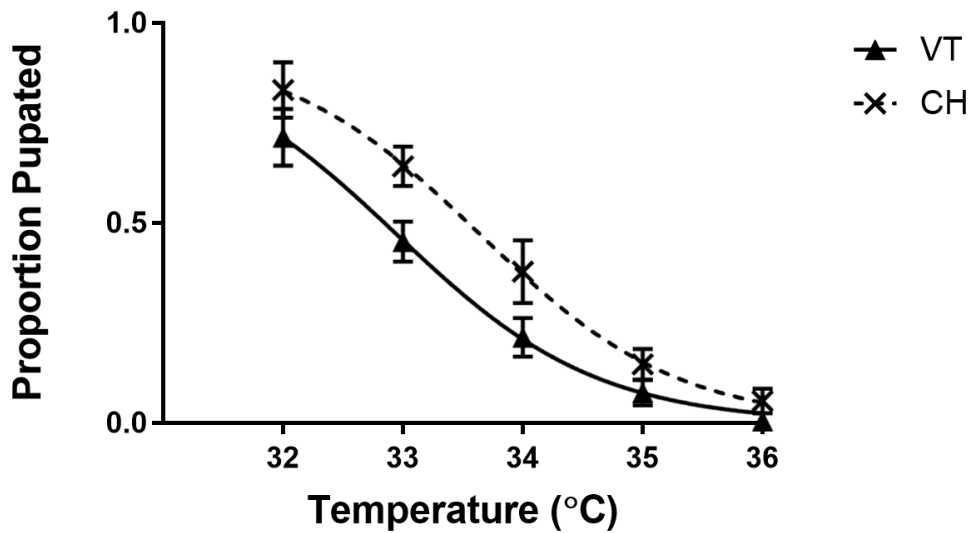


Figure 2: Proportion of individuals that pupated from the Vermont and Chiapas genotypes across the temperature range of 32 °C to 36 °C. Points represent the average proportion of individuals that pupated in each vial for each genotype and temperature. The error bars represent the 95% confidence intervals. The LT_{50} for CH was significantly higher than VT (Non-linear regression logistic model, $F_{(1, 124)} = 5.021$, $p = 0.0268$).

The LT_{50} for pupation success is representative of the number of larvae that successfully pupated. The LT_{50} for the Chiapas genotype was significantly higher than for the Vermont genotype, the LT_{50} values being 33.64 ± 0.18 °C and 32.95 ± 0.27 °C, respectively (Figure 2; Non-linear regression logistic model, Fisher's exact test, $F_{(1, 124)} = 5.021$, $p = 0.0268$). The proportion of individuals that pupated in each vial from the Vermont lineage was lower than the proportion that pupated from the Chiapas lineage. As temperature increased, the proportion of individuals that pupated also decrease. Across the temperature range the Vermont flies pupated at a lower rate compared to the Chiapas lineage.

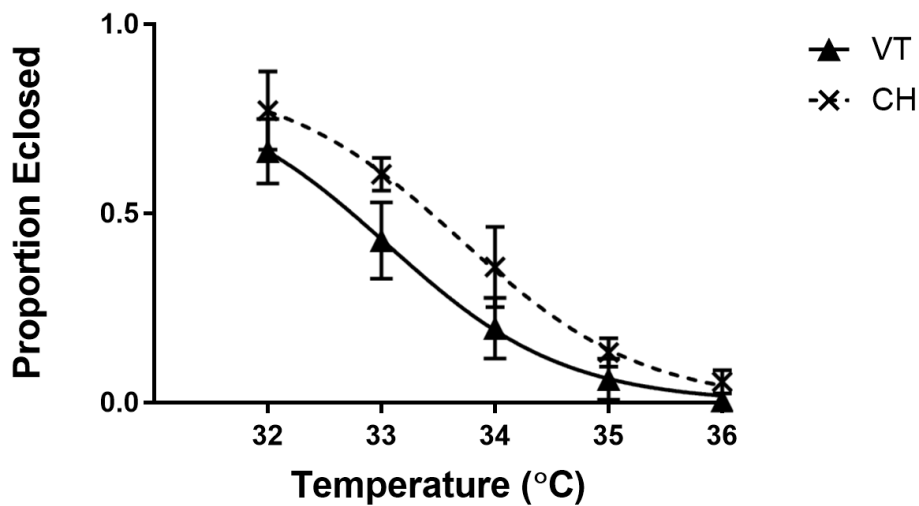


Figure 3: The proportion of individuals that successfully eclosed from Vermont and Chiapas across a 5 °C temperature range: 32 °C, 33 °C, 34 °C, 35 °C and 36 °C. Points represent the average proportion of adults that successful eclosed in each vial for each genotype at specific temperatures. The error bars represent the 95% confidence interval. The LT_{50} for CH was significantly higher than VT (Non-linear regression logistic model, $F_{(1, 121)} = 4.169$, $p = 0.0433$).

The LT_{50} for eclosion success is representative of the number of adult flies that successfully eclosed. The LT_{50} for the Vermont genotype was significantly lower compared to the Chiapas genotype, the LT_{50} values being 33.06 ± 0.22 °C and 33.70 ± 0.21 °C, respectively (Figure 3; Non-linear regression logistic model, Fisher's exact test, $F_{(1, 121)} = 4.169$, $p = 0.0433$). As the temperature of the embryonic heat shock increased, the proportion of flies that successful eclosed decreased. Additionally, the proportion of individuals from the Vermont genotype that eclosed was lower than the proportion of individuals that eclosed from the Chiapas genotype. The trend showed the eclosion proportion of individuals from Vermont was lower than the proportion of individuals that eclosed from Chiapas across the temperature range.

Performance

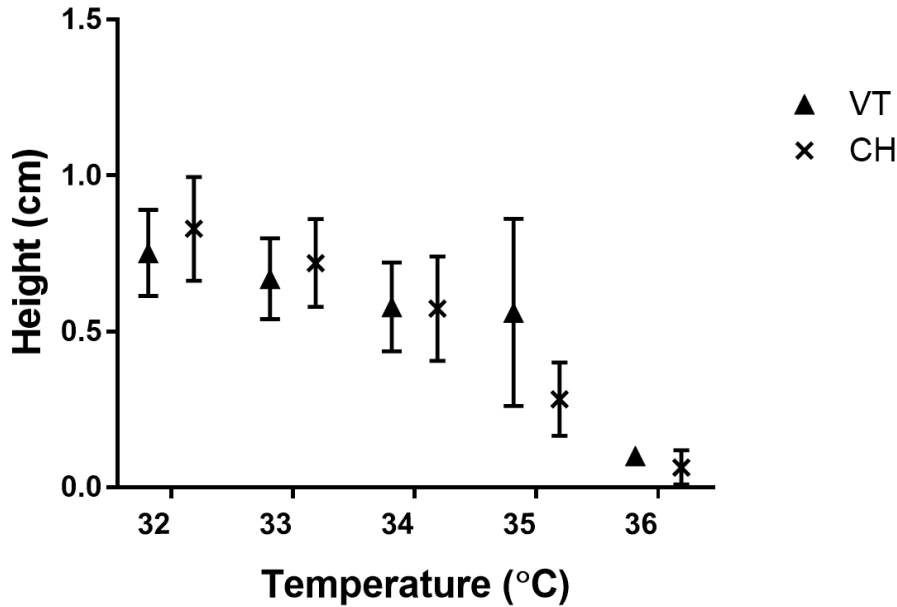


Figure 4: Average pupation height of fly larvae from Vermont and Chiapas after a 45 minute exposure to an embryonic heat shock across 5 temperatures: 32 °C, 33 °C, 34 °C, 35 °C and 36 °C. Points represent the average pupation height for each lineage at each temperature. The error bars represent the 95% confidence interval. There was a significant effect of temperature but not genotype on larval pupation height (2-way ANOVA, genotype $F_{(1, 830)} = 0.014$, $p = 0.9050$, temperature $F_{(1, 830)} = 22.6018$, $p < 0.0001$, interaction $F_{(1, 830)} = 2.4429$, $p = 0.1184$).

There was no significant difference in pupation height observed between the two genotypes (Figure 4; 2-way ANOVA, genotype main effect, $F_{(1, 830)} = 0.014$, $p = 0.9050$). The confidence intervals for each genotype greatly overlapped at each temperature. There was a significant decrease in larval pupation height as the temperature of the embryonic thermal shock increased (Figure 4; 2-way ANOVA, temperature main effect, $F_{(1, 830)} = 22.6018$, $p < 0.0001$). There was no interaction effect between the genotype of the larvae and temperature. In regard to the pupation height, both genotypes responded similarly to the temperature increase (Figure 4; 2-way ANOVA, interaction effect, $F_{(1, 830)} = 2.4429$, $p = 0.1184$).

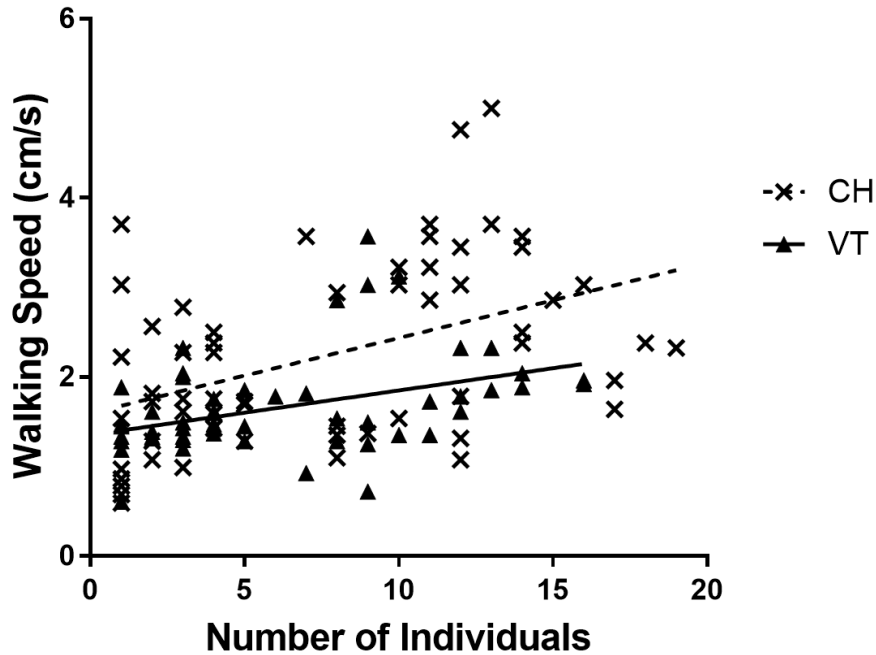


Figure 5: Average walking speed per vial based on the number of adult flies in each vial ranging from 1 to 19 adults. The symbols represent the average walking speed in each vial. The dashed and solid lines represent the linear regression for each genotype. Walking speed increased with increasing number of individuals in the vial (3-way ANOVA, individuals, $F_{(1, 108)} = 26.2897$, $p < 0.0001$).

There was a significant increase in walking speed as the number of individuals in a vial increased (Figure 5; 3-way ANOVA reduced model, individuals main effect, $F_{(1, 108)} = 26.2897$, $p < 0.0001$). In comparing the response of the two genotypes to embryonic heat shock, a reduced model was used as there was no interaction found between the number of individuals in the vial and the genotype, temperature, or all three main effects (3-way ANOVA, genotype x individuals interaction, $F_{(1, 105)} = 1.7828$, $p = 0.1847$; temperature x individuals interaction, $F_{(1, 105)} = 2.2526$, $p = 0.1364$, genotype x temperature x individuals interaction, $F_{(1, 105)} = 0.0476$, $p = 0.8277$). There was no significant difference between the two genotypes in the positive relationship between walking speed and the number of individuals in a vial (Figure 5; Linear regression, CH $y = 0.05862 * x + 1.823$, $R^2 = 0.1691$, VT $y = 0.04566 * x + 1.372$, $R^2 = 0.517$, $F_{(1, 29)} = 0.7607$, $p = 0.09455$). Across the range of numbers of individuals in a vial, the Chiapas genotype walked at a

consistently faster pace than the Vermont genotype (Figure 5; Linear regression, $F_{(1, 30)} = 7.564$, $p = 0.0100$).

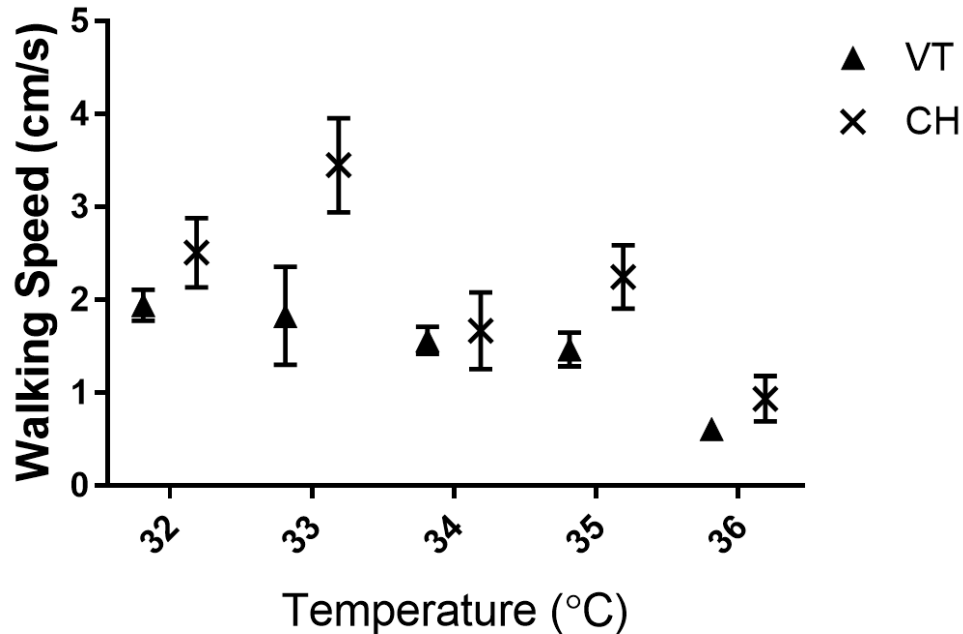


Figure 6: Average walking speed of adult *D. melanogaster* from Vermont and Chiapas after enduring an embryonic thermal shock at 5 temperatures: 32 °C, 33 °C, 34 °C, 35 °C and 36 °C. Bars represent the average walking speed and error bars represent the 95% confidence interval. As temperature increased, there was a pattern of decreasing walking speed in both genotypes (3-way ANOVA reduced model; genotype, $F_{(1, 108)} = 17.7395$, $p < 0.0001$, temperature, $F_{(1, 108)} = 8.7949$, $p < 0.0001$, genotype*temperature interaction, $F_{(1, 108)} = 3.9971$, $p < 0.0481$).

As temperature of the embryonic heat shock increased, the average adult walking speed decreased significantly (Figure 6; 3-way ANOVA reduced model, temperature main effects, $F_{(1, 108)} = 8.7949$, $p < 0.0001$). Flies from Vermont walked at a significantly slower speed compared to flies from Chiapas (Figure 6; 3-way ANOVA reduced model, genotype main effects, $F_{(1, 108)} = 17.7395$, $p < 0.0001$). There was a significant interaction between genotype and temperature in reference to walking speed (Figure 6; 3-way ANOVA reduced model, genotype*temperature interaction, $F_{(1, 108)} = 3.9971$, $p = 0.0481$). The Vermont genotype showed a consistent pattern of decreasing in walking speed as embryonic heat shock temperature increased. The Chiapas

genotype did not consistently decrease in average walking speed with each embryonic thermal shock temperature increase. At a 33 °C embryonic thermal shock temperature, the adult flies from the Chiapas genotype walked at a significantly faster pace compared to the Vermont genotype (Figure 6; 2-way ANOVA, Sidak post-hoc, $t = 7.085$, $p < 0.0001$). A similar difference in walking speed also occurred at 35 °C (Figure 6; 2-way ANOVA, Sidak post-hoc, $t = 3.394$, $p < 0.0049$).

Discussion

The goal of this research was to determine if there was an effect of embryonic thermal stress on larval and adult performance in *D. melanogaster*. To test this, I performed a 0-1 hour embryonic thermal shock over a temperature range from 32 °C to 36 °C in a tropical and a temperate genotype. I predicted that with increasing embryonic thermal shock survival rates across the embryonic, pupation, and adult life stages would decrease, and that the LT_{50} would be lower in the temperate Vermont genotype compared to the tropical Chiapas genotype. Additionally, I expected that as the embryonic thermal stress temperature increased there would be a plastic response in both genotypes resulting in a decreased pupation height and adult walking speed.

The survival rates of embryonic, larval and adult *D. melanogaster*, measured by proportion of individuals hatched, pupated and eclosed, respectively, decreased as the temperature of the embryonic thermal shock increased, supporting the hypothesis that with increasing thermal-stress temperatures, survival rates would decline. Previously Lockwood *et al.* (2018) had found that *D. melanogaster* from temperate environments had a lower LT_{50} compared to tropical species (Lockwood *et al.* 2018). When comparing the LT_{50} for embryos in the Vermont and Chiapas genotypes from this experiment, there was no significant difference between the two genotypes. However, the general trend of the tropical embryos surviving at a higher rate compared to the temperate genotype was present in these data. Although there was no significant difference in LT_{50}

specifically, previous research has found that natural selection can lead to embryos from Chiapas better being able to survive an early life heat stress compared to embryos from Vermont (Lockwood *et al* 2018).

After experiencing a thermal shock during the earlier embryonic stage, pupae showed a decrease in survival as the temperature of the previous thermal stress increased. The pupal LT_{50} for the Chiapas genotype was significantly higher than that of the Vermont genotype. Given previous trends found in embryonic success, it makes sense that pupal survival rates be higher in the tropical genotype. Similar to findings on embryonic thermal tolerance from Lockwood *et al.* (2018), previous research has shown that during the pupation stage, *Drosophila pseudoobscura*, another species of fruit fly from warmer climates, have a higher thermal tolerance compared to more temperate species (Coyne *et al.* 1983). Pupae in this experiment were reared at the same temperature before being exposed to an acute thermal shock. They found that the individuals from the warmer climate had higher survivorship compared to those from colder habitats, after the thermal stress (Coyne *et al.* 1983). Although tropical populations have a higher thermal tolerance compared to temperate species during the immobile pupa stage, they still suffered negative effects on survival after the embryonic thermal stress.

Adult survival rates (eclosion success), showed the same trend as I found for hatching and pupation success. The adult LT_{50} for the flies from Vermont was significantly lower than the LT_{50} for flies from Chiapas. Similar to the pupation success, despite being returned to a 25 °C environment, which is within normal temperature ranges for both Vermont and Chiapas, the flies were not able to recover and survive. A possible mechanism causing the decreased pupal and adult survivorship could be the acute heat stress resulted in decreased energy stores during the embryonic stage, and thus the individuals not being able to successfully pupate or eclose. Klepsatel

et al. (2016) showed that a 45-minute heat shock at 38 °C resulted in a depletion of fat stores in adult *D. melanogaster*, reducing the amount of reserved energy (Klepsatel *et al.* 2016). Although I did not test for this in our embryos, there is the potential for energy-store depletion, which could explain the decrease in survival during the later life stages.

Contrary to my predictions, there was no significant difference in pupation height between the temperate and tropical genotypes. Both genotypes showed a similar plastic response: as temperature of the embryonic heat shock increased, larval pupation height decreased. In the laboratory setting, decreased pupation height has been linked to increased mortality rates, especially for individuals who pupate on the food directly (Casares, P. *et al.*, 1997). While both genotypes showed similar effects of pupation height in response to the 0-1 hour thermal stress, the increased thermal tolerance in tropical flies does not act as a protective factor against negative phenotypic changes in pupation height resulting from the temperature stress. Decreased pupation heights have also been found to occur when larvae were exposed to a heat stress as they were preparing to pupate (Schnebel, & Grossfield. 1992). They hypothesized that lower pupation heights were caused by the individuals attempting to avoid the heat stress being experienced during pupation (Schnebel, & Grossfield. 1992).

In my research I did not directly account for any possible effects on pupation height due to larval density. Previous studies have found that there is a positive correlation between larval density and pupation height (Sokolowski, & Hansell, 1983). In the present study, survival rates, and thus population sizes, were higher at lower embryonic thermal shock temperatures. Given the higher larval densities at the lower shock temperatures, it is possible that this could in part have been driving the pattern of higher pupation heights at lower embryonic shock temperatures. In the studies conducted by Sokolowski and Hansell (1983), larval densities ranged from twenty to over

one hundred individuals per vial (Sokolowski, & Hansell, 1983). In my research the maximum population size was twenty individuals, making the effect of larval density on pupation height unclear. In future research, larval density should be controlled to negate the possibility of this effect.

There was a positive correlation between adult walking speed and population size. When comparing the two genotypes, I found that, in general, the Chiapas genotype walked faster than the Vermont genotype; however, there was no significant difference in the rate at which the average walking speed increased between the genotypes. In contrast to my results, Graves and Mueller (1993) found a negative correlation between population density and flight performance in *D. melanogaster*, however these experiments were run with populations between 32 and 200 individuals (Graves & Mueller 1993). Their findings were explained as a result of population density leading to decreased energy availability, however as the population densities in the present study were much smaller, resource availability likely did not have an effect on adult performance (Graves & Mueller 1993).

I found that there was a negative correlation between embryonic thermal shock temperature and the average adult walking speed, suggesting that the thermal shock resulted in a performance cost extending into adulthood. Over the temperature range, flies from Vermont walked at a slower pace compared to the flies from Chiapas. The difference in walking speed between the two genotypes was driven by the increased speed of the Chiapas genotype after the 33 °C and 35 °C embryonic thermal shocks. Outside of these two temperatures, the overall difference in walking speed between the two genotypes was insignificant. The difference in walking speeds between the two genotypes could be due to a difference in plastic response to the embryonic heat shock. Compared to the Chiapas embryos, the Vermont embryos may have suffered a more significant

effect due to their lower thermal tolerance (Lockwood et al., 2018). When comparing adult walking speed in flies that developed at 25 °C compared to 29 °C, Gilbert *et al.* found that the lower development temperature yielded higher adult walking speeds (Gibert *et al.*, 2001). Although these results were observed under conditions in which the flies were reared at a constant temperature instead of experiencing a brief thermal shock early in development, the pattern is consistent with my results, in that higher temperatures resulted in lower walking speeds (Gibert *et al.* 2001). However, it is unknown whether the same mechanism is resulting in the lowered walking speeds at higher temperatures in both studies.

When tropical and temperate genotypes were reared under different thermal conditions, the environment of origin had little effect on walking speed. The minor effect of genotype on walking speed found by Gibert et al. (2001) could explain the slight difference in walking speed between the Vermont and Chiapas genotypes found in my experiment (Gibert *et al.* 2001). When *D. melanogaster* lines were developed in a lab setting at 16 °C, 25 °C and 29°C for at least 100 generations, the differences in walking speed patterns when tested across a temperature range only varied minimally (Gilchrist et al., 1997). However, in that study, the line evolved at the lowest temperature walked at a faster rate compared to the other two lines, when tested at temperatures between 15 °C and 25 °C (Gilchrist et al., 1997). These results contrast to my findings as the flies from Chiapas, comparable to the flies reared at the warmer temperature, were faster than the flies from Vermont. Overall, there seems to be minimal effects, if any at all, of genotype on walking speed given the variation in results.

The embryonic thermal shock resulted in a lasting plastic response in the adult flies shown as a decrease in walking speed. Walking speed is a physiological trait used to assess performance in adult flies as it can be related to overall performance of an individual (Gibert *et al.*, 2001).

Specifically, walking speed in males has been found to correlate with reproductive success (Partridge *et al.*, 1987). I did not specifically measure whether there were any differences in walking speed in males and females. A future experiment could determine if there are any sex-specific effects of an embryonic thermal shock on walking speed and reproductive success.

In conclusion, I demonstrated that embryonic thermal shock resulted in a cost which extended across the life span. As temperature of the thermal shock increased, the phenotypic costs became more severe. Although the higher embryonic thermal tolerance previously found in tropical genotypes helped increase survival across development, both the flies from Chiapas and Vermont showed decrease in survival as shock temperature increased (Lockwood *et al.*, 2018). Despite divergence between tropical and temperate genotypes resulting in higher tropical embryonic LT₅₀, phenotypic costs that resulted from the heat shock were similar between both genotypes. This suggests that embryonic thermal tolerance, although leading to increased survival, does not protect against phenotypic changes resulting in decreased performance. As global temperatures are on the rise with increasing instances of temperature extremes (Meehl *et al.*, 2000), the effects of naturally occurring embryonic heat shock will affect tropical and temperate populations of *D. melanogaster*. Although Gilchrist *et al.* (1997) showed that evolutionary changes could occur over a shorter time frame, we cannot predict the outcomes of natural selection in wild populations, for example, as to whether or not *D. melanogaster* will adapt such that populations will not adapt to reduce the costs of acute thermal stress during early embryonic development.

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